Draft Assessment Report

Evaluation of Active Substances

Plant Protection Products

Prepared according to **Regulation (EC) 1107/2009**
as it applies in Great Britain

Pydiflumetofen (SYN545974)

Volume 3 – B.5 (AS)

Methods of Analysis

Great Britain

June 2023

Version History

When	What
October 2022	Initial DAR
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B.5. METHODS OF ANALYSIS

B.5.1. METHODS USED FOR THE GENERATION OF PRE-AUTHORISATION DATA

B.5.1.1. Methods for the analysis of the active substance as manufactured

Report:	KCA 4.1.1/2 [redacted] 2015
Evaluation status:	**New data, submitted for purpose of review**
Title:	Analytical Method SA-97/1
Testing facility:	Syngenta Crop Protection AG. CH-4002 Basie Switzerland
Report No.:	300029020
Document No:	VV-128116, SYN545974_10168
Guidelines:	Commission Regulation (EU) 283/2013 in accordance with Regulation (EC) 1107/2009 and SANCO/3030/99 rev.5
Deviations:	None
GLP:	No

Report:	KCA 4.1.1/3 [redacted] 2015a
Evaluation status:	**New data, submitted for purpose of review**
Title:	Validation of Analytical Method SA-97/1
Testing facility:	GLP Testing Facility WMU Syngenta Crop Protection Münchwilen AG Im Breitenloh 5 4333 Münchwilen, Switzerland
Report No.:	CHMU140778
Document No:	VV-410836, SYN545974_10148
Guidelines:	Commission Regulation (EU) 283/2013 in accordance with Regulation (EC) 1107/2009 and SANCO/3030/99 rev.5
Deviations:	None
GLP:	Yes

The analytical method SA-97/1 has been validated for the determination of pydiflumetofen in pydiflumetofen technical. The analytical method determines pydiflumetofen via HPLC with UV detection.

Preparation of Test Item Solutions

Weigh (to the nearest 0.1 mg) 65 - 75 mg test substance into a 100 ml volumetric flask. Add 90 ml of acetonitrile and place the flask in an ultrasonic apparatus for 2 minutes. Make up to the mark with acetonitrile at room temperature. Dilute the solution 1 + 9 with acetonitrile. The nominal concentration of test item is approximately 0.065-0.075 mg/mL (65-75 µg/mL).

Chromatographic Analysis

The following details are examples of suitable equipment and conditions for its use.

Table B.5.1.1-1: Analytical conditions for method SA-97/1

Chromatograph:	Agilent Technologies 1290 Infinity		
Detector:	Agilent Technologies 1260 infinity DAD detection wavelength: 230 nm (UV detection) layer thickness: 10 mm bandwidth: 8 nm slit: 4 nm output voltage: 1 V data acquisition rate: 20 Hz		
Chromatography Data system:	EZ Chrom Elite		
Column:	Zorbax SB-C18 RRHD particle size: 1.8 µm column length: 50 mm column i.d.: 3.0 mm		
Column temperature:	30°C		
Size of sample:	5 µl of reference / test solution		
Flow rate:	1..2 ml/minute		
Duration of chromatography:	approx. 2.5 minutes		
Gradient program:	time [minutes]	0.1 % v/v aqueous phosphoric acid* [%]	acetonitrile [%]
	0	60	40
	2.0	10	90
	2.5	10	90
	2.6	60	40
	3.5	60	40
Retention time:	Approx. 1.86 mins		

*phosphoric acid ≥ 85 %

Validation of Method SA-97/1

Precision/Repeatability

12 determinations (6 weightings, double injection each) of pydiflumetofen technical (batch no.: SMU2EP12007) were analysed using the method described above, and the %RSD was calculated. The relative standard deviation obtained was within the guideline requirements of a HORRAT (Hr) of ≤ 1

Linearity

Linearity was demonstrated by the analysis of six standards, in duplicate, of increasing concentration. The range of standard concentrations used was 36 – 106 µg/mL. Assuming a nominal concentration of 70 µg/mL in the test item solutions, the linear range is equivalent to approx. 50 – 150 % of the nominal concentration in test item solutions and approx. 500 – 1500 g/kg in TGAI. The analytical calibration extends over the lowest and highest nominal concentration of the analyte in relevant analytical solutions with an appropriate range of ±at least 20%. The response was linear with a correlation coefficient (r) of 0.99999. This linear range is sufficient to cover the 5-batch analysis data for pydiflumetofen.

Specificity and Interference

Specificity was demonstrated by retention time match with a reference standard, as shown in 5-batch analysis study reports submitted (e.g., study CHMU170048). Chromatograms for pydiflumetofen technical material and a chromatogram of a mixture of by-products present in technical material at levels equal or above 0.1% w/w showed no interference between the active ingredient and the by-products (study CHMU140778).

Pydiflumetofen technical is a racemic mixture, and the applicant has provided a statement (See section A.9 in the Volume 4 at HSE Internal Reference: W 002007945) to demonstrate that the synthetic steps do not promote the formation of a specific enantiomer. Therefore, an enantiomer-selective method is not required.

Conclusion

The method is acceptably validated in accordance with SANCO/3030/99 rev.5 and is suitable for the determination of pydiflumetofen in the technical material.

Table B.5.1.1-2: Analytical validation data for the determination of the active in the technical material

Matrix	Analyte	LOQ (%w/w)	Recovery	Repeatability % RSD (n)	Linearity	Specificity
pydiflumetofen technical	pydiflumetofen	Not required.		0.10 @ 98.17 %w/w (n=6x2) Modified Horwitz = 1.34 Hr = 0.07	36 – 106 µg/mL (approx. 500 – 1500 g/kg in TGAI) (n=6x2) Y= 0.992*X + 0.590 r = 0.99999	Retention time match to reference standard. No significant interfering peaks observed.

B.5.1.2. Methods for risk assessment

B.5.1.2.1. Methods in soil, water, sediment, air, and any additional matrices used in support of environmental fate studies

Method GRM061.04A

Fate studies supported by method GRM061.04A:

Data point	Author(s)	Year	Title Company Report No.
KCA1 7.1.2.2.1-16	[redacted]	2015	SYN545974 – Bare Soil Plot Soil Dissipation Study in Italy in 2013-2015 Report number: S13-02241
KCA1 7.1.2.2.1-12	[redacted]	2015a	SYN545974 – Bare Soil Plot Soil Dissipation Study in Northern France in 2013-2015 Report number: S13-02238
KCA1 7.1.2.2.1-13	[redacted]	2015b	SYN545974 – Bare Soil Plot Soil Dissipation Study in Southern France in 2013-2015 Report number: S13-02239
KCA1 7.1.2.2.1-14	[redacted]	2015c	SYN545974 – Bare Soil Plot Soil Dissipation Study in Spain in 2013-2015 Report number: S13-02240
KCA1 7.1.2.2.1-11	[redacted]	2015d	SYN545974 – Bare Soil Plot Soil Dissipation Study in Germany in 2013-2015 Report number: S13-02237
KCA1 7.1.2.2.1-15	[redacted]	2015e	SYN545974 – Bare Soil Plot Soil Dissipation Study in UK in 2013-2015 Report number: S13-02236
KCA1 7.1.2.2.1-02	[redacted]	2019b	SYN545974 – Soil Dissipation Study in Germany in 2016-2017 Report number: S16-01816
KCA1 7.1.2.2.1-06	[redacted]	2020b	SYN545974 – Soil Dissipation Study in Northern France in 2016-2017 Report number: S16-02708
KCA1 7.1.2.2.1-08	[redacted]	2020d	SYN545974 – Soil Dissipation Study in Southern France in 2016-2017 Report number: S16-02711
KCA1 7.1.2.2.1-07	[redacted]	2020e	SYN545974 – Soil Dissipation Study in Portugal in 2016-2017 Report number: S16-02712
KCA1 7.1.2.2.1-09	[redacted]	2020	SYN545974 - Additional Soil Sampling and Analysis at Five Historical Field Dissipation Sites in Northern Germany, Northern France, and UK in 2020. Report Number S20-06491

Method GRM061.04A has been used for data generation purposes in support of fate studies to determine residues of pydiflumetofen in soil. This method is also proposed as a method for post authorisation control and is discussed in detail in Section B.5.2.3. Procedural recoveries from the fate studies are reported in Table B.5.1.2.1-1. Additional validation data has also been provided in studies S13-01816, S16-02708, S16-02711 and S16-02712 which is reported in Table B.5.1.2.1-2. The mean recoveries are within the acceptable range (70-110%) and the %RSD is <20%.

Table B.5.1.2.1-1: Procedural recoveries of pydiflumetofen in soil dissipation studies

Study	Matrix	Analyte	Recovery fortification level (µg/kg)	Recoveries % range (mean)	Repeatability % RSD (n)
S13-02241 KCA 7.1.2.2.1-16	Soil	pydiflumetofen	0.5	83 – 108 (97)	7 (25)
			5.0	93 – 109 (101)	6 (7)
			2000	102 – 103 (102)	1 (3)
			Overall	83 – 109 (98)	7 (35)
S13-02238 KCA 7.1.2.2.1-12	Soil	pydiflumetofen	0.5	88 – 105 (97)	5 (22)
			5.0	77 – 100 (92)	11 (7)
			2000	81 – 88 (86)	4 (4)
			Overall	77 – 105 (94)	8 (33)
S13-02239 KCA 7.1.2.2.1-13	Soil	pydiflumetofen	0.5	70 – 109 (95)	10 (18)
			5.0	90 – 102 (96)	4 (6)
			2000	91 – 102 (96)	5 (4)
			Overall	70 – 109 (95)	8 (28)
S13-02240 KCA 7.1.2.2.1-14	Soil	pydiflumetofen	0.5	86 – 108 (97)	6 (18)
			5.0	97 – 118 (105)	8 (5)
			2000	101 – 106 (103)	2 (4)
			Overall	86 – 118 (99)	7 (27)
S13-02237 KCA 7.1.2.2.1-11	Soil	pydiflumetofen	0.5	90 – 103 (95)	4 (9)
			5.0	89 – 94 (92)	3 (3)
			2000	96 – 100 (98)	2 (4)
			Overall	89 – 103 (95)	4 (16)
S13-02236 KCA 7.1.2.2.1-15	Soil	pydiflumetofen	0.5	69 – 102 (80)	12 (17)
			5.0	83 – 94 (88)	5 (5)
			2000	68 – 73 (70)	3 (4)
			Overall	68 – 102 (80)	12 (26)
S13-01816 KCA 7.1.2.2.1-02	Soil	pydiflumetofen	0.5	99	- (1)
			5.0	89 – 100 (94)	4 (5)
			5000	96 – 97 (96)	- (2)

Study	Matrix	Analyte	Recovery fortification level (µg/kg)	Recoveries % range (mean)	Repeatability % RSD (n)
			Overall	89 – 100 (96)	4 (8)
S16-02708 KCA 7.1.2.2.1-06	Soil	pydiflumetofen	0.5	108	- (1)
			5.0	91 – 105 (97)	7 (5)
			5000	99 (99)	- (2)
			Overall	91 – 108 (99)	6 (8)
S16-02711 KCA 7.1.2.2.1-08	Soil	pydiflumetofen	0.5	106	- (1)
			5.0	84 – 104 (98)	8 (5)
			5000	94 - 96 (95)	- (2)
			Overall	84 – 106 (98)	7 (8)
S16-02712 KCA 7.1.2.2.1-07	Soil	pydiflumetofen	0.5	103	- (1)
			5.0	75 – 98 (91)	11 (5)
			5000	95 - 98 (97)	- (2)
			Overall	75 – 103 (94)	9 (8)
S20-06491 KCA 7.1.2.2.1-09	Soil	pydiflumetofen	0.5	79 – 113 (101)	9 (12)
			5	89 – 105 (97)	8 (6)
			Overall	79 – 113 (100)	9 (18)

Table B.5.1.2.1-2: Additional validation data of SNY545974 from the soil dissipation studies

Study	Matrix	Analyte	Recovery fortification level (µg/kg)	Recoveries % range (mean)	Repeatability % RSD (n)
S13-01816 KCA 7.1.2.2.1-02	Soil	pydiflumetofen	0.5	77 – 105 (96)	11 (5)
			6000	89 – 99 (94)	4 (5)
			Overall	77 – 105 (95)	9 (10)
S16-02708 KCA 7.1.2.2.1-06	Soil	pydiflumetofen	0.5	75 – 101 (95)	12 (5)
			6000	94 – 99 (96)	2 (5)
			Overall	75 – 101 (95)	8 (10)
S16-02711 KCA 7.1.2.2.1-08	Soil	pydiflumetofen	0.5	89 – 105 (96)	7 (5)
			6000	92 – 99 (95)	2 (5)
			Overall	89 – 105 (96)	5 (10)

Study	Matrix	Analyte	Recovery fortification level (µg/kg)	Recoveries % range (mean)	Repeatability % RSD (n)
S16-02712 KCA 7.1.2.2.1-07	Soil	pydiflumetofen	0.5	88 – 110 (97)	9 (5)
			6000	93 – 97 (96)	2 (5)
			Overall	88 – 110 (96)	6 (10)

B.5.1.2.2. Methods in soil, water and any additional matrices used in support of efficacy studies

No methods of analysis to support efficacy studies for the active substance have been submitted.

B.5.1.2.3. Methods in feed, body fluids and tissues, air and any additional matrices used in support of toxicological studies

Study overview:

Data point	Author(s)	Year	Title Company Report No.	Conclusion
KCA1 4.1.2/03	[redacted]	2014	SYN545974 - Supplementary Validation of the Assay for the Determination of SYN545974 in VRF-1 Fine Ground Rodent Diet Report number: BFI0231	Method code: BFI012LC Acceptable method LOQ: 50 mg/kg
KCA1 4.1.2/04	[redacted]	2014a	SYN545974 - Validation of the Formulation Procedure for SYN545974 in VRF-1 Fine Ground Rodent Diet and Assessment of Formulation Stability Report number: BFI0232	
KCA1 4.1.2/10	[redacted]	2013	SYN545974 - Validation of the Assay for the Determination of SYN545974 in R&M No. 3 Fine Ground Diet Report number: BFI0111	
KCA1 4.1.2/06	[redacted]	2012	SYN545974 - Validation of the Assay for the Determination of SYN545974 in 1 % w/v Aqueous Carboxymethylcellulose Report number: BFI0048	Method code: BFI0007LC Acceptable method LOQ: 1 mg/mL
KCA1 4.1.2/07	[redacted]	2012	SYN545974 - Validation of the Formulation Procedure of SYN545974 in 1 % w/v Aqueous Carboxymethylcellulose and Assessment of Formulation Stability Report number: BFI0049	
KCA1 4.1.2/08	[redacted]	~~2021~~2020	CA6519 - Validation of the Formulation Procedure for CA6519 in Corn Oil and Assessment of Formulation Stability Report number: BFI1026 Document number: VV-884148	Method code: BFI095LC Acceptable method LOQ: 5 mg/mL
KCA1 4.1.2/09	[redacted]	~~2021~~2020	CA6519 - Validation of the Assay for the Determination of CA6519 in Corn Oil Report number: BFI1026	
KCA1 4.1.2/01	[redacted]	2021	CA6519 - Validation of an Analytical Method Using HPLC Report number: AG23LM.GTCHEM.BTL; tk0527779	Acceptable method LOQ: 10 µg/mL
KCA1 4.1.2/22	[redacted]	2012	SYN545974 - Validation of Methodologies for the Analysis of SYN545974 in Dietary Formulations Report number: 32657	Method code: No. 2794 Acceptable method LOQ: 1 mg/mL
KCA1 4.1.2/23	[redacted]	2013	SYN545974-Validation of Methodologies for the Analysis of SYN545974 in RM1 Dietary Formulations Report number: 33720	Method code: No. 2962 Acceptable method LOQ: 1 mg/mL

Data point	Author(s)	Year	Title Company Report No.	Conclusion
KCA1 4.1.2/14	[redacted]	2012	SYN545974 - Validation of an Analytical Method for the Determination of SYN545974 in Rat and Mouse Blood by LC-MS/MS Report number: 33236	Method code: AP.2009.02 Acceptable method LOQ: 12.5 ng/mL
KCA1 4.1.2/05	[redacted]	2013	SYN545974 - Partial Validation of a Bioanalytical Method for the Determination of SYN545974 in Rabbit Blood: Water Report number: BFI0127	Method code: BFI013MS Acceptable method LOQ: 5 ng/mL
KCA1 4.1.2/33	[redacted]	2018	SYN508272 - Validation of a Bioanalytical Method for the Determination of SYN508272 in Rat Blood: Water [1:1 (v/v)] by LC-MS/MS Report No 0057/002	Method code: 0001/052 Acceptable method LOQ: 60 ng/ml (fully validated) 20 ng/mL (fit for purpose)
KCA1 4.1.2/18	[redacted]	2021	2,4,6 -trichlorophenol The Validation of a Bioanalytical Method for the Determination of 2,4,6 - trichlorophenol in Rat Whole Blood (K_2EDTA) by LC-MS/MS Report number: (0029/0027) BFI1034	Method code: 0001/186 Acceptable method LOQ: 60 ng/ml (fully validated) 20 ng/mL (fit for purpose)

Report:	**KCA1 4.1.2/03 [redacted] (2014)**
Title	SYN545974 - Supplementary Validation of the Assay for the determination of SYN545974 in VRF-1 Fine Ground Rodent Diet Report number: BFI0231 Document number: VV-28419, SYN545974_10103
Guidelines:	SANCO/3029/99 rev. 4
GLP:	Yes
Deviations	N/A
Previous evaluation:	None

Report:	**KCA1 4.1.2/04 [redacted] (2014)**
Title	SYN545974 - Validation of the Formulation Procedure for SYN545974 in VRF-1 Fine Ground Rodent Diet and Assessment of Formulation Stability Report Number: BFI0232 Document Number: VV-410268, SYN545974_10109
Guidelines:	SANCO/3029/99 rev. 4
GLP:	Yes
Deviations	N/A
Previous evaluation:	None

Report:	**KCA1 4.1.2/10 [redacted], [redacted] (2013)**
Title	SYN545974 – Validation of the Assay for the Determination of SYN545974 in R&M No. 3 Fine Ground Diet Report No. BFI0111 Document No. VV-404895, SYN545974_10079
Guidelines:	SANCO/3029/99 rev. 4
GLP:	Yes
Deviations	N/A
Previous evaluation:	None

The studies used the same HPLC-UV method (BFI012LC) to determine the concentration of pydiflumetofen in test samples.

Sample preparation:

To a Duran bottle, accurately weigh 10 g of test item. Add 50 mL of acetonitrile, mechanically shake for approximately 30 minutes then filter using a Whatman autovial. The dilute diet extract is then further diluted with acetonitrile to achieve a final injection concentration of approximately 0.5 µg/mL pydiflumetofen.

Samples are analysed by HPLC-UV under the conditions below.

HPLC-UV conditions:

Column:	Waters X-Bridge C18, 50 mm x 3.0 mm 3.5 µm
Detector:	UV @ 260 nm
Flow rate:	0.75 mL/min
Column temperature:	45 °C
Autosampler temperature:	25 °C
Injection volume:	10 µL
Run time:	8 minutes
Mobile phase A:	1:1 (v/v) formic acid: UHP water
Mobile phase B:	0.1% formic acid in 1:1 (v/v) acetonitrile: water
Needle wash:	10% acetonitrile
Gradient:	

Time (minutes)	0	2.0	2.1	5.0	5.1	8
% mobile phase A	35	35	5	5	35	35
% mobile phase B	65	65	95	95	65	65

A summary of the method validation data is given in Table B.5.1.2.3-1.

B.5.1.2.3-1: Summary of method validation data for determination of pydiflumetofen residues in fine ground rodent diet

Study	**Matrix**	**Analyte**	**LOQ (µg/mL)**	**Recovery**			**Linearity**
				fortification level (µg/mL) *	**% range (mean)**	**Repeatability % RSD (n)**	
KCA1 4.1.2/03 KCA1 4.1.2/04 KCA1 4.1.2/10	Fine ground rodent diet	pydiflumetofen	0.5	10	93 – 115 (107)	5.6 (21)	0.25 – 0.75 µg/mL (n = 5) y = 4845x + 28.7 R = 0.9982
				200	86 – 104 (97)	4.8 (21)	
				2000	98 – 106 (101)	2.3 (21)	
				Overall	93 – 115 (101)	4.8 (18)	

* Dilutions were performed as necessary to be within the linear calibration range

Specificity

Chromatograms were presented for the low level and high-level calibration standards, matrix blank and fortified samples at 50, 1000 and 10000 ppm respectively. No significant interference was observed at the retention time of interest and the retention time of pydiflumetofen matched with reference standards.

Linearity

Linearity was demonstrated by the analysis of six standards of increasing concentration in triplicate. The range of standard concentrations used was 0.25 to 0.75 µg/mL. Samples were diluted to be within the linear calibration range. The response was linear with a coefficient of determination of at least 0.9982. It is noted samples are diluted to within the linear range.

Accuracy

For each of the studies, samples were fortified at 50, 1000 and 10000 ppm. It is noted each of the fortification levels were diluted to within the linear range. 21 determinations were made at each fortification level (Combined from the three studies - 6 determinations in KCA1 4.1.2/03, 6 determinations in KCA1 4.1.2/04 and 9 determinations in KCA1 4.1.2/10). The mean recoveries were within the acceptable range (70-110%).

Precision

Precision was determined from the accuracy recovery data for pydiflumetofen. The %RSD was ≤20% for each of the three fortification levels. 21 determinations were made at each fortification level (Combined from the three studies - 6 determinations in KCA1 4.1.2/03, 6 determinations in KCA1 4.1.2/04 and 9 determinations in KCA1 4.1.2/10).

Matrix effects

Matrix effects were not specifically addressed in each of the three studies.

LOQ

According to SANCO/3029/99 rev. 4, the LOQ is defined as the lowest concentration tested, at which an acceptable mean recovery (70-110%) with an acceptable RSD (≤20%) is obtained. Therefore, the LOQ of the method is 50 mg/kg in fine ground rodent diet.

Conclusion

The method is acceptably validated in accordance with SANCO/3029/99 rev. 4 for the determination of pydiflumetofen in fine ground rodent diet.

Report:	**KCA1 4.1.2/06 [redacted], [redacted] (2012)**
Title	SYN545974 - Validation of the Assay for the Determination of SYN545974 in 1 % w/v Aqueous Carboxymethylcellulose Report No. BFI0048 Document No. VV-402591, SYN545974_10019
Guidelines:	SANCO/3029/99 rev. 4
GLP:	Yes
Deviations	N/A
Previous evaluation:	None

Report:	**KCA1 4.1.2/07 [redacted], [redacted] (2012)**
Title	SYN545974 - Validation of the Formulation Procedure of SYN545974 in 1 % w/v Aqueous Carboxymethylcellulose and Assessment of Formulation Stability Report No. BFI0049 Document No. VV-402593, SYN545974_10020
Guidelines:	SANCO/3029/99 rev. 4
GLP:	Yes
Deviations	N/A
Previous evaluation:	None

The studies used the same HPLC-UV method (BFI007LC) to determine the concentration of pydiflumetofen in test samples:

Sample preparation:

Measure an appropriate quantity of test item formulation samples into volumetric flasks, make up to volume with acetonitrile and mix well. If necessary, further dilute with acetonitrile to achieve final injection concentration of approximately 10 µg/mL pydiflumetofen. Samples are analysed by HPLC-UV under the conditions below.

HPLC-UV conditions:

Column:	Waters XBridge C18, 150 mm x 4.6 mm 3.5 µm
Detector:	UV @ 260 nm
Flow rate:	1 mL/minute
Column temperature	45 °C
Injection volume:	10 µL
Needle wash:	10% acetonitrile aq.
Mobile phase:	0.2% formic acid: acetonitrile 40:60 (isocratic)
Run time:	10 minutes
Retention time:	Approximately 8.66 min.

A summary of the method validation data is given in Table B.5.1.2.3-2.

Table B.5.1.2.3-2: Summary of method validation data for determination of pydiflumetofen in 1 % w/v aqueous carboxymethylcellulose

Study	Analyte	LOQ (mg/mL)	Recovery fortification level (mg/mL) *	Recoveries % range (mean)	Repeatability % RSD (n)	Linearity
KCA1 4.1.2/06 KCA1 4.1.2/07	pydiflumetofen	1	1	87 – 100 (100)	5.0 (24)	5 – 15 µg/mL (n = 5) y = 60.08x+1.37 R = 0.9974
			20	95 – 102 (98)	2.4 (15)	
			200	96 – 103 (99)	1.4 (15)	
			Overall	87 – 103 (97)	4.1 (54)	

* Dilutions were performed as necessary to be within the linear calibration range

Specificity

Chromatograms were presented for the solvent blank and fortified samples at 1, 20 and 200 mg/mL respectively. No significant interference was observed at the retention time of interest and the retention time of pydiflumetofen matched with reference standards.

Linearity

Linearity was demonstrated by the analysis of five solvent standards of increasing concentration in triplicate. The range of standard concentrations used was 0.5-15 µg/mL. The response was linear with a coefficient of determination of at least 0.9974. It is noted samples are diluted to within the linear range.

Accuracy

The samples were fortified at 1, 20 and 200 mg/mL 21 determinations were made at 1 mg/mL and 15 determinations were made 20 and 200 mg/mL respectively (these are combined across KCA1 4.1.2/06 and KCA1 4.1.2/07). The fortification levels were diluted as necessary to be in the calibration range. The mean recoveries were within the acceptable range (70-110%).

Precision

Precision was determined from the accuracy recovery data for pydiflumetofen. The %RSD was ≤20% for each fortification level. 21 determinations were made at 1 mg/mL and 15 determinations were made 20 and 200 mg/mL respectively (these are combined across KCA1 4.1.2/06 and KCA1 4.1.2/07).

Matrix effects

Matrix effects were not investigated and are missing.

LOQ

The validated LOQ of the method is 1 mg/mL, this is the lowest concentration tested, at which an acceptable mean recovery (70-110%) with an acceptable RSD (≤20%) is obtained.

Conclusion

The method is fit for purpose in accordance with SANCO/3029/99 rev. 4 for the determination of pydiflumetofen in 1% w/v aqueous carboxymethylcellulose. It should be noted for KCA1 4.1.2/06 and KCA1 4.1.2/07 matrix effects are not investigated. However, due to the matrix being mostly water (1% w/v aqueous carboxymethylcellulose) it is unlikely that matrix effects would be significant in this matrix.

Report:	**KCA1 4.1.2/08 [redacted] (~~2021~~2020)**
Title	CA6519 - Validation of the Formulation Procedure for CA6519 in Corn Oil and Assessment of Formulation Stability Report number: BFI1026 Document No. VV-884148
Guidelines:	SANCO/3029/99 rev. 4
GLP:	Yes
Deviations	N/A
Previous evaluation:	None

Report:	**KCA1 4.1.2/09 [redacted] (~~2021~~2020)**
Title	CA6519 - Validation of the Assay for the Determination of CA6519 in Corn Oil Report No. BFI1024 Document No. VV-884147
Guidelines:	SANCO/3029/99 rev. 4
GLP:	Yes
Deviations	N/A
Previous evaluation:	None

The studies use the same HPLC-UV method (BFI095LC) to determine the concentration of CA6519 (2.4.6-trichlorophenol) in test samples:

Sample preparation (BFI095LC):

A required quantity of test item formulation is dissolved into a volumetric flask using acetonitrile. Complete dissolution was achieved (sonicate if necessary). Acetonitrile is then added up to the mark of the volumetric flask and mixed well. Further dilutions were made to achieve final injection concentrations of approximately 100 µg/mL CA6519.

Samples are analysed by HPLC-UV under the conditions below.

HPLC-UV conditions:

LC column packing:	Waters Xbridge C18 3.5 µm, 50 mm x 3.0 mm
Detector:	UV @ 218 nm
LC flow rate:	0.75 mL/min
LC column temperature:	40°C
Mobile phase	1% formic acid in a 40:60 acetonitrile: water solution (v/v)
Injection mode:	5 µL
Run time:	5 min.
Pump mode:	Isocratic
Retention time:	Approx. 2.5 min.

A summary of the method validation data is given in Table B.5.1.2.3-3.

Table B.5.1.2.3-3: Summary of method validation data for determination of CA6519 in corn oil

Study	**Matrix**	**Analyte**	**LOQ (mg/mL)**	**Recovery fortification level* (mg/mL)**	**Recoveries % range (mean)**	**Repeatability % RSD (n)**	**Linearity**
KCA1 4.1.2/08 KCA1 4.1.2/09	corn oil	CA6519	5	5	99 - 107 (102.8)	3.1 (9)	52 – 160 µg/mL (n = 6) y = 20740 x + 24277 R = 0.9995
				200	101 – 107 (103.4)	2.1 (9)	
				Overall	99 – 107 (103.1)	2.6 (18)	

* Dilutions were performed as necessary to be within the linear calibration range

Specificity

Chromatograms were presented for the low level and high-level calibration standards, solvent blank, and fortified samples at 5 and 200 mg/mL (diluted into the calibration range). No significant interference was observed at the retention time of interest and the retention time of CA6519 matched with reference standards.

Linearity

Linearity was demonstrated by the analysis of six standards of increasing concentration in triplicate. The range of standard concentrations used was 52-160 µg/mL. The response was linear with a coefficient of determination of at least 0.9995. It is noted samples are diluted to be within the linear range.

Accuracy

Corn oil samples were fortified at 5 and 200 mg/mL. 9 determinations were made at each fortification level (3 in KCA1 4.1.2/08 and 6 in KCA1 4.1.2/09). The mean recoveries were within the acceptable range (70-110%).

Precision

Precision was determined from the accuracy recovery data for CA6519. The %RSD was ≤20% for each fortification level. 9 determinations were made for each fortification level (3 in KCA1 4.1.2/08 and 6 in KCA1 4.1.2/09).

Matrix effects

Matrix effects were not specifically addressed.

LOQ

The validated LOQ of the method is 5 mg/mL, this is the lowest concentration tested, at which an acceptable mean recovery (70-110%) with an acceptable RSD (≤20%) is obtained.

Conclusion

The method is acceptably validated in accordance with SANCO/3029/99 rev. 4 for the determination of CA6519 in corn oil.

Report:	**KCA1 4.1.2/01 [redacted] (2021)**
Title	CA6519 - Validation of an Analytical Method Using HPLC Report number: AG23LM.GTCHEM.BTL Document No. VV-898124
Guidelines:	SANCO/3029/99 rev. 4
GLP:	Yes
Deviations	N/A
Previous evaluation:	None

The following studies rely on the method to determine the concentration of CA6519 (2,4,6-TCP) in test samples:

KCA1 5.8.1-04_[redacted]_2021-04-09

KCA1 5.8.1-03_[redacted]._2021-04-07

Sample preparation:

Method validation part 1:

The test samples were prepared from a quality control formulation 1 and 2 (QCF-1 and QCF-2), with concentrations of 200 and 2.0 mg/mL CA6519 in corn oil respectively. These were diluted to the same final test sample concentration through the following dilutions:

- 0.1 mL of QCF-1 (200 mg/mL) is dissolved into 50 mL of acetonitrile giving a solution concentration of 400.0 µg/mL. 0.5 mL of this solution is combined with 0.499 mL of corn oil and made up to 10 mL with acetonitrile. This gives a final test sample concentration of 20 µg/mL.

- 10 mL of QCF-2 (2.0 mg/mL) is dissolved into 50 mL of acetonitrile giving a solution concentration of 400.0 µg/mL. 0.5 mL of this solution is combined with 0.400 mL of corn oil and made up to 10 mL with acetonitrile. This gives a final test sample concentration of 20 µg/mL

Method validation part 2:

Method validation part 2 was started following successful completion of part one. The method validation part two was not conducted by the technician that completed part one and instead was completed by a technician not involved in part one. Fresh standards, stocks, samples, diluent, and mobile phase were prepared by the second technician. No standards, stocks, samples, diluent, and mobile phase were reused from the method validation part one. Solvent standards were prepared as per the method validation part one. QC samples were prepared as per the method validation part one.

Samples are analysed by HPLC-UV under the conditions below.

HPLC-UV conditions:

Chromatographic system:	Agilent 1200 HPLC
Detector:	UV 210 nm[1], bandwidth 2 nm, Reference off
Software:	Agilent ChemStation with Open Lab CDS
(MPA) Mobile phase A:	0.1% acetic acid in deionized water
(MPB) Mobile phase B:	0.1% acetic acid in acetonitrile
Column:	ACE Excel 3, Super C18, 75 mm x 3 mm, with an inline filter
Column temperature:	40 °C
Autosampler temperature:	Ambient
Injection volume:	5 µL
Needle wash:	80:20 (v/v) acetonitrile: deionized water
Flow rate:	0.6 mL/min
Retention time:	~3.782 minutes
Run time:	13 minutes
Elution mode:	Gradient (see below)

Gradient:

Time (minutes)	0	1	7	10	10.1	13
% MPA	50	50	0	0	50	50
% MPB	50	50	100	100	50	50

[1]A UV scan was performed to optimize the wavelength used in the method validation.
[1]The method was also analysed using wavelengths of 200, 205, 220 and 296 nm, but was not evaluated at those wavelengths.

A summary of the method validation data is given in Table B.5.1.2.3-4.

Table B.5.1.2.3-4 Summary of method validation data for determination of CA6519 in corn oil

Study	Matrix	Analyte	LOQ (µg/mL)	Recovery fortification level (µg/mL)	Recoveries % range (mean)	Repeatability % RSD (n)	Linearity
KCA1 4.1.2/01 Method validation 1	Corn Oil	CA6519	20	20	96.4 – 101.6 (99)	3.71 (2)	10 – 60 µg/mL (100-600 mg/mL QCF-1 and 1-6 mg/mL QCF-2) (n = 6) y = 67.2x + 44.2 R = 0.9998
KCA1 4.1.2/01 Method validation 2	Corn Oil	CA6519	20	20	99.9-100 (100)	0.07 (2)	10 – 60 µg/mL (100-600 mg/mL QCF-1 and 1-6 mg/mL QCF-2) (n = 6) y = 67.2x + 31.7 R = 0.9999

Specificity

Chromatograms were presented for standards, matrix blank, matrix standards and fortified samples at 20 µg/mL. No significant interference was observed at the retention time of interest and the retention time of CA6519 matched with reference standards.

Linearity

Linearity was demonstrated by the analysis of six matrix matched standards of increasing concentration. The range of standard concentrations used was 10-60 µg/mL (equivalent to 100-600 mg/mL QCF-1 and 1-6 mg/mL QCF-2). The response was linear with a coefficient of determination of at least 0.9998 across the two method validations. It is noted samples are diluted to within the linear range.

Accuracy

Corn oil samples were fortified with CA6519 at 20 µg/mL and analysed concurrently with the samples. These fortification levels are appropriate to the concentrations used in the toxicology studies. Two determinations were made at the fortification level for each method validation. The mean recoveries were within the acceptable range (70-110%). Method validation was conducted at the same time as analysis of the samples from the residue trials, (i.e., validation recoveries are procedural recoveries). Matrix standards were tested at 10 and 60 µg/mL in triplicate with recoveries in an acceptable range. These provide more confidence in the accuracy of the method.

Stability

CA6519 in corn oil was found to be stable for at least 48 hours when stored at room temperature and for at least 11 days when stored at 2-8ºC.

Precision

Precision was determined from the accuracy recovery data for CA6519. The %RSD was ≤20% for the fortification level. However, for this study only two determinations were reported. In accordance with SANCO/3029/99 rev. 4, a minimum of five determinations are required at each fortification level.

Matrix effects

Matrix effects were not specifically addressed. However, matrix matched standards were used for calibration.

LOQ

The LOQ stated by the applicant is 10 µg/mL, determined from the lowest concentration matrix standard with a recovery of 90-110% and an RSD of ≤ 5%. According to SANCO/3029/99 rev. 4, the LOQ is defined as the lowest concentration tested, at which an acceptable mean recovery (70-110%) with an acceptable RSD (≤20%) is obtained. Recovery data were only provided at 20 µg/mL. As the sample preparation involves dissolution of samples in acetonitrile an estimate of recovery and precision can be made from the matric matched standards. The LOQ of 10 µg/mL can be consider supported

Conclusion

The method is not fully validated in accordance with SANCO/3029/99 rev. 4 for the determination of CA6519 in corn oil as there is an insufficient number of recovery determinations at the fortification level. However, further recovery data is available in matrix standards with acceptable recovery and precision. Therefore, the method can be considered fit for purpose.

Report:	**KCA1 4.1.2/22 [redacted], [redacted] and [redacted] (2012)**
Title	SYN545974 - Validation of Methodologies for the Analysis of SYN545974 in Dietary Formulations Report No. 32657 Document No. VV-400860, SYN545974_10006
Guidelines:	SANCO/3029/99 rev. 4
GLP:	Yes
Deviations	N/A
Previous evaluation:	None

Sample preparation (analytical method No. 2794):

Low level (ca. 10 ppm):

Accurately weigh ca. 10 g of diet formulation into an 8 oz. amber glass jar. Extract in 100 mL of 10 µg/mL nifedipine (as internal standard) in methanol by shaking at 300 rpm for 30 min on an orbital shaker.

High level (ca. 16000 ppm):

Accurately weigh ca. 2 g of diet formulation into an 8 oz. amber glass jar. Extract in 200 mL of 10 µg/mL nifedipine (as internal standard) in methanol by shaking at 300 rpm for 30 min on an orbital shaker. Further dilute this aliquot 0.62 to 10 mL in 10 µg/mL nifedipine in methanol.

Samples are analysed by HPLC-UV under the conditions below.

HPLC-UV conditions:

Chromatographic system: Phenomenex Kinetex C_{18}, 150 x 4.6 mm, 2.6 µm
Mobile phase A: 75/25 (v/v) Milli-Q H_2O/ Acetonitrile +0.1% formic acid
Mobile phase B: 20/80 (v/v) Milli-Q H_2O/ Acetonitrile +0.1% formic acid
Flow rate: 1 mL/minute
UV detector: UV @ 260 nm
Gradient:

Time (minute)	% A	% B	Curve
0	100	0	-
30	0	100	6
33	100	0	1

Column temperature: 45 °C
Autosampler temperature: Ambient
Retention time: Approximately 20.3 minutes

A summary of the method validation data is given in Table B.5.1.2.3-5.

Table B.5.1.2.3-5: Summary of method validation data for determination of pydiflumetofen residues in rodent dietary formulation

Study	Matrix	Analyte	LOQ (mg/mL)	Recovery fortification level (ppm)	Recoveries % range (mean)	Repeatability % RSD (n)	Linearity
KCA1 4.1.2/22	Certified rodent diet	pydiflumetofen	0.01	0.01	100.9 – 102.8 (102.4)	1.6 (5)	0.001 – 0.1 mg/mL (n = 6) y = 37.3x + 0.0006 R = 0.9998
				16*	101.3 – 102.1 (101.7)	0.4 (5)	
				Overall	100.9 – 102.8 (102.1)	1.1 (10)	

* Dilutions were performed as necessary to be within the linear calibration range

Specificity

Chromatograms were presented for the high-level calibration standard, blank solvent sample (10 µg/mL nifedipine in methanol) and a blank PMI nutrition international certified rodent diet No. 5CR4 (14% protein) sample prepared and diluted in the same manner as the lowest concentration of pydiflumetofen under investigation. No significant interference was observed at the retention time of interest and the retention time of pydiflumetofen matched with reference standards.

Linearity

Linearity was demonstrated by the analysis of six matrix matched standards of increasing concentration. The range of standard concentrations used was 0.001 – 0.1 mg/mL. The response was linear with a coefficient of determination of at least 0.9998.

Accuracy

Rodent diet samples were fortified at 0.01 and 16 mg/mL and analysed concurrently with the samples. These fortification levels are appropriate to the concentrations used in the toxicology studies. Five determinations were made at each fortification level. The mean recoveries were within the acceptable range (70-110%).

Precision

Precision was determined from the accuracy recovery data for pydiflumetofen. The %RSD was ≤20% for each fortification level. 5 determinations were made at each fortification level.

Matrix effects

Matrix effects were not specifically addressed.

LOQ

The validated LOQ of the method is 0.01 mg/mL, this is the lowest concentration tested, at which an acceptable mean recovery (70-110%) with an acceptable RSD (≤20%) is obtained.

Conclusion

The method is fit for purpose in accordance with SANCO/3029/99 rev. 4 for the determination of pydiflumetofen in certified rodent diet formulation. Matrix effects were not accounted for in the study.

Report:	**KCA1 4.1.2/23 [redacted] (2013)**
Title	SYN545974 - Validation of Methodologies for the Analysis of SYN545974 in RM1 Dietary Formulations Report No. 33720 Document No. VV-405904, SYN545974_10087
Guidelines:	SANCO/3029/99 rev. 4
GLP:	Yes
Deviations	N/A
Previous evaluation:	None

Sample preparation (analytical method No. 2962):

Low level (ca. 10 ppm pydiflumetofen):

Accurately weigh ca. 10 g of diet formulation into a 250 mL amber glass jar. Extract in 100 mL of acetonitrile by shaking at 300 rpm for 30 min on an orbital shaker. This gives a sample concentration of 0.1 g/mL.

High level (ca. 1600 ppm pydiflumetofen):

Accurately weigh ca. 2 g of diet formulation into a 250 mL amber glass jar. Extract in 200 mL of acetonitrile by shaking at 300 rpm for 30 min on an orbital shaker. Further dilute 20-fold in acetonitrile to within range of the calibration curve. This gives a sample concentration of 0.5 mg/mL.

Samples are analysed by HPLC-UV under the conditions below.

HPLC-UV conditions:

Chromatographic system: Waters Xbridge C18 XP. 3 x 50 mm, 2.6 µm
Mobile phase A: 0.1% formic acid
Mobile phase B: Acetonitrile
Flow rate: 0.8 mL/minute
UV detector: UV @ 260 nm
Gradient:

Time (minute)	% A	% B	Curve
0	70	30	-
8	30	70	6
10	70	30	1

Column temperature: 50 °C
Autosampler temperature: Ambient
Injection volume: 10 µL (in duplicate)
Run time: 10 min.
Retention time: Approximately 6.6 minutes

A summary of the method validation data is given in Table B.5.1.2.3-6.

Table B.5.1.2.3-6: Summary of method validation data for determination of pydiflumetofen residues in RM1 dietary formulation

Study	**Matrix**	**Analyte**	**LOQ (mg/mL)**	**Recovery fortification level (mg/mL)**	**Recoveries % range (mean)**	**Repeatability % RSD (n)**	**Linearity**
KCA1 4.1.2/23	Rat and mouse No. 1 (RM1) dietary formulation	pydiflumetofen	0.01	0.01	106.7 – 108.6 (107.6)	0.6 (5)	0.001 – 0.05 mg/mL (n = 8) y = 4652x + 0.1318 R = 0.9998
				16*	101.2 – 104.4 (102.8)	1.1 (5)	
				Overall	101.2 – 108.6 (105.2)	2.56 (10)	

* Dilutions were performed as necessary to be within the linear calibration range

Specificity

Chromatograms were presented for the high-level calibration standards, blank solvent sample, blank matrix sample and fortified samples at 0.1 mg/mL (low level fortification). No significant interference was observed at the retention time of interest and the retention time of pydiflumetofen matched with reference standards.

Linearity

Linearity was demonstrated by the analysis of eight matrix matched standards of increasing concentration. The range of standard concentrations used was 0.001 – 0.05 mg/mL. The response was linear with a coefficient of determination of at least 0.9998. It is noted samples are diluted to within the linear range.

Accuracy

For the RM1 dietary formulation samples were fortified at 0.01 and 16 mg/mL. Five determinations were made at each fortification level. The mean recoveries were within the acceptable range (70-110%).

Precision

Precision was determined from the accuracy recovery data for pydiflumetofen. The %RSD was ≤20% for each fortification level. 5 determinations were made at each fortification level.

Matrix effects

Matrix effects were not addressed.

LOQ

The validated LOQ of the method is 0.01 mg/mL, this is the lowest concentration tested, at which an acceptable mean recovery (70-110%) with an acceptable RSD (≤20%) is obtained.

Conclusion

The method is fit for purpose in accordance with SANCO/3029/99 rev. 4 for the determination of pydiflumetofen in certified rodent diet formulation. Matrix effects were not accounted for in the study.

Report:	**KCA1 4.1.2/14 [redacted] (2012)**
Title	SYN545974 - Validation of an Analytical Method for the Determination of SYN545974 in Rat and Mouse Blood by LC-MS/MS Report No. 33236 Document No. VV-402650, SYN545974_10009
Guidelines:	SANCO/3029/99 rev. 4
GLP:	Yes
Deviations	N/A
Previous evaluation:	None

Sample preparation (Method AP.2009.02):

A volume of 20 µL of rat or mouse blood: water (50:50, v/v) is transferred to a matrix tube. This is then fortified with 10 µL of fortification solution containing pydiflumetofen (concentrations between 5 – 100000 ng/mL when spiked into 20 µL of blood: water). 150 µL of acetonitrile is added to this solution and it is mixed well. The sample is centrifuged at 3500 rpm for 5 minutes (ambient temperature). An aliquot of 300 µL is transferred to an HPLC glass vial for analysis.

Samples are analysed by HPLC-MS/MS under the conditions below.

HPLC conditions:

Analytical column:	ACE C18-HL 30 x 2.1 mm I.D, 3µ
Guard column:	Phenomenex Security Guard C18 4 x 2 mm
Mobile phase A:	Water: Formic acid (100: 0.1, v/v)
Mobile phase B:	Acetonitrile: formic acid (100: 0.1, v/v)
Flow rate:	1000 µL/minute
Column temperature:	~40 °C
Autosampler temperature:	~4 °C

Injection volume: 10 µL (may be adjusted)
Solvent samples: Acetonitrile
Run time: 5 min.
Gradient:

Time (minute)	% A	% B
0.0	85	15
0.2	85	15
2.5	5	95
3.5	5	95
4.0	85	15
5.0	85	15

Retention time: Approximately 2.1 min.

MS/MS conditions:

Ionisation mode: Turbo ion spray, positive
Parameter: pydiflumetofen
m/z 426.1 → 192.9

A summary of the method validation data is given in Table B.5.1.2.3-7.

Table B.5.1.2.3-7: Summary of method validation data for determination of pydiflumetofen residues in rat and mouse blood

Study	**Matrix**	**Analyte**	**LOQ (ng/mL)**	**Recovery fortification level (ng/mL)**	**Recoveries % range (mean)**	**Repeatability % RSD (n)**	**Linearity**
KCA1 4.1.2/14	Rat blood	pydiflumetofen	12.5	12.5	94 – 110 (100.2)	5.17 (18)	5 – 1000 ng/mL (n = 10) y = 1127 x - 207 R = 0.9983
				150	85 – 103 (95.9)	4.02 (18)	
				800	95 – 104 (99.2)	3.10 (18)	
				Overall	85 – 110 (98.4)	4.58 (54)	
KCA1 4.1.2/14	Mouse blood	pydiflumetofen	12.5	12.5	85 – 114 (109.0)	9.14 (18)	5 – 1000 ng/mL (n = 5) y = 1601 x + 833 R = 0.9978
				150	95 – 103 (99.0)	3.07 (18)	
				800	89 – 110 (102.1)	7.86 (18)	
				Overall	85 – 114 (101.3)	7.54 (54)	

Specificity

Chromatograms were presented for the low level and high-level calibration standards (5 and 1000 ng/mL for rat and mouse blood) and matrix blank (for both rat and mouse blood). No significant interference was observed at the retention time of interest and the retention time of pydiflumetofen matched with reference standards.

Linearity

Linearity was demonstrated by the analysis of 10 matrix matched standards of increasing concentration. The range of standard concentrations used was 5 – 1000 ng/mL. The response was linear with a coefficient of determination of at least 0.9998. It is noted samples are diluted to within the linear range.

Accuracy

For rat and mouse blood samples were fortified at 12.5, 150 and 800 ng/mL. 18 determinations were made at each fortification level. The mean recoveries were within the acceptable range (70-110%).

Precision

Precision was determined from the accuracy recovery data for pydiflumetofen. The %RSD was ≤20% for each fortification level. 18 determinations were made at each fortification level.

Matrix effects

Matrix effects were observed for pydiflumetofen in rat (5 of the 6 tests observed matrix effects) and mouse blood (6 of the 6 tests observed matrix effects), but these were compensated for when using a matrix-matched calibration.

LOQ

The validated LOQ of the method is 12.5 mg/mL, this is the lowest concentration tested, at which an acceptable mean recovery (70-110%) with an acceptable RSD (≤20%) is obtained.

Conclusion

The method is acceptably validated in accordance with SANCO/3029/99 rev. 4 for the determination of pydiflumetofen in rat and mouse blood.

Report:	**KCA1 4.1.2/05 [redacted] (2013)**
Title	SYN545974 - Partial Validation of a Bioanalytical Method for the Determination of SYN545974 in Rabbit Blood Water Report No. BFI0127 Document No. VV-415358, SYN545974_10372
Guidelines:	SANCO/3029/99 rev. 4
GLP:	Yes
Deviations	N/A
Previous evaluation:	None

Sample preparation (Analytical method BFI013MS):

Samples are prepared through the following steps:

- An aliquot of 20 µL of sample into a polypropylene tube.
- Add 150 µL of 10 mM ammonium bicarbonate buffer pH 10.0 to the tube and vortex mix briefly.

Samples are extracted by supported liquid extraction (SLE) according to the procedure below:

- Transfer the pre-mixed samples onto the Isolute SLE + 200 mg plate. Tap plate to ensure even distribution of the sample across the surface of the sorbent.
- Apply vacuum for 5-15 seconds to allow the sample to be absorbed into the sorbent. Leave to stand for approximately 5 minutes to allow the sample to soak in.
- Elute the analyte from the SLE sorbent with 1mL of methyl-tert-butyl ether (MTBE). Allow the MTBE to drip through under gravity for approximately 10 minutes, collecting the eluents into polypropylene tubes or a deep well plate. Apply vacuum to draw through any residual sample.
- Evaporate the eluents to dryness under nitrogen at approximately 40 °C.

Re-dissolve the residues in 200 µL of 50 % (v/v) methanol, cap and vortex mix the tubes, centrifuge the samples if necessary, and inject a portion into the LC-MS/MS system.

- Validation samples are prepared by aliquoting volumes of solutions of 0.1 – 25 µg/mL pydiflumetofen in in methanol: DMSO [1:1 (v/v)], into polypropylene tubes and adding the appropriate volume of rabbit blood: water [1:1 (v/v)], the blood containing EDTA as anticoagulant. The samples are then mixed briefly, before preparation and extraction as outlined above. The validation samples are prepared between 2.5 – 1250 ng/mL pydiflumetofen (blood: water concentration), equivalent to 5.0 – 2500 ng/mL pydiflumetofen (blood concentration), and a final sample concentration for measurement of 0.5 – 250 ng/mL pydiflumetofen.

Samples are analysed by HPLC-UV under the conditions below.

HPLC conditions:

HPLC column:	50 x 2 mm Gemini NX C18, 5 µm
Column temperature:	Ambient
Flow rate:	0.8 mL/min
Mobile phase A:	10 mM ammonium bicarbonate pH 10.0
Mobile phase B:	Methanol
HPLC mode:	Gradient
Typical injection volume:	2-15 µL

Gradient programme:

Time (minute)	% A	% B
0.0	50	50
1.5	5	95
3.5	5	95
3.6	50	50
4.7	50	50

Mass spectrometer conditions:

Ionisation mode:	Electrospray
Polarity:	Positive
Source/ auxiliary gas temperature:	450 °C
Analyte:	pydiflumetofen
Precursor ion (m/z):	426
Product ion (m/z):	193
Dwell time (ms):	100
Retention time:	~2.5

A summary of the method validation data is given in Table B.5.1.2.3-8.

Table B.5.1.2.3-8: Summary of method validation data for determination of pydiflumetofen in rabbit blood: water

Study	**Matrix**	**Analyte**	**LOQ (ng/mL)**	**Recovery fortification level (ng/mL)**	**Recoveries % range (mean)**	**Repeatability % RSD (n)**	**Linearity**
KCA1 4.1.2/05	Rabbit blood: water	pydiflumetofen	5	5	89 – 115 (97.8)	9.52 (6)	5 – 2500 ng/mL (equivalent to 0.1 – 25 µg/mL) (n = 8) $y = 0.0092x^2 + 604 x + 310$ R = 0.9947
				10	94 – 119 (104.8)	9.93 (6)	
				100	91 – 116 (102.8)	8.28 (6)	
				2000	90 – 112 (100.1)	7.90 (6)	
				2500	94 – 110 (96)	7.17 (6)	
				Overall	89 – 119 (100.4)	8.63 (30)	

Specificity

Chromatograms were presented for the low level and high-level calibration standards and matrix blank sample. No significant interference was observed at the retention time of interest and the retention time of pydiflumetofen matched with reference standards.

Linearity

Linearity was demonstrated by the analysis of eight matrix matched standards of increasing concentration. The range of standard concentrations used was 5 – 10 ng/mL (equivalent to 0.1 – 25 µg/mL). The response was linear with a coefficient of determination of at least 0.9947. It is noted samples are diluted to within the linear range.

Accuracy

Rabbit blood: water samples were fortified at concentrations of 5, 10, 100, 2000 and 2500 µg/mL respectively and analysed concurrently with the samples. Six determinations were made at each fortification level. The mean recoveries were within the acceptable range (70-110%).

Stability study

pydiflumetofen was considered stable in biological matrix following three freeze-thaw cycles if the mean response of the stability samples was within 15 % of the mean of the original data. pydiflumetofen is stable in biological matrix when stored at room temperature for up to 24 hours, and when stored at approximately -80 °C for up to 31 days.

Precision

Precision was determined from the accuracy recovery data for pydiflumetofen. The %RSD was ≤20% for each of the five fortification levels. 6 determinations were made at each fortification level.

Matrix effects

Matrix effects were originally found to be > 15 % and therefore not acceptable at 10 ng/mL for pydiflumetofen. The variation of the peak areas of pydiflumetofen at 2000 ng/mL was ≤ 15 % and therefore acceptable. The determination at the low concentration level was repeated on two further occasions, with variation ≤ 15 % on each occasion.

LOQ

The LOQ of the method is validated at 5.0 ng/mL in rabbit blood. This is the lowest concentration tested, at which an acceptable mean recovery (70-110%) with an acceptable RSD (≤20%) is obtained.

Conclusion

The method is acceptably validated in accordance with SANCO/3029/99 rev. 4 for the determination of pydiflumetofen in rabbit blood.

Report:	**KCA1 4.1.2/33 [redacted] (2018)**
Title	SYN508272 – Validation of a Bioanalytical Method for the Determination of SYN508272 in Rat Blood: Water [1:1 (v/v)] by LC-MS/MS Report No. 0057/002 Document No. VV-489573
Guidelines:	SANCO/3029/99 rev. 4
GLP:	Yes
Deviations	N/A
Previous evaluation:	None

Whole blood samples are diluted 1:1 with water to mimic the matrix treatment of toxicology samples upon collection. An aliquot of 20 µL of the study sample is transferred to a polypropylene plate. 150 µL of internal standard is added to the study sample. Mix the plate at full speed using plate shaker (AA-INST-0007) for approximately 30 seconds. Transfer 50 µL of supernatant to a 2 mL collection plate and add 50 µL 0.1% formic acid. Seal and mix plate using a multi-plate shaker for approx. 10 minutes. This gives a sample concentration of 0.11 Transfer to the LC-MS/MS system for analysis.

Samples are analysed by HPLC-MS/MS under the conditions below.

HPLC-MS/MS conditions:

Chromatographic system:	Hypersil Gold – C18 5 µ x 100 x 3mm column
Column temperature	40 °C

Mobile phase A: 0.1% formic acid in water
Mobile phase B: Acetonitrile
Injection volume: not stated
Flow rate: 0.75 mL/minute

Gradient:

Time (minute)	% A	% B	Curve
0.00	95	5	6
0.50	95	5	6
3.50	5	95	6
3.60	5	95	6
4.10	95	5	6
4.50	95	5	6

Retention time: Approximately 1.7 minutes
MS method
Polarity: Positive
Ionisation mode: ESI
Ion transitions: 176 → 136
290.1 → 198.1

A summary of the method validation data is given in Table B.5.1.2.3-9.

Table B.5.1.2.3-9: Summary of method validation data for determination of pydiflumetofen residues in rat blood: water [1:1 (v/v)]

Study	**Matrix**	**Analyte**	**LOQ (ng/mL)**	**Recovery fortification level (ng/mL)**	**Recoveries % range (mean)**	**Repeatability % RSD (n)**	**Linearity**
KCA1 4.1.2/33	Rat blood: water [1:1 (v/v)]	pydiflumetofen	10	10	86 – 119 (106.0)	8.8 (18)	10 – 10000 ng/mL (n = 5) y = 0.000129 x + 0.00752 R = 0.9983
				30	88 – 127 (105.1)	9.9 (18)	
				2000	95 – 119 (103.8)	7.7 (18)	
				4000	94 – 116 (102.7)	5.9 (18)	
				5000	100 – 109 (106.5)	3.4 (6)	
				Overall	86 – 127 (104.6)	7.9 (78)	

Specificity

Chromatograms were presented for the low level and high-level calibration standards and reagent blank sample of rat blood: water [1:1 (v/v)]. No significant interference was observed at the retention time of interest and the retention time of pydiflumetofen matched with reference standards.

Linearity

Linearity was demonstrated by the analysis of eight matrix matched standards of increasing concentration. The range of standard concentrations used was 10 - 5000 ng/mL (pydiflumetofen). The response was linear with a coefficient of determination of at least 0.991. It is noted samples are diluted to within the linear range.

Accuracy

Rat blood: water [1:1 (v/v)] samples were fortified at 10, 30, 2000, 4000 and 5000 ng/mL. 18 determinations were made at each fortification level except for 5000 ng/mL (6 determinations). The mean recoveries were within the acceptable range (70-110%).

Precision

Precision was determined from the accuracy recovery data for pydiflumetofen. The %RSD was ≤20% for each fortification level. At least 6 determinations were made at each fortification level.

Matrix effects

Matrix effects were determined to be not significant for the matrix tested. Two concentrations (30 ng/mL and 4000 ng/mL) were tested and were shown to be not significant, with acceptable precision data provided for both concentrations.

LOQ

The validated LOQ of the method is 30 ng/mL in blood: water [1:1 v/v], this is the lowest concentration tested, at which an acceptable mean recovery (70-110%) with an acceptable RSD (≤20%) is obtained. This is equivalent to 60 ng/mL in whole blood. It is noted the applicant claimed the LOQ to be 10 ng/mL in blood: water [1:1 v/v] (20 ng/mL in blood). Whilst acceptable precision and accuracy data were achieved at this level it is also the lowest calibration standard concentration measured, therefore samples fortified at 10 ng/ml have the potential to give concentrations that are below the lowest calibration standard.

Conclusion

The method is fully validated in accordance with SANCO/3029/99 rev. 4 for the determination of pydiflumetofen in rat blood, with an LOQ of 30 ng/mL in blood: water [1:1 v/v]. This is equivalent to 60 ng/mL in whole blood.

It is considered fit for purpose to determine concentrations at 10 ng/ml in blood: water [1:1 v/v], equivalent to 20 ng/mL in blood).

Report:	**KCA1 4.1.2/18 [redacted] (2021)**
Title	The Validation of a Bioanalytical Method for the Determination of 2,4,6 -trichlorophenol in Rat Whole Blood (K_2EDTA) by LC-MS/MS Report No. 0029/027 Document No. VV-899602
Guidelines:	SANCO/3029/99 rev. 4
GLP:	Yes
Deviations	N/A
Previous evaluation:	None

Sample preparation (Analytical method 0001/186):

Whole blood samples are diluted 1:1 with water to mimic the matrix treatment of toxicology samples upon collection. Study samples are thawed to an ambient temperature. A 20 µL aliquot is measured into a 1.5 mL microcentrifuge tubes, and 20 µL internal standard solution [$^{13}C_6$]-2,4,6 -trichlorophenol added. The tube is then capped, and vortex mixed for 15s. 150 µL of acetonitrile is added to the tube, the tube recapped, and vortex mixed followed by centrifugation. 100 µL of the supernatant from the tube is transferred to a 96-deep well plate. 50 µL of derivatization agent (1 mg/mL of dansyl chloride) is added, with 10 µL of 1% triethylamine. The plate is then sealed, mixed for 15 seconds at 1800 rpm, and then incubated at 60 °C for 10 mins. Post incubation, extracts are cooled on ice for 10 mins, 100 µL of mobile phase A is added and the solution mixed for 15 seconds at 1800 rpm.

Samples are analysed by HPLC-MS/MS under the conditions below.

HPLC-MS/MS conditions:

MS instrument: Sciex API 6500+

LC method

Mobile phase A: 0.1% aq. formic acid

Mobile phase B: 0.1% formic acid in acetonitrile

Autosampler wash solvent: 9:1 water/ methanol
1:9 water/ acetonitrile

Column: Kinetex XB-C18 2.6 µm, 2.1 x 50 mm column

Column temperature: 50 °C

LC runtime: 2.4

Flow rate: 0.8 mL/min

Gradient:

Time (minute)	% A	% B
0.00	35	65
0.10	35	65
1.20	25	75
1.30	5	95
1.80	5	95
1.90	35	65
2.40	35	65

MS method

Polarity: Positive

Scan type: MRM

	Q1 mass	Q3 mass
2,4,6-trichlorophenol	429	171
2,4,6-[13C6]-trichlorophenol	438	171

Retention time 1.27 min.

A summary of the method validation data is given in Table B.5.1.2.3-10.

Table B.5.1.2.3-10: Summary of method validation data for determination of 2,4,6-trichlorophenol residues in rat blood

Study	Matrix	Analyte	LOQ (ng/mL)	Recovery fortification level (ng/mL)	Recoveries % range (mean)	Repeatability % RSD (n)	Linearity
KCA1 4.1.2/18	Rat blood	2,4,6-trichlorophenol	10	10	97.8 – 107 (102)	3.6 (6)	10 – 10000 ng/mL (n = 10) y = 0.00242 x + 0.00695 R = 0.9991
					82 – 123 (107)	13.6 (6)	
					88.1 – 127 (108)	14.4 (6)	
					81.3 – 124 (93.7)	17.7 (6)	
				Overall	82 – 127 (103)	13.6 (24)	
				30	97 – 101 (99.6)	1.5 (6)	
					105 – 113 (107)	2.7 (6)	
					102 – 115 (107)	4.6(6)	
					97 – 109 (102)	3.8 (6)	
				Overall	97 – 115 (104)	4.9 (24)	
				3000	84 – 100 (96)	5.9 (6)	
					93 – 106 (97)	2.9 (6)	
					98 – 102 (100)	1.6 (6)	
					97 – 103 (100)	2.1 (6)	
				Overall	84 – 106 (98.0)	4.8 (24)	
				8000	81 – 98 (93)	6.3 (6)	
					84 – 96 (93)	4.9 (6)	
					91 – 101 (96)	4.5 (6)	
					95 – 99 (97)	1.3 (6)	
				Overall	81 – 101 (94.6)	4.4 (24)	

Table B.5.1.2.3-10 cont'd: Summary of method validation data for determination of 2,4,6-trichlorophenol residues in rat blood

Study	Matrix	Analyte	LOQ (ng/mL)	Recovery fortification level (ng/mL)	Recoveries % range (mean)	Repeatability % RSD (n)	Linearity
KCA1 4.1.2/18	Rat blood	2,4,6-trichlorophenol	10	10000	94 – 98 (96)	2.0 (6)	10 – 10000 ng/mL (n = 10) y = 0.00242 x + 0.00695 R = 0.9991
					92 – 97 (95)	1.8 (6)	
					89 – 100 (97)	4.2 (6)	
					95 – 98 (97)	1.1 (6)	
				Overall	81 – 100 (96)	2.4 (24)	

Specificity

Chromatograms were presented for the low level and high-level calibration standards, matrix blank and fortified samples at 30, 3000 and 8000 ng/mL. No significant interference was observed at the retention time of interest and the retention time of 2,4,6-trichlorophenol matched with reference standards.

Linearity

Linearity was demonstrated by the analysis of 10 matrix matched standards of increasing concentration in duplicate. The range of standard concentrations used was 10 – 10000 ng/L. The response was linear with a coefficient of determination of at least 0.9991.

Accuracy

Rat blood samples were fortified at 10, 30, 3000 and 10000 ng/mL. 24 determinations were made at each fortification level (6 determinations at each level analysed on 4 separate occasions). The mean recoveries were within the acceptable range (70-110%).

Precision

Precision was determined from the accuracy recovery data for 2,4,6-trichlorophenol. The %RSD was ≤20% for each fortification level. 24 determinations were made at each fortification level.

Extract stability

Acceptable stability was demonstrated for up to 78 hours storage at nominally 10 °C for injected extracts, acceptable stability was demonstrated for up to 76 hours storage at nominally 10 °C for processed extracts prior to injection of the analytical run.

Matrix effects

No significant matrix effect was demonstrated in blank blood: water (1:1) rat. The method recommends that carry-over/back-ground interference/contamination should be routinely assessed within each run and an assessment of the significance of potential carry-over on the following sample should be performed. Blanks should be run after predicted high concentration samples.

LOQ

The validated LOQ of the method is 30 ng/mL in blood: water [1:1 v/v], this is the lowest concentration tested, at which an acceptable mean recovery (70-110%) with an acceptable RSD (≤20%) is obtained. This is equivalent to 60 ng/mL in whole blood. It is noted the applicant claimed the LOQ to be 10 ng/mL in blood: water [1:1 v/v] (20 ng/mL in blood). Whilst acceptable precision and accuracy data were achieved at this level it is also the lowest

calibration standard concentration measured, therefore samples fortified at 10 ng/ml have the potential to give concentrations that are below the lowest calibration standard.

Conclusion

The method is acceptably validated in accordance with SANCO/3029/99 rev. 4 for the determination of 2,4,6-trichlorophenol in rat blood with an LOQ of 30 ng/mL in blood: water [1:1 v/v]. This is equivalent to 60 ng/mL in whole blood. It is considered fit for purpose to determine concentrations at 10 ng/ml in blood: water [1:1 v/v], equivalent to 20 ng/mL in blood).

B.5.1.2.4. Methods in body fluids, air and any additional matrices used in support of operator worker, resident, and bystander exposure studies

No methods of analysis to support operator exposure studies for the active substance have been submitted.

B.5.1.2.5. Methods in or on plants, plant products, processed food commodities, food of plant and animal origin, feed and any additional matrices used in support of residue studies

Summary overview:

Methods used in support of residue studies determine the following analytes:

Code Name	Synonyms	IUPAC Name
pydiflumetofen	CSCD678790	3-difluoromethyl-1-methyl-1H-pyrazole-4-carboxylic acid methoxy-[1-methyl-2-(2,4,6-trichloro-phenyl)-ethyl]amide
2,4,6-trichlorophenol	2,4,6-TCP CSCA08329	2,4,6-Trichlorophenol
SYN548263	CSCZ159698	2-[[3-(difluoromethyl)-1-methyl-pyrazole-4-carbonyl]-methoxy-amino]propanoic acid
SYN548264	CSCD548196	2-[[3-(difluoromethyl)-1-methyl-pyrazole-4-carbonyl]amino]propanoic acid
SYN547897	CSCV764146	3-difluoromethyl-1-methyl-1H-pyrazole-4-carboxylic acid methoxy-[1-methyl-2-(2,4,6-trichloro-3-hydroxy-phenyl)-ethyl]-amide
SYN508272	CSCC210616 R423363	3-difluoromethyl-1-methyl-1H-pyrazole-4-carboxylic acid amide

Table B.5.1.2.5-1: Summary of pre-registration methods for residues

Analyte (s)	Limit of quantification (mg/kg)	Commodities used for validation	Commodity types represented	Method	Method Reference
Pre-registration methods for crop commodities					
pydiflumetofen	0.01	Wheat (forage, straw, grain), apple (fruit), lettuce (head), tomato (fruit), cabbage (head), fresh peas (seed), grape (fruit), potato (tuber), dried bean (seed), oilseed rape (seed)	Acidic commodities; Commodities with high oil content; Commodities with high water content; Commodities with high protein content; Commodities with high starch content; Dry commodities	LC-MS/MS	GRM061.03A [redacted] (2015)
Pre-registration methods for animal commodities					
pydiflumetofen	0.01	Bovine meat, liver, kidney, fat, milk Chicken eggs	Animal commodities	LC-MS/MS	GRM061.06A [redacted] (2015)
SYN548264 SYN508272	0.01	Milk	Animal commodities	LC-MS/MS	GRM061.08A [redacted] (2015)
SYN547897 (free + conjugated) SYN548263 (free + conjugated)	0.01	Liver, kidney	Animal commodities	LC-MS/MS	GRM061.09A [redacted] (2015)
2,4,6-trichlorophenol (free + conjugated)	0.01	Bovine meat, liver, kidney, fat, milk Chicken eggs	Animal commodities	LC-MS/MS	GRM061.07A [redacted] and [redacted] (2015) Also proposed as a post-registration method

Method GRM061.03A

For method GRM061.03A, method validation data was provided for additional matrices in the following studies:

- Frozen storage stability in crops,
- Magnitude of residues in crops trials,
- Rotational crop field studies; and
- Magnitude of residues in honey.

Residues studies supported by method GRM061.03A:

Data point	Author(s)	Year	Title Company Report No.
KCA1 6.1	[redacted]	2015	SYN545974 – Storage Stability in Crops Stored Frozen for up to 23 months Report number: S13-02224 Document No. VV-414120 , SYN545974_10278
KCA1 6.3.14-1	[redacted]	2017	SYN545974 – Residue Study on Barley in North France, Germany, Poland, Hungary, and the UK in 2016 Report number: 38034 Syngenta File No. A21857B_10013 (VV-467584)
KCA1 6.5.3-7	[redacted]	2017	SYN545974 – Residue Study on Barley and Processed Specimens in Northern France, Germany, and Poland in 2013 Report number: S13-02518 Document No. VV-463141 , A17573A_10004
KCA1 6.3.13-4	[redacted]	2016	SYN545974 – Residue Study on Oilseed Rape in the United Kingdom and Northern France in 2013 Report number: S13-02259 Document No. VV-415279 , A19649B_10230
KCA1 6.3.13-2	[redacted]	2017	SYN545974 – Residue Study on Oilseed Rape and Processed Products in Northern France, Germany & UK in 2014 Report number: CEMR-6531 Document No. VV-468119 , A19649B_10334
KCA1 6.3.13-3	[redacted]	2016	SYN545974 – Residue Study on Oilseed Rape in Southern France, Italy, and Spain in 2013 Report number: S13-02260 Syngenta File No. A19649B_10231 (VV-415280)
KCA1 6.3.13-1	[redacted]	2015	SYN545974 – Residue Study on Oilseed Rape in Southern France, Spain, and Italy in 2014 Report number: CEMR-6532 Syngenta File No. A19649B_10106 (VV-412280)
Appendix C 3.1.2.04-1	[redacted]	2017	SYN545974 – Residue Study on Carrot in Northern France, Germany, Poland, and the United Kingdom in 2016 Report number: CEMR-7597
Appendix C 3.1.2.04-2	[redacted]	2017	SYN545974 – Residue Study on Carrot in Southern France, Greece, Spain, and Italy in 2016 Report number: CEMR-7598
KCA1 6.6.2/1	[redacted]	2018	Adepidyn – Residue Study on Rotational Crops in Northern France and Germany during 2016-2017 Report number: CEMR-7709 Document No. VV-469769 , A19649B_10353
KCA1 6.6.2/2	[redacted]	2018	Pydiflumetofen – Residue Study on Rotational Crops in Southern France and Spain During 2016-2014 Report number: CEMR-7710 Document No. VV-470802 , A19649B_10359
KCA1 6.6.2/3	[redacted]	2016	SYN545974 – Residue Study on Rotational Crops in Southern France and Italy during 2013-2015 Report number: S13-01023 Document No. VV-415410 , A19649B_10235

KCA1 6.6.2/4	[redacted]	2016	SYN545974 – Residue Study on Rotational Crops in the United Kingdom and Germany during 2013-2014 Report number: S13-01022 Document No. VV-415357 , A19649B_10234
KCA1 6.10.1	[redacted]	2017	SYN545974 and Fludioxonil – Residues in Honey Following Exposure of Bees to Treated Winter Oilseed Rape in Germany during 2016 Report number: S16-02006 Document No. VV-466889 , A8240D_12181
KCA1 6.3.15	[redacted]	2017	SYN545974 – Residue Study on Wheat in Northern France and the United Kingdom in 2013 Report number: S13-02516 Document No. VV-467692 , A17573A_10005

Report:	**KCA1 4.1.2/17 [redacted] (2015)**
Title	SYN545974 – Analytical Method for Determination of SYN545974 in Crops by LC-MS/MS with Validation Data Report number: GRM061.03A Document No. VV-618773 , SYN545974_50054
Guidelines:	SANCO/3029/99 rev. 4
GLP:	No
Deviations	N/A
Previous evaluation:	None

Report:	**KCA1 4.1.2/31 [redacted], [redacted] (2015)**
Title	SYN545974 – Validation of the Syngenta Method GRM061.03A for the Determination of Residues of SYN545974 in Crop Matrices Report number: S14-05352 Document No. VV-412456 , SYN545974_10180
Guidelines:	SANCO/3029/99 rev. 4
GLP:	Yes
Deviations	N/A
Previous evaluation:	None

LC-MS/MS method GRM061.03A was developed and validated for the determination of pydiflumetofen in the following crop groups:

- high water content commodities (apples, lettuce, tomato, cabbage, fresh peas, and cereal forage),
- high protein content commodities (dry beans),
- high starch content commodities (wheat grain, potato),
- high oil content commodities (oilseed rape seed),
- high acid content commodities (grape); and
- dry commodities (wheat straw).

Reference items:

pydiflumetofen, batch AMS 1432/1, purity 99.5 %w/w, CoA provided, expiry December 2017

Sample preparation:

Commodities except high oil content:

Samples (10 g) were extracted by homogenisation with 100 mL acetonitrile/ultra-pure water (80/20, v/v). Dry samples were allowed to soak in extraction solvent for about 20 minutes. All sample extracts were centrifuged at 15 °C for 5 minutes at 3500 rpm. An additional 100 mL of 80/20 (v/v) acetonitrile/ultra-pure water water was transferred to the supernatants. The supernatants were mixed well to produce a homogenous extract.

High oil content commodities:

Samples (10 g) were extracted by homogenisation with 100 mL acetonitrile and left at room temperature for 15 minutes before centrifugation at 15°C for 5 minutes at 3500 rpm. An additional 100 mL of acetonitrile was added

to the supernatant. The supernatants were mixed well to produce a homogenous extract. 2.5 mL of the resulting extract was transferred into a clean glass vial containing approximately 100 mg of Bakerbond Octadecyl C18 (40 µm). After this step, the mixture was gently mixed for 30 seconds, and the vial was allowed to sit with occasional swirling for approximately 5 minutes. The mixture was transferred into a syringe and filtered through a 0.45 µm PTFE syringe filter into an appropriate size glass vial.

SPE Clean up:

An SPE cartridge (OasisTM HLB 200 mg, 6 mL) was conditioned by loading the SPE cartridge successively with 1 mL methanol, 1 mL acetonitrile and 1 mL acetonitrile/ultra-pure water (80/20, v/v). 1 mL of the final extracts were diluted with 4 mL HPLC grade water and mixed well. The diluted extracts were loaded portion-wise and quantitatively onto the SPE cartridge. During loading the flow rate was less than 20 drops per minute. Slight vacuum was applied if necessary. The eluents were discarded. The cartridge was washed by rinsing the sample tubes with 1.0 mL of acetonitrile/water (20/80, v/v) and transferring the rinsate to the SPE cartridge. The cartridge was washed four times with 1.0 mL of acetonitrile/water (20/80, v/v).

The cartridge was eluted three times with 1.0 mL acetonitrile (HPLC grade) into a graduated tube. The solution was evaporated to a volume of 0.2 mL under gentle N_2 stream at a bath temperature of 40°C. The sample was re-constituted to a final volume of 5.0 mL with acetonitrile/water (20/80, v/v). The samples were mixed and transferred into HPLC vials for analysis by high performance liquid chromatography (LC-MS/MS).

It is noted sample preparation has been reported for soybean forage in study GRM061.03A (KCA1 4.1.2/17, [redacted]). However, this matrix hasn't been used in the validation study (KCA1 4.1.2/31, [redacted], [redacted]) and hence the sample preparation has not been reported here.

LC-MS/MS conditions:

Chromatographic system:	Agilent 1200 Series
Analytical column:	Discovery C8, 5 x 4.6 mm, 5 µm particle size
Injection volume:	30 µL
Column temperature:	30°C
Mobile phase A:	Acetonitrile + 0.1% formic acid
Mobile phase B:	Water + 0.1% formic acid
Flow rate:	0.6 mL/minute

Gradient:

Time (minute)	% A	% B
0.00	20	80
3.00	90	10
5.00	90	10
5.10	20	80
7.00	20	80

Retention time:	Approximately 4.4 minutes
Tandem mass spectrometer:	API 4000 Mass Spectrometer
Ionisation type:	Electrospray (ESI, TurboIon Spray)
Polarity:	Positive ion mode
Scan type:	MS/MS, multiple reaction monitoring (MRM)
Mass transitions:	pydiflumetofen

Ion monitored	Declustering Potential	Collision Energy	Collisions Cell Exit Potential	Dwell time
m/z 426→193 (quantification)	90 eV	41 eV	10 eV	0.15 s
m/z 428→195 (confirmation)	90 eV	43 eV	10 eV	0.15 s

A summary of the method validation data is given in Table B.5.1.2.5-2 and B.5.1.2.5-3.

Table B.5.1.2.5-2: Summary of method validation data for determination of residues of pydiflumetofen in plant commodities – primary transition *m/z* 426 →193

Matrix	Analyte	LOQ (mg/kg)	Recovery fortification level (mg/kg)	Recoveries % range (mean)	Repeatability % RSD (n)	Linearity
Apples	pydiflumetofen	0.01	0.01	79 – 108 (91)	12 (5)	0.03 - 10 ng/mL (equivalent to 0.003 – 1 mg/kg) (n = 7) y = 12696x-9 R^2 = 1.0000
			0.2	90 – 96 (93)	3 (5)	
			Overall	79 – 108 (92)	8 (10)	
Lettuce	pydiflumetofen	0.01	0.01	73 – 97 (82)	11 (5)	0.03 - 20 ng/mL (equivalent to 0.003 –2 mg/kg) (n = 8) y = 21582x+2526 R^2 = 0.9989
			10	88 – 101 (93)	6 (5)	
			Overall	73 – 101 (88)	10 (10)	
Tomato	pydiflumetofen	0.01	0.01	78 – 111 (96)	12 (5)	0.03 - 10 ng/mL (equivalent to 0.003 – 1 mg/kg) (n = 7) y = 16510x-51 R^2 = 0.9989
			1.4	90 – 96 (93)	3 (5)	
			Overall	78 – 111 (95)	9 (10)	
Cabbage	pydiflumetofen	0.01	0.01	91 – 103 (94)	5 (5)	0.03 - 20 ng/mL (equivalent to 0.003 –2 mg/kg) (n = 8) y = 21582x+2526 R^2 = 0.9989
			10	102 – 108 (105)	3 (5)	
			Overall	91 – 108 (100)	7 (10)	
Peas (fresh)	pydiflumetofen	0.01	0.01	95 – 101* (98)	3 (4)	0.03 - 5 ng/mL (equivalent to 0.003 – 0.5 mg/kg) (n = 6) y = 15431x+251 R^2 = 1.0000
			0.2	90 – 119 (108)	10 (5)	
			Overall	90 – 119 (104)	9 (9)	
Bean (dried)	pydiflumetofen	0.01	0.01	87 – 107 (96)	8 (5)	0.03 - 20 ng/mL (equivalent to 0.003 –2 mg/kg) (n = 8) y = 21582x+2526 R^2 = 0.9989
			0.2	65 – 87 (76)	13 (5)	
			Overall	65 – 107 (86)	16 (10)	
Wheat (grain)	pydiflumetofen	0.01	0.01	90 – 108 (97)	8 (5)	0.03 - 20 ng/mL (equivalent to 0.003 –2 mg/kg) (n = 8) y = 21582x+2526 R^2 = 0.9989
			0.2	92 – 101 (96)	4 (5)	
			Overall	90 – 108 (96)	6 (10)	
Wheat (straw)	pydiflumetofen	0.01	0.01	100 – 123 (110)	8 (5)	0.03 - 10 ng/mL** (equivalent to 0.003 –1 mg/kg) (n = 7) y = 14967x+22 R^2 = 1.0000
			10	82 – 113 (102)	14 (5)	
			Overall	82 – 123 (106)	12 (10)	
Cereal (forage)	pydiflumetofen	0.01	0.01	93 – 111 (101)	7 (5)	0.03 - 20 ng/mL (equivalent to 0.003 –2 mg/kg) (n = 8) y = 21582x+2526 R^2 = 0.9989
			10	92 – 118 (108)	11 (5)	
			Overall	92 – 118 (104)	10 (10)	
Potato	pydiflumetofen	0.01	0.01	81 – 101	8 (5)	0.03 - 10 ng/mL

Matrix	Analyte	LOQ (mg/kg)	Recovery fortification level (mg/kg)	Recoveries % range (mean)	Repeatability % RSD (n)	Linearity
				(92)		(equivalent to 0.003 – 1 mg/kg) (n = 7) y = 15155x-55 R^2 = 0.9999
			10	89 – 103 (97)	5 (5)	
			Overall	81 – 103 (94)	7 (10)	
Rape seed	pydiflumetofen	0.01	0.01	82 – 110 (92)	12 (5)	0.03 - 10 ng/mL (equivalent to 0.003 – 1 mg/kg) (n = 7) y = 25985x+1905 R^2 = 0.9998
			0.2	89 – 100 (95)	5 (5)	
			Overall	82 – 110 (93)	9 (10)	
Grape	pydiflumetofen	0.01	0.01	74 – 104 (88)	18 (5)	0.03 - 20 ng/mL (equivalent to 0.003 –2 mg/kg) (n = 8) y = 21582x+2526 R^2 = 0.9989
			1.4	79 – 95 (95)	7 (5)	
			Overall	74 – 104 (88)	12 (10)	

*At 0.01 mg/kg for pydiflumetofen in fresh peas a 65% recovery was determined. This is considered an outlier according to the Grubbs test. Therefore, this recovery has not been considered in the statistical evaluation.
**Linearity data for wheat straw using matrix matched standards is presented in a separate study report (S13-02224).

Table B.5.1.2.5-3 : Summary of method validation data for determination of residues of pydiflumetofen in plant commodities – confirmatory transition *m/z* 426 →195

Matrix	Analyte	LOQ (mg/kg)	Recovery fortification level (mg/kg)	Recoveries % range (mean)	Repeatability % RSD (n)	Linearity
Apples	pydiflumetofen	0.01	0.01	82 – 97 (92)	7 (5)	0.03 - 10 ng/mL (equivalent to 0.003 – 1 mg/kg) (n = 7) y = 13206x-38 R^2 = 1.0000
			0.2	90 – 96 (93)	2 (5)	
			Overall	82 – 97 (93)	5 (10)	
Lettuce	pydiflumetofen	0.01	0.01	78 – 92 (83)	7 (5)	0.03 - 20 ng/mL (equivalent to 0.003 –2 mg/kg) (n = 8) y = 22113x+3016 R^2 = 0.9989
			10	84 – 96 (90)	6 (5)	
			Overall	78 – 96 (86)	7 (10)	
Tomato	pydiflumetofen	0.01	0.01	79 – 116 (98)	15 (5)	0.03 - 10 ng/mL (equivalent to 0.003 – 1 mg/kg) (n = 7) y = 17229x+269 R^2 = 0.9985
			1.4	97 – 103 (99)	3 (5)	
			Overall	79 – 116 (98)	10 (10)	
Cabbage	pydiflumetofen	0.01	0.01	82 – 102 (93)	8 (5)	0.03 - 20 ng/mL (equivalent to 0.003 –2 mg/kg) (n = 8) y = 22113x+3016 R^2 = 0.9989
			10	96 – 99 (97)	1 (5)	
			Overall	82 – 102 (95)	6 (10)	
	pydiflumetofen	0.01	0.01	81 – 91*	6 (4)	0.03 - 5 ng/mL

Matrix	Analyte	LOQ (mg/kg)	Recovery fortification level (mg/kg)	Recoveries % range (mean)	Repeatability % RSD (n)	Linearity
Peas (fresh)				(86)		(equivalent to 0.003 – 0.5 mg/kg) (n = 6) y = 16059x+205 R^2 = 0.9999
			0.2	93 – 119 (110)	9 (5)	
			Overall	81 – 119 (99)	16 (9)	
Bean (dried)	pydiflumetofen	0.01	0.01	95 – 104 (99)	4 (5)	0.03 - 20 ng/mL (equivalent to 0.003 –2 mg/kg) (n = 8) y = 22113x+3016 R^2 = 0.9989
			0.2	70 – 88 (80)	10 (5)	
			Overall	70 – 104 (89)	13 (10)	
Wheat (grain)	pydiflumetofen	0.01	0.01	82 – 103 (90)	10 (5)	0.03 - 20 ng/mL (equivalent to 0.003 –2 mg/kg) (n = 8) y = 22113x+3016 R^2 = 0.9989
			0.2	89 – 98 (93)	4 (5)	
			Overall	82 – 103 (92)	7 (10)	
Wheat (straw)	pydiflumetofen	0.01	0.01	88 – 117 (105)	10 (5)	0.03 - 20 ng/mL** (equivalent to 0.003 –2 mg/kg) (n = 8) y = 22113x+3016 R^2 = 0.9989
			10	81 – 121 (106)	16 (5)	
			Overall	81 – 121 (105)	12 (10)	
Cereal (forage)	pydiflumetofen	0.01	0.01	88 – 120 (103)	12 (5)	0.03 - 20 ng/mL (equivalent to 0.003 –2 mg/kg) (n = 8) y = 22113x+3016 R^2 = 0.9989
			10	93 – 120 (110)	11 (5)	
			Overall	88 – 110 (107)	11 (10)	
Potato	pydiflumetofen	0.01	0.01	73 – 109 (90)	16 (5)	0.03 - 10 ng/mL (equivalent to 0.003 – 1 mg/kg) (n = 7) y = 15735x-280 R^2 = 0.9998
			10	93 – 108 (100)	6 (5)	
			Overall	73 – 109 (95)	12 (10)	
Rape seed	pydiflumetofen	0.01	0.01	86 – 99 (93)	5 (5)	0.03 - 10 ng/mL (equivalent to 0.003 – 1 mg/kg) (n = 7) y = 29447x+315 R^2 = 0.9999
			0.2	86 – 102 (95)	6 (5)	
			Overall	86 – 102 (94)	5 (10)	
Grape	pydiflumetofen	0.01	0.01	75 – 112 (92)	19 (5)	0.03 - 20 ng/mL (equivalent to 0.003 –2 mg/kg) (n = 8) y = 22113x+3016 R^2 = 0.9989
			1.4	86 – 97 (93)	5 (5)	
			Overall	75 – 112 (92)	13 (10)	

*At 0.01 mg/kg for pydiflumetofen in fresh peas a 50% recovery was obtained. This is considered an outlier according to the Grubbs test. Therefore, this recovery has not been considered in the statistical evaluation.
**The study report states matrix matched standards have been used for wheat straw, but no linearity data has been presented. Matrix effects are not considered to be significant. Therefore, the linearity data using solvent matched standards has been presented for wheat straw.

Specificity and Confirmation of Analyte Identity

LC-MS/MS with primary and confirmatory ion transitions is considered a highly specific technique. Therefore, additional methods to confirm the identity of the analytes are not considered necessary. Chromatograms for standards, reagent blank, control samples and samples fortified at the LOQ and at the higher level have been provided for all matrices. No significant interference (>30% of the LOQ) between the peak pydiflumetofen and any of the plant commodity matrices was observed. Analyte identity was confirmed by retention time match with pydiflumetofen reference standard.

Linearity

The linearity of the LC-MS/MS detector was tested using matrix matched standards in rape seed, potatoes, tomatoes, apples, and fresh peas (0.03-10 ng/mL except fresh peas 0.03-5 ng/mL). For grape, lettuce, cabbage, dry beans, cereal forage and wheat grain, solvent matched standards were used in the range 0.03-20 ng/mL. At least six standards at different concentrations were analysed. The response was linear for both MS/MS transitions with a coefficient of determination (R^2) ranging from 0.9985-1.0000. It is noted that if a residue is beyond the concentration range expected, samples are diluted appropriately to be within the linear range.

The study report states matrix matched standards are used for wheat straw, but no calibration data have been provided. However, the lack of linearity data in matrix-matched standards is considered acceptable as matrix effects are deemed to not be significant in wheat straw. Therefore, the solvent matched standard linearity data will cover wheat straw. Furthermore, acceptable linearity data have been presented in study S13-02224 for the quantitative transition only using matrix matched standards for wheat straw.

Accuracy

Fortified samples were analysed in quintuplet at the limit of quantification (LOQ: 0.01 mg/kg) and at a higher level (0.2 mg/kg for dry beans, rape seed, apples, fresh peas and wheat grain, 1.4 mg/kg for tomato and grape and 10 mg/kg for lettuce, cabbage, wheat straw, cereal forage, and potato). Some individual recoveries were outside of the acceptable range; however, mean recoveries for all levels were within the acceptable range. It should be noted an outlier was identified according to the Grubbs test at 0.01 mg/kg for fresh peas for each mass transitions. Nevertheless, the accuracy of the method is acceptable.

Precision

Precision was determined from the accuracy recovery data for pydiflumetofen. The %RSD was ≤20% with five samples prepared at each fortification level. However, it is noted for fresh peas for both mass transitions at 0.01 mg/kg an outlier was identified according to the Grubbs test and therefore only four determinations were used to calculate the %RSD. This is considered acceptable.

Matrix effects

No significant matrix effects were observed in cereal forage, wheat grain, dry beans, cabbage, grape, and lettuce. Matrix effects on detector response caused by apple, tomato, wheat straw and potato were also considered insignificant, but matrix matched standards were used for quantification. Significant matrix effects were observed in oilseed rape seed and fresh peas tested during the method validation therefore matrix matched standards were used for quantification.

LOQ

0.01 mg/kg for pydiflumetofen in all matrices.

Storage Stability of Final Extracts

Residues of pydiflumetofen were demonstrated to be stable in final extracts from apple, lettuce, tomato, cabbage, peas (fresh), bean (dried), wheat (grain, straw, forage), potato, rape seed and grape when stored at 5 ± 4 °C for between 8 and 21 days. Samples were fortified at LOQ level (0.01 mg/kg) and the day 0 and day 8-21 days recoveries were compared. At each interval and for each matrix, the mean recoveries were between 70-110% with a %RSD ≤20%. There was no significant difference between the day 0 and day 8-21 results (results were within ±10% of the initial values when re-analysed).

Storage Stability of Standard Solutions

The stability of standard solutions of pydiflumetofen was checked after a storage period of 122 days in a refrigerator at 5 ± 4 °C against freshly prepared calibration standards. The concentrations were within ±10 % of the initial values. The standard solutions can thus be considered as stable.

Extraction Efficiency

Pydiflumetofen has been shown to be efficiently extracted from oilseed rape, tomatoes, and wheat (forage, hay, straw, and grain) using the conditions described in GRM061.03A in radiolabelled metabolism studies (refer to section B.5.2.1). This covers high water content and dry commodities (high protein/starch content) extracted using acetonitrile/ultra-pure water (80/20, v/v) and high oil content commodities extracted using 100% acetonitrile. Furthermore, extraction efficiency is satisfactorily addressed for high acid commodities as tomatoes are slightly acidic. Therefore, data in tomatoes (high water content commodities) can be bridged to high acid commodities.

Conclusion

The method GRM061.03A is acceptably validated in accordance with SANCO/3029/99 rev. 4 for the determination of pydiflumetofen residues in high water (apples, lettuce, tomato, cabbage, fresh peas, and cereal forage), high acid (grape), high oil (oilseed rape), high starch (cereal grain and potato), high protein (dry beans) and dry (cereal straw) commodities, with an LOQ of 0.01 mg/kg.

Report:	**KCA1 6.10.1 [redacted] (2017)**
Title	SYN545974 and Fludioxonil – Residues in Honey Following Exposure of Bees to Treated Winter Oilseed Rape in Germany during 2016 Report number: S16-02006 Document No. VV-466889, A8240D_12181
Guidelines:	SANCO/3029/99 rev. 4
GLP:	Yes
Deviations	N/A
Previous evaluation:	None

Method GRM061.03A was also validated for the determination of SNY545974 in honey.

Sample preparation:

2 g of honey were weighed into a 50 mL Sarstedt tube. Fortification was carried out at this step by adding the fortification solution and allowing the solvent to evaporate for approximately 10 minutes. Then 20 mL acetonitrile/ultra-pure water (60/40, v/v) were added to the sample. The sample was extracted by homogenisation for 3-5 minutes and centrifuged at 15°C for 3 minutes at 3500 rpm. The supernatant was decanted into a clean 50 mL Sarstedt tube. An additional 20 mL of acetonitrile/ultra-pure water were added to the residual fraction. The sample was extracted by homogenisation for 3-5 minutes and centrifuged at 15°C for 3 minutes at 3500 rpm. The supernatants were mixed to produce a homogenous extract.

Samples are then cleaned up by SPE before analysis by LC-MS/MS. The SPE clean-up procedure and the LC-MS/MS conditions are the same as that given in study S14-05352 for method GRM061.03A, which are presented earlier in this report.

A summary of the method validation data is given in Table B.5.1.2.5-7.

Table B.5.1.2.5-7: Summary of method validation data for determination of residues of pydiflumetofen in honey – primary transition *m/z* 426 →193

Matrix	**Analyte**	**LOQ (mg/kg)**	**Recovery fortification level (mg/kg)**	**Recoveries % range (mean)**	**Repeatability % RSD (n)**	**Linearity**
Honey	pydiflumetofen	0.01	0.01	85 – 99 (93)	6 (5)	0.03 - 20 ng/mL (equivalent to 0.003 – 2 mg/kg) (n = 7) y = 45796.3x+8377.2 R^2 = 0.9986
			0.1	86 – 92 (89)	3 (5)	
			Overall	85 – 99 (91)	5 (10)	

Table B.5.1.2.5-8: Summary of method validation data for determination of residues of pydiflumetofen in honey – confirmatory transition *m/z* 428 →195

Matrix	Analyte	LOQ (mg/kg)	Recovery fortification level (mg/kg)	Recoveries % range (mean)	Repeatability % RSD (n)	Linearity
Honey	pydiflumetofen	0.01	0.01	88 – 102 (93)	6 (5)	0.03 - 20 ng/mL (equivalent to 0.003 – 2 mg/kg) (n = 7) y = 45981.8x+7385.7 R^2 = 0.9989
			0.1	83 – 90 (86)	3 (5)	
			Overall	83 – 102 (90)	6 (10)	

Specificity and Confirmation of Analyte Identity

LC-MS/MS with primary and confirmatory ion transitions is considered a highly specific technique. Therefore, additional methods to confirm the identity of the analytes are not considered necessary. Chromatograms for standards, reagent blank, control samples and samples fortified at the LOQ and at the higher level have been provided for honey. No significant interference (>30% of the LOQ) was observed at the retention time of interest.

Linearity

Linearity was demonstrated by the analysis of at seven standards of increasing concentration. The range of standard concentrations used was 0.03-20 ng/mL, equivalent to 0.003-2 mg/kg. Hence, the analytical calibration extends over a range appropriate to the lowest and highest nominal concentration of the analyte in relevant analytical solutions + at least 20%, in line with SANCO/3029/99 rev. 4. The response was linear for both mass transitions with a coefficient of determination (R^2)>0.99.

Accuracy

Fortified samples were analysed at the limit of quantification (LOQ: 0.01 mg/kg) and at a higher level (10 x LOQ: 0.1 mg/kg). Five samples were prepared at each fortification level and mean recoveries were within the acceptable range (70-110%). One procedural recovery was also reported at each fortification level. These were within the acceptable range.

Precision

Precision was determined from the accuracy recovery data for pydiflumetofen. The %RSD was ≤20% with five samples prepared at each fortification level.

LOQ

The LOQ is 0.01 mg/kg for honey. This is the lowest fortification level with acceptable precision and recovery data.

Matrix effects

No significant matrix effects were observed in honey. Therefore, solvent-based standard solutions were used for quantification.

Storage Stability of Final Extracts

Residues of pydiflumetofen were demonstrated to be stable in final extracts from honey when stored at 1-10 °C for 11 days. Samples were fortified at LOQ level (0.01 mg/kg) and the day 0 and day 11 days recoveries were compared. The mean recoveries were between 70-110% with a %RSD ≤20%. There was no significant difference between the day 0 and day 11 results (results were within ±20% of the initial values when re-analysed).

Storage Stability of Standard Solutions

The stability of standard solutions of pydiflumetofen was checked after a storage period of 11 days at 1-10°C. The concentrations were within ±10 % of the initial values. The standard solutions can therefore be considered as stable.

Conclusion

The method GRM061.03A is acceptably validated in accordance with SANCO/3029/99 rev. 4 for the determination of pydiflumetofen residues in honey with an LOQ of 0.01 mg/kg.

Method GRM061.06A

Residues studies supported by method GRM061.06A:

Data point	Author(s)	Year	Title Company Report No.
KCA1 6.1/01	[redacted]	2017	SYN545974 – Storage Stability of SYN545974 in Bovine Muscle, Liver, Milk, Fat and Chicken Eggs Report number: 36552 Document No. VV-414208 , SYN545974_10291
KCA1 6.4.1/01	[redacted]	2015	SYN545974 – Magnitude of the Residues in Tissue and Eggs Resulting from the Feeding of Three Dose Levels to Poultry 2014 Report number: TK0103796 Document No. VV-414618 , SYN545974_50189
KCA1 6.4.2/01	[redacted]	2017	SYN545974 – Magnitude of Residues in Milk and Tissues of Dairy Cows Following Multiple Oral Administration of SYN545974 Report number: 35775 Document No. VV-414196 , SYN545974_10288

Report:	**KCA1 4.1.2/29 [redacted] (2015)**
Title	SYN545974 – Analytical Method (GRM061.06A) for the Determination of SYN545974 in Bovine Milk, Liver, Kidney, Muscle, Fat, Blood, and Hen Eggs by LC-MS/MS Report number: GRM061.06A Document No. VV-132524 , SYN545974_50123
Guidelines:	SANCO/3029/99 rev. 4
GLP:	No
Deviations	N/A
Previous evaluation:	None

Report:	**KCA1 4.1.2/15 [redacted] (2015)**
Title	SYN545974 – Validation of an Analytical Method for the Determination of SYN545974 in Bovine Meat, Liver, Kidney, Fat, Milk, Blood, and Chicken Eggs Report number: 36383 Document No. VV-413066 , SYN545974_10247
Guidelines:	SANCO/3029/99 rev. 4
GLP:	Yes
Deviations	The mobile phases used for run 35 were indicated to expire on 13 March 2015 but were used on 16 March 2015. This is not expected to impact the study.
Previous evaluation:	None

LC-MS/MS method GRM061.06A was developed and validated for the determination of pydiflumetofen in bovine meat, liver, kidney, fat, milk, blood, and chicken eggs. It is noted the method is not required to be validated in blood, therefore these data have not been assessed.

Reference items:

pydiflumetofen, batch SMU2EP12007, purity 98.5 %w/w, CoA provided, expiry June 2016

Sample preparation:

Bovine Muscle, Liver, Kidney, Milk, Blood, and Hen Eggs

For the extraction of bovine muscle, liver, kidney, milk, blood and chicken eggs, a 10 g subsample is homogenised with 100 mL acetonitrile: water (80:20, v/v) for 2-5 minutes. The samples are centrifuged for 5 minutes and are then either analysed directly or are diluted before analysis if the sensitivity of the instrument allows (1 mL aliquot diluted to 5 mL ultra-pure water containing 0.1% acetic acid). If the sensitivity of the instrument is inadequate or interference is expected or observed, samples are subjected to further clean-up by SPE.

Bovine Fat

For the extraction of bovine fat, a 10 g subsample is dissolved into 40 mL *n*-hexane by sonication and shaking. The mixture is centrifuged for 5 minutes and then the upper layer is transferred into a separating funnel. The extraction process is repeated twice more with 10 mL hexane, to give a combined volume of 60 mL. 50 mL acetonitrile: water (80:20, v/v) is added to the separating funnel and the mixture is shaken vigorously for 3 minutes. The phases are allowed to separate and then the lower layer is transferred to a graduated cylinder. This process is repeated, and the combined lower layers are adjusted to 100 mL. The samples are then either analysed directly or are diluted before analysis if the sensitivity of the instrument allows (1 mL aliquot diluted to 5 mL ultra-pure water containing 0.1% acetic acid). If the sensitivity of the instrument is inadequate or interference is expected or observed, samples are subjected to further clean-up by SPE.

SPE Clean-Up

10 mL of sample extract are transferred to a 15 mL polypropylene tube. The acetonitrile content is removed by evaporation to reduce the volume to <2 mL. The sample volume is adjusted to 5 mL using 0.4 M sodium acetate buffer (pH 5) and the sample is then mixed thoroughly. The sample is then centrifuged for 2-3 minutes. The sample is transferred to the SPE cartridge and is passed over the cartridge under gravity or slight vacuum. The eluate is collected and then volume adjusted to 10 mL with ultrapure water. The samples are vortexed to mix thoroughly and then centrifuged for 2-3 minutes. The final sample composition is acetonitrile/ultra-pure water containing 0.1% acetic acid (20/80 v/v). The sample is transferred to a suitable vial for LC-MS/MS analysis. The final sample concentration is 0.1 g/mL.

No hydrolysis step is required for the analysis of pydiflumetofen in animal matrices for method GRM061.06A.

LC-MS/MS conditions:

Chromatographic system:	Agilent Infinity 1290 HPLC system
Analytical column:	Agilent Poroshell 120 EC-C18 50 mm x 2.1 mm, 2.7 µm
Injection volume:	10 µL
Column temperature:	40°C
Mobile phase A:	0.1% formic acid in water
Mobile phase B:	0.1% formic acid in acetonitrile
Flow rate:	0.6 mL/minute

Gradient:

Time (minute)	% A	% B
0.0	80	20
0.2	80	20
1.5	10	90
2.5	10	90
2.6	80	20
2.8	5	95
3.0	80	20
3.2	5	95
3.4	80	20
3.6	5	95
3.8	80	20
5.0	80	20

Retention time:	Approximately 2 minutes
Detector:	Sciex API 5500QTRAP triple quadrupole mass spectrometer
Ionisation type:	APCI
Polarity:	Positive
Scan type:	MS/MS, multiple reaction monitoring (MRM)
Mass transitions:	pydiflumetofen

Ion monitored	Declustering Potential (DP)	Collision Energy (CE)	Collisions Cell Exit Potential (CXP)	Dwell time
m/z 426→193 (quantification)	70 eV	40 eV	15 eV	150 ms
m/z 426→166 (confirmation)	70 eV	36 eV	15 eV	150 ms

A summary of the method validation data is presented in Table B.5.1.2.5-9 – 12.

Table B.5.1.2.5-9: Summary of method validation data for determination of residues of pydiflumetofen in animal matrices – primary transition *m/z* 426 →193 (direct analysis)

Matrix	**Analyte**	**LOQ (mg/kg)**	**Recovery fortification level (mg/kg)**	**Recoveries % range (mean)**	**Repeatability % RSD (n)**	**Linearity**
Bovine Meat	pydiflumetofen	0.01	0.01	107 – 116 (111)	4 (5)	Standards in acetonitrile: water (80:20, v/v) 0.25 – 12.5 ng/mL (equivalent to 0.0025-0.125 mg/kg) (n = 6*2) y = 6926x+643 R^2 = 0.9978
			0.1	78 – 119 (108)	16 (5)	
			Overall	78 – 119 (110)	11 (10)	
Bovine Liver	pydiflumetofen	0.01	0.01	105 – 112 (108)	3 (5)	
			0.1	111 – 118 (115)	3 (5)	
			Overall	105 – 118 (111)	4 (10)	
Bovine Kidney	pydiflumetofen	0.01	0.01	101 – 110 (105)	4 (5)	
			0.1	100 – 107 (104)	3 (5)	
			Overall	100 – 110 (104)	3 (10)	
Bovine Fat	pydiflumetofen	0.01	0.01	84 – 95 (88)	5 (5)	
			0.1	93 – 109 (102)	7 (5)	
			Overall	84 – 109 (95)	10 (10)	
Bovine Milk	pydiflumetofen	0.01	0.01	99 – 109 (104)	5 (5)	
			0.1	105 – 113 (109)	3 (5)	
			Overall	99 – 113 (107)	5 (10)	
Chicken Eggs	pydiflumetofen	0.01	0.01	99 – 105** (101)	3 (4)	
			0.1	101 – 108 (104)	3 (5)	
			Overall	99 – 108 (103)	3 (9)	

**For eggs at 0.01 mg/kg a recovery was obtained at 150%. This was identified as an outlier and excluded in calculations.

Table B.5.1.2.5-10: Summary of method validation data for determination of residues of pydiflumetofen in animal matrices – confirmatory transition *m/z* 426 →166 (direct analysis)

Matrix	Analyte	LOQ (mg/kg)	Recovery fortification level (mg/kg)	Recoveries % range (mean)	Repeatability % RSD (n)	Linearity
Bovine Meat	pydiflumetofen	0.01	0.01	110 – 120 (115)	3 (5)	Standards in acetonitrile: water (80:20, v/v) 0.25 – 12.5 ng/mL (equivalent to 0.0025-0.125 mg/kg) (n = 6*2) y = 3764x+261 R^2 = 0.9989
			0.1	77 – 116 (106)	16 (5)	
			Overall	77 – 120 (111)	11 (10)	
Bovine Liver	pydiflumetofen	0.01	0.01	93 – 112 (105)	7 (5)	
			0.1	110 – 122 (116)	4 (5)	
			Overall	93 – 122 (110)	7 (10)	
Bovine Kidney	pydiflumetofen	0.01	0.01	Not validated due to significant interference – SPE clean-up recommended		
			0.1			
			Overall			
Bovine Fat	pydiflumetofen	0.01	0.01	84 – 103 (91)	8 (5)	
			0.1	95 – 107 (102)	6 (5)	
			Overall	84 – 107 (97)	9 (10)	
Bovine Milk	pydiflumetofen	0.01	0.01	95 – 111 (104)	6 (5)	
			0.1	106 – 112 (110)	2 (5)	
			Overall	95 – 112 (107)	5 (10)	
Chicken Eggs	pydiflumetofen	0.01	0.01	93 – 112** (102)	8 (4)	
			0.1	105 – 109 (107)	2 (5)	
			Overall	93 – 112 (105)	6 (9)	

**For eggs at 0.01 mg/kg a recovery was obtained at 159%. This was identified as an outlier and excluded in calculations.

Table B.5.1.2.5-11: Summary of method validation data for determination of residues of pydiflumetofen in animal matrices – primary transition *m/z* 426 →193 (SPE Clean up)

Matrix	Analyte	LOQ (mg/kg)	Recovery fortification level (mg/kg)	Recoveries % range (mean)	Repeatability % RSD (n)	Linearity
Bovine Meat	pydiflumetofen	0.01	0.01	86 – 103 (93)	8 (5)	Standards in acetonitrile: water (80:20, v/v) 0.25 – 12.5 ng/mL (equivalent to 0.0025-0.125 mg/kg) (n = 6*2) y = 6926x+643 R^2 = 0.9978
			0.1	70 – 99 (87)	12 (5)	
			Overall	70 – 103 (90)	10 (10)	
Bovine Liver	pydiflumetofen	0.01	0.01	92 – 106 (97)	5 (5)	
			0.1	100 – 105 (103)	2 (5)	
			Overall	92 – 106 (100)	5 (10)	
Bovine Kidney	pydiflumetofen	0.01	0.01	84 – 104 (92)	10 (5)	
			0.1	84 – 100 (91)	7 (5)	
			Overall	84 – 104 (91)	8 (10)	
Bovine Fat	pydiflumetofen	0.01	0.01	77 – 89 (81)	5 (5)	
			0.1	86 – 91 (89)	2 (5)	
			Overall	77 – 91 (85)	6 (10)	
Bovine Milk	pydiflumetofen	0.01	0.01	84 – 109 (93)	11 (5)	
			0.1	82 – 99 (90)	8 (5)	
			Overall	82 – 109 (91)	9 (10)	
Chicken Eggs	pydiflumetofen	0.01	0.01	91 – 103 (95)	6 (5)	
			0.1	94 – 100 (97)	3 (5)	
			Overall	91 – 103 (96)	4 (10)	

Table B.5.1.2.5-12: Summary of method validation data for determination of residues of pydiflumetofen in animal matrices – confirmatory transition *m/z* 426 →166 (SPE Clean up)

Matrix	Analyte	LOQ (mg/kg)	Recovery fortification level (mg/kg)	Recoveries % range (mean)	Repeatability % RSD (n)	Linearity
Bovine Meat	pydiflumetofen	0.01	0.01	89 – 100 (96)	5 (5)	Standards in acetonitrile: water (80:20, v/v) 0.25 – 12.5 ng/mL (equivalent to 0.0025-0.125 mg/kg) (n = 6*2) y = 3764x+261 R^2 = 0.9989
			0.1	73 – 98 (88)	11 (5)	
			Overall	73 – 100 (92)	9 (10)	
Bovine Liver	pydiflumetofen	0.01	0.01	94 – 103 (97)	4 (5)	
			0.1	99 – 105 (103)	3 (5)	
			Overall	94 – 105 (100)	4 (10)	
Bovine Kidney	pydiflumetofen	0.01	0.01	86 – 117 (98)	13 (5)	
			0.1	86 – 104 (92)	8 (5)	
			Overall	86 – 117 (95)	11 (10)	
Bovine Fat	pydiflumetofen	0.01	0.01	78 – 85 (81)	4 (5)	
			0.1	85 – 91 (88)	2 (5)	
			Overall	78 – 91 (85)	5 (10)	
Bovine Milk	pydiflumetofen	0.01	0.01	88 – 105 (94)	7 (5)	
			0.1	82 – 95 (89)	6 (5)	
			Overall	82 – 105 (91)	7 (10)	
Chicken Eggs	pydiflumetofen	0.01	0.01	92 – 107 (97)	6 (5)	
			0.1	96 – 100 (98)	2 (5)	
			Overall	92 – 107 (98)	4 (10)	

Recovery, precision, linearity, and specificity data have been presented in the study report using solvent standards and matrix matched standards. Data are presented in the tables above for the solvent standards only as matrix effects were not significant. The data using matrix matched standards are also acceptable.

Specificity and Confirmation of Analyte Identity

LC-MS/MS with primary and confirmatory ion transitions is considered a highly specific technique. Therefore, additional methods to confirm the identity of the analyte are not considered necessary. Chromatograms for standards, reagent blank, control samples and samples fortified at the LOQ and at the higher level have been provided for all matrices for direct analysis and for SPE clean up. No significant interference (>30% of the LOQ) between the peak pydiflumetofen and any of the animal commodities was observed, except for direct analysis of bovine kidney for the confirmatory transition. However, using SPE clean-up no significant interference is observed for bovine kidney.

Linearity

Linearity was demonstrated by the analysis of at least six standards in duplicate. The range of standard concentrations used was 0.25-12.5 ng/mL, equivalent to 0.0025-0.125 mg/kg (25%-1250% of the LOQ). Hence, the analytical calibration extends over a range appropriate to the lowest and highest nominal concentration of the analyte in relevant analytical solutions +/- at least 20%, in line with SANCO/3029/99 rev. 4. The response was linear for both mass transitions with a coefficient of determination (R^2)>0.99. Higher concentration samples are diluted to within the linear range if required. It should be noted linearity data is available for solvent standards and matrix matched standards.

Accuracy

Fortified samples were analysed in quintuplet at the limit of quantification (LOQ: 0.01 mg/kg) and at a higher level (10 x LOQ: 0.1 mg/kg). Recovery data has been presented for direct analysis and using an SPE clean-up prior to analysis. Mean recoveries were within the acceptable range (70-110%), except for bovine meat and liver for both mass transitions by direct analysis. For bovine meat at 0.01 mg/kg a mean recovery of 111% and 115% is reported for the quantitative and confirmatory mass transitions respectively. For bovine liver at 0.1 mg/kg a mean recovery of 115% and 116% is observed for the quantitative and confirmatory mass transitions respectively. However, the mean recoveries using SPE clean up are within the acceptable range for bovine meat and liver. Therefore, the accuracy of the method is acceptable.

Precision

Precision was determined from the accuracy recovery data for pydiflumetofen. The %RSD was ≤20% with five samples prepared at each fortification level. However, an outlier was identified in chicken eggs for both mass transitions using direct analysis, so only four determinations have been used to calculate the %RSD.

Matrix effects

No significant matrix effects were observed in bovine meat, liver, kidney, fat, milk, and chicken eggs. Solvent and matrix matched standards were used for quantification.

LOQ

0.01 mg/kg for pydiflumetofen in all matrices. This is the lowest fortification level with acceptable precision and recovery data.

Storage Stability of Extracts

Residues of pydiflumetofen were demonstrated to be stable in final extracts from animal matrices when stored at 4 °C for 6-7 days. Samples were fortified at LOQ level (0.01 mg/kg) and the day 0 and day 6–7-day recoveries were compared. The mean recoveries were between 70-110% with a %RSD ≤20%. There was no significant difference between the day 0 and day 6-7 results.

Storage Stability of Standard Solutions

The stability of the stored working standard solutions of pydiflumetofen was checked after a storage period of 105 days (1000 µg/mL), 102 days (6.25 and 0.125 µg/mL), 38 days (0.025 µg/mL) and 9 days (2 and 20 µg/mL). Samples were stored in a refrigerator at a target temperature of <4°C and analysed against freshly prepared calibration standards. The results demonstrated that pydiflumetofen residues were stable in the stored working standard solutions during the duration of the study.

Extraction Efficiency

Two animal metabolism studies were submitted that describe the metabolism of radiolabelled pydiflumetofen in lactating goats and laying hen. The extraction solvents used in the metabolism studies extracted >70% of the TRR from milk, kidney, muscle, and fat. However, residues extractability was lower in liver (50.4 and 47.4% TRR for phenyl and pyrazole in ruminant liver, respectively). Details of the extractable %TRR were given following each extraction. The extraction solvents used in the animal metabolism studies are as follows:

- Milk: samples were extracted by homogenizing with acetonitrile (500 mL) and hexane (100 mL). The majority of the %TRR was extracted in acetonitrile.
- Liver, kidney, muscle, eggs: samples were extracted by homogenizing with acetonitrile: water (80:20, v/v) twice followed by acetonitrile: water (1:1, v/v). For kidney, muscle and egg whites, the majority of the %TRR was extracted with the first acetonitrile: water (80:20, v/v) extraction. For liver and egg yolk, the levels extracted were low. The unextractable residues for liver and egg yolk were investigated further by solubilization either with the surfactant sodium dodecyl sulphate (SDS) or proteolytic enzyme hydrolysis.

Results show most of the extractable residue for liver and egg yolk were extracted following the first extraction with acetonitrile: water (80:20, v/v).

- Fat: samples were extracted by homogenizing with acetonitrile: water (80:20, v/v) + heptane twice followed by acetonitrile: water (1:1, v/v). The majority of the %TRR was extracted with the first extraction in acetonitrile: water (80:20, v/v).

Method GRM061.06A has been developed for risk assessment purposes and has been used to determine residues of pydiflumetofen in the livestock feeding studies and storage stability studies. Method GRM061.06A uses the following extraction solvents:

- Milk, liver, kidney, muscle, and eggs: samples were extracted by homogenizing with acetonitrile: water (80:20, v/v)
- Fat: samples were extracted by homogenizing with acetonitrile: water (80:20, v/v) + heptane

Overall, for liver, kidney muscle, eggs and fat, the same extraction solvent was used to extract residues in the animal metabolism studies as is used in Method GRM061.06A (acetonitrile: water (80:20, v/v)). Residue extractabilities were generally high using the solvent extraction described in the metabolism studies. On this basis, the extraction efficiency of Method GRM061.06A is considered satisfactorily addressed.

For milk, different extraction solvents were used to extract residues in the animal metabolism studies (acetonitrile) and Method GRM061.06A (acetonitrile: water (80:20, v/v)). In line with SANTE/2017/10632, solvent mixtures are considered as being identical if their composition varies by not more than 20%. Hence, the extraction efficiency is considered acceptable for milk since samples were extracted with 100% acetonitrile in the metabolism studies, whereas Method GRM061.06A uses 80% acetonitrile: 20% water for the extraction.

Conclusion

The method GRM061.06A is acceptably validated in accordance with SANCO/3029/99 rev. 4 for the determination of pydiflumetofen residues in bovine meat, liver, kidney, fat, milk, and chicken eggs with an LOQ of 0.01 mg/kg.

Method GRM061.08A

Residues studies supported by method GRM061.08A:

Data point	Author(s)	Year	Title Company Report No.
KCA1 6.1/01	[redacted]	2016	SYN545974 – Frozen Storage Stability of Residues of SYN508272, SYN548264, SYN547897 and SYN548263 in Animal Matrices Report number: CEMR-7064 Document No. VV-412637 , SYN508272_10915
KCA1 6.4.2/01	[redacted]	2017	SYN545974 – Magnitude of Residues in Milk and Tissues of Dairy Cows Following Multiple Oral Administration of SYN545974 Report number: 35775 Document No. VV-414196 , SYN545974_10288

Report:	**KCA1 4.1.2/27 [redacted] (2015a)**
Title	SYN545974 – Analytical Method (GRM061.08A) for the Determination of SYN548264 and SYN508272 in Bovine Milk by LC-MS/MS Report number: GRM061.08A Document No. VV-132522 , SYN548264_50000
Guidelines:	SANCO/3029/99 rev. 4
GLP:	No
Deviations	N/A
Previous evaluation:	None

Report:	**KCA1 4.1.2/13 [redacted], [redacted], [redacted] (2017)**
Title	SYN545974 – Magnitude of Residues in Milk and Tissues of Dairy Cows Following Multiple Oral Administrations of SYN545974 Report number: 35775 Document No. VV-414196 , SYN545974_10288
Guidelines:	SANCO/3029/99 rev. 4
GLP:	Yes
Deviations	N/A
Previous evaluation:	None

LC-MS/MS method GRM061.08A was developed and validated for the determination of metabolites SYN548264 and SYN508272 in bovine milk.

Reference items:

SYN548264, batch MES 335/1, CoA provided, expiry April 2016

SYN508272, batch MES 114/1, CoA provided, purity 97%, expiry May 2017

Sample preparation:

Milk samples (10 g) were extracted by shaking with acetonitrile (40 mL). The samples were then centrifuged. A 50 µL aliquot was transferred into an autosampler vial and ultra-pure water (440 µL) and acetonitrile (10 µL) were added. The samples were then mixed thoroughly. SYN548264 and SYN508272 were analysed by high-performance liquid chromatography with triple quadrupole mass spectrometric detection (LC-MS/MS), monitoring for the primary transitions (*m/z* 246-131 and 176-136) and confirmatory transitions (*m/z* 246-111 and 176-156) respectively. The final sample concentration is 0.02 g/mL, taking into account the water content of the milk.

LC-MS/MS conditions:

Chromatographic system:	Agilent Infinity 1290 HPLC
Analytical column:	Agilent Poroshell C18 50 mm x 2.1 mm, 2.7 µm
Injection volume:	10 µL
Column temperature:	40°C

Mobile phase A: 0.1% formic acid in water
Mobile phase B: 0.1% formic acid in acetonitrile
Flow rate: 0.6 mL/minute

Gradient:

Time (minute)	% A	% B
0	95	5
0.2	95	5
2.0	10	90
3.5	10	90
3.6	95	5
5.0	95	5

Retention time: SYN508272: approximately 1.5 minutes
SYN548264: approximately 1.6 minutes

Detector: Sciex API 5500QTRAP triple quadrupole mass spectrometer

SYN548264

Ionisation type: ESI
Polarity: Negative

Ion monitored	Declustering Potential	Collision Energy	Collisions Cell Exit Potential	Dwell time
m/z 246→131 (quantification)	-110 V	-24 V	-18 V	200 ms
m/z 246→111 (confirmation)	-110 V	-24 V	-18 V	200 ms

SYN508272

Ionisation type: ESI
Polarity: Positive
Mass transitions:

Ion monitored	Declustering Potential (DP)	Collision Energy (CE)	Collisions Cell Exit Potential (CXP)	Dwell time
m/z 176→136 (quantification)	80 V	24 V	18 V	200 ms
m/z 176→156 (confirmation)	80 V	14 V	18 V	200 ms

A summary of the method validation data is presented in Table B.5.1.2.5-13 and B.5.1.2.5-14 for SYN548264 and SYN508272 respectively.

Table B.5.1.2.5-13: Summary of method validation data for determination of residues of SYN548264 in milk (solvent calibration standards)

Matrix	**Analyte**	**LOQ (mg/kg)**	**Recovery fortification level (mg/kg)**	**Recoveries % range (mean)**	**Repeatability % RSD (n)**	**Linearity**
Bovine Milk	Primary SYN548264 *m/z* 246 → 131	0.01	0.01	82 – 97 (89)	6 (5)	0.05 – 2.5 ng/mL (equivalent to 0.0025-0.125 mg/kg) (n = 7*2) y = 88260x-231 R^2 = 0.9991
			0.1	92 – 102 (95)	5 (5)	
			Overall	82– 102 (92)	6 (10)	
	Confirmatory: SYN548264 *m/z* 246 → 111	0.01	0.01	88 – 93 (92)	2 (5)	0.05 – 2.5 ng/mL[a] (equivalent to 0.0025-0.125 mg/kg) (n = 7*2) y = 13700x-105 R^2 = 0.9992
			0.1	95 – 105 (97)	5 (5)	
			Overall	88 – 105 (94)	5 (10)	

a) One of the calibration standards at 0.05 ng/mL was outside of the acceptance so was not included in the calibration.

Table B.5.1.2.5-14: Summary of method validation data for determination of residues of SYN508272 in milk (solvent calibration standards)

Matrix	Analyte	LOQ (mg/kg)	Recovery fortification level (mg/kg)	Recoveries % range (mean)	Repeatability % RSD (n)	Linearity
Bovine Milk	Primary Transition: SYN508272 *m/z* 176 → 136	0.01	0.01	75 – 94 (85)	9 (5)	0.05 – 2.5 ng/mL[a] (equivalent to 0.0025-0.125 mg/kg) (n = 7*2) y = 121700x-1331 R^2 = 0.9977
			0.1	78 – 92 (85)	8 (5)	
			Overall	75 – 94 (85)	8 (10)	
	Confirmatory Transition SYN508272 *m/z* 176 → 156	0.01	0.01	81 – 92 (87)	5 (5)	0.05 – 2.5 ng/mL[a] (equivalent to 0.0025-0.125 mg/kg) (n = 7*2) y = 82030x-620 R^2 = 0.9974
			0.1	77 – 92 (85)	7 (5)	
			Overall	77 – 92 (86)	6 (10)	

a) One of the calibration standards at 0.05 ng/mL and 2.0 ng/mL were outside of the acceptance so were not included in the calibration.

Recovery, precision, and linearity data have been presented in the study report using solvent standards and matrix matched standards. Data is presented in the tables above for the solvent standards only as matrix effects were not significant. The data using matrix matched standards is also acceptable.

Specificity and Confirmation of Analyte Identity

LC-MS/MS with primary and confirmatory ion transitions is considered a highly specific technique. Therefore, additional methods to confirm the identity of the analytes are not considered necessary. Chromatograms for standards in water/acetonitrile, reagent blanks, control samples and samples fortified at the LOQ (0.01 mg/kg) and at 10 x LOQ (0.1 mg/kg) have been provided for milk for SYN548264 and SYN508272 for both mass transitions. No significant interference (>30% of the LOQ) at the retention time of interest was observed.

Linearity

Linearity was demonstrated by the analysis of at least seven standards in duplicate for SY548264 and SYN508272. The range of standard concentrations used was 0.05-2.5 ng/mL, equivalent to 0.0025-0.125 mg/kg (25-1250% of the LOQ). The response was linear for both mass transitions with a coefficient of determination (R^2)>0.99. It is noted for the confirmatory transitions the following calibration standards were outside of the acceptable range and were not included in the calibration: 0.05 ng/mL for SYN548264 and 0.05 and 2.0 ng/mL for SYN508272. This is acceptable as a sufficient number of standards are still used for the calibration. In addition to this, samples are diluted appropriately to ensure they are within the linear range.

Accuracy

Fortified samples were analysed in quintuplet at the limit of quantification (LOQ: 0.01 mg/kg) and at a higher level (10x LOQ: 0.1 mg/kg) for SYN548264 and SYN508272. Mean recoveries were within the acceptable range (70-110%) for both mass transitions for each metabolite.

Precision

Precision was determined from the accuracy recovery data for SYN548264 and SYN508272. The %RSD was ≤20% with five samples prepared at each fortification level.

Matrix effects

No significant matrix effects were observed in milk for SYN548264 and SYN508272. Both solvent and matrix matched standards were used for quantification. Data is presented in the tables above for the solvent standards only as matrix effects were not significant.

LOQ

0.01 mg/kg for SYN548264 and SYN508272 in milk. This is the lowest fortification level with acceptable precision and recovery data.

Storage Stability of Extracts

Residues of SYN548264 and SYN508272 were demonstrated to be stable in final extracts from milk when stored at 4 °C for at least 11 days. Samples were fortified at LOQ level (0.01 mg/kg) and the day 0 and day 11 recoveries were compared. The mean recoveries were between 70-110% with a %RSD ≤20%. There was no significant difference between the day 0 and day 6-7 results. `

Storage Stability of Standards

Standard solutions of SYN548264 and SYN508272 were stable over the course of the study when stored at 4 °C.

Extraction Efficiency

The metabolism of radiolabelled pydiflumetofen has been described in a study in lactating goats. Milk samples were extracted by homogenizing with acetonitrile (500 mL) and hexane (100 mL). The majority of the %TRR was extracted in acetonitrile (>70% of the TRR).

Method GRM061.08A has been developed for risk assessment purposes and has been used to determine residues of pydiflumetofen in the livestock feeding studies and storage stability studies. As part of GRM061.08A, milk samples are extracted by shaking with acetonitrile.

Hence, the same extraction solvent was used to extract residues in the animal metabolism studies as is used in Method GRM061.08A (acetonitrile). On this basis, the extraction efficiency of GRM061.08A is considered satisfactorily addressed.

Hydrolysis

Metabolites SYN548264 and SYN508272 were only found in their free ‘unconjugated’ form in milk in the metabolism study, and therefore a hydrolysis step is not required.

Conclusion

The method GRM061.08A is acceptably validated in accordance with SANCO/3029/99 rev. 4 for the determination of SYN548264 and SYN508272 in milk with an LOQ of 0.01 mg/kg.

Method GRM061.09A

Residues studies supported by method GRM061.09A:

Data point	Author(s)	Year	Title Company Report No.
KCA1 6.1/01	[redacted]	2016	SYN545974 – Frozen Storage Stability of Residues of SYN508272, SYN548264, SYN547897 and SYN548263 in Animal Matrices Report number: CEMR-7064 Document No. VV-412637 , SYN508272_10915
KCA1 6.4.2/01	[redacted]	2017	SYN545974 – Magnitude of Residues in Milk and Tissues of Dairy Cows Following Multiple Oral Administration of SYN545974 Report number: 35775 Document No. VV-414196 , SYN545974_10288

Report:	**KCA1 4.1.2/28 [redacted] (2015b)**
Title	SYN545974 – Analytical Method (GRM061.09A) for the Determination of Free and Conjugated SYN547897 and SYN548263 in Kidney and Liver by LC-MS/MS Report number: GRM061.09A Document No. VV-132523 , SYN547897_50000
Guidelines:	SANCO/3029/99 rev. 4
GLP:	No
Deviations	N/A
Previous evaluation:	None

Report:	**KCA1 4.1.2/13 [redacted], [redacted], [redacted] (2017)**
Title	SYN545974 – Magnitude of Residues in Milk and Tissues of Dairy Cows Following Multiple Oral Administrations of SYN545974 Report number: 35775 Document No. VV-414196 , SYN545974_10288
Guidelines:	SANCO/3029/99 rev. 4
GLP:	Yes
Deviations	N/A
Previous evaluation:	None

LC-MS/MS method GRM061.09A was developed and validated for the determination of free and conjugated SYN547897 and SYN548263 in bovine kidney and liver.

Reference items:

SYN547897, batch MES 311/1, CoA provided, expiry May 2020

SYN548263, batch MES 346/1, CoA provided, expiry August 2016

Sample preparation:

10 g bovine liver and kidney samples were extracted with 100 mL acetonitrile: water (80/20, v/v) using a homogeniser at high speed. The extracts were then centrifuged to remove solid material. A 10 mL aliquot of supernatant (equivalent to 0.1 g sample) was filtered through a C18 Sep-Pak cartridge. The supernatant was then evaporated to aqueous only (acetonitrile completely removed). Samples were buffered to pH 5 with 0.4M sodium acetate containing β-glucuronidase and hydrolysed at 37°C for 18 hours. After hydrolysis, samples were diluted to 10 mL with 50 mM KH_2PO_4 and cleaned-up by solid phase extraction (SPE) using an Oasis MAX cartridge. The final sample consists of ultra-pure water, ethanol/formic acid (60/40/0.4 v/v/v). SYN547897 and SYN548263 were analysed by high performance liquid chromatography with triple quadrupole mass spectrometric detection (LC-MS/MS), monitoring for the primary transitions (m/z 442-210 and 276-131) and confirmatory transitions (m/z 442-360 and 276-200) respectively. The final sample concentration is 0.1 g/mL.

LC-MS/MS conditions:

Chromatographic system: Agilent Infinity 1290 HPLC system

Analytical column: Ascentis Express C18 50 mm x 3 mm, 2.7 µm
Injection volume: 30 µL
Column temperature: 30°C
Mobile phase A: 0.1% acetic acid in water
Mobile phase B: 0.1% acetic acid in acetonitrile
Flow rate: 0.5 mL/minute

Gradient:

Time (minute)	% A	% B
0.0	80	20
2.0	80	20
2.5	10	90
3.5	10	90
4.0	80	20
6.0	80	20

Retention time: SYN548263: approximately 1.6 minutes
SYN547897: approximately 3.3 minutes
Detector: Sciex API 5500QTRAP triple quadrupole mass spectrometer
Ionisation type: ESI
Polarity: Negative
Scan type: MS/MS, multiple reaction monitoring (MRM)
Mass transitions: **SYN547897**

Ion monitored	Declustering Potential	Collision Energy	Collisions Cell Exit Potential	Dwell time
m/z 442→210 (quantification)	-110 V	-43 V	-15 V	100 ms
m/z 442→360 (confirmation)	-110 V	-36 V	-15 V	100 ms

SYN548263

Ion monitored	Declustering Potential	Collision Energy	Collisions Cell Exit Potential	Dwell time
m/z 276→131 (quantification)	-60 V	-26 V	-15 V	100 ms
m/z 276→200 (confirmation)	-60 V	-15 V	-15 V	100 ms

A summary of the method validation data is presented in Table B.5.1.2.5-15 and B.5.1.2.5-16 for SYN547897 and SYN548263 respectively.

Table B.5.1.2.5-15: Summary of method validation data for determination of residues of SYN547897 in liver and kidney

Matrix	Analyte	LOQ (mg/kg)	Recovery fortification level (mg/kg)	Recoveries % range (mean)	Repeatability % RSD (n)	Linearity
Bovine Liver	Primary Transition: SYN547897 *m/z* 442 → 210	0.01	0.01	86 – 125 (108)	16 (5)	0.25 – 12.5 ng/mL (equivalent to 0.0025-0.125 mg/kg) (n = 6*2) y = 197500x-4210 R^2 = 0.9965
			0.1	88 – 99 (94)	5 (5)	
			Overall	86– 125 (101)	14 (10)	
	Confirmatory Transition: SYN547897 *m/z* 442 → 360	0.01	0.01	81 – 125 (106)	18 (5)	0.25 – 12.5[a] ng/mL (equivalent to 0.0025-0.125 mg/kg) (n = 6*2) y = 10400x-700 R^2 = 0.9961
			0.1	83 – 96 (92)	6 (5)	
			Overall	81 – 125 (99)	18 (10)	
Bovine Kidney	Primary Transition: SYN547897 *m/z* 442 → 210	0.01	0.01	95 – 115 (106)	7 (5)	0.25 – 12.5 ng/mL (equivalent to 0.0025-0.125 mg/kg) (n = 6*2) y = 125100x+3945 R^2 = 0.9979
			0.1	109 – 119 (113)	4 (5)	
			Overall	95– 119 (110)	6 (10)	
	Confirmatory Transition: SYN547897 *m/z* 442 → 360	0.01	0.01	98 – 113 (105)	6 (5)	0.25 – 12.5 ng/mL (equivalent to 0.0025-0.125 mg/kg) (n = 6*2) y = 6197x-334 R^2 = 0.9985
			0.1	107 – 119 (113)	4 (5)	
			Overall	98 – 119 (109)	6 (10)	

(a) One of the calibration standards at 2.5 ng/mL is outside of the acceptable limits, therefore this has not been included in the calibration.

Table B.5.1.2.5-16: Summary of method validation data for determination of residues of SYN548263 in liver and kidney

Matrix	Analyte	LOQ (mg/kg)	Recovery fortification level (mg/kg)	Recoveries % range (mean)	Repeatability % RSD (n)	Linearity
Bovine Liver	Primary Transition: SYN548263 *m/z* 276 → 131	0.01	0.01	83 – 88 (85)	2 (5)	0.25 – 12.5 ng/mL (equivalent to 0.0025-0.125 mg/kg) (n = 6*2) y = 26940x-586 R^2 = 0.9995
			0.1	88 – 92 (91)	2 (5)	
			Overall	83– 92 (88)	4 (10)	
	Confirmatory Transition: SYN548263 *m/z* 276 → 200	0.01	0.01	78 – 86 (82)	4 (5)	0.25 – 12.5 ng/mL (equivalent to 0.0025-0.125 mg/kg) (n = 6*2) y = 48710x-314 R^2 = 0.9996
			0.1	84 – 95 (90)	5 (5)	
			Overall	78 – 95 (86)	6 (10)	
Bovine Kidney	Primary Transition: SYN548263 *m/z* 276 → 131	0.01	0.01	75 – 85 (78)	5 (5)	0.25 – 12.5 ng/mL (equivalent to 0.0025-0.125 mg/kg) (n = 6*2) y = 35960x+800 R^2 = 0.9988
			0.1	77 – 85 (80)	5 (5)	
			Overall	75– 85 (79)	5 (10)	
	Confirmatory Transition: SYN548263 *m/z* 276 → 200	0.01	0.01	74 – 83 (80)	5 (5)	0.25 – 12.5 ng/mL (equivalent to 0.0025-0.125 mg/kg) (n = 6*2) y = 63290x+644 R^2 = 0.9975
			0.1	79 – 85 (81)	3 (5)	
			Overall	74 –85 (81)	4 (10)	

Specificity and Confirmation of Analyte Identity

LC-MS/MS with primary and confirmatory ion transitions is considered a highly specific technique. Therefore, additional methods to confirm the identity of the analytes are not considered necessary. Chromatograms for standards in bovine liver and kidney, reagent blanks, control samples and samples fortified at the LOQ (0.01 mg/kg) and at 10 x LOQ (0.1 mg/kg) have been provided for bovine liver and kidney for SYN547897 and SYN548263 for both mass transitions. No significant interference (>30% of the LOQ) at the retention time of interest was observed.

Linearity

Linearity was demonstrated by the analysis of at least six standards in duplicate for SY547897 and SYN548263. The range of standard concentrations used was 0.25-12.5 ng/mL, equivalent to 0.0025-0.125 mg/kg (25-1250% of the LOQ). The response was linear for both mass transitions with a coefficient of determination (R^2)>0.99. Samples are diluted appropriately to ensure they are within the linear range. It is noted for SYN547897 confirmatory transition, one of the calibration standards at 2.5 ng/mL is outside of the acceptable range and therefore was not included in the calibration.

Accuracy

Fortified samples were analysed in quintuplet at the limit of quantification (LOQ: 0.01 mg/kg) and at a higher level (10x LOQ: 0.1 mg/kg) for SYN547897 and SYN548263. Mean recoveries were within the acceptable range (70-110%) for both mass transitions for SYN548263. For SYN547897, mean recoveries were within the

acceptable range for liver but not for kidney. Mean recoveries at 113% were obtained for kidney at 0.1 mg/kg for both mass transitions. However, these are only slightly outside of the acceptable range.

Precision

Precision was determined from the accuracy recovery data for SYN547897 and SYN548263. The %RSD was ≤20% with five samples prepared at each fortification level.

Matrix effects

Matrix effects were variable (3-44% enhancement) in the liver and kidney tested. Therefore, matrix matched standards were used for quantification.

LOQ

0.01 mg/kg for SYN547897 and SYN548263 in bovine liver and kidney. This is the lowest fortification level with acceptable precision and recovery data.

Storage Stability of Extracts

Residues of SYN547897 and SYN548263 were demonstrated to be stable in final extracts from bovine kidney and liver when stored at 4 °C for 7-8 days. Samples were fortified at LOQ level (0.01 mg/kg) and the day 0 and day 7-8 recoveries were compared. The mean recoveries were between 70-110% with a %RSD ≤20%. There was no significant difference between the day 0 and day 7-8 results.

Storage Stability of Standards

Standard solutions of SYN547897 and SYN548263 were stable over the course of the study when stored at 4 °C.

Extraction Efficiency

The metabolism of radiolabelled pydiflumetofen has been described in a study in lactating goats. Liver and kidney samples were extracted by homogenizing with acetonitrile: water (80:20, v/v) twice followed by acetonitrile: water (1:1, v/v). The extraction solvents used in the metabolism studies extracted >70% of the TRR from kidney. For kidney, the majority of the %TRR was extracted with the first acetonitrile: water (80:20, v/v) extraction. However, residues extractability was lower in liver (50.4 and 47.4% TRR for phenyl and pyrazole in ruminant liver, respectively). For liver, most of the extractable residue was extracted following the first extraction with acetonitrile: water (80:20, v/v).

Method GRM061.09A has been developed for risk assessment purposes and has been used to determine residues of pydiflumetofen in the livestock feeding studies and storage stability studies. Method GRM061.09A uses acetonitrile: water (80/20, v/v) for extraction of residues from kidney and liver samples.

Hence, the same extraction solvent was used to extract residues in the animal metabolism studies as is used in Method GRM061.09A (acetonitrile: water (80/20, v/v)). On this basis, the extraction efficiency of GRM061.08A is considered satisfactorily addressed.

Hydrolysis

The metabolism of radiolabelled pydiflumetofen has been described in a study in lactating goats. Enzyme hydrolysis procedures were undertaken to release metabolites from their conjugated forms. Enough sodium acetate was weighed into the extract to produce a 0.2M solution, and the sample was then adjusted to pH using acetic acid before the addition of β-glucuronidase. The resulting mixture was incubated overnight in a shaking water bath at 37 °C (~18 hours).

As part of Method GRM061.09A, enzyme hydrolysis is performed using 2 mL of 5 mg/mL β-glucuronidase solution prepared in 0.4 M sodium acetate buffer (pH 5). Hence, similar hydrolysis conditions were used to release conjugated as part of Method GRM061.09A. As such, by implication the metabolism study fully validates the hydrolysis step used within the residues method.

Conclusion

The method GRM061.09A is acceptably validated in accordance with SANCO/3029/99 rev. 4 for the determination of SYN547897 and SYN548263 in bovine liver and kidney with an LOQ of 0.01 mg/kg.

Method GRM061.07A

Residues studies supported by method GRM061.07A:

Data point	Author(s)	Year	Title Company Report No.
KCA1 6.1/01	[redacted]	2016	SYN545974 – Storage Stability of Residues of Conjugated 2,4,6-trichlorophenol in Animal Matrices Stored Frozen for up to Twelve Months Report number: PTRL Europe ID P 3669 G Document No. VV-414155 , SYN545974_10280
KCA1 6.4.1/01	[redacted]	2015	SYN545974 – Magnitude of the Residues in Tissue and Eggs Resulting from the Feeding of Three Dose Levels to Poultry 2014 Report number: TK0103796 Document No. VV-414618 , SYN545974_50189
KCA1 6.4.2/01	[redacted]	2017	SYN545974 – Magnitude of Residues in Milk and Tissues of Dairy Cows Following Multiple Oral Administration of SYN545974 Report number: 35775 Document No. VV-414196 , SYN545974_10288

Method GRM061.07A has been used for data generation purposes in support of animal studies where pydiflumetofen metabolite 2,4,6-trichlorophenol was analysed. This method is also proposed as a method for post authorisation control and is discussed in detail in Section B.5.2.2.

B.5.1.2.6. Methods used in support of ecotoxicological studies

Study overview:

Data point	Author(s)	Year	Title Company Report No.	Conclusion
KCA1 8.1.1.2-02	[redacted]	2013	SYN545974 – A Dietary LC_{50} Study with the Northern Bobwhite Report number: 528-391	Acceptable method LOQ: 120 ppm in avian diet
KCA1 8.1.1.2-03	[redacted]	2013a	SYN545974 – A Dietary LC_{50} Study with the Mallard Report number: 528-392	
KCA1 8.1.1.3-02	[redacted]	2015	SYN545974 – A Reproduction Study with the Northern Bobwhite Report number: 528-396	Acceptable method LOQ: 100 ppm in 2% w/w corn oil fortified avian diet
KCA1 8.1.1.3-03	[redacted]	2014	SYN545974 – A Reproduction Study with the Mallard Report number: 528-397	
KCA1 8.2.1-07	[redacted]	2012	SYN545974 – Acute Toxicity to Rainbow Trout (*Oncorhynchus mykiss*) Under Flow-Through Conditions Report number: 1781.6840	Fit for regulatory purposes but the method is not fully validated in accordance with SANCO/3029/99 rev. 4. LOQ: 0.3 µg/L in aqueous matrices
KCA1 8.2.1-05	[redacted]	2013	SYN545974 – Acute Toxicity to Fathead Minnow (*Pimephales promelas*) Under Flow-Through Conditions Report number: 1781.6883	See 8.2.1-07
KCA1 8.2.1-04	[redacted]	2013a	SYN545974 – Acute Toxicity to Carp (*Cyprinus carpio)* Under Flow-Through Conditions Report number: 1781.6882	See 8.2.1-07
KCA1 8.2.1-06	[redacted]	2013b	SYN545974 – Acute Toxicity to Sheepshead Minnow (*Cyprinodon variegatus)* Under Flow-Through Conditions Report number: 1781.6884	See 8.2.1-07
KCA1 8.2.1-03	[redacted]	2014	SYN545974 – Acute Toxicity to Bluegill Sunfish (*Lepomis macrochirus*) Under Flow-Through Conditions Report number: 1781.7025	See 8.2.1-07
KCA1 8.2.2.1-03	[redacted]	2020	SYN545974 – Early Life-Stage toxicity Test with Fathead Minnow (P*imephales promelas)* Report number: 1781.6843	See 8.2.1-07
KCA1 8.2.2.1-04	[redacted]	2015	SYN545974 – Early Life-Stage toxicity Test with Sheepshead Minnow, *Cyprinodon variegatus* Final Report Report number: 1781.6979	See 8.2.1-07
KCA1 8.2.4.1-03	[redacted]	2017	SYN545974 – Acute Toxicity to Water Fleas (*Daphnia magna*) Under Static Conditions Report number: 1781.6839	See 8.2.1-07

Data point	Author(s)	Year	Title Company Report No.	Conclusion
KCA1 8.2.4.2-11	[redacted]	2016	SYN545974 – Acute Toxicity to Mysid (*Americamysis bahia)*, Under Static Conditions Report number: 1781.6838	See 8.2.1-07
KCA1 8.2.4.2-12	[redacted]	2014a	SYN545974 – Toxicity to Eastern Oyster (*Crassotrea virginica)* Under Flow-Through Conditions Report number: 1781.6885	See 8.2.1-07
KCA1 8.2.5.1-01	[redacted]	2016	SYN545974 – Full Life-Cycle Toxicity Test with Water Fleas, *Daphnia magna*, Under Static Renewal Conditions Report number: 1781.6842	See 8.2.1-07
KCA1 8.2.5.2-02	[redacted]	2015	SYN545974 – Life-Cycle Toxicity Test with Mysids (*Americamysis bahia*) Report number: 1781.6886	See 8.2.1-07
KCA1 8.2.6.1-03	[redacted]	2013	SYN545974 – 96-hour toxicity test with freshwater green alga, *pseudokirchneriella subcapitata* Report number: 1781.6841	See 8.2.1-07 It should be noted unacceptable recoveries were determined at a test concentration of 10,000 µg/L. The applicant has justified this as the solubility limit of the test substance in AAP medium has been reached.
KCA1 8.2.6.2-01	[redacted]	2013	SYN545974 – Toxicity Test to the Freshwater Blue-Green Alga, *Anabaena flos-aquae* Report number: 1781.6881	See 8.2.1-07 It should be noted unacceptable recoveries were determined at a test concentration of 10,000 µg/L. The applicant has justified this as the solubility limit of the test substance in AAP medium has been reached
KCA1 8.2.6.2-03	[redacted]	2015	SYN545974 – 96-Hour Toxicity Test with the Freshwater Diatom, *Navicula pelliculosa* Report number: 1781.6879	See 8.2.1-07 It should be noted unacceptable recoveries were determined at a test concentration of 10,000 µg/L. The applicant has justified this as the solubility limit of the test substance in AAP medium has been reached
KCA1 8.2.6.2-02	[redacted]	2014	SYN545974 – 96-Hour Toxicity Test with Marine Diatom, *Skeletonema costatum* Report number: 1781.6880	See 8.2.1-07 It should be noted unacceptable recoveries were determined at a test concentration of 10,000 µg/L. The applicant has justified this as the solubility limit of the test substance in

Data point	Author(s)	Year	Title Company Report No.	Conclusion
				artificially enriched sea water has been reached
KCA1 8.2.7-01	[redacted]	2015a	SYN545974 – 7-day Toxicity Test with Duckweed (*Lemna gibba*) Report number: 1781.6878	See 8.2.1-07 It should be noted unacceptable recoveries were determined at a test concentration of 10,000 µg/L. The applicant has justified this as the solubility limit of the test substance in AAP medium has been reached
KCA1 8.2.1-02	[redacted]	2015	SYN545547 – Acute Toxicity Test with Rainbow Trout (*Oncorhynchus mykiss*) Under Static Conditions Report number: 1781.7096	Fit for regulatory purposes but the method is not fully validated in accordance with SANCO/3029/99 rev. 4. LOQ: 0.015 mg/L in freshwater and AAP medium
KCA1 8.2.4.1-02	[redacted]	2015a	SYN545547 – Acute Toxicity to Water Fleas (*Daphnia magna)* Under Static Conditions Report number: 1781.7095	See 8.2.1-02
KCA1 8.2.6.1-02	[redacted]	2015	SYN545547 – 96-Hour Toxicity Test with the Freshwater Green Alga, *Pseudokirchneriella subcapitata* Report number: 1781.7094	See 8.2.1-02
KCA1 8.2.1-08	[redacted]	2016	SYN548261 – Acute Toxicity to *Oncorhnchus mykiss* Report number: 3201085	Acceptable method LOQ: 0.05 µg/mL in treated mains water
KCA1 8.2.4.1-04	[redacted]	2016a	SYN548261 – Acute toxicity to Water Fleas, (*Daphnia magna)* under static conditions Report number: 3201086	Acceptable method LOQ: 0.05 µg/mL in Elendt M4 water
KCA1 8.2.6.1-04	[redacted]	2016b	SYN548261 – Inhibition of Growth to the Alga *Pseudokirchneriella supcapitata* in a 96-hour test Report number: 3201084	Acceptable method LOQ: 0.05 µg/mL in EC medium
KCA1 8.2.1-01	[redacted]	2009	M700F001 (metabolite of BAS 700 F) Acute Toxicity for Rainbow Trout BASF DocID 2009/1021591	Acceptable method LOQ: 0.05 mg/L in water
KCA1 8.2.4.1-01	[redacted]	2009a	M700F001 (metabolite of BAS 700 F) Daphnia Magna, Acute Immobilization Test BASF DocID 2009/1021592	See 8.2.1-01
KCA1 8.2.6.1-01	[redacted]	2009b	M700F001 (metabolite of BAS 700 F) *Pseudokirchneriella subcapitata* SAG.61.81 Growth Inhibition Test BASF Doc ID 2009/1021953	See 8.2.1-01

Data point	Author(s)	Year	Title Company Report No.	Conclusion
KCA1 8.2.3-01	[redacted]	2020	Pydiflumetofen – Amphibian Metamorphosis Assay with African Clawed Frog (*Xenopus laevis*) Report number: 1781.7310	Acceptable method LOQ: 0.05 µg/L in well water
KCA1 8.2.3-04	[redacted]	2020a	Pydiflumetofen – Fish Short-Term Reproduction Assay with Fathead Minnow (*Pinephales promelas*) Report number: 1781.7303	See 8.2.3-01
KCA1 8.2.4.2-01	[redacted]	2015	SYN545974 – Acute toxicity of SYN545974 to *Asellus aquaticus* Report number: CEA.1644	Fit for regulatory purposes but the method is not fully validated in accordance with SANCO/3029/99 rev. 4. LOQ: 0.05 µg/L
KCA1 8.2.4.2-02	[redacted]	2015	SYN545974 – Acute Toxicity of SYN545974 to *Chaoborus crystallinus* Report number: CEA.1666	See 8.2.4.2-01
KCA1 8.2.4.2-03	[redacted]	2015a	SYN545974 – Acute Toxicity of SYN545974 to *Chironomus riparius* Report number: CEA.1667	See 8.2.4.2-01
KCA1 8.2.4.2-09	[redacted]	2015a	SYN545974 – Acute Toxicity of SYN545974 to *Cloeon dipterum* Report number: CEA.1664	See 8.2.4.2-01
KCA1 8.2.4.2-04	[redacted]	2015b	SYN545974 – Acute Toxicity of SYN545974 to *Crangonyx pseudogracilis* Report number: CEA.1661	See 8.2.4.2-01
KCA1 8.2.4.2-05	[redacted]	2015b	SYN545974 – Acute Toxicity of SYN545974 to *Cyclops agilis speratus* Report number: CEA.1662	See 8.2.4.2-01
KCA1 8.2.4.2-07	[redacted]	2015c	SYN545974 – Acute Toxicity of SYN545974 to *Lumbriculus variegatus* Report number: CEA.1642	See 8.2.4.2-01
KCA1 8.2.4.2-06	[redacted]	2015d	SYN545974 – Acute Toxicity of SYN545974 to *Lymnaea stagnalis* Report number: CEA.1645	See 8.2.4.2-01
KCA1 8.2.4.2-10	[redacted]	2015	SYN545974 – A 48-Hour Static Acute Toxicity Test with the Freshwater Amphipod (Hyalella azteca) Report number 528A-287	Acceptable method LOQ: is 0.0025 mg/L in water
KCA1 8.2.5.3-01	[redacted]	2015	SYN545547 - A Prolonged Sediment Toxicity Test with the Midge (Chironomus riparius) Using Spiked Sediment Report number: 528A-286	Acceptable method LOQ: 0.2 mg/Lin water samples and 2.49 mg/kg dry sediment
KCA1 8.2.5.4-03	[redacted]	2015a	SYN545974 – 42-Day Toxicity Test Exposing Freshwater Amphipods (Hyalella azteca) to Spiked Sediment Report number: 1781.6890	Acceptable method LOQ: 0.151 µg/Lin water samples and 0.021 mg/kg dry sediment

Data point	Author(s)	Year	Title Company Report No.	Conclusion
KCA1 8.2.5.4-04	[redacted]	2015b	SYN545974 - Life-Cycle Toxicity Test Exposing Midges (Chironomus dilutus) to Spiked Sediment Report number: 1781.6889	Acceptable method LOQ: 0.151 µg/Lin water samples and 0.021 mg/kg dry sediment
KCA1 8.2.5.4-05	[redacted]	2015	SYN545974 - 10-Day Toxicity Test Exposing Estuarine Amphipods (Leptocheirus plumulosus) to a Test Substance Applied to Sediment under Static Conditions Report number 1781.7069	Acceptable method LOQ: 0.151 µg/Lin water samples and 0.021 mg/kg dry sediment
KCA1 8.3.1.3-05	[redacted]	2015	SYN545974 SC (A19649B) – Chronic toxicity to the honeybee larvae Apis mellifera L. under laboratory conditions (in vitro) Report number: 14 10 48 005 B	Acceptable method LOQ: 832 mg/L in test diet (aqueous sugar solution)
KCA1 8.3.1.3-06	[redacted]	2015	SYN545974 - A laboratory study to determine the chronic effects on the brood of the honeybee Apis Report Number: 037SRFR15C06	The method itself is considered acceptable, however the analytical results for dose verification indicate that the target doses in the study will not have been achieved. This will need to be considered in the main study.
KCA1 8.3.1.3-02	[redacted]	2015a	SYN545974 SC (A19649B) - A laboratory study to determine the chronic effects on the brood of the honeybee Apis mellifera L. (Hymenoptera: Apidae). Report Number: 037SRFR15C07	Acceptable method LOQ: 0.26 mg/L in test diet (aqueous sugar solution)
KCA1 8.3.1.3-05	[redacted]	2018	Pydiflumetofen - Effects on the honeybee brood Apis mellifera L. following chronic oral exposure under field conditions Report number: 17 48 BFB 0001	Acceptable method LOQ: 0.005 mg/kg
KCA1 8.6.2-01	[redacted]	2015	SYN545974 SC (A19649B) - Toxicity Effects on the Seedling Emergence of Ten Species of Plants Report number: 528P-124	Acceptable method LOQ: 500 mg/L in test spray solutions
KCA1 8.6.2-02	[redacted]	2015a	SYN545974 SC (A19649B) - Toxicity Effects on the Seedling Emergence of Ten Species of Plants Report Number: 528P-115	See 8.6.2-01
KCA1 8.6.2-03	[redacted]	2015b	SYN545974 SC (A19649B) - Toxicity Effects on the Vegetative Vigour of Ten Species of Plants Report Number: 528P-116	See 8.6.2-01

Report:	**KCA1 8.1.1.2-02 [redacted], [redacted], [redacted]. (2013)**
Title	SYN545974 – A Dietary LC_{50} Study with the Northern Bobwhite Report number: 528-391
Guidelines:	SANCO/3029/99 rev. 4
GLP:	Yes
Deviations	N/A
Previous evaluation:	None

Report:	**KCA1 8.1.1.2-03 [redacted], [redacted], [redacted]. (2013a)**
Title	SYN545974 – A Dietary LC_{50} Study with the Mallard Report number: 528-392
Guidelines:	SANCO/3029/99 rev. 4
GLP:	Yes
Deviations	N/A
Previous evaluation:	None

Report:	**KCA1 8.1.1.3-02 [redacted] et al. (2015)**
Title	SYN545974 – A Reproduction Study with the Northern Bobwhite Report number: 528-396
Guidelines:	SANCO/3029/99 rev. 4
GLP:	Yes
Deviations	N/A
Previous evaluation:	None

Report:	**KCA1 8.1.1.3-03 [redacted] et al. (2014)**
Title	SYN545974 – A Reproduction Study with the Mallard Report number: 528-397
Guidelines:	SANCO/3029/99 rev. 4
GLP:	Yes
Deviations	N/A
Previous evaluation:	None

The following studies rely on the same HPLC-UV method to determine the concentration of SYN545974 in test samples:

- KCA1 8.1.1.2-02
- KCA1 8.1.1.2-03
- KCA1 8.1.1.3-02
- KCA1 8.1.1.3-03

Sample preparation:

A sample of avian feed (10 g) is weighed into 8 oz. French square bottles (or equivalent). 100 mL of acetonitrile is added using a graduated cylinder to each sample. The samples are sonicated for 60 minutes in a water bath and then shaken at approximately 250 rpm for 60 minutes using a tabletop shaker. A 20 mL aliquot is taken and centrifuged at approximately 1500 rpm for 10 minutes. The samples are then diluted with acetonitrile as follows:

- Study 528-391 and 528-392:

  0-562 ppm: dilute 1 mL to 10 mL, 1000-1780 ppm: dilute 1 mL to 25 mL, 3160-6000: dilute 1 mL to 100 mL)

- Study 528-396 and 528-397:
  0-200 ppm: dilute 1 mL to 5mL, 1000 ppm: dilute 1 mL to 25 mL, 5000-6000 ppm: dilute 1 mL to 100 mL

Samples are analysed by HPLC-UV under the conditions below.

HPLC-UV conditions:

Chromatographic system:	Agilent Series 1100/1200 High Performance Liquid Chromatograph (HPLC) equipped with an Agilent 1100 variable wavelength detector (VWD)/ Waters Alliance HPLC equipped with a Waters 2489 VWD
Analytical column:	YMC ODS-AM (150 mm x 4.6 mm I.D., 5 µm particle size)
Injection volume:	25 µL
Oven temperature	40°C
Mobile phase A:	0.1% phosphoric acid
Mobile phase B:	Acetonitrile
Flow rate:	1 mL/minute

Gradient:

Time (minute)	% A	% B
0.00	90	10
1.00	90	10
10.0	5	95
12.00	5	95
12.10	90	10
15.00	90	10

Retention time: Approximately 11.7 minutes
Detection wavelength: 230 nm

A summary of the method validation data is given in Table B.5.1.2.6-1. Procedural recoveries for each study have been presented in Table B.5.1.2.6-2.

Table B.5.1.2.6-1: Summary of method validation data for determination of pydiflumetofen residues in avian diet

Study	**Matrix**	**Analyte**	**LOQ (ppm)**	**Recovery fortification level (ppm)**	**Recoveries % range (mean)**	**Repeatability % RSD (n)**	**Linearity**
Study 528-391 and 528-392 KCA 8.1.1.2-02; KCA 8.1.1.2-03	Avian diet	pydiflumetofen	120	120	98 – 101 (100)	2.1 (2)	0.5 – 10 µg/mL (equivalent to 50-1000 ppm) (n = 5) y = 60.08x+1.37 R^2 = 0.9998
				6000	100 – 102 (101)	1.4 (2)	
				Overall	98 – 102 (100)	1.7 (4)	
Study 528-396 and 528-397 KCA 8.11.1.3-02; KCA 8.1.1.3-03	2% w/w corn oil fortified avian diet	pydiflumetofen	100	100	89 - 108 (97)	5.9 (8)	1 – 10 µg/mL (equivalent to 50-500 ppm) (n = 5) y = 60.15x+0.14 R^2 = 0.9999
				6000	99 – 103 (101)	1.7 (8)	
				Overall	89 – 108 (99)	4.5 (16)	

Table B.5.1.2.6-2: Summary of dose verification of pydiflumetofen concentrations in avian diet

Study	Matrix	Analyte	Nominal concentration (ppm)	Sample Interval	% Recovery
Study 528-391 and 528-392 KCA1 8.1.1.2-02 and KCA1 8.1.1.2-03	Avian diet	pydiflumetofen	1000	Day 0	99 - 102 (100)
			1780	Day 0	99 - 102 (100)
			3160	Day 0	100 - 100 (100)
Study 528-396 and 528-397 KCA1 8.11.1.3-02 and KCA1 8.1.1.3-03	2% w/w corn oil fortified avian diet	pydiflumetofen	200	Day 2-20	92 – 104 (100)
			1000	Day 2-20	96 - 116 (105)
			5000	Day 2-20	94 - 115 (103)

Specificity

Chromatograms were presented for the low level and high-level calibration standards, matrix blank and fortified samples at 120 ppm and 562 ppm (study 528-391 and 528-392) and at 100 ppm and 200 ppm (study 528-396 and 528-397). No significant interference was observed at the retention time of interest and the retention time of pydiflumetofen matched with reference standards.

Linearity

Linearity was demonstrated by the analysis of five matrix matched standards of increasing concentration. The range of standard concentrations used was 0.5-10 µg/L (equivalent to 50-1000 ppm) for study 528-391 and 528-392 and 1-10 µg/L (equivalent to 50-500 ppm) for study 528-396 and 528-397. The response was linear with a coefficient of determination of at least 0.9998. It is noted samples are diluted to within the linear range.

Accuracy

For study 528-391 and 528-392, avian diet samples were fortified at 120 and 6000 ppm and analysed concurrently with the samples. These fortification levels are appropriate to the concentrations used in the ecotoxicology studies (Study 528-391 and 528-392: 562-5620 ppm). Two determinations were made at each fortification level with the first determination at Day 0 and the second at Day 5 of the study. The mean recoveries were within the acceptable range (70-110%). Acceptable procedural recoveries were available at test initiation at nominal concentrations of 1000, 1780 and 3160 ppm.

For study 528-396 and 528-397, 2% w/w corn oil fortified avian diet samples were fortified at 100 and 6000 ppm and analysed concurrently with the samples. These fortification levels are appropriate to the concentrations used in the above ecotoxicology studies (Study 528-396 and 528-397: 200-5000 ppm). One determination was made at the following intervals for each fortification level:

- Day 0 Week 1
- Day 7 Week 1
- Day 0 Week 2
- Day 0 Week 3 & 4
- Day 0 Week 8
- Day 0 & 7 Week 12
- Day 0 Week 16 & 20
- Day 7, Week 20

Overall, this gives eight determinations at each fortification level and the mean recoveries were within the acceptable range (70-110%). Procedural recoveries were available at various intervals in the test from Day 2-20 at nominal concentrations of 200, 1000 and 5000 ppm. Some of the individual procedural recoveries are outside of the acceptable range (70-110%) but the mean recoveries are within the acceptable range. This is acceptable.

Precision

Precision was determined from the accuracy recovery data for pydiflumetofen. The %RSD was ≤20% for each fortification level. However, for study 528-391 and 528-392 only two determinations were reported. In accordance with SANCO/3029/99 rev. 4, a minimum of five determinations are required at each fortification level.

Matrix effects

Matrix effects were not specifically addressed. However, matrix matched standards were used for calibration.

LOQ

The LOQ stated by the applicant is 50 ppm, determined from the limit of quantification of the instrument and the dilution factor of the control samples. According to SANCO/3029/99 rev. 4, the LOQ is defined as the lowest concentration tested, at which an acceptable mean recovery (70-110%) with an acceptable RSD (≤20%) is obtained. Therefore, the LOQ of the method is 120 ppm in avian diet and 100 ppm in 2% w/w corn oil fortified avian diet.

Conclusion

The method is acceptably validated in accordance with SANCO/3029/99 rev. 4 for the determination of pydiflumetofen in avian diet. It should be noted for study 528-391 and 528-392 there is an insufficient number of recovery determination at each fortification level. However, further recovery data is available in a very similar matrix (2% w/w corn oil fortified avian diet) in study 528-396 and 528-397. Therefore, the accuracy and precision of the method is acceptable.

Report:	**KCA1 8.2.1-07 [redacted] (2012)**
Title	SYN545974 – Acute Toxicity to Rainbow Trout (*Oncorhynchus mykiss*) Under Flow-Through Conditions Report number: 1781.6840
Guidelines:	SANCO/3029/99 rev. 4
GLP:	Yes
Deviations	N/A
Previous evaluation:	None

Report:	**KCA1 8.2.1-05 [redacted] (2013)**
Title	SYN545974 – Acute Toxicity to Fathead Minnow (*Pimephales promelas*) Under Flow-Through Conditions Report number: 1781.6883
Guidelines:	SANCO/3029/99 rev. 4
GLP:	Yes
Deviations	N/A
Previous evaluation:	None

Report:	**KCA1 8.2.1-04 [redacted] (2013a)**
Title	SYN545974 – Acute Toxicity to Carp (*Cyprinus carpio)* Under Flow-Through Conditions Report number: 1781.6882
Guidelines:	SANCO/3029/99 rev. 4
GLP:	Yes
Deviations	N/A
Previous evaluation:	None

Report:	**KCA1 8.2.1-06 [redacted] (2013b)**
Title	SYN545974 – Acute Toxicity to Sheepshead Minnow (*Cyprinodon variegatus)* Under Flow-Through Conditions Report number: 1781.6884
Guidelines:	SANCO/3029/99 rev. 4
GLP:	Yes
Deviations	N/A
Previous evaluation:	None

Report:	**KCA1 8.2.1-03 [redacted] (2014)**
Title	SYN545974 – Acute Toxicity to Bluegill Sunfish (*Lepomis macrochirus*) Under Flow-Through Conditions Report number: 1781.7025
Guidelines:	SANCO/3029/99 rev. 4
GLP:	Yes
Deviations	N/A
Previous evaluation:	None

Report:	**KCA1 8.2.2.1-03 [redacted] (2020)**
Title	SYN545974 – Early Life-Stage toxicity Test with Fathead Minnow (P*imephales promelas)* Report number: 1781.6843
Guidelines:	SANCO/3029/99 rev. 4
GLP:	Yes
Deviations	N/A
Previous evaluation:	None

Report:	**KCA1 8.2.2.1-04 [redacted] (2015)**
Title	SYN545974 – Early Life-Stage toxicity Test with Sheepshead Minnow, *Cyprinodon variegatus* Final Report Report number: 1781.6979
Guidelines:	SANCO/3029/99 rev. 4
GLP:	Yes
Deviations	N/A
Previous evaluation:	None

Report:	**KCA1 8.2.4.1-03 [redacted] (2017)**
Title	SYN545974 – Acute Toxicity to Water Fleas (*Daphnia magna*) Under Static Conditions Report number: 1781.6839
Guidelines:	SANCO/3029/99 rev. 4
GLP:	Yes
Deviations	N/A
Previous evaluation:	None

Report:	**KCA1 8.2.4.2-11 [redacted] (2016)**
Title	SYN545974 – Acute Toxicity to Mysid (*Americamysis bahia),* Under Static Conditions Report number: 1781.6838
Guidelines:	SANCO/3029/99 rev. 4
GLP:	Yes
Deviations	N/A

Previous evaluation:	None

Report:	**KCA1 8.2.4.2-12 [redacted] (2014a)**
Title	SYN545974 – Toxicity to Eastern Oyster (*Crassotrea virginica)* Under Flow-Through Conditions Report number: 1781.6885
Guidelines:	SANCO/3029/99 rev. 4
GLP:	Yes
Deviations	N/A
Previous evaluation:	None

Report:	**KCA1 8.2.5.1-01 [redacted] (2016)**
Title	SYN545974 – Full Life-Cycle Toxicity Test with Water Fleas, *Daphnia magna*, Under Static Renewal Conditions Report number: 1781.6842
Guidelines:	SANCO/3029/99 rev. 4
GLP:	Yes
Deviations	N/A
Previous evaluation:	None

Report:	**KCA1 8.2.5.2-02 [redacted] (2015)**
Title	SYN545974 – Life-Cycle Toxicity Test with Mysids (*Americamysis bahia*) Report number: 1781.6886
Guidelines:	SANCO/3029/99 rev. 4
GLP:	Yes
Deviations	N/A
Previous evaluation:	None

Report:	**KCA1 8.2.6.1-03 [redacted] (2013)**
Title	SYN545974 – 96-hour toxicity test with freshwater green alga, *pseudokirchneriella subcapitata* Report number: 1781.6841
Guidelines:	SANCO/3029/99 rev. 4
GLP:	Yes
Deviations	N/A
Previous evaluation:	None

Report:	**KCA1 8.2.6.2-01 [redacted] (2013)**
Title	SYN545974 – Toxicity Test to the Freshwater Blue-Green Alga, *Anabaena flos-aquae* Report number: 1781.6881
Guidelines:	SANCO/3029/99 rev. 4
GLP:	Yes
Deviations	N/A
Previous evaluation:	None

Report:	**KCA1 8.2.6.2-03 [redacted] (2015)**
Title	SYN545974 – 96-Hour Toxicity Test with the Freshwater Diatom, *Navicula pelliculosa* Report number: 1781.6879
Guidelines:	SANCO/3029/99 rev. 4

GLP:	Yes
Deviations	N/A
Previous evaluation:	None

Report:	**KCA1 8.2.6.2-02 [redacted] (2014)**
Title	SYN545974 – 96-Hour Toxicity Test with Marine Diatom, *Skeletonema costatum* Report number: 1781.6880
Guidelines:	SANCO/3029/99 rev. 4
GLP:	Yes
Deviations	N/A
Previous evaluation:	None

Report:	**KCA1 8.2.7-01 [redacted] (2015a)**
Title	SYN545974 – 7-day Toxicity Test with Duckweed (*Lemna gibba*) Report number: 1781.6878
Guidelines:	SANCO/3029/99 rev. 4
GLP:	Yes
Deviations	N/A
Previous evaluation:	None

A summary of the studies that use the following LC-MS/MS method are shown in Table B.5.1.2.6-3. Any differences between the method used in the studies and validation data are reported. Procedural recoveries demonstrate these differences do not have a negative impact on the study. Filtered sea watered was used as the matrix in the method validation data as a representative complex aqueous matrix.

Table B.5.1.2.6-3: Summary of the studies relying on the LC-MS/MS method reported below

Data point	Study number	Differences to method used in validation data
KCA1 8.2.1-07	Study 1781.6840	None
KCA1 8.2.1-05	Study 1781.6883	Well water was used as the matrix instead of filtered seawater. The calibration standards used in the study were 0.05-1.00 µg/L, compared to 0.015-1.00 µg/L in the method validation.
KCA1 8.2.1-04	Study 1781.6882	Well water was used as the matrix instead of filtered seawater. The calibration standards used in the study were 0.05-1.00 µg/L, compared to 0.015-1.00 µg/L in the method validation.
KCA1 8.2.1-06	Study 1781.6884	The calibration standards used in the study were 0.05-1.00 µg/L, compared to 0.015-1.00 µg/L in the method validation.
KCA1 8.2.1-03	Study 1781.7025	Well water was used as the matrix instead of filtered seawater.
KCA1 8.2.2.1-03	Study 1781.6843	Bedrock well and dechlorinated well water was used as the matrix instead of filtered seawater.
KCA1 8.2.2.1-04	Study 1781.6979	None
KCA1 8.2.4.1-03	Study 1781.6839	None
KCA1 8.2.4.2-11	Study 1781.6838	None
KCA1 8.2.4.2-12	Study 1781.6885	None
KCA1 8.2.5.1-01	Study 1781.6842	Well water was used as the matrix instead of filtered seawater.

Data point	Study number	Differences to method used in validation data
KCA1 8.2.5.2.-02	Study 1781.6886	None
KCA1 8.2.6.1-03	Study 1781.6841	AAP medium was used as the matrix instead of filtered seawater.
KCA1 8.2.6.2-01	Study 1781.6881	AAP medium was used as the matrix instead of filtered seawater. Samples after 96 hours were centrifuged.
KCA1 8.2.6.2-03	Study 1781.6879	AAP medium was used as the matrix instead of filtered seawater. The calibration standards used in the study were 0.05-1.00 µg/L due to higher test concentrations, compared to 0.015-1.00 µg/L in the method validation.
KCA1 8.2.6.2-02	Study 1781.6880	Artificially enriched seawater (AES) was used as the matrix instead of filtered seawater. Samples after 96 hours were centrifuged prior to analysis to remove algal biomass.
KCA1 8.2.7-01	Study 1781.6878	20 x AAP medium was used as the matrix instead of filtered seawater.

Sample preparation:

A method validation was performed to quantify the amount of pydiflumetofen present in filtered seawater. Recovery samples were initially diluted with 20:80 acetonitrile: purified reagent water to a final composition of 18:10:72 acetonitrile: filtered sea water: purified reagent water (v/v/v). The mid and high concentration recovery samples were further diluted into the calibration standard range 18:10:72 acetonitrile: filtered seawater: purified reagent water (v/v/v). All samples were analysed by liquid chromatography/mass spectrometry (LC-MS/MS).

LC-MS/MS conditions:

Chromatographic system: MDS Sciex/ API 5000 mass spectrometer equipped with a Sciex Turbo V Source ESI, a Shimadzu Model 20AD vacuum degasser, Shimadzu Model 20AD solvent delivery pumps, a Shimadzu 20AD column compartment, a Shimadzu 20AD autoinjector, and Analyst 1.4.2 software for data acquisition

Analytical column: XBridge C18, 2.5 µm, 2.1 mm x 50 mm

Injection volume: 20 µL

Mobile phase A: 0.1% formic acid in purified reagent water

Mobile phase B: 0.1% formic acid in acetonitrile

Flow rate: 0.35 mL/minute

Gradient:

Time (minute)	% A	% B
1.00	70	30
3.00	10	90
5.00	10	90
5.10	70	30

Retention time: Approximately 4.1 minutes

Detection system: MS

Ionisation mode: Positive

Scan type: MRM

Q1/Q3 mass: 426.2/193.0 amu

Dwell time: 300 milliseconds

Source temperature: 400°C

A summary of the method validation data is given in Table B.5.1.2.6-4. Procedural recoveries for each study have been presented in Table B.5.1.2.6-5 – B.5.1.2.6-21.

Table B.5.1.2.6-4: Summary of method validation data for determination of pydiflumetofen residues in filtered sea water

Matrix	Analyte	LOQ (µg/L)	Recovery fortification level (µg/L)	Recoveries % range (mean)	Repeatability % RSD (n)	Linearity
Filtered seawater	pydiflumetofen	0.3	0.3	97.2 - 103 (100)	3 (3)	0.015 – 1.00 µg/L (n = 6*2) y = -5552x^2+237040x+329 R^2 = 0.9994
			30	102 - 114 (106)	7 (3)	
			10,000	91.5-98.7 (95)	4 (3)	
			Overall	91.5 – 114 (101)	6 (9)	

Table B.5.1.2.6-5: Procedural recoveries of pydiflumetofen from a 96-hour flow through acute exposure test of rainbow trout (*Oncorhynchus mykiss)*

Study	Matrix	Analyte	Nominal concentration (µg/L)	Sample Interval	% Recovery
Study 1781.6840 KCA 8.2.1-07	Well water	pydiflumetofen	32	0-hour	111*
				48-hour	117*
				96-hour	118*
			250	0-hour	104
				48-hour	109
				96-hour	105
			1000	0-hour	95
				48-hour	105
				96-hour	99

*Recoveries at a concentration of 32 µg/L at 0-hour, 48-hours and 96-hours were slightly outside of the acceptable range (70-110%).

Table B.5.1.2.6-6: Procedural recoveries of pydiflumetofen from an acute toxicity to fathead minnow (*Pimephales promelas)* test under flow-through conditions

Study	Matrix	Analyte	Nominal concentration (µg/L)	Sample Interval	% Recovery
Study 1781.6683 KCA 8.2.1-05	Well water	pydiflumetofen	30	0-hour	102
				48-hour	100
				96-hour	91
			200	0-hour	103
				48-hour	102
				96-hour	107
			1000	0-hour	101
				48-hour	102
				96-hour	98

Table B.5.1.2.6-7: Procedural recoveries of pydiflumetofen from an acute toxicity test to carp (*Cyprinus carpio)* under flow-through conditions

Study	Matrix	Analyte	Nominal concentration (µg/L)	Sample Interval	% Recovery
Study 1781.6682 KCA 8.2.1-04	Well water	pydiflumetofen	30	0-hour	105
				48-hour	91
				96-hour	96
			200	0-hour	109
				48-hour	95
				96-hour	104
			1000	0-hour	104
				48-hour	91
				96-hour	96

Table B.5.1.2.6-8: Procedural recoveries of pydiflumetofen from an acute toxicity test to sheepshead minnow (*Cyprinodon variegatus)* under flow-through conditions

Study	Matrix	Analyte	Nominal concentration (µg/L)	Sample Interval	% Recovery
Study 1781.6884 KCA 8.2.1-06	Filtered sea water	pydiflumetofen	30	0-hour	100
				48-hour	110
				96-hour	89
			200	0-hour	99
				48-hour	104
				96-hour	103
			1000	0-hour	94
				48-hour	103
				96-hour	94

Table B.5.1.2.6-9: Procedural recoveries of pydiflumetofen from an acute toxicity test to Bluegill sunfish (*Lepomis macrochirus)* under flow-through conditions

Study	Matrix	Analyte	Nominal concentration (µg/L)	Sample Interval	% Recovery
Study 1781.7025 KCA 8.2.1-03	Well water	pydiflumetofen	30	0-hour	104
				48-hour	102
				96-hour	89
			250	0-hour	107
				48-hour	104
				96-hour	86
			1000	0-hour	108
				48-hour	98
				96-hour	82

Table B.5.1.2.6-10: Procedural recoveries of pydiflumetofen from an early life-stage toxicity test with fathead minnow (*pimephales promelas)*

Study	Matrix	Analyte	Nominal concentration (µg/L)	Sample Interval	% Recovery
Study 1781.6843 KCA 8.2.2.1-03	Bedrock well and dechlorinated well water	pydiflumetofen	5	Day 0	108
				Day 4	88
				Day 11	90
				Day 20	102
				Day 27	102
				Day 32	102
			64	Day 0	108
				Day 4	95
				Day 11	94
				Day 20	81
				Day 27	102
				Day 32	102
			400	Day 0	108
				Day 4	97
				Day 11	95
				Day 20	99
				Day 27	112
				Day 32	96

Table B.5.1.2.6-11: Procedural recoveries of pydiflumetofen from an early life-stage toxicity test with sheepshead minnow (*cyprinodon variegatus)*

Study	Matrix	Analyte	Nominal concentration (µg/L)	Sample Interval	% Recovery
Study 1781.6979 KCA 8.2.2.1-04	Filtered seawater	pydiflumetofen	20	Day 0	81
				Day 6	96
				Day 13	94
				Day 20	88
				Day 28	98
				Day 33	97
			130	Day 0	96
				Day 6	92
				Day 13	88
				Day 20	95
				Day 28	103
				Day 33	98
			500	Day 0	96
				Day 6	95
				Day 13	99
				Day 20	96
				Day 28	86
				Day 33	96

Table B.5.1.2.6-12: Procedural recoveries of pydiflumetofen from an acute toxicity test to water fleas (*Daphnia magna)* under static conditions

Study	Matrix	Analyte	Nominal concentration (µg/L)	Sample Interval	% Recovery
Study 1781.6839 KCA 8.2.4.1-03	Well water	pydiflumetofen	30	0-hour	103
				48-hour	104
			250	0-hour	104
				48-hour	104
			1000	0-hour	96
				48-hour	98

Table B.5.1.2.6-13: Procedural recoveries of SYN 545974 from an acute toxicity test to mysid (*americamysis bahia*) under static conditions

Study	Matrix	Analyte	Nominal concentration (µg/L)	Sample Interval	% Recovery
Study 1781.6383 KCA 8.2.4.2-11	Filtered sea water	pydiflumetofen	30	0-hour	102
				96-hour	120*
			200	0-hour	104
				96-hour	110
			1000	0-hour	95
				96-hour	97

*Recovery at 30 µg/L after 96 hours is slightly outside of the acceptable range (70-110%)

Table B.5.1.2.6-14: Procedural recoveries of pydiflumetofen from a toxicity test to eastern oyster (*Crassostrea virginica*) under flow-through conditions

Study	Matrix	Analyte	Nominal concentration (µg/L)	Sample Interval	% Recovery
Study 1781.6885 KCA 8.2.4.2-12	Filtered sea water	pydiflumetofen	30	0-hour	94
				96-hour	93
			200	0-hour	94
				96-hour	101
			1000	0-hour	86
				96-hour	89

Table B.5.1.2.6-15: Procedural recoveries of pydiflumetofen from a full life-cycle toxicity test with water fleas, *daphnia magna*, under static renewal conditions

Study	Matrix	Analyte	Nominal concentration (µg/L)	Sample Interval	% Recovery
Study 1781.6842 KCA 8.2.5.1-01	Well water	pydiflumetofen	2.4	Day 0	113*
				Day 2	106
				Day 5	101
				Day 16	100
				Day 19	109
				Day 21	105
			30	Day 0	89
				Day 2	93
				Day 5	88
				Day 16	90
				Day 19	102
				Day 21	98
			300	Day 0	110
				Day 2	106
				Day 5	94
				Day 16	96
				Day 19	117*
				Day 21	102

*Recoveries at 2.4 µg/L on day 0 and at 300 µg/L on day 19 are slightly outside of the acceptable range (70-110%).

Table B.5.1.2.6-16: Procedural recoveries of pydiflumetofen from a life-cycle toxicity test with mysids (*americamysis bahia)*

Study	Matrix	Analyte	Nominal concentration (µg/L)	Sample Interval	% Recovery
Study 1781.6886 KCA 8.2.5.2-02	Filtered sea water	pydiflumetofen	1.25	Day 0	103
				Day 7	89
				Day 14	91
				Day 21	111*
				Day 28	95
			10	Day 0	98
				Day 7	89
				Day 14	102
				Day 21	108
				Day 28	94
			80	Day 0	97
				Day 7	80
				Day 14	95
				Day 21	108
				Day 28	91

*Recovery at 1.25 µg/L on day 21 is slightly outside of the acceptable range (70-110%).

Table B.5.1.2.6-17: Procedural recoveries of pydiflumetofen from a 96-hour toxicity test with the freshwater green alga, *pseudokirchneriella subcapitata*

Study	Matrix	Analyte	Nominal concentration (µg/L)	Sample Interval	% Recovery
Study 1781.6841 KCA 8.2.6.1-03	AAP medium	pydiflumetofen	5	0-hour	96
				96-hour	94
			100	0-hour	96
				96-hour	106
			10000	0-hour	99
				96-hour	96

Table B.5.1.2.6-18: Procedural recoveries of pydiflumetofen from a toxicity test to the Freshwater Blue-Green Alga, *Anabaena flos-aquae*

Study	Matrix	Analyte	Nominal concentration (µg/L)	Sample Interval	% Recovery
Study 1781.6881 KCA 8.2.6.2-01	AAP medium	pydiflumetofen	50	0-hour uncentrifuged	94
				96-hour uncentrifuged	101
				96-hour centrifuged	92
			1000	0-hour uncentrifuged	94
				96-hour uncentrifuged	97
				96-hour centrifuged	93
			10000	0-hour uncentrifuged	97
				96-hour uncentrifuged	107
				96-hour centrifuged	97

Table B.5.1.2.6-19: Procedural recoveries of pydiflumetofen from a 96-hour toxicity test with freshwater diatom, *navicular pelliculosa*

Study	Matrix	Analyte	Nominal concentration (µg/L)	Sample Interval	% Recovery
Study 1781.6879 KCA 8.2.6.2-03	AAP medium	pydiflumetofen	17	0-hour uncentrifuged	106
				96-hour uncentrifuged	101
				96-hour centrifuged	105
			1000	0-hour uncentrifuged	103
				96-hour uncentrifuged	95
				96-hour centrifuged	95
			10000	0-hour uncentrifuged	102
				96-hour uncentrifuged	98
				96-hour centrifuged	87

Table B.5.1.2.6-20: Procedural recoveries of pydiflumetofen from a 96-hour toxicity test with the marine diatom, *skeletonema costatum*

Study	Matrix	Analyte	Nominal concentration (µg/L)	Sample Interval	% Recovery
Study 1781.6880 KCA 8.2.6.2-02	Artificially enriched seawater (AES)	pydiflumetofen	50	0-hour uncentrifuged	92
				96-hour uncentrifuged	99
				96-hour centrifuged*	76
			1000	0-hour uncentrifuged	96
				96-hour uncentrifuged	106
				96-hour centrifuged*	88
			10000	0-hour uncentrifuged	100
				96-hour uncentrifuged	101
				96-hour centrifuged*	75

*Centrifuging samples seems to decrease the recovery, but samples centrifuged after 96 hours are still within the acceptable range (70-110%).

Table B.5.1.2.6-21: Procedural recoveries of SYN 545974 from a 7-day toxicity test with duckweed (*Lemna gibba*)

Study	Matrix	Analyte	Nominal concentration (µg/L)	Sample Interval	% Recovery
Study 1781.6878 KCA 8.2.7-01	20 x AAP medium	pydiflumetofen	50	Day 0 (new)	105
				Day 3 (aged)	100
			1000	Day 0 (new)	101
				Day 3 (aged)	100
			10000	Day 0 (new)	106
				Day 3 (aged)	100

Specificity

Chromatograms were presented for the calibration solution at 0.1 µg/L, fortified sample at 30 µg/L and the control sample for filtered seawater. No chromatograms have been provided for the test solutions or of the control for other matrices e.g., well water and AAP medium used in various studies. However, it is noted filtered sea water is used as a representative complex aqueous matrix and demonstrates suitability of the analytical method in other aqueous matrices. No significant interference was observed at the retention time of interest.

Linearity

Linearity was demonstrated by the analysis of six matrix matched standards of increasing concentration in duplicate. The range of standard concentrations used was 0.015 – 1.00 µg/L. The response was quadratic with a coefficient of determination of 0.9994. It is noted samples are diluted to within the linear range.

Accuracy

Fortified samples of filtered seawater were analysed at low, mid, and high levels (0.3, 30 and 10,000 µg/L). This is equivalent to the LOQ level, 30x LOQ and 33,333x LOQ. These fortification levels are appropriate to the concentrations in the ecotoxicology studies (see Table B.5.1.2.6-22 below). Three samples were prepared at each fortification level and the mean recoveries were within the acceptable range (70-110%). Acceptable procedural recoveries were also reported in the acceptable range (70-110%) at test initiation except for study 1781.6840 and study 1781.6842 where one recovery in each report is slightly outside of the range. Further recoveries at each test concentration are presented in the reports. It should be noted for study 1781.6841, 1781.6881, 1781.6879, 1781.6880 and 1781.6878 the test samples at 10,000 µg/L show recoveries outside of the acceptable range. The applicant has justified this as the solubility limit of the test substance in the matrix has been reached.

Table B.5.1.2.6-22: Test concentrations used in the ecotoxicology studies

Study report	**Nominal test concentrations (µg/L)**
KCA1 8.2.1-07 [redacted] (2012) Study 1781.6840	63-1000
KCA1 8.2.1-05 [redacted] (2013) Study 1781.6883	63-1000
KCA1 8.2.1-04 [redacted] (2013a) Study 1781.6882	63-1000
KCA1 8.2.1-06 [redacted] (2013b) Study 1781.6884	63-1000
KCA1 8.2.1-03 [redacted] (2014) Study 1781.7025	63-1000
KCA1 8.2.2.1-03 [redacted] (2020) Study 1781.6843	10-400
KCA1 8.2.2.1-04 [redacted] (2015) Study 1781.6979	31-500
KCA1 8.2.4.1-03 [redacted] (2017) Study 1781.6839	63-1000
KCA1 8.2.4.2-11 [redacted] (2016) Study 1781.6838	63-1000
KCA1 8.2.4.2-12 [redacted] (2014a) Study 1781.6885	63-1000
KCA1 8.2.5.1-01 [redacted] (2016) Study 1781.6842	4.8-300
KCA1 8.2.5.2-02 [redacted] (2015) Study 1781.6886	2.5-80
KCA1 8.2.6.1-03 [redacted] (2013) Study 1781.6841	9.3-10,000
KCA1 8.2.6.2-01 [redacted] (2013) Study 1781.6881	100-10,000
KCA1 8.2.6.2-03 [redacted] (2015) Study 1781.6879	34-10,000
KCA1 8.2.6.2-02 [redacted] (2014) Study 1781.6880	100-10,000
KCA1 8.2.7-01 [redacted] (2015a) Study 1781.6878	10-10,000

Precision

Precision was determined from the accuracy recovery data for pydiflumetofen. The %RSD was ≤20% with three samples prepared at each fortification level. However, in accordance with SANCO/3029/99 rev. 4 a minimum of five determinations are required.

Matrix effects

Matrix matched standards were not specifically addressed. However, matrix matched standards were used for quantification

LOQ

The LOQ stated by the applicant is 0.151 µg/L, determined from the limit of quantification of the instrument and the dilution factor of the control samples. According to SANCO/3029/99 rev. 4, the LOQ is defined as the lowest concentration tested, at which an acceptable mean recovery (70-110%) with an acceptable RSD (≤20%) is obtained. Therefore, the LOQ of the method is 0.3 µg/L.

Conclusion

The method is not fully validated in accordance with SANCO/3029/99 rev. 4 for the determination of pydiflumetofen in aqueous matrices. The following deficiencies have been noted:

- Only three recovery determination have been reported at each fortification level but SANCO/3029/99 rev. 4 states a minimum of five determinations are required.

- Control chromatograms for well water and AAP medium have not been presented.

Nevertheless, all samples are diluted to within the linear range and there is data reported at three fortification levels. The method of analysis is intended to simply determine the concentration of the active substance in solution. Not all the studies relying on this method use filtered sea water. However, the method validation has been undertaken with filtered sea water as a representative complex aqueous matrix. Therefore, this demonstrates suitability of the analytical method of analysis of pydiflumetofen in aqueous matrices. On this basis, the method can be considered fit for regulatory purposes with an LOQ of 0.3 µg/L.

Report:	**KCA1 8.2.1-02 [redacted] (2015)**
Title	SYN545547 – Acute Toxicity Test with Rainbow Trout (*Oncorhynchus mykiss*) Under Static Conditions Report number: 1781.7096
Guidelines:	SANCO/3029/99 rev. 4
GLP:	Yes
Deviations	N/A
Previous evaluation:	None

Report:	**KCA1 8.2.4.1-02 [redacted] (2015a)**
Title	SYN545547 – Acute Toxicity to Water Fleas (*Daphnia magna)* Under Static Conditions Report number: 1781.7095
Guidelines:	SANCO/3029/99 rev. 4
GLP:	Yes
Deviations	N/A
Previous evaluation:	None

Report:	**KCA1 8.2.6.1-02 [redacted] (2015)**
Title	SYN545547 – 96-Hour Toxicity Test with the Freshwater Green Alga, *Pseudokirchneriella subcapitata* Report number: 1781.7094
Guidelines:	SANCO/3029/99 rev. 4
GLP:	Yes
Deviations	N/A
Previous evaluation:	None

A summary of the studies that use the following HPLC-UV method to determine the concentration of the metabolite SYN545547 are shown in Table B.5.1.2.6-23. Any differences between the method used in the study and the validation data are reported. Acceptable procedural recoveries demonstrate these differences do not have a negative impact on the study.

Table B.5.1.2.6-23: Summary of the studies relying on the HPLC-UV method reported below

Data point	Study number	Differences to method used in validation data
KCA1 8.2.1-02	1781.7096	None
KCA1 8.2.4.1-02	1781.7095	None
KCA1 8.2.6.1-02	1781.7094	AAP medium was used in the study and test samples at test termination were centrifuged prior to analysis.

Sample preparation:

A method validation was performed to quantify the amount of the metabolite SYN545547 present in recovery samples prepared in freshwater (reconstituted for hardness). Recovery samples were initially diluted with acetonitrile to a composition of 20:80 acetonitrile: freshwater (reconstituted for hardness) (v/v). The mid- and high-level recovery samples were further diluted into the calibration standard range with 20:80 acetonitrile: purified reagent water (v/v). All samples were analysed by automated injection on a high-performance chromatographic system equipped with ultraviolet detection (HPLC-UV).

HPLC-UV conditions:

Chromatographic system:	Hewlett-Packard Series 1100 quaternary solvent pump, Hewlett-Packard Series 1100 autosampler, Hewlett-Packard 1100 series variable wavelength detector, Hewlett-Packard Series 1100 vacuum degasser and ChemStation Version B.04.02 for data acquisition
Analytical column:	Agilent Zorbax SB-C18, 3.5 µm, 75 mm x 4.6 mm
Injection volume:	500 µL
Mobile phase A:	0.05% phosphoric acid in purified reagent water
Mobile phase B:	100% acetonitrile
Flow rate:	1.4 mL/minute

Gradient:

Time (minute)	% A	% B
0.00	80	20
1.00	80	20
10.0	0	100
12.0	0	100
13.0	80	20

Retention time:	Approximately 7.8 minutes
Detection wavelength:	225 nm
Run time:	13 minutes
Equilibration delay:	3 minutes

A summary of the method validation data is given in Table B.5.1.2.6-24.

Table B.5.1.2.6-24: Summary of method validation data for determination of SYN545547 in freshwater

Matrix	**Analyte**	**LOQ (mg/L)**	**Recovery fortification level (mg/L)**	**Recoveries % range (mean)**	**Repeatability % RSD (n)**	**Linearity**
Freshwater	SYN545547	0.015	0.015	97 – 98 (97)	0.5 (3)	0.005 – 0.1 mg/L (n = 6*2) y = 588x+0.6 R^2 = 0.9998
			1.00	100 – 101 (101)	0.6 (3)	
			15.0	102 – 103 (102)	0.6 (3)	
			Overall	97 – 103 (100)	2.4 (9)	

Table B.5.1.2.6-25: Procedural recoveries of SYN545547 from an acute toxicity test with rainbow trout (*Oncorhynchus mykiss*) under static conditions

Study	Matrix	Analyte	Nominal concentration (mg/L)	Sample Interval	% Recovery
Study 1781.7096 KCA 8.2.1-02	Freshwater	SYN545547	0.125	0-hour	104
				96-hour	932*
			2.50	0-hour	104
				96-hour	95
			10.0	0-hour	104
				96-hour	96

*At test termination at 0.125 mg/L the recovery is significantly outside the acceptable range (70-110%). The applicant has stated this is likely due to a fortification error.

Table B.5.1.2.6-26: Procedural recoveries of SYN545547 from an acute toxicity test to water fleas (*Daphnia magna*) under static conditions

Study	Matrix	Analyte	Nominal concentration (mg/L)	Sample Interval	% Recovery
Study 1781.7095 KCA 8.2.4.1-02	Freshwater	SYN545547	0.125	0-hour	98
				48-hour	104
			2.50	0-hour	103
				48-hour	105
			10.0	0-hour	99
				48-hour	104

Table B.5.1.2.6-27: Procedural recoveries of SYN545547 from a 96-hour toxicity test with the freshwater green alga, *pseudokirchneriella subcapitata*

Study	Matrix	Analyte	Nominal concentration (mg/L)	Sample Interval	% Recovery
Study 1781.7094 KCA 8.2.6.1-02	AAP medium	SYN545547	0.25	0-hour uncentrifuged	106
				0-hour centrifuged	95*
				96-hour uncentrifuged	102
			2.50	0-hour uncentrifuged	101
				0-hour centrifuged	86*
				96-hour uncentrifuged	103
			10.0	0-hour uncentrifuged	100
				0-hour centrifuged	75*
				96-hour uncentrifuged	92

*Centrifuged samples showed a slight decrease in the recovery but are still within the acceptable range (70-110%).

Specificity

Chromatograms were presented for the calibration solution at 0.05 mg/L, fortified sample at 1 mg/L and the control sample. No chromatograms have been provided for the test solutions or for the control of the AAP medium. Nevertheless, no significant interference was observed at the retention time of interest.

Linearity

Linearity was demonstrated by the analysis of six matrix matched standards of increasing concentration in duplicate. The range of standard concentrations used was 0.005 – 0.1 mg/L. The response was linear with a coefficient of determination of 0.9998. It is noted samples are diluted to within the linear range.

Accuracy

Fortified samples of freshwater were analysed at low, mid, and high levels (0.015, 1 and 15 mg/L). This is equivalent to the LOQ level, 67xLOQ and 1000xLOQ. These fortification levels are appropriate to the concentrations in the ecotoxicology studies (Study 1781.7096 and 1781.7095: 0.31-10 mg/L, Study 1781.7094: 0.63-10 mg/L). Three samples were prepared at each fortification level and the mean recoveries were within the acceptable range (70-110%). Procedural recoveries have also been reported for each study within the acceptable range (70-110%) except for study 1781.7096 at 0.125 mg/L after 96 hours where a recovery of 932% was observed. The applicant has stated this is likely due to a fortification error.

Precision

Precision was determined from the accuracy recovery data for SYN545547. The %RSD was ≤20% with three samples prepared at each fortification level. However, in accordance with SANCO/3029/99 rev. 4 a minimum of five determinations are required.

Matrix effects

Matrix matched standards were not specifically addressed. However, matrix matched standards were used for calibration.

LOQ

The LOQ stated by the applicant is 0.00606 mg/L, determined from the limit of quantification of the instrument and the dilution factor of the control samples. According to SANCO/3029/99 rev. 4, the LOQ is defined as the lowest concentration tested, at which an acceptable mean recovery (70-110%) with an acceptable RSD (≤20%) is obtained. Therefore, the LOQ of the method is 0.015 mg/L.

Conclusion

The method is not fully validated in accordance with SANCO/3029/99 rev. 4 for the determination of SYN545547 in freshwater and AAP medium. The following deficiencies are noted:

- Only three recovery determination have been reported but SANCO/3029/99 rev. 4 states a minimum of five determinations are required.
- A chromatogram of the control of AAP medium has not been provided. Only a chromatogram of the freshwater control has been presented.

Nevertheless, all samples are diluted to within the linear range and there is data reported at three fortification levels which are appropriate to the test concentrations. The method of analysis is intended to simply determine the concentration of the active substance in solution. Overall, the method can be considered fit for regulatory purposes with an LOQ of 0.015 mg/L.

Report:	**KCA1 8.2.1-08 [redacted] and [redacted] (2016)**
Title	SYN548261 – Acute Toxicity to *Oncorhnchus mykiss* Report number: 3201085
Guidelines:	SANCO/3029/99 rev. 4
GLP:	Yes
Deviations	N/A
Previous evaluation:	None

Report:	**KCA1 8.2.4.1-04 [redacted] and [redacted] (2016a)**
Title	SYN548261 – Acute toxicity to Water Fleas, (*Daphnia magna)* under static conditions Report number: 3201086
Guidelines:	SANCO/3029/99 rev. 4
GLP:	Yes
Deviations	N/A
Previous evaluation:	None

Report:	**KCA1 8.2.6.1-04 [redacted] and [redacted] (2016b)**
Title	SYN548261 – Inhibition of Growth to the Alga *Pseudokirchneriella supcapitata* in a 96-hour test Report number: 3201084
Guidelines:	SANCO/3029/99 rev. 4
GLP:	Yes
Deviations	N/A
Previous evaluation:	None

The studies that use the following HPLC-UV method to determine the concentration of the metabolite SYN548261 are as follows:

- KCA 8.2.1-08: Study 3201085
- KCA 8.2.4.1-04: Study 3201086
- KCA 8.2.6.1-04: Study 3201084

Sample preparation:

Aqueous test samples (7 mL) were initially diluted with acetonitrile (3 mL) and orthophosphoric acid (20 µL), then, if necessary, further diluted with (7:3 v/v) of the appropriate test water: acetonitrile and 0.2% orthophosphoric acid to bring the expected concentration to within the calibration range. Samples were analysed by HPLC-UV.

HPLC-UV conditions:

Chromatographic system:	Agilent 1100 series HPLC system
Analytical column:	Phenomenex Luna, C18, 150 x 4.6 mm, 5 µm
Injection volume:	100 µL
Column temperature:	25°C
Mobile phase A:	70% 0.2% orthophosphoric acid in HPLC water
Mobile phase B:	30% 0.2% orthophosphoric acid in acetonitrile
Flow rate:	1.0 mL/minute
Retention time:	Approximately 3.6 minutes
Detection wavelength:	228 nm
Run time:	7 minutes

A summary of the method validation data is given in Table B.5.1.2.6-28.

Table B.5.1.2.6-28: Summary of method validation data for determination of SYN548261 in Elendt M4 water

Matrix	Analyte	LOQ (µg/mL)	Recovery fortification level (µg/mL)	Recoveries % range (mean)	Repeatability % RSD (n)	Linearity
Treated mains water	SYN548261	0.05	0.05	99 – 103 (101)	1.3 (5)	0.005 – 0.1 µg/mL (n = 7) R^2 >0.98
			1.0	103 – 104 (103)	0.4 (5)	
			10	99 – 101 (100)	0.8 (5)	
			Overall	99 – 104 (100)	1.6 (15)	
Elendt M4 water	SYN548261	0.05	0.05	105 – 110 (107)	1.6 (5)	0.005 – 0.1 µg/mL (n = 7) R^2 >0.98
			1.0	104 – 105 (104)	0.5 (5)	
			10	103 – 105 (104)	0.8 (5)	
			Overall	103 – 110 (105)	1.7 (15)	
EC medium	SYN548261	0.05	0.05	101 – 106 (103)	1.8 (5)	0.005 – 0.1 µg/mL (n = 7) R^2 >0.98
			1.0	104 – 105 (104)	0.5 (5)	
			10	103 – 103 (103)	0 (5)	
			Overall	101 – 106 (104)	1.2 (15)	

Table B.5.1.2.6-29: Procedural recoveries of SYN548261 from an acute toxicity test to *Oncorhynchus mykiss*

Study	Matrix	Analyte	Nominal concentration (µg/mL)	Sample Interval	% Recovery
Study 3201085 KCA 8.2.1-08	Treated mains water	SYN548261	100	0 hours	98
				96 hours	100

Table B.5.1.2.6-30: Procedural recoveries of SYN548261 from an acute toxicity test to water fleas, *Daphnia magna*, under static conditions

Study	Matrix	Analyte	Nominal concentration (µg/mL)	Sample Interval	% Recovery
Study 32010186 KCA 8.2.4.1-04	Elendt M4 water	SYN548261	100	0 hours	101
				24 hours	101
				48 hours	101

Table B.5.1.2.6-31: Procedural recoveries of SYN548261 from an inhibition growth to the alga *pseudokirchneriella subcapitata* in a 96-hour test

Study	Matrix	Analyte	Nominal concentration (µg/mL)	Sample Interval	% Recovery
Study 32010184 KCA 8.2.6.1-04	EC medium	SYN548261	100	0 hours	100
				96 hours	109

Specificity

Chromatograms were presented for the control sample, 100 µg/mL test sample at day 0 and fortified sample at 0.5 µg/mL for each matrix. No significant interference was observed at the retention time of interest.

Linearity

Linearity was demonstrated by the analysis of seven matrix matched standards of increasing concentration for each matrix. The range of standard concentrations used was 0.005 – 0.1 µg/mL. A calibration plot has been presented for each matrix, showing the response is linear with a coefficient of determination (R^2) > 0.98. However, an equation of the line has not been reported. It is noted samples are diluted to within the linear range.

Accuracy

Fortified samples of treated mains water, Elendt M4 water and EC medium were analysed at low, mid, and high levels (0.05, 1.0 and 10 µg/mL). This is equivalent to the LOQ level, 20x LOQ and 200x LOQ. Five samples were prepared at each fortification level and the mean recoveries were within the acceptable range (70-110%). However, the highest fortification level is 10 times lower than the concentration used within the ecotoxicology tests (test concentration: 100 µg/mL). Procedural recoveries at a nominal concentration of 100 µg/mL have been reported for each study within the acceptable range (70-110%). This demonstrates the method is sufficiently accurate at 100 µg/mL.

Precision

Precision was determined from the accuracy recovery data for SYN548261. The %RSD was ≤20% with five samples prepared at each fortification level for treated mains water, Elendt M4 medium and EC medium.

Matrix effects

Matrix matched standards were not specifically addressed. However, matrix matched standards were used for calibration.

LOQ

The LOQ of the method is 0.05 µg/mL for all matrices. This is the lowest fortification level with acceptable accuracy and precision.

Conclusion

The method is acceptably validated in accordance with SANCO/3029/99 rev. 4 for the determination of SYN548261 in treated mains water, Elendt M4 water and EC medium with an LOQ of 0.05 µg/mL.

Report:	**KCA1 8.2.1-01 [redacted] (2009)**
Title	M700F001 (metabolite of BAS 700 F) Acute Toxicity for Rainbow Trout BASF DocID 2009/1021591
Guidelines:	SANCO/3029/99 rev. 4
GLP:	Yes
Deviations	N/A
Previous evaluation:	None

Report:	**KCA1 8.2.4.1-01 [redacted] (2009a)**
Title	M700F001 (metabolite of BAS 700 F) Daphnia Magna, Acute Immobilization Test BASF DocID 2009/1021592
Guidelines:	SANCO/3029/99 rev. 4
GLP:	Yes
Deviations	N/A
Previous evaluation:	None

Report:	**KCA1 8.2.6.1-01 [redacted] (2009b)**
Title	M700F001 (metabolite of BAS 700 F) *Pseudokirchneriella subcapitata* SAG.61.81 Growth Inhibition Test BASF Doc ID 2009/1021953
Guidelines:	SANCO/3029/99 rev. 4
GLP:	Yes
Deviations	N/A
Previous evaluation:	None

The studies that use the following HPLC-UV method to determine the concentration of the metabolite M700F001 (also known as NOA449410) are:

- KCA 8.2.1-01: BASF DocID 2009/1021591
- KCA 8.2.4.1-01: BASF DocID 2009/1021592
- KCA 8.2.6.1-01: BASF DocID 2009/1021953

A letter of co-ownership has been submitted for these three studies, showing Syngenta co-owns the studies with BASF.

Sample preparation:

Each sample of 10 mL volume (i.e., control sample, test sample, sample fortified with the test item) was mixed thoroughly with 0.01 mL of concentrated orthophosphoric acid. If necessary, the sample was diluted in 0.1% orthophosphoric acid. Samples were analysed by HPLC-UV.

HPLC-UV conditions:

Chromatographic system:	Varian ProStar HPLC with Varian ProStar UV-Vis detector
Analytical column:	Microsorb-MV 100-5, C18, 250 x 4.6 mm
Injection volume:	20 µL
Mobile phase:	Acetonitrile: universal buffer (40:60, v/v)
Flow rate:	1.0 mL/minute
Retention time:	Approximately 3.5 minutes
Detection wavelength:	220 nm

A summary of the method validation data is given in Table B.5.1.2.6-32.

Table B.5.1.2.6-32: Summary of method validation data for determination of M700F001 in water

Matrix	Analyte	LOQ (mg/L)	Recovery fortification level (mg/L)	Recoveries % range (mean)	Repeatability % RSD (n)	Linearity
Water	M700F001 (also known as NOA449410)	0.05	0.05	96 – 106 (100)	4.7 (5)	0.01 – 10 mg/L (n = 7) y = 4.93e+5x R^2 = 0.9998
			1.0	98 – 105 (101)	2.3 (5)	
			10	91 – 99 (95)	3.3 (5)	
			Overall	91 – 106 (98)	4.4 (15)	

Table B.5.1.2.6-33: Procedural recoveries of M700F001 from an acute toxicity test for rainbow trout

Study	Matrix	Analyte	Nominal concentration (mg/L)	Sample Interval	Mean % Recovery (n=3)
BASF DocID 2009/1021591 KCA 8.2.1-01	Filtered tap water	M700F001 (also known as NOA449410)	100	0 hours	91
				96 hours	85

Table B.5.1.2.6-34: Procedural recoveries of M700F001 from an acute immobilization test for *daphnia magna*

Study	Matrix	Analyte	Nominal concentration (mg/L)	Sample Interval	Mean % Recovery (n=3)
BASF DocID 2009/1021592 KCA 8.2.4.1-01	Elendt M7 water	M700F001 (also known as NOA449410)	10	0 hours	93
				48 hours	94
			18	0 hours	95
				48 hours	98
			32	0 hours	96
				48 hours	96
			56	0 hours	97
				48 hours	98
			100	0 hours	98
				48 hours	99

Table B.5.1.2.6-35: Procedural recoveries of M700F001 from a growth inhibition test for *psurdokirchneriella subcapitata*

Study	Matrix	Analyte	Nominal concentration (mg/L)	Sample Interval	Mean % Recovery (n=3)
BASF DocID 2009/1021593 KCA 8.2.6.1-01	AAP medium	M700F001 (also known as NOA449410)	10	0 hours	87
				72 hours	85
			18	0 hours	96
				72 hours	102
			32	0 hours	92
				72 hours	92
			56	0 hours	97
				72 hours	97
			100	0 hours	87
				72 hours	90

Specificity

Chromatograms were presented for the control sample for each study and for 100 mg/L test sample at test initiation and test termination. No significant interference was observed at the retention time of interest. It is noted a chromatogram for the calibration solution has not been presented. However, this is acceptable as the test sample is M700F001 standard in the test matrix.

Linearity

Linearity was demonstrated by the analysis of seven standards of increasing concentration. The range of standard concentrations used was 0.01-10 mg/L. The response is linear with a coefficient of determination (R^2) of 0.9998. It is noted samples are diluted to within the linear range.

Accuracy

Fortified samples of water were analysed at low, mid, and high levels (0.05, 1.0 and 10 mg/L). This is equivalent to the LOQ level, 20xLOQ and 200xLOQ. Five samples were prepared at each fortification level and the mean recoveries were within the acceptable range (70-110%). The concentrations used in the ecotoxicology tests using this method range from 10-100 mg/L. The highest fortification level is 10 times lower than the highest concentration used in the tests. Procedural recoveries at the test concentrations (10-100 mg/L) have been reported within the acceptable range (70-110%). This demonstrates the method is sufficiently accurate at 10-100 mg/L.

Precision

Precision was determined from the accuracy recovery data for M700F001. The %RSD was ≤20% with five samples prepared at each fortification level.

Matrix effects

Matrix matched standards were not specifically addressed, however, the chromatograms for the control demonstrate no significant matrix effects are expected. Matrix matched standards have not been used for calibration.

LOQ

The LOQ of the method is 0.05 mg/L. This is the lowest fortification level with acceptable accuracy and precision.

Conclusion

The method is acceptably validated in accordance with SANCO/3029/99 rev. 4 for the determination of M700F001 (also known as NOA449410) in aqueous matrices with an LOQ of 0.05 mg/L.

Report:	**KCA1 8.2.3-01 [redacted] (2020)**
Title	Pydiflumetofen – Amphibian Metamorphosis Assay with African Clawed Frog (*Xenopus laevis*) Report number: 1781.7310
Guidelines:	SANCO/3029/99 rev. 4
GLP:	Yes
Deviations	N/A
Previous evaluation:	None

Report:	**KCA1 8.2.3-04 [redacted] (2020a)**
Title	Pydiflumetofen – Fish Short-Term Reproduction Assay with Fathead Minnow (*Pinephales promelas*) Report number: 1781.7303
Guidelines:	SANCO/3029/99 rev. 4
GLP:	Yes
Deviations	N/A
Previous evaluation:	None

The following studies use method ECO-066-01A to determine the concentration of pydiflumetofen:

- KCA 8.2.3-01: Study 1781.7310

- KCA 8.2.3-04: Study 1781.7303

Sample preparation:

Recovery samples were prepared by fortifying laboratory well water in disposable glass vials with the test substance to obtain concentration of 0.05 and 100,000 µg/L. Recovery samples were initially diluted with 0.2% acetic acid in acetonitrile to a final composition of 20/80/0.04 acetonitrile/laboratory well water/acetic acid (v/v). The high-level recovery samples were subsequently diluted into the calibration standard range with 20/80/0.04 acetonitrile/purified reagent water/acetic acid (v/v/v). All samples were analysed using liquid chromatography with tandem mass spectrometry detection (LC-MS/MS). The analysis was conducted using solvent-based standards.

LC-MS/MS conditions:

Chromatographic system:	Shimadzu SIL-20ACHT autosampler, Shimadzu DGU-20A5R and Shimadzu DGU-20A3 vacuum degassers, Shimadzu LC-20AD solvent delivery pumps, Shimadzu CTO-20A column oven
Analytical column:	Ace C18 3 µm, 50 × 2.1 mm
Column oven temperature:	40°C
Autosampler temperature:	5°C
Injection volume:	10 µL
Mobile phase:	A: 0.1% formic acid in reagent grade water B: 100% acetonitrile

Gradient:

Time (minutes)	% solvent A	% solvent B
0.01	70	30
3.00	20	80
4.00	20	80
4.10	70	30
7.00	70	30

Flow rate:	0.5 mL/minute
Retention time:	Approximately 3.3 minutes
Stop time:	7.0 minutes
Detector:	AB Sciex API 5000 mass spectrometer equipped with an AB Sciex ESI Turbo V Ion source
Ionisation mode:	Positive
Scan type:	MRM
Mass transitions:	*m/z* 426 → 192.9 (primary) *m/z* 428 → 194.9 (confirmatory)

A summary of the method validation data is given in Table B.5.1.2.6-36.

Table B.5.1.2.6-36: Summary of method validation data for determination of pydiflumetofen in laboratory well water

Matrix	Analyte	LOQ (µg/L)	Recovery fortification level (µg/L)	Recoveries % range (mean)	Repeatability % RSD (n)	Linearity
Laboratory well water	pydiflumetofen *m/z* 426 → 192.9	0.05	0.05	96 – 104 (99)	3.3 (5)	0.01 – 0.25 µg/L (n = 6*2) y = 394456x-458 R^2 = 0.999
			100, 000	89 – 113 (107)	9.5 (5)	
			Overall	89 – 113 (103)	8 (10)	
	pydiflumetofen *m/z* 428 → 194.9	0.05	0.05	99 – 106 (103)	2.5 (5)	0.01 – 0.25 µg/L (n = 6*2) y = 356514x-76 R^2 = 0.999
			100, 000	88 – 110 (105)	9.3 (5)	
			Overall	88 – 110 (104)	6.6 (10)	

Table B.5.1.2.6-37: Procedural recoveries of pydiflumetofen from a 21-day exposure test of African clawed frog tadpoles to pydiflumetofen

Study	Matrix	Analyte	Nominal concentration (µg/L)	Sample Interval	% Recovery
Study 1781.7310 KCA 8.2.3-01	Well water	pydiflumetofen	15	Day 0	102
				Day 7	101
				Day 14	116*
				Day 21	92
			100	Day 0	96
				Day 7	102
				Day 14	102
				Day 21	106
			375	Day 0	97
				Day 7	95
				Day 14	104
				Day 21	101

*Recovery at 15 µg/L on day 14 is slightly outside of the acceptable range (70-110%).

Table B.5.1.2.6-38: Procedural recoveries of pydiflumetofen from a 21-day exposure test of fathead minnow to pydiflumetofen

Study	Matrix	Analyte	Nominal concentration (µg/L)	Sample Interval	% Recovery
Study 1781.7303 KCA 8.2.3-04	Well water	pydiflumetofen	0.75	Day 0	84
				Day 5	105
				Day 7	107
				Day 14	97
				Day 21	105
			15	Day 0	74
				Day 5	95
				Day 7	105
				Day 14	95
				Day 21	103
			150	Day 0	85
				Day 5	104
				Day 7	103
				Day 14	97
				Day 21	108

Specificity

Chromatograms were presented for the control sample, reagent blank, 0.075 µg/L pydiflumetofen standard and recovery samples at 0.05 µg/ and 100,000 µg/L for both mass transitions. No significant interference was observed at the retention time of interest.

Linearity

Linearity was demonstrated by the analysis of six standards of increasing concentration in duplicate. The range of standard concentrations used was 0.01-0.25 µg/L. The response is linear with a coefficient of determination (R^2) of 0.999 for both mass transitions. It is noted samples are diluted to within the linear range.

Accuracy

Fortified samples of laboratory well water were analysed at 0.05 and 100,000 µg/L with five samples prepared at each fortification level. One individual recovery was outside of the acceptable range, but the mean recoveries were within the acceptable range (70-110%). The concentrations used in the ecotoxicology tests using this method range from 1.3-320 µg/L. Therefore, the highest fortification level is not appropriate to the test concentrations.

Procedural recoveries at the test concentrations have been reported within the acceptable range (70-110%), except in study 1781.7310 at 15 µg/L on day 14 which is slightly outside of the acceptable range. It is noted unacceptable results are observed at 13 µg/L for the test sample over the 21-days with a mean recovery of 130%. The applicant has noted the recoveries of the 13 µg/L treatment level remained slightly higher above the nominal concentration but the standard deviation for the recoveries at this level was ≤20%. Furthermore, acceptable recoveries were demonstrated at a similar level (15 µg/L) for the QC samples.

Precision

Precision was determined from the accuracy recovery data for pydiflumetofen. The %RSD was ≤20% with five samples prepared at each fortification level.

Matrix effects

Matrix matched standards were not specifically addressed; however, the applicant has stated interference arising from the matrices tested has not been observed. Matrix matched standards have not been used for calibration.

LOQ

The LOQ of the method is 0.05 µg/L. This is the lowest fortification level with acceptable accuracy and precision.

Conclusion

The method is acceptably validated in accordance with SANCO/3029/99 rev. 4 for the determination of pydiflumetofen in well water with an LOQ of 0.05 µg/L.

Report:	**KCA1 8.2.4.2-01 [redacted] (2015)**
Title	SYN545974 – Acute toxicity of SYN545974 to *Asellus aquaticus* Report number: CEA.1644
Guidelines:	SANCO/3029/99 rev. 4
GLP:	Yes
Deviations	N/A
Previous evaluation:	None

Report:	**KCA1 8.2.4.2-02 [redacted] (2015)**
Title	SYN545974 – Acute Toxicity of SYN545974 to *Chaoborus crystallinus* Report number: CEA.1666
Guidelines:	SANCO/3029/99 rev. 4
GLP:	Yes
Deviations	N/A
Previous evaluation:	None

Report:	**KCA1 8.2.4.2-03 [redacted] (2015a)**
Title	SYN545974 – Acute Toxicity of SYN545974 to *Chironomus riparius* Report number: CEA.1667
Guidelines:	SANCO/3029/99 rev. 4
GLP:	Yes
Deviations	N/A
Previous evaluation:	None

Report:	**KCA1 8.2.4.2-09 [redacted] (2015a)**
Title	SYN545974 – Acute Toxicity of SYN545974 to *Cloeon dipterum* Report number: CEA.1664
Guidelines:	SANCO/3029/99 rev. 4
GLP:	Yes
Deviations	N/A
Previous evaluation:	None

Report:	**KCA1 8.2.4.2-04 [redacted] (2015b)**
Title	SYN545974 – Acute Toxicity of SYN545974 to *Crangonyx pseudogracilis* Report number: CEA.1661
Guidelines:	SANCO/3029/99 rev. 4
GLP:	Yes
Deviations	N/A
Previous evaluation:	None

Report:	**KCA1 8.2.4.2-05 [redacted] (2015b)**
Title	SYN545974 – Acute Toxicity of SYN545974 to *Cyclops agilis speratus* Report number: CEA.1662
Guidelines:	SANCO/3029/99 rev. 4
GLP:	Yes
Deviations	N/A
Previous evaluation:	None

Report:	**KCA1 8.2.4.2-07 [redacted] (2015c)**
Title	SYN545974 – Acute Toxicity of SYN545974 to *Lumbriculus variegatus* Report number: CEA.1642
Guidelines:	SANCO/3029/99 rev. 4
GLP:	Yes
Deviations	N/A
Previous evaluation:	None

Report:	**KCA1 8.2.4.2-06 [redacted] (2015d)**
Title	SYN545974 – Acute Toxicity of SYN545974 to *Lymnaea stagnalis* Report number: CEA.1645
Guidelines:	SANCO/3029/99 rev. 4
GLP:	Yes
Deviations	N/A
Previous evaluation:	None

Method GRM061.01A for the determination of pydiflumetofen in aqueous matrices is also used for monitoring purposes. This method is reported in full under section B.5.2.4. The following studies rely on this method:

- KCA1 8.2.4.2-01 – Study CEA.1644
- KCA1 8.2.4.2-02 – Study CEA.1666
- KCA1 8.2.4.2-03 – Study CEA.1667
- KCA1 8.2.4.2-09 – Study CEA.1664
- KCA1 8.2.4.2-04 – Study CEA.1661
- KCA1 8.2.4.2-05 – Study CEA.1662
- KCA1 8.2.4.2-07 – Study CEA.1642
- KCA1 8.2.4.2-06 – Study CEA.1645

Method validation data and procedural recoveries have been presented in these studies for mesocosm water. A summary of this data is given in Table B.5.1.2.6-39 and B.5.1.2.6-40.

Table B.5.1.2.6-39: Summary of additional method validation data for determination of pydiflumetofen in water

Matrix	Analyte	LOQ (µg/L)	Recovery fortification level (µg/L)	Recoveries % range (mean)	Repeatability % RSD (n)	Linearity
Mesocosm water	pydiflumetofen	0.05	0.05	76 – 97 (84)	13.5 (3)	0.01 – 5 µg/L (n = 7) $R^2 > 0.998$
			12,000	83 – 95 (87)	7.6 (3)	
			Overall	76 – 97 (84)	10 (6)	

Table B.5.1.2.6-40: Summary of procedural recoveries of pydiflumetofen from ecotoxicology tests

Study	Matrix	Analyte	Nominal concentration (µg/L)	% Recovery
Study CEA.1644 KCA1 8.2.4.2-01	Mesocosm water	pydiflumetofen	0.05	90
			12000	113*
Study CEA.1666 KCA1 8.2.4.2-02			0.05	70
			12000	84
Study CEA.1667 KCA1 8.2.4.2-03			0.05	86
			12000	104
Study CEA.1664 KCA1 8.2.4.2-09			0.05	92, 102, 100
			12000	101, 105, 94
Study CEA.1661 KCA1 8.2.4.2-04			0.05	102
			12000	105
Study CEA.1662 KCA1 8.2.4.2-05			0.05	90
			12000	110
Study CEA.1642 KCA1 8.2.4.2-07			0.05	79
			12000	103
Study CEA.1645 KCA1 8.2.4.2-06			0.05	102
			12000	102

*Recovery at 12,000 µg/L in study CEA.1644 is slightly outside of the acceptable range (70-110%).

Specificity

Additional chromatograms were presented for 0.04 µg/L matrix matched standard, mesocosm water control and fortified samples at 0.05 µg/L and 12000 µg/L. However, significant interference was observed in all the studies (approximately 44-61% of the LOQ peak), except study CEA.1644, at the retention time of interest. The applicant has provided a justification for the significant interference. They have stated pydiflumetofen detected in test control samples is not considered to be detrimental to the study outcome as the measured levels were approximately 50-5000 times less than the respective NOECs. It is noted matrix matched standards have been used which should account for the interference. Furthermore, this method is validated in aqueous matrices for monitoring purposes and the method validation data doesn't show significant interference.

Linearity

Further linearity data has been presented using at least seven matrix matched standards of increasing concentration. The range of standard concentrations used was 0.01-5 µg/L. The response is linear with a coefficient of determination >0.998. It is noted samples are diluted to within the linear range.

Accuracy

Additional accuracy data has been reported with fortified samples of mesocosm water analysed at 0.05 and 12,000 µg/L. Three samples were prepared at each fortification level and mean recoveries were within the acceptable range (70-110%). These fortification levels are appropriate to the concentrations used in the ecotoxicology tests (4-10,000 µg/L). Procedural recoveries have been reported at 0.05 and 12,000 µg/L. These are within the acceptable range except for study CEA.1644 at 12,000 µg/L which is slightly outside of the acceptable range.

Control and reagent blank samples have been analysed but show levels of pydiflumetofen above the LOQ. The applicant has addressed this stating since the immobility and mortality in the pooled controls did not exceed the allowable limits of validity criteria (≤15% in controls) and the measured levels were significantly less than the NOEC, this is not considered to have an impact on the outcome of the study.

Precision

Additional precision was determined from the accuracy recovery data for pydiflumetofen. The %RSD was ≤20% with three samples prepared at each fortification level. It is noted in accordance with SANCO/3030/99 rev. 5, a minimum of five determinations are required. However, extra validation data is presented in section B.5.2.4 for this method with five determinations at each fortification level. This is acceptable.

Matrix effects

Matrix matched standards were not specifically addressed; however, matrix matched standards have been used for calibration.

LOQ

The LOQ of the method is 0.05 µg/L. This is the lowest fortification level with acceptable accuracy and precision.

Conclusion

GRM061.01A method is not acceptably validated in accordance with SANCO/3029/99 rev. 4 for the determination of pydiflumetofen in mesocosm water as significant interference is observed at the retention time of interest. However, matrix matched standards have been used which should account for the interference and the measured levels in the study are significantly greater than the LOQ. This method is also proposed as a monitoring method and no significant interference was observed in the method validation data for aqueous matrices. On this basis, the method can be considered fit for regulatory purposes with an LOQ of 0.05 µg/L.

Report:	**KCA1 8.2.4.2-10 [redacted] et al (2015)**
Title	SYN545974 – A 48-Hour Static Acute Toxicity Test with the Freshwater Amphipod (Hyalella azteca) Report number: 528A-287
Guidelines:	SANCO/3029/99 rev. 4
GLP:	Yes
Deviations	N/A
Previous evaluation:	None

Sample preparation:

Samples of fresh water were diluted as necessary, with 20: 80 (v/v) methanol: freshwater before analysis by LC/MS/MS under the conditions below.

LC-MS/MS conditions:

Chromatographic system:	Applied Biosystems/MDS Sciex API 3000 mass spectrometer + Agilent 1260 series HPLC system
Analytical column:	Thermo Betasil C-18 (50 mm × 2.1 mm, 5-µm particle size)
Injection volume:	50 µL
Mobile phase A:	0.1% formic acid in purified reagent water
Mobile phase B:	0.1% formic acid in acetonitrile
Flow rate:	350 µL/minute

Gradient:

Time (minute)	% A	% B
0	30	70
4.0	30	70

Retention time:	Approximately 1.9 minutes
Ion source:	Turbo ion spray
Polarity:	Positive
Transition monitored:	*m/z* 428.087 → 408.100
Mode:	MRM

A summary of the method validation data is given in Table B.5.1.2.6-41.

Table B.5.1.2.6-41: Summary of method validation data for determination of pydiflumetofen residues in fresh water

Matrix	Analyte	LOQ (mg a.s/L)	Recovery fortification level (mg a.s/L)	Recoveries % range (mean)	Repeatability % RSD (n)	Linearity
Fresh Water	pydiflumetofen	0.00125	0.0025	97.2 101 (99.1)	n =2	0.001 – 0.01 mg/L (n = 5) y = 41258700x - 4234.35 R^2 = 0. 0.9970
			0.5	97.7 102 (99.8)	n =2	
			1.25	99.0 99.4 (99.2)	n =2	
			Overall	99.4	1.87 (6)	

Specificity

Specificity was demonstrated by retention time match with a reference standard and the absence of interfering peaks in the chromatogram of a blank freshwater sample.

Linearity

Linearity was demonstrated by the analysis of 5 standards of increasing concentration. The range of standard concentrations used was 0.001 – 0.01 mg a.s/L. The response was linear with a R^2 value of 0.9970.

Accuracy

Recovery samples were prepared by spiking freshwater with active substance standard at concentrations of 0.0025, 0.5 and 1.25 mg/Land analysing them by the method described. 2 samples were prepared at each fortification level. Recovery values were calculated as a percentage of measured concentration relative to fortified concentration. Mean recovery levels were within the range 99.1-99.8 %.

Precision

2 replicate samples at each fortification level were prepared and analysed using the method described above. The combined RSD of all 6 values obtained were within the guideline requirements of a %RSD ≤20%. Although there weren't enough replicates per fortification level, the combined 6 values from all levels can be deemed sufficient.

Matrix effects

Matrix matched standards were not specifically addressed. However, matrix matched standards were used for calibration.

LOQ

The LOQ of the method is 0.0025 mg./L based on the lowest fortification level with acceptable accuracy.

Conclusion

The method is acceptably validated in accordance with SANCO/3029/99 rev.4 and is suitable for the determination of pydiflumetofen in fresh water

Report:	**KCA1 8.2.5.3-01 [redacted] et al. (2015)**
Title	SYN545547 - A Prolonged Sediment Toxicity Test with the Midge (Chironomus riparius) Using Spiked Sediment Report Number: 528A-286
Guidelines:	SANCO/3029/99 rev. 4
GLP:	Yes
Deviations	N/A
Previous evaluation:	None

Sample preparation:

Separation of overlying water, pore water and sediment

An aliquot (approximately 20 mL) of each overlying water sample was removed from approximately mid-depth for analysis. The remainder of each sediment sample was transferred to polypropylene centrifuge tubes and centrifuged for approximately 10 minutes. The pore water from each centrifuged sample was transferred into graduated cylinder and its volume measured. The sediment samples remaining in the centrifuge tube were transferred to specimen cups.

Overlying water and pore water samples were centrifuged for 10 minutes. Aliquots of each centrifuged overlying water and pore water sample were then transferred to scintillation vials, to which an equal volume of 0.2% phosphoric acid in acetonitrile was added. Samples were capped and mixed with vortex action. Dilutions were performed, as necessary, with 50:50:0.1 (v/v/v) acetonitrile: HPLC-grade water: phosphoric acid prior to analysis HPLC-UV under the conditions described below.

Aliquots of the sediment (1.00 g) were weighed into polypropylene centrifuge tubes to which 10.0 mL of 50:50:0.1 (v/v/v) acetonitrile: HPLC-grade water: phosphoric acid was added. The samples were mixed with vortex action for five minutes and then centrifuged. The extracts were transferred to separate polypropylene centrifuge tubes, the extraction was repeated, and the extracts combined. The final volume of the combined extracts was adjusted to 20 mL with 50:50:0.1 (v/v/v) acetonitrile: HPLC-grade water: phosphoric acid. Dilutions were made, as necessary, with 50:50:0.1 (v/v/v) acetonitrile: HPLC-grade water: phosphoric acid prior to analysis HPLC-U V under the conditions described below.

HPLC conditions:

Chromatographic system	Agilent Model 1200 high performance liquid chromatograph (HPLC) with an Agilent Series 1200 variable wavelength detector
Analytical column:	YMC-PACK ODS-AM (150 mm x 4.6 mm, 3 µm particle size)
Oven temperature:	40ºC
Injection volume:	100 µL
Mobile phase A:	0.1% H3PO4 in HPLC-grade water
Mobile phase B:	CH_3CN
Flow rate:	1.00 mL/minute

Gradient:

Time (minute)	% A	% B
1.00	90	10
9.00	2	98
10.00	2	98
10.10	90	10
14.00	90	10

Retention time:	Approximately 11.4 minutes
Detector wavelength:	220 nm

A summary of the method validation data is given in Table B.5.1.2.6-42.

Table B.5.1.2.6-42: Summary of method validation data for determination of SYN545547 in waters and sediment

Matrix	Analyte	LOQ	Recovery fortification level (mg a.s/kg)	Recoveries % range (mean)	Repeatability % RSD (n)	Linearity
Water	SYN545547	0.2 mg/L	0.5	101 – 106 (103)	n = 3	0.100 – 1.00 mg/L (n = 5) y = 325.3x -0.08917 R^2 = 0. 0.9998
			5	101 – 105 (102)	n = 3	
			75	100 – 104 (102)	n = 3	
			Overall	100 – 106 (102)	2.1 (9)	
Sediment	SYN545547	2.5 mg/kg	7.5	98 - 99 (99)	n = 3	
			100	97 - 99 (98)	n = 3	
			1100	100 - 105 (102)	n = 3	
			Overall	97 – 105 (100)	2.33 (9)	

Specificity

Specificity was demonstrated by retention time match with a reference standard and the absence of interfering peaks in the chromatogram of a blank water and sediment samples.

Linearity

Linearity was demonstrated by the analysis of 5 standards of increasing concentration. The range of standard concentrations used was 0.1 – 1.0 mg/L. The response was linear with a R^2 value of 0. 0.9998.

Accuracy

Recovery samples were prepared a low level, mid-level, and a high level by spiking blank water or sediment samples formulation with active substance standard and analysing them by the method described. 3 samples were prepared at each fortification level. Recovery values were calculated as a percentage of measured concentration relative to fortified concentration. Mean recovery levels were within the range 98 to 103 %.

Precision

3 replicate samples were prepared at 3 different fortification levels The combined RSD of all 9 values obtained were within the guideline requirements of a %RSD ≤20% for each matrix. Although there weren't enough replicates per fortification level, the combined values from all levels can be deemed sufficient.

Matrix effects

Matrix matched standards were not specifically addressed.

LOQ

The LOQ of the method is 0.2 mg/Lin water samples and 2.49 mg/kg dry sediment The LOQ was established based on the lowest calibration level and dilution factors applied during sample preparation.

Conclusion

The method is acceptably validated and is suitable for the determination of SYN545547 in water and sediment.

Report:	**KCA1 8.2.5.4-03 [redacted] (2015a)**
Title	SYN545974 – 42-Day Toxicity Test Exposing Freshwater Amphipods (Hyalella azteca) to Spiked Sediment Report Number: 1781.6890
Guidelines:	SANCO/3029/99 rev. 4
GLP:	Yes
Deviations	N/A
Previous evaluation:	None

Report:	**KCA1 8.2.5.4-04 [redacted] (2015b)**
Title	SYN545974 - Life-Cycle Toxicity Test Exposing Midges (Chironomus dilutus) to Spiked Sediment Report Number: 1781.6889
Guidelines:	SANCO/3029/99 rev. 4
GLP:	Yes
Deviations	N/A
Previous evaluation:	None

Report:	**KCA1 8.2.5.4-05 [redacted] (2015)**
Title	SYN545974 - 10-Day Toxicity Test Exposing Estuarine Amphipods (Leptocheirus plumulosus) to a Test Substance Applied to Sediment under Static Conditions Report Number: 1781.7069
Guidelines:	SANCO/3029/99 rev. 4
GLP:	Yes
Deviations	N/A
Previous evaluation:	None

The following studies rely on the same LC/MS-MS method to determine the concentration of pydiflumetofen:
- KCA1 8.2.5.4-03
- KCA1 8.2.5.4-04
- KCA1 8.2.4.4-05

Separation of overlying water, pore water and sediment

Overlying water was decanted, and its volume measured. Pore water samples were collected by removing the entire sediment sample from each test vessel and centrifuging for 15 to 30 minutes. The resulting pore water was

removed from the centrifuge tube and its volume measured. Sediment samples were collected from the centrifuge tube, following centrifugation and removal of the pore water sample. To achieve homogeneous sub-samples, sediment samples were mixed well after the removal of pore water.

Sample preparation

Water

To minimize the potential for losses of the test substance, the aqueous test samples were not sub-sampled prior to dilution. Samples were either analysed directly or diluted with 20:80 acetonitrile: purified reagent water prior to analysis LC-MS/MS under the conditions described below.

Sediment

A 35.0-mL aliquot of 0.1% formic acid in acetonitrile is added to sediment samples (5 g dry weight). The samples are placed on a shaker table for 30 minutes at 150 rpm, and then centrifuged at 3000 rpm for 10 minutes. The supernatant is transferred to a 100-mL volumetric flask and the extraction procedure repeated with another 35.0 mL aliquot of 0.1% formic acid in acetonitrile. The extracts arere combined and diluted to a volume of 100 mL with 0.1% formic acid in acetonitrile and mixed well. All samples were diluted into the calibration standard range with 20:80 acetonitrile: purified reagent water (v/v) prior to analysis by LC/MS/MS under the conditions described below.

LC conditions:

Analytical column:	XBridge C18, 2.5 µm, 2.1 × 50 mm
Injection volume:	20 µL
Mobile phase A:	0.1% formic acid in purified reagent water
Mobile phase B:	0.1% formic acid in acetonitrile
Flow rate:	0.35 mL/minute

Gradient:

Time (minute)	% A	% B
1.00	70	30
3.0	10	90
5.00	10	90
5.10	70	30

Retention time: Approximately 4.1 minutes

MS conditions:

Instrument:	MDS API 5000 mass spectrometer
Ionization mode:	positive turbo spray
Q1/Q3 mass:	426.20/193.00 amu
Dwell time:	300 milliseconds
Source temperature:	400 °C
Scan type:	MRM

A summary of the method validation data is given in Table B.5.1.2.6-43. Marine sediment and seawater were used for method validation.

Table B.5.1.2.6-43: Summary of method validation data for determination of pydiflumetofen residues in marine sediment

Matrix	Analyte	LOQ (mg/kg)	Recovery fortification level (mg/kg)	Recoveries % range (mean)	Repeatability % RSD (n)	Linearity
Sediment	pydiflumetofen	0.021	0.1	101 98.4 95.6 (98.3)	2.7 (3)	0.1 – 2 µg/L (n = 6 x 2) $y = -7628.5x^2 + 116530x - 1148.2$ $R^2 = 0.99443$
			5.0	99.3 96.9 91.8 (96)	4.0 (3)	
			100	89.9 95.9 98.7 (94.8)	4.7 (3)	
			Overall	96.4	3.77 (9)	
Water	pydiflumetofen		0.3 µg/L	100 97.2 103 (100)	n=3	0.015 – 1 µg/L (n = 6 x 2) $y = -5551.8x^2 + 237040x + 328.72$ $R^2 = 0.9994$
			30 µg/L	114 103 102 (106)	n=3	
			10000 µg/L	95.5 98.7 91.5 (95.2)	n=3	
			Overall	101	6.2 (9)	

Specificity

Specificity was demonstrated by retention time match with a reference standard and the absence of interfering peaks in the chromatogram of a control sediment sample.

Linearity

Linearity was demonstrated by the analysis of 6 standards of increasing concentration in duplicate. The range of standard concentrations used was 0.1 – 2 ug/ml. The response was linear with a R^2 value of 0. 0.9944.

Accuracy

Recovery samples were prepared by spiking blank marine sediment or seawater with active substance standard at 3 different concentrations and analysing them by the method described. 3 samples were prepared at each fortification level. Recovery values were calculated as a percentage of measured concentration relative to fortified concentration. Mean recovery levels were within the range 95to 106%.

Precision

3 replicate samples at 3 different fortification levels were prepared and analysed using the method described above. The combined RSD of all 9 values obtained were within the guideline requirements of a %RSD ≤20% for each matrix. Although there weren't enough replicates per fortification level, the combined values from all levels can be deemed sufficient.

Matrix effects

Matrix matched standards were not specifically addressed.

LOQ

The LOQ of the method is 0.0210 mg/kg for sediment and 0.151 ug/L for water. The LOQ was established based on the lowest calibration level and dilution factors applied during sample preparation.

Procedural recovery data (three aqueous and three sediment samples) were prepared at each sampling interval and in sediment and water at relevant concentrations of pydiflumetofen in each study. These data are summarised in Table B.5.1.2.6-44.

Table B.5.1.2.6-44: Summary of procedural recovery data for determination of pydiflumetofen residues in sediment and water

Study	Matrix	Analyte	Recovery fortification level (µg/kg)	Recoveries % range (mean)	Repeatability % RSD (n)
1781.6890 KCA 8.2.5.4-03	sediment		2.0 mg/kg	96.0 101 95.8	
			20 mg/kg	99.1 103 90.4	
			100 mg/kg	98.9 103 97.4	
	Water		0.0003 mg/L	110 95.7 101	
			0.03 mg/L	95.3 94.0 99.0	
			10 mg/L	108 102 105	
1781.6889 KCA 8.2.5.4-04	sediment		1.3 mg/kg	98.3, 95.4	
			15 mg/kg	94.5 99.9 92.3	
			100 mg/kg	97.4 103 118	
	Water		0.0003 mg/L	97.7 97 102	
			0.03 mg/L	101 101	
			10 mg/L	100 111 107	
1781.7069 KCA 8.2.5.4-05	sediment		0.3 mg/kg	90 93	
			25 mg/kg	97.2 103	
			100 mg/kg	104 105	
	Water		0.0003 mg/L	115 110	
			0.03 mg/L	9838 110	
			10 mg/L	104 101	

Conclusion

The method is acceptably validated and is suitable for the determination of pydiflumetofen in sediment and water. Procedural recovery data demonstrate the applicability of the method to the water and sediment types tested in the studies.

Report:	**KCA1 8.3.1.3-05 [redacted] (2015)**
Title	SYN545974 SC (A19649B) – Chronic toxicity to the honeybee larvae Apis mellifera L. under laboratory conditions (in vitro) Report No: 14 10 48 005 B
Guidelines:	SANCO/3029/99 rev. 4
GLP:	Yes
Deviations	N/A
Previous evaluation:	None

Sample preparation:

Samples of aqueous sugar solution (test diets) were diluted by a factor of 200 with methanol/water (1:1 v/v) before analysis by HPLC-UV under the conditions below.

HPLC-UV conditions:

Chromatographic system: Shimadzu LC-10 HPLC system equipped with a diode-array detector
Analytical column: Macherey Nagel Nuicleoshell RP18, (2.7 mm x 100mm, 2.7 µm particle size)
Over temperature Ambient
Mobile phase A: 0.1% phosphoric acid in purified water
Mobile phase B: 0.1% phosphoric acid in acetonitrile
Flow rate: 0.4 mL/minute

Gradient:

Time (minute)	% A	% B
0	85	15
7	10	90
8	10	90
8.01	85	15

Retention time: Approximately 6.5 minutes
Detection wavelength 225 nm

A summary of the method validation data is given in Table B.5.1.2.6-45.

Table B.5.1.2.6-45: Summary of method validation data for determination of pydiflumetofen residues in aqueous sugar solution

Matrix	**Analyte**	**LOQ (mg a.s/L)**	**Recovery fortification level (mg a.s/L)**	**Recoveries % range (mean)**	**Repeatability % RSD (n)**	**Linearity**
Aqueous sugar solution	pydiflumetofen	832.1	832.1	102 - 103, (102)	0.2 (5)	3.222 – 9.764 mg/L (n = 5) y = 65392.1x – 4616.5 R^2 = 0. 0.9999
			1672	103 - 104, (104)	0.3 (5)	

Specificity

Specificity was demonstrated by retention time match with a reference standard and the absence of interfering peaks in the chromatogram in the blank test medium.

Linearity

Linearity was demonstrated by the analysis of 5 standards of increasing concentration. The range of standard concentrations used was 3.222 – 9.764 mg/L. The response was linear with a R^2 value of 0. 0.9999.

Accuracy

Recovery samples were prepared by spiking test medium with active substance at 2 different concentrations and analysing them by the method described. 5 samples were prepared at each fortification level. Recovery values were calculated as a percentage of measured concentration relative to fortified concentration. Mean recovery levels were within the range 102-104%.

Precision

5 replicate samples at each fortification level were prepared and analysed using the method described above, and the %RSD was calculated. The relative standard deviation of each fortification level was within the guideline requirements of a %RSD ≤20%.

Matrix effects

Matrix matched standards were not specifically addressed, as samples were diluted by a factor of 200 before analysis, therefore matrix effects were negligible.

LOQ

The LOQ of the method is 832.1 mg a.s/L.

Conclusion

The method is acceptably validated in accordance with SANCO/3029/99 rev.4 and is suitable for the determination of pydiflumetofen in aqueous sugar solutions.

Report:	**KCA1 8.3.1.3-01 [redacted] (2015)**
Title	SYN545974 - A laboratory study to determine the chronic effects on the brood of the honeybee Apis Report Number: 037SRFR15C06
Guidelines:	SANCO/3029/99 rev. 4
GLP:	Yes
Deviations	N/A
Previous evaluation:	None

Report:	**KCA1 8.3.1.3-02 [redacted] (2015a)**
Title	SYN545974 SC (A19649B) - A laboratory study to determine the chronic effects on the brood of the honeybee Apis mellifera L. (Hymenoptera: Apidae). Report Number: 037SRFR15C07
Guidelines:	SANCO/3029/99 rev. 4
GLP:	Yes
Deviations	N/A
Previous evaluation:	None

The following studies rely on the same HPLC/UV method to determine the concentration of pydiflumetofen in artificial sugar solution diet:

- KCA1 8.3.1.3-01
- KCA1 8.3.1.3-02

Sample preparation:

Samples of the test item (aqueous sugar solutions of pydiflumetofen with a target concentration of 0.0015 g a.s/L or 3.231 g a.s/L, depending on the study) were either analysed directly after ultrasonication, or were diluted with water to be within the calibration range prior to analysis by HPLC-UV under the conditions described below.

HPLC conditions:

Instrument:	Merck Lichrospher 100 RP18 (12.5cm x 4.0mm x 5.0µm particle size)
Oven temperature:	40ºC
Injection volume:	100 µL
Mobile phase A:	Water + 0.1% orthophosphoric acid
Mobile phase B:	Acetonitrile
Flow rate:	1.5 mL/minute

Gradient:

Time (minute)	% A	% B
0.0	50	50
8.0	20	80
10.0	10	90
10.1	50	50
14.0	50	50

Retention time:	Approximately 5.7 minutes
Detection wavelength:	230nm

A summary of the results of the verification of the solution concentrations is given in Table B.5.1.2.6-46.

Table B.5.1.2.6-46: Summary of analytical data for determination of pydiflumetofen in aqueous sugar solutions

Study reference	**Analyte**	**LOQ (mg/L)**	**Nominal solution concentration (mg/L)**	**Recoveries % range (mean)**	**Repeatability % RSD (n)**	**Linearity**
Report no. 037SRFR15C07 KCA1 8.3.1.3-01	pydiflumetofen	0.26	1.5	42 – 43 (43)	0.9 (4)	0.51 – 10.0 mg/L (n = 5*2)
			1.5	65 – 70 (67)	3.8 (4)	y = 1.78E+06x – 1.09E+05 R^2 = 0.9998
Report no. 037SRFR15C07 KCA1 8.3.1.3-02			3.23	94 – 102 (98)	4.2 (4)	

Specificity

Specificity was demonstrated by retention time match with a reference standard and the absence of interfering peaks in the chromatogram of a reagent blank.

Linearity

Linearity was demonstrated by the analysis of 5 standards of increasing concentration in duplicate. The range of standard concentrations used was 0.51 – 10 mg/L. The response was linear with a R^2 value of 0.9998.

Accuracy

No specific recovery data were provided. The results provided are based on the does verification data. For the study [redacted] (2015) (037SRFR15C06) the amount measured in the test solution was significantly lower than the target nominal value (mean recoveries of 43% and 67% of nominal concentration). Therefore, the target doses in the study will not have been achieved. This will need to be considered in the main study.

Precision

Four determinations were made for each concentration level, the RSD vales were <20%, indicating acceptable precision

Matrix effects

Matrix matched standards were not specifically addressed.

LOQ

The LOQ of the method is 0.26 mg/L, calculated based on the 10-x signal noise of the lowest calibration level.

Conclusion

The method is not validated in accordance with SANCO/3029/99 rev.4. The method itself is considered acceptable for the determination of SYN545874, however the analytical results were used for dose verification and indicate that the target doses in the study [redacted] (2015) (037SRFR15C06) will not have been achieved. This will need to be considered in the main study.

Report:	**KCA1 8.3.1.3-04 [redacted] (2018)**
Title	Pydiflumetofen - Effects on the honeybee brood Apis mellifera L. following chronic oral exposure under field conditions Report Number: 17 48 BFB 0001
Guidelines:	SANCO/3029/99 rev. 4
GLP:	Yes
Deviations	N/A
Previous evaluation:	None

Report:	**KCP 10.3.1.5-01 [redacted] (2018)**
Title	Pydiflumetofen SC (A19649B) – A Semi-Field Study to Evaluate Side Effects on Honeybees (*Apis mellifera* L.) in Germany in 2017 Report number: 17 48 BTB 0003
Guidelines:	SANCO/3029/9 rev. 4
GLP:	Yes
Deviations	N/A
Previous evaluation:	None

Report:	**KCA 4.1.2 [redacted] (2017)**
Title	SYN545974 – Analytical method ECO_066_03A and Validation for the Determination of SYN545974 in Pollinator Matrices (Pollen, Nectar, Foliage and Flowers and in Feeding Solutions (Sucrose) from Honeybee Oral Laboratory Studies Report number: 17 35 CRB 0148
Guidelines:	SANCO/3029/9 rev. 4
GLP:	Yes
Deviations	N/A
Previous evaluation:	None

Method ECO_066_03A using LC-MS/MS was used to determine residues of pydiflumetofen in feeding solution, flowers, foliage, nectar, and pollen samples in support of studies conducted using the active substance and the representative product.

Sample preparation:

- Samples were thawed and homogenized as necessary. Aliquots of 0.3 g were weighed into 10 mL polypropylene tubes and fortified if necessary. 3 mL of acetonitrile and ultrapure water were added to each (in the case of pollen and flowers: additionally add 1 mL of hexane) as well as 0.7 g salt mixture (0.5 g magnesium sulphate and 0.2 g sodium chloride). The samples were vortexed at 2500 rpm for 10 minutes followed by centrifugation at 3000 rpm for 5 minutes for phase separation. In the case of nectar samples, the upper acetonitrile phase was diluted with acetonitrile into autosampler vials and analysed by HPLC-MS/MS. Extracts of pollen, flowers and foliage were purified by dispersive SPE and diluted with acetonitrile prior to analysis by HPLC-MS/MS according to the conditions below:

LC-MS/MS conditions:

Chromatographic system:	Agilent 1200 HPLC System
Analytical column:	Zorbax Eclipse Plus C18, 2.1 x 50 mm, 1.8 µm
Column temperature:	35°C
Injection volume:	10 µL

Mobile phase: A: HPLC water containing 0.1% formic acid
B: acetonitrile containing 0.1% formic acid

Gradient:

Time (minutes)	% solvent A	% solvent B
0.00	75	25
5.0	0	100
7.0	0	100

Flow rate: 0.35 mL/minute
Run time: 10 minutes
Retention time: Approximately 4.6 minutes
Detector: 6470 triple quadrupole mass spectrometric detector
Ionisation mode: ESI positive
Scan type: MRM
Mass transitions: *m/z* 426 → 193 (quantifier)
m/z 426 → 171
m/z 426 → 166 (confirmatory)

A summary of the method validation data is given in Tables B.5.1.2.6-47 to Table B.5.1.2.6-50.

Table B.5.1.2.6-47: Summary of method validation data for determination of pydiflumetofen residues in honeybee feeding solution ([redacted], 2017)

Matrix	Analyte	LOQ (mg a.s /kg)	Recovery fortification level (mg a.s/kg)	Recoveries % range (mean)	Repeatability % RSD (n)	Linearity
Feeding solution	pydiflumetofen Primary Transition *m/z* 426 → 193	0.005	0.005	79 – 90 (86)	5.3 (5)	0.07 – 23.8 µg a.s/L (n = 8) Quadratic $y = -8.15x^2+1544x+145$ $R^2 = 0.9977$
			0.1	80 – 86 (82)	2.9 (5)	
			36	87 – 93 (89)	3.0 (5)	
			Overall	79 – 93 (86)	4.8 (15)	
	pydiflumetofen Confirmatory Transition *m/z* 426 → 166	0.005	0.005	79 – 99 (91)	9.3 (5)	0.07 – 23.8 µg a.s/L (n = 8) Quadratic $y = -4.5x^2+786x+76$ $R^2 = 0.9981$
			0.1	80 – 87 (82)	3.2 (5)	
			36	86 – 94 (89)	3.3 (5)	
			Overall	79 – 99 (87)	7.2 (15)	

Table B.5.1.2.6-48: Summary of method validation data for determination of pydiflumetofen residues in honeybee feeding solution ([redacted], 2018)

Matrix	Analyte	LOQ (mg a.s/kg)	Recovery fortification level (mg/kg)	Recoveries % range (mean)	Repeatability % RSD (n)	Linearity
Feeding solution	pydiflumetofen	0.005	0.005	84 – 87 (86)	1.6 (3)	0.07 – 24.5 ug/L (n = 8) Quadratic: $y = -13.51x^2 + 1832.66x + 90.85$ $R^2 = 0.$ 0.999
			0.034	79 -82 (81)	n=2	
			39.79	80 – 94 (88)	2.7 (3)	

Table B.5.1.2.6-49: Summary of procedural recovery data for determination of pydiflumetofen in flowers, leaves, nectar, and pollen matrices ([redacted], 2018)

Matrix	Analyte	LOQ (mg a.s /kg)	Recovery fortification level (mg a.s/kg)	Recoveries % range (mean)	Repeatability % RSD (n)	Linearity
Nectar	pydiflumetofen Primary Transition *m/z* 426 → 193	0.005	0.005	93 – 93 (93)	- (2)	0.07 – 4 µg a.s/L (n = 8)
			0.1	87 – 88 (88)	- (2)	
			Overall	87 – 93 (90)	4 (4)	y = 1961x+81 R^2 = 0.9974
Pollen	pydiflumetofen Primary Transition *m/z* 426 → 193	0.005	0.005	96 – 96 (96)	- (2)	0.07 – 4 µg a.s/L (n = 8)
			0.1	103 – 106 (105)	- (2)	
			Overall	96 – 106 (100)	5 (4)	y = 1582x+16 R^2 = 0.9996
Flowers	pydiflumetofen Primary Transition *m/z* 426 → 193	0.005	0.005	92 – 100 (96)	- (2)	0.07 – 4 µg a.s/L (n = 8)
			0.1	93 – 95 (94)	- (2)	
			Overall	92 – 100 (95)	4 (4)	y = 1858x+35 R^2 = 0.9992
Foliage	pydiflumetofen Primary Transition *m/z* 426 → 193	0.005	0.005	77 – 78 (78)	- (2)	0.07 – 4 µg a.s/L (n = 8) y = 2042x+33 R^2 = 0.9989

Table B.5.1.2.6-50: Summary of method validation data for the determination of pydiflumetofen in flowers, leaves, nectar, and pollen matrices ([redacted], 2017)

Matrix	Analyte	LOQ (mg a.s /kg)	Recovery fortification level (mg a.s/kg)	Recoveries % range (mean)	Repeatability % RSD (n)	Linearity
Nectar	pydiflumetofen Primary Transition *m/z* 426 → 193	0.005	0.005	76 – 101 (87)	10.6 (5)	0.07 – 4 µg a.s/L (n = 8)
			0.1	82 – 88 (85)	3.1 (5)	
			Overall	76 – 101 (86)	7.7 (10)	y = 716x+65 R^2 = 0.9932
	pydiflumetofen Confirmatory Transition *m/z* 426 → 166	0.005	0.005	75 – 88 (82)	6.6 (5)	0.07 – 4 µg a.s/L (n = 8)
			0.1	79 – 85 (83)	3.1 (5)	
			Overall	75 – 88 (83)	5.0 (10)	y = 361x+32 R^2 = 0.9932
Pollen	pydiflumetofen Primary Transition *m/z* 426 → 193	0.005	0.005	76 – 104 (88)	15.5 (5)	0.07 – 4 µg a.s/L (n = 8)
			0.1	80 – 104 (88)	9.9 (5)	
			Overall	76 – 104 (91)	12.3 (10)	y = 615x+31 R^2 = 0.9991
	pydiflumetofen Confirmatory Transition *m/z* 426 → 166	0.005	0.005	71 – 106 (88)	16.9 (5)	0.07 – 4 µg a.s/L (n = 8)
			0.1	80 – 101 (91)	9.2 (5)	
			Overall	71 – 106 (90)	12.9 (10)	y = 311x+34 R^2 = 0.9998
Flowers	pydiflumetofen Primary Transition *m/z* 426 → 193	0.005	0.005	69 – 111 (93)	16.9 (5)	0.07 – 4 µg a.s/L (n = 8)
			0.1	87 – 104 (99)	6.8 (5)	
			Overall	69 – 111 (96)	12.2 (10)	y = 979x+112 R^2 = 0.9993
	pydiflumetofen Confirmatory Transition *m/z* 426 → 166	0.005	0.005	59 – 91 (73)	17 (5)	0.07 – 4 µg a.s/L (n = 8)
			0.1	89 – 105 (99)	5.8 (5)	
			Overall	59 – 105 (86)	19.0 (10)	y = 500x+65 R^2 = 0.9989
Foliage	pydiflumetofen Primary Transition *m/z* 426 → 193	0.005	0.005	79 – 88 (82)	4.8 (5)	0.07 – 4 µg a.s/L (n = 8)
			0.1	82 – 91 (85)	4.1 (5)	
			Overall	79 – 91 (84)	4.4 (10)	y = 1071x+71 R^2 = 0.9992
	pydiflumetofen Confirmatory Transition *m/z* 426 → 166	0.005	0.005	82 – 93 (87)	5.6 (5)	0.07 – 4 µg a.s/L (n = 8)
			0.1	81 – 91 (85)	4.1 (5)	
			Overall	81 – 93 (86)	4.8 (10)	y = 545x+43 R^2 = 0.9987
			0.1	92 – 94 (93)	- (2)	
			Overall	77 – 94 (85)	11 (4)	

Specificity

Chromatograms were presented for the lowest and highest calibration standard (0.07 and 4 µg a.s/L), blank, control, fortified samples (0.005 and 0.1 mg/kg) and treated samples for each matrix. Specificity was demonstrated by a retention time match between the test samples and standards. No significant interference was observed at the retention time of interest.

Linearity

Linearity was demonstrated by the analysis of eight standards of increasing concentration for both mass transitions. The range of standard concentrations used was ~0.07-24 µg a.s/L. A quadratic calibration line was reported in both reports, the coefficient of determination (R^2) > 0.997. It is noted samples are diluted to within the linear range.

Accuracy

Samples of nectar, pollen, flowers, and foliage were fortified with pydiflumetofen at 0.005 (LOQ) and 0.1 mg a.s/kg (20 x LOQ) with five samples prepared at each level. Some individual recoveries are outside of the linear range; however, the mean recoveries were within the acceptable range (70-110%). The fortification levels are low compared to some of the levels of residues of pydiflumetofen detected in the study. However, this is acceptable as samples are diluted appropriately. Acceptable procedural recoveries have also been reported.

Precision

Precision was determined from the accuracy recovery data for pydiflumetofen. The overall %RSD was ≤20% for each matrix with five determinations at each fortification level.

Matrix effects

Matrix matched standards we not specifically addressed. However, matrix matched standards were used for quantification.

LOQ

The LOQ of the method is 0.005 mg a.s/kg for feeding solutions nectar, pollen, flowers, and foliage. This is the lowest fortification level with acceptable accuracy and precision.

Conclusion

The method is acceptably validated and is suitable for the determination of pydiflumetofen in feeding solutions, nectar, pollen, flower, and foliage samples with an LOQ of 0.005 mg/kg.

Report:	**KCA1 8.6.2-01 [redacted] et al. (2015)**
Title	SYN545974 SC (A19649B) - Toxicity Effects on the Seedling Emergence of Ten Species of Plants Report number: 528P-124
Guidelines:	SANCO/3029/99 rev. 4
GLP:	Yes
Deviations	N/A
Previous evaluation:	None

Report:	**KCA1 8.6.2-02 [redacted], [redacted], [redacted], [redacted] (2015a)**
Title	SYN545974 SC (A19649B) - Toxicity Effects on the Seedling Emergence of Ten Species of Plants Report Number: 528P-115
Guidelines:	SANCO/3029/99 rev. 4
GLP:	Yes
Deviations	N/A
Previous evaluation:	None

Report:	**KCA1 8.6.2-03 [redacted] et al (2015b)**
Title	SYN545974 SC (A19649B) - Toxicity Effects on the Vegetative Vigour of Ten Species of Plants Report Number: 528P-116
Guidelines:	SANCO/3029/99 rev. 4
GLP:	Yes
Deviations	N/A
Previous evaluation:	None

The following studies rely on the same HPLC/UV method to determine the concentration of pydiflumetofen in spray mixtures:

- KCA1 8.6.2-03
- KCA1 8.6.2-02
- KCA1 8.6.2-01

Sample preparation:

Samples were diluted as necessary with 50:50 acetonitrile: HPLC grade water before analysis by HPLC-UV under the conditions described below. Reverse osmosis (RO) water was used as a matrix blank.

HPLC conditions:

Instrument:	Agilent Series 1100/1200 high performance liquid chromatograph (HPLC) equipped with an Agilent Series1100 variable wavelength detector (VWD)
Analytical column:	YMC-PACK ODS-AM (150 mm x 4.6 mm I.D. x 3 µm particle size)
Oven temperature:	40ºC
Injection volume:	25 µL
Mobile phase A:	0.1% Phosphoric Acid
Mobile phase B:	Acetonitrile
Flow rate:	1.00 mL/minute

Gradient:

Time (minute)	% A	% B
0.00	90	10
1.00	90	10
10.00	5	95
12.00	5	95
12.10	90	10
15.00	90	10

Retention time:	Approximately 11.7 minutes
Detection wavelength:	230nm

A summary of the method validation data is given in Table B.5.1.2.6-51.

Table B.5.1.2.6-51: Summary of method validation data for determination of pydiflumetofen in spray mixtures

Matrix	**Analyte**	**LOQ (mg a.s/L)**	**Study reference**	**Recovery fortification level (mg a.s/L)**	**Recoveries % range (mean)**	**Repeatability % RSD (n)**	**Linearity**
Spray solution (in water)	pydiflumetofen	500	Report Number: 528P-116	900	97	n=1	1.00 – 10.0 µg/mL (n = 5) y = 62.1589x + 1.1333 R^2 = 1.0000
				1000	97- 99 (98)	8 (3)	
				1100	98	n=1	
			Report Number: 528P-115	900	108	n=1	
				1000	101 – 103 (101)	1.5 (3)	
				1100	102	n=1	
			Report Number: 528P-124	1800	100 – 104 (102)	n=2	
				2000	84 - 100 (96)	6.4 (6)	
				2200	101 – 107 (104)	n=2	

Specificity

Specificity was demonstrated by retention time match with a reference standard and the absence of interfering peaks in the chromatogram of blank matrix.

Linearity

Linearity was demonstrated by the analysis of 5 standards of increasing concentration. The range of standard concentrations used was 1.00 – 10 ug/ml. The response was linear with a R^2 value of 1.000.

Accuracy

Recovery samples were prepared at a low level and high level appropriate to the analysis in each study. Recovery values were calculated as a percentage of measured concentration relative to fortified concentration. Mean recovery levels were within the range 96 to 104 %.

Precision

The relative standard deviation (RSD) was not determined for every level due to limited sample numbers, however when it could be determined, the relative standard deviation obtained was within the guideline requirements of a %RSD ≤20%.

Matrix effects

Matrix matched standards were not specifically addressed.

LOQ

The LOQ of the method is 500 µg/mL., calculated as the product of the lowest calibration standard (1.00 µg a.s/mL) and the dilution factor used (500).

Conclusion

The method is acceptably validated in accordance with SANCO/3029/99 rev.4 and is suitable for the determination of pydiflumetofen.

B.5.1.2.7. Methods used in support of physical and chemical properties studies

Study Overview

Data point	Author(s)	Year	Title Company Report No.	Conclusion
KCA1 2.2/1	[redacted]	2017	SYN545974 Vapour Pressure Report number: SMG11739	Method SD-1643/1 is fit for regulatory purposes.
KCA1 2.5/1	[redacted]	2012	SYN545974 Solubility in Water Report number: SMG11737	Method SD-1640/1 is fit for regulatory purposes.
KCA1 2.6/1	[redacted]	2012a	SYN545974 Solubility in Organic Solvents Report number: SMG11891	Method SD-1638/1 is fit for regulatory purposes.
KCA1 2.7/6	[redacted]	2012	SYN545974 Octanol/Water Partition Coefficient Report number: SMG11738	Method SD-1645/1 is fit for regulatory purposes.
KCA1 2.7/7	[redacted]	2009	SYN545974 Octanol/Water Partition Coefficient Report number: SMG10197	Method SD-1262/1 is fit for regulatory purposes.

Report:	**KCA1 2.2/1 [redacted] (2017)**
Title	SYN545974 Vapour Pressure Report number: SMG11739
Guidelines:	SANCO/3029/99 rev. 4
GLP:	Yes
Deviations	N/A
Previous evaluation:	None

Method SD-1643/1 was used to determine the content of pydiflumetofen in the vapour pressure test.

Sample preparation

The trapped test item from the U-tubes was extracted using mobile phase and diluted further with the mobile phase to reach final concentrations compatible with the concentration of pydiflumetofen in the reference solutions (1.015-4.02 mg/L for the analysis of samples at an experimental temperature of 60°C, 0.995-4.08 mg/L for the analysis of samples at an experimental temperature of 70°C and 80°C).

HPLC-UV conditions

Chromatographic system:	HPLC-UV
Analytical column:	Ace C18 (3 µm), length 150 mm, i.d. 4.6 mm
Injection volume:	20 µL
Column temperature:	40°C
Mobile phase:	Acetonitrile: 0.2% aqueous acetic acid (65:35, v/v)
Flow rate:	1 mL/minute
Retention time:	Approximately 6.6 minutes
Detection wavelength:	220 nm
Run time:	30 minutes

Summary

A trapping efficiency test was carried out by using two condensation tubes in series and by quantifying the amount of transferred test item in each tube separately. There was insignificant amount of test item in the second U-tubes, hence the efficiency of trapping is demonstrated. A recovery test was also performed at two levels (7.36 mg/mL and 205 mg/mL) giving acceptable recoveries of 99.3 and 100.6%. Chromatograms have been presented for pydiflumetofen reference solution and control (mobile phase). A chromatogram of the test solution has not been presented. Nevertheless, there is no significant interference observed at the retention time of interest.

Limited method validation data has been provided – no linearity or precision data. However, the method is simply to determine the content of the active substance in the mobile phase. On this basis, the method can be considered fit for regulatory purposes.

Report:	**KCA1 2.5/1 [redacted] (2012)**
Title	SYN545974 Solubility in water Report number: SMG11737
Guidelines:	SANCO/3029/99 rev. 4
GLP:	Yes
Deviations	N/A
Previous evaluation:	None

Method SD-1640/1 was used in the solubility in water test to determine the content of pydiflumetofen in clear, saturated solutions.

Sample preparation

Aliquot of 1.0 mL of saturated solutions were diluted to 2.0 mL with acetonitrile to reach concentrations within those of the reference solutions (0.505-3.96 mg/L and 0.501-4.02 mg/L for the analysis of samples at an experimental flow rate of 25 mL/hr and 12.5 mL/hr respectively).

HPLC-UV conditions

HPLC-UV conditions are the same as those described above for KCA1 2.2 except for the injection volume and run time:

- An injection volume of 50 µL is used instead of 20 µL
- The run time is 10 minutes instead of 30 minutes

Summary

Chromatograms have been presented for pydiflumetofen reference solution, control (water) and test solution. Specificity was demonstrated by a retention time match between the test solution and reference standard. No significant interference was observed at the retention time of interest.

No data has been submitted in the areas of linearity, accuracy, and precision. However, the method is simply to determine the content of the active substance in water. On this basis, the method can be considered fit for regulatory purposes.

Report:	**KCA1 2.6/1 [redacted] (2012a)**
Title	SYN545974 Solubility in Organic Solvents Report number: SMG11891
Guidelines:	SANCO/3029/99 rev. 4
GLP:	Yes
Deviations	N/A
Previous evaluation:	None

Method SD-1638/1 was used in the solubility in organic solvents test to determine the content of pydiflumetofen in centrifuged saturated solutions.

Sample preparation

Aliquots from the centrifuged saturated solutions were diluted appropriately with acetonitrile and mobile phase to reach concentrations within those of the reference solutions (20.3-79.8 mg/L). For hexane, aliquots from the centrifuged saturated solutions in hexane were concentrated to dryness and diluted with mobile phase to reach concentrations within those of the reference solutions.

HPLC-UV conditions

HPLC-UV conditions are the same as those described above for KCA1 2.2 except for the injection volume and run time:

- An injection volume of 5 µL is used instead of 20 µL
- The run time is 10 minutes instead of 30 minutes

Summary

Chromatograms have been presented for pydiflumetofen reference solution and test solution (toluene). Specificity was demonstrated by a retention time match between the test solution and reference standard. However, a chromatogram of the control solution and other test solutions (acetone, dichloromethane, ethyl acetate, hexane, methanol, and octanol) have not been provided. There is a retention time match between the reference solutions and test solution (toluene). Furthermore, no significant interference is observed in the chromatograms.

No data has been submitted in the areas of linearity, accuracy, and precision. However, the method is simply to determine the content of the active substance in different organic solvents. On this basis, the method can be considered fit for regulatory purposes.

Report:	**KCA1 2.7/6 [redacted] (2012)**
Title	SYN545974 Octanol/ Water Partition Coefficient Report number: SMG11738
Guidelines:	SANCO/3029/99 rev. 4
GLP:	Yes
Deviations	N/A
Previous evaluation:	None

Method SD-1645/1 was used to determine the content of pydiflumetofen in aliquots removed from separated octanol and aqueous phases during the determination of the octanol/water partition coefficient.

Sample preparation

Analysis of the octanol phase test solution:

1.0 mL aliquots from the clear, centrifuged octanol/water test mixture from the upper phase (octanol) were diluted into 50 mL volumetric flask. 10 mL of methanol was added, and the solution was diluted up to the mark with mobile phase to reach concentration within those of the reference solutions (20.1-820 mg/L). After having taken the aliquot for analysis the rest of the octanol phase was quantitatively removed.

Analysis of the aqueous phase test solution:

4.0 mL aliquots from the remaining aqueous solutions were diluted into 5 mL volumetric flask with acetonitrile to reach concentrations within those of the reference solutions (402-1600 µg/L). When taking the aliquot from the aqueous phase air was gently expelled through the pipette during its introduction into the solution and the pipette did not touch the walls of the centrifuge tube. This prevented the accidental inclusion of any residual traces of the octanol phase in this aliquot.

HPLC-UV conditions

HPLC-UV conditions are the same as those described above for KCA1 2.2 except for the injection volume and run time:

- Injection volume: 10 µL of the reference and test solution of octanol phase and 90 µL of the reference and test solution of aqueous phase
- The run time is 10 minutes instead of 30 minutes

Summary

Chromatograms have been presented for pydiflumetofen reference solution and test solution for both the octanol and aqueous phase. A retention time match is observed between the reference solution and test solution for both phases. No significant interference is observed at the retention time of interest. However, it should be noted a chromatogram of the control has not been provided.

No data has been submitted in the areas of linearity, accuracy, and precision. However, the method is simply to determine the content of the active substance in solution. On this basis, the method can be considered fit for regulatory purposes.

Report:	**KCA1 2.7/7 [redacted] (2009)**
Title	CA4312 Octanol/water partition coefficient Report number: SMG10197
Guidelines:	SANCO/3029/99 rev. 4
GLP:	Yes
Deviations	N/A
Previous evaluation:	None

Method SD-1262/1 was used to determine the content of CA4312 in aliquots removed from separated octanol and aqueous phases during the determination of the octanol/water partition coefficient.

To investigate any dependency of the octanol/water partition coefficient upon pH, experiments were carries out in three standard aqueous buffer solutions rather than pure water: citrate buffer (pH 5.0), phosphate buffer (pH 7.0) and borax buffer (pH 9.0).

Sample preparation

Analysis of the octanol phase test solution:

An aliquot from the centrifuged upper phase (octanol/aqueous mixture) of CA4312, saturated with buffer solution was pipetted into a 10 mL volumetric flask, diluted, and made up to the mark with diluent to reach concentrations within those of the reference solutions (20.2-79.9 mg/L for buffer solution pH 5.0, 0.4-1.6 mg/L for buffer solution pH 7.0 and pH 9.0). After having taken the aliquot for analysis the rest of the octanol phase was quantitatively removed.

Analysis of the aqueous phase test solution:

2.0 mL aliquots from the remaining aqueous solutions were diluted to 50 mL with the diluent to reach concentrations within those of the reference solutions (~20-80 mg/L for buffer solution pH 5.0, 7.0 and 9.0). When taking the aliquot from the aqueous phases air was gently expelled through the pipette during its introduction into the solution and the pipette did not touch the walls of the centrifuge tube. This prevented the accidental inclusion of any residual traces of the octanol phase in this aliquot.

HPLC-UV conditions

Chromatographic system:	HPLC-UV
Analytical column:	Nucleosil C18, 10 µm, 250 x 4.6 mm
Injection volume:	20 µL of the reference and test solutions of octanol phase 10 µL of the reference and test solutions of aqueous phase
Column temperature:	40°C
Mobile phase:	Acetonitrile: 0.1% aqueous trifluoroacetic acid (20:80, v/v)
Flow rate:	1 mL/minute
Retention time:	Approximately 5.6 minutes
Detection wavelength:	230 nm
Run time:	12 minutes

Summary

Chromatograms have been presented for CA4312 reference solution in the octanol phase and test solution for the octanol and aqueous phase. No chromatograms have been presented for the control or the reference solution in the aqueous phase. Nevertheless, a retention time match is observed between the reference solution and test solutions and there is no significant interference.

No data has been submitted in the areas of linearity, accuracy, and precision. However, the method is simply to determine the content of the active substance in solution. On this basis, the method can be considered fit for regulatory purposes.

B.5.2. METHODS FOR POST-APPROVAL CONTROL AND MONITORING PURPOSES

B.5.2.1. Methods for residues in or on food and feed of plant origin

Summary Overview:

The QuEChERS method EN 15662 has been proposed as the enforcement method for food and feed of plant origin. The method of analysis has been validated for the parent active substance only (pydiflumetofen). pydiflumetofen is the only component of the residue definition for enforcement in food/feed of plant origin. The method has been validated in various commodities belonging to the four main matrix groups and in a difficult to analyse matrix.

Cross validation data was provided to address extraction efficiency for the QuEChERS method EN 15662 as a different solvent system was used in the plant metabolism studies. Extraction efficiency was addressed for high acid content, high water content and dry commodities (high starch/high protein) for QuEChERS method EN 15662. **A data gap has been identified for extraction efficiency in high oil commodities.**

An independent laboratory validation of the QuEChERS method was undertaken for high oil content, high water content, high starch content and difficult to analyse commodities.

Table B.5.2.1-1: Summary of analytical methods for monitoring residues of pydiflumetofen in or on food and feed of plant origin

Reference	Author	Study scope	Method Reference	Commodities used for validation	Commodity types represented	Limit of quantification (mg/kg)
Food/feed of plant origin						
KCA1 4.2/12	[redacted] (2015)	Primary validation study in crops	QuEChERS method EN 15662	Oranges (fruit), wheat (grain), lettuce (head), oilseed rape (seed), dried bean (seed), coffee beans (roasted)	High acid content commodities High oil content commodities High water content commodities Dry commodities (high protein/high starch content) Difficult to analyse matrix (coffee beans)	0.01
KCA1 4.2/6	[redacted] (2017)	Extraction efficiency	GRM061.03A and QuEChERS method	-	-	-
KCA 4.2/5	[redacted] (2015)	ILV for KCA1 4.2/12	QuEChERS method EN 15662	Wheat (grain), lettuce (head), oilseed rape (seed), coffee beans (roasted)	High oil content commodities High water content commodities High starch content commodities Difficult to analyse matrix (coffee beans)	0.01

Report:	**KCA1 4.2/12, [redacted] (2015)**
Title	Validation of QuEChERS Method for the Determination of Residues of SYN545974 in Crop Matrices by LC-MS/MS Report number: S14-05402 (Document No.: VV-412200)
Guidelines:	SANCO/825/00 rev.8.1
GLP:	Yes
Deviations	N/A
Previous evaluation:	None

Report:	**KCA1 4.2/6, [redacted] (2017)**
Title	Pydiflumetofen – Evaluation of the Extraction Efficiency of two Analytical Methods Used for the Determination of Pydiflumetofen Residues in Crop Matrices Report number: CEMR-8368 (Document No.: VV-468712)
Guidelines:	SANCO 825/00 rev 8.1 and SANTE 2017/10632 rev. 3
GLP:	Yes
Deviations	N/A
Previous evaluation:	None

The purpose of this study was the validation of QuEChERS method for the determination of pydiflumetofen in crop matrices (lettuce, oilseed rape, dried broad beans, wheat grain, orange fruit and coffee bean) by LC-MS/MS. The primary method was conducted at Eurofins Agroscience Services Chem SAS, 75 Chemin de Sommieres, 30310 Vergeze, France.

Reference items:

pydiflumetofen, batch AMS 1432/1, purity 99.5 %w/w, CoA provided, expiry December 2017

Sample preparation:

Specimen material (5 g) was extracted by agitation with acetonitrile in presence of ultra-pure water (1:1 v/v). After addition of a buffer salt mixture, containing magnesium sulphate, sodium chloride and sodium citrate, the extract was shaken. After centrifugation, an aliquot of the acetonitrile phase was cleaned by dispersive solid phase extraction (SPE), containing a mixture of primary secondary amine (PSA), C18EC and magnesium sulphate. After centrifugation, an aliquot (200 µL) was diluted in ultra-pure water (800 µL) and analysed for pydiflumetofen by high-performance liquid chromatography with mass-spectrometric detection (LC-MS/MS), monitoring for the primary transition (m/z 426.1→193.1) and the confirmatory transition (m/z 426.1→171.1).

LC-MS/MS conditions:

Chromatographic system:	LC20AD, shimadzu +SIL20AC, Shimadzu or HTC Pal, CTC Analytics
Analytical column:	Discovery C8: 4.6 mm x 50 mm, Particle size 5 µm
Target column temperature:	40 °C
Injection volume:	10 µL, 30 µL, 40 µL
Mobile phase A:	0.1% formic acid in ultra-pure water
Mobile phase B:	0.1% formic acid in acetonitrile
Flow rate:	0.6 mL/minute

Gradient:

Time (minute)	% A	% B
0.0	80	20
3.0	10	90
5.0	10	90
5.1	80	20
7.0	80	20

Detection system:	API 4000 (AB Sciex)
Ionisation:	ESI^+
Scan type:	MRM
Retention time:	Pydiflumetofen: approximately 4.1 minutes
Ions monitored:	*m/z* 426 → 193 quantitative *m/z* 426 → 171 confirmatory

A summary of the method validation data is given in Table B.5.2.1-2.

Table B.5.2.1-2: Summary of method validation data for determination of pydiflumetofen residues in plant matrices

Matrix	Analyte	LOQ (mg/kg)	Recovery fortification level (mg/kg)	Recoveries % range (mean)	Repeatability % RSD (n)	Linearity
High water group: Lettuce (head)	Pydiflumetofen Primary Transition *m/z* 426 → 193	0.01	0.01	81 - 101 (94)	9 (5)	0.05 - 10 ng/mL (0.0025 – 0.5 mg/kg) (n = 8) y = 2344x-6 R^2 = 0.9996
			5.0	99 - 111 (103)	5 (5)	
			Overall	81 - 111 (98)	8 (10)	
	Pydiflumetofen Confirmatory Transition *m/z* 426 → 171	0.01	0.01	88 - 112 (101)	10 (5)	As above y = 1194x-12 R^2 = 0.9988
			5.0	101 - 110 (105)	3 (5)	
			Overall	88-112 (103)	7 (10)	
High oil group: Oilseed rape (seed)	Pydiflumetofen Primary Transition *m/z* 426 → 193	0.01	0.01	76 - 84 (80)	4 (5)	0.05 - 10 ng/mL (0.0025 – 0.5 mg/kg) (n = 8) y = 45409x+687 R^2 = 0.9932
			0.2	71 - 86 (81)	8 (5)	
			Overall	71 - 86 (81)	6 (10)	
	Pydiflumetofen Confirmatory Transition *m/z* 426 → 171	0.01	0.01	74 – 82 (78)	5 (5)	As above y = 28266x+129 R^2 = 0.9930
			0.2	71 - 88 (82)	8 (5)	
			Overall	71 – 88 (80)	7 (10)	
High starch group: Wheat (grain)	Pydiflumetofen Primary Transition *m/z* 426 → 193	0.01	0.01	86 - 99 (94)	6 (5)	0.05 - 10 ng/mL (0.0025 – 0.5 mg/kg) (n = 8) y = 2355x-9 R^2 = 0.9990
			1.0	70 – 92 (85)	10 (5)	
			Overall	70 – 99 (89)	10 (10)	
	Pydiflumetofen Confirmatory Transition *m/z* 426 → 171	0.01	0.01	92 - 107 (98)	6 (5)	As above y = 1235x-25 R^2 = 0.9991
			1.0	69 - 95 (86)	12 (5)	
			Overall	69 – 107 (92)	11 (10)	
High protein group: Dried broad beans (seed)	Pydiflumetofen Primary Transition *m/z* 426 → 193	0.01	0.01	83 - 90 (87)	3 (5)	0.05 - 10 ng/mL (0.0025 – 0.5 mg/kg) (n = 8) y = 2237x+6 R^2 = 0.9995
			0.2	80 - 87 (83)	3 (5)	
			Overall	80 – 90 (85)	4 (10)	
	Pydiflumetofen Confirmatory Transition *m/z* 426 → 171	0.01	0.01	65 – 90 (78)	13 (5)	As above y = 3794x+21 R^2 = 0.9994
			0.2	77 - 84 (81)	3 (5)	
			Overall	65 – 90 (80)	9 (10)	

Matrix	Analyte	LOQ (mg/kg)	Recovery fortification level (mg/kg)	Recoveries % range (mean)	Repeatability % RSD (n)	Linearity
High acid group: Oranges (fruit)	Pydiflumetofen Primary Transition *m/z* 426 → 193	0.01	0.01	70 – 101 (87)	13 (5)	0.05 - 10 ng/mL (0.0025 – 0.5 mg/kg) (n = 8) y = 10410x-23 R^2 = 0.9969
			1.0	67 – 81 (74)	7 (5)	
			Overall	67 - 101 (81)	13 (10)	
	Pydiflumetofen Confirmatory Transition *m/z* 426 → 171	0.01	0.01	71 - 101 (85)	15 (5)	As above n = 7* y = 5311x-54 R^2 = 0.9971
			1.0	70 – 80 (74)	6 (5)	
			Overall	70 – 101 (79)	14 (10)	
Difficult to analyse: Coffee bean	Pydiflumetofen Primary Transition *m/z* 426 → 193	0.01	0.01	81 - 117 (95)	14 (5)	0.05 - 10 ng/mL (0.0025 – 0.5 mg/kg) (n = 8) y = 2169x+29 R^2 = 0.9982
			0.2	61 - 78 (70)	10 (5)	
			Overall	61-117 (83)	20 (10)	
	Pydiflumetofen Confirmatory Transition *m/z* 426 → 171	0.01	0.01	58 - 97 (77)	19 (5)	As above y = 6397x-90 R^2 = 0.9988
			0.2	102 - 108 (105)	2 (5)	
			Overall	58 - 108 (91)	(10)	

* For the confirmatory transition for oranges the calibration standard at 0.5 ng/mL was excluded from the linearity as it deviated more than 30%.

Specificity and Confirmation of Analyte Identity

LC-MS/MS with primary and confirmatory ion transitions is considered to be a highly specific technique. Therefore, additional methods to confirm the identity of the analytes are not considered necessary. Chromatograms have been provided showing a retention time match with standards. No significant interference (>30% of the LOQ) was observed at the retention time of interest. It is noted significant matrix effects are observed for lettuce and coffee beans, but matrix matched standards were used so this is acceptable. The ion transitions monitored are appropriate.

Linearity

Linearity was demonstrated by the analysis of eight matrix matched standards of increasing concentration. The range of standard concentrations used was 0.05-10 ng/mL, equivalent to 0.0025-0.5 mg/kg. The lowest concentration injected was at 25% of the LOQ of the method and the highest concentration injected was equivalent to 50x LOQ. The response was linear for both MS/MS transitions with a coefficient of determination (R^2) ranging from 0.9930-0.9996.

It is noted for oranges, lettuce and wheat matrices the highest fortification levels are outside of the linear range (1.0, 5.0 and 1.0 mg/kg respectively). However, samples were diluted to within the linear and therefore this is acceptable (10-fold, 50-fold and 10-fold dilutions respectively).

Accuracy and Precision

Fortified samples were analysed in quintuplet at the limit of quantification (LOQ 0.01 mg/kg) and at a higher level (0.2 mg/kg for oilseed rape, dried broad bean, and coffee bean (20 x LOQ), 1 mg/kg for wheat grain and oranges (100 x LOQ) and 5 mg/kg for lettuce (500 x LOQ)). Some individual recoveries were outside of the acceptable range; however, mean recoveries for all levels were within the acceptable range (60 – 120 at 0.01 mg/kg, 70 – 110 at 0.2 mg/kg and 1.0 mg/kg and 5 mg/kg). Therefore, the accuracy of the method is acceptable. The %RSD for all matrices at each level was acceptable (≤30% at 0.01 mg/kg, ≤15% at 0.2 mg/kg and 1.0 mg/kg, ≤10% at 5 mg/kg).

Matrix effects

No significant matrix effects (>20%) were observed in oilseed rape, dried broad beans, wheat grain and oranges matrices tested during method validation. Significant matrix effects (suppression) were observed in coffee bean and lettuce matrices tested during method validation. Matrix matched linearity standards were used for quantification for all matrices.

LOQ

The LOQ is defined as the lowest validated level. This corresponds to 0.01 mg/kg for pydiflumetofen in all matrices.

Storage Stability of Extracts

Residues of pydiflumetofen were demonstrated to be stable in final extracts from lettuce, oilseed rape, dried broad beans, wheat grain, coffee bean and oranges when stored at 4°C for at least 8 or 9 days. Samples were fortified at LOQ level (0.01 mg/kg) and the day 0 and day 8-9 recoveries were compared. At each interval and for each matrix, the mean recoveries were between 70-110% with a %RSD ≤20%. There was no significant difference between the day 0 and day 8/9 results.

Stability of Standard Solutions

The stability of stored working standard solutions of pydiflumetofen was not performed during this study however they have been proved to be stable for 4 months when stored at a target temperature of 4°C (see Method GRM061.03A).

Extraction Efficiency

Three plant metabolism studies were submitted that describe the metabolism of pydiflumetofen in wheat (cereals), oilseed rape (pulses and oilseeds) and tomatoes (fruits and fruiting vegetables). As part of these studies, residues were generally extracted using acetonitrile: water (80:20 v/v). In all cases, >70% of the TRR was extracted using this solvent mixture. A detailed summary of the extractability of residues in the metabolism studies is described in table B.5.2.1-3.

Table B.5.2.1-3: Extractability and distribution of radioactive residues in plant matrices with acetonitrile/ water (80/20, v/v) according to the plant metabolism studies

Radiolabel	Crop commodity	Extractable radioactivity		Non-extractable radioactivity		TRR*
		% TRR	mg / kg	% TRR	mg / kg	mg / kg
[Phenyl-U-^{14}C]-pydiflumetofen	Wheat forage	96.5	0.327	3.5	0.012	0.338
	Wheat hay	94.2	0.920	5.8	0.057	0.977
	Wheat straw	95.8	1.232	4.6	0.059	1.286
	Wheat grain	90.4	0.033	9.6	0.004	0.037
	Tomato fruit (1DAA2)**	100.0	0.0520	0.1	0.001	0.521
	Tomato fruit (14DAA2)**	99.7	0.640	0.3	0.002	0.642
	Oilseed rape (seed)	74.5	0.015	25.5	0.005	0.020
[Pyrazole-5-^{14}C]-pydiflumetofen	Wheat forage	95.6	0.445	4.4	0.020	0.465
	Wheat hay	94.2	1.311	5.7	0.079	1.391
	Wheat straw	94.5	1.443	6.1	0.093	1.527
	Wheat grain	84.9	0.048	15.2	0.009	0.057
	Tomato fruit (1DAA2)**	98.4	0.473	1.6	0.008	0.481
	Tomato fruit (14DAA2)**	100.0	0.632	0.1	0.001	0.633
	Oilseed rape (seed)	71.8	0.014	28.2	0.005	0.019

* mg/kg calculated directly from summation of the radioactivity present in the extracted radioactivity in the debris and specific activity

** 1DAA2 = 1 days after second application, 14DAA2 = 14 days after second application

The plant metabolism studies demonstrated that >70% of the TRR was extracted using acetonitrile: water (80:20 v/v). However, the proposed QuEChERS method for enforcement in food/feed of plant origin uses a different solvent system for extraction (acetonitrile: water 50/50 v/v). Therefore, additional data have been provided to address the extraction efficiency using this different solvent system (KCA 4.2/6; [redacted], 2017). The residues data generation method GRM061.03A uses the same extraction solvents as those used in the metabolism studies (acetonitrile: water 80:20 v/v). Therefore, the GRM061.03A method (using 80:20 v/v) and the QuEChERS method (using 50:50 v/v) have been compared using triplicate extractions of field incurred residue samples from completed field studies. The samples were taken from field studies using strawberries, barley grain, barley straw and carrots. For the purpose of analytical methods, these crops correspond to high acid, dry and high water/high starch content commodities, respectively.

The results of the extraction using the GRM061.03A method (using 80:20 v/v) and the QuEChERS method (using 50:50 v/v) are compared in Table B.5.2.1-4.

Table B.5.2.1-4: Cross validation residues data comparing acetonitrile/water (80/20, v/v) and acetonitrile/water (50/50, v/v)

Extraction Solvent		**Acetonitrile/Water (80/20, v/v)**	**Acetonitrile/Water (50/50, v/v)**	**% Difference in incurred residue**
Method		**GRM061.03A**	**QuEChERS**	
Matrix		**pydiflumetofen (mg/kg)**	**pydiflumetofen (mg/kg)**	
Strawberry	Replicate 1	0.36	0.43	+19.4
	Replicate 2	0.41	0.47	+14.6
	Replicate 3	0.39	0.38	-2.6
	Mean	**0.39**	**0.43**	+10.3
Barley Grain	Replicate 1	1.55	1.78	+14.8
	Replicate 2	1.57	1.60	+1.9
	Replicate 3	1.66	1.59	-4.2
	Mean	**1.59**	**1.66**	+4.4
Barley Straw*	Replicate 1	2.01	2.01	0
	Replicate 2	2.43	2.17	-10.6
	Replicate 3	2.34	2.11	-9.8
	Mean	**2.26**	**2.10**	-7.1
Carrot	Replicate 1	0.11	0.11	0
	Replicate 2	0.10	0.11	+10
	Replicate 3	0.11	0.11	0
	Mean	**0.11**	**0.11**	0

*For the QuEChERS method for straw it was necessary to increase the water content beyond 50/50 acetonitrile/water (v/v) to ensure acceptable procedural recoveries were obtained. A solvent composition of 29/71 acetonitrile/water (v/v) gave acceptable procedural recoveries and extraction efficiency was comparable to 80/20 acetonitrile/water (v/v).

The cross-validation study demonstrates residues extracted with acetonitrile/water (80/20, v/v) and acetonitrile/water (50/50, v/v) differ by less than 30% for strawberries (high acid commodity), barley grain and straw (dry commodities: high protein/ high starch) and carrots (high water/high starch). Hence, the results with acetonitrile/water (80/20, v/v) and acetonitrile/water (50/50, v/v) are comparable. In line with SANTE/2017/10632, bridging between high water content and acidic matrices is acceptable for slightly acidic matrices e.g., apples, tomato, grapes. Extraction efficiency has been demonstrated in the metabolism studies for tomato and therefore the data for tomatoes (high water commodity) can be bridged to high acid commodities. Residues data for a high acid commodity is presented in the cross-validation study. Hence, extraction efficiency is sufficiently proven for high acid commodities, high water commodities and dry commodities (high protein/high starch).

However, no residues data for high oil has been presented in the cross-validation study. In line with SANTE/2017/10632, solvent mixtures are considered as being identical if their composition varies by not more than 20%. Hence, based on the information from the metabolism studies the extraction efficiency is addressed for solvent extractions with ratios of acetonitrile: water from 100:0 to 80:20. As the QuEChERS method utilised acetonitrile/water (50/50, v/v) the efficiency is not addressed for high oil commodities. It is noted however that the method used for data generation purposes method GRM061.03A used 100% acetonitrile for the extraction of

high oil content commodities, therefore the efficiency of GRM061.03A is addressed. **A data gap has been identified for extraction efficiency for high oil commodities extracted using acetonitrile/water (50/50, v/v).**

Conclusion

The method is acceptably validated in accordance with SANCO/825/00 rev. 8.1. for the determination of pydiflumetofen residues via LC-MS/MS in high water (lettuce), high acid (orange), high oil (oilseed rape), high starch (wheat grain), high protein (dried broad beans) and difficult to analyse (coffee beans) commodities, with an LOQ of 0.01 mg/kg. **A data gap has been identified for extraction efficiency for high oil commodities extracted using acetonitrile/water (50/50, v/v).**

Report:	**KCA 4.2/5 [redacted], [redacted] (2015)**
Title	SYN545974: Independent Laboratory Validation of the QuEChERS method for the Determination of Residues of SYN545974 in Crop Matrices by LC-MS/MS Report number: S14-05729 (Document No.: VV-412466)
Guidelines:	SANCO/825/00 rev.8.1
GLP:	Yes
Deviations	N/A
Previous evaluation:	None

The objective of this study was to perform an independent laboratory validation (ILV) of the analytical multi-residue method QuEChERS as described in study S14-05402 for the determination of pydiflumetofen in plant matrices. The ILV was carried out by Eurofins Agroscience Services Chem Ltd, Derbyshire, UK, a different test facility to the primary method.

Quantification was performed using LC-MS/MS with two ion transitions (quantitative transition *m/z* 426 → 193 and confirmatory transition *m/z* 426 → 171 for pydiflumetofen). A summary of the method validation data is given in Table B.5.2.1-5.

Deviations from the primary method validation study:

- The primary method uses a Discovery C8, 4.6 x 50 mm, 5.0 µm column. The column used in the ILV method is Zorbax SB-C8 4.6 x 7.5 mm, 3.5 µm. The same stationary phase is used in the ILV, but it has different dimensions slightly affecting the retention time only. The retention time for pydiflumetofen in the ILV method is approximately 4.8 minutes compared to 4.1 minutes for the primary method.
- In the sample preparation for the primary method 5 g of matrix is used. However, for the ILV for oilseed rape a sample weight of 2 g was used. The volumes of solvent were kept the same as the primary method. The modification was necessary to avoid a high matrix influence during the extraction.
- In the ILV, oilseed rape sample extracts were placed in the freezer for minimum 30 minutes to settle. This modification was required because the centrifuge was not equipped with a temperature-controlled function.

Table B.5.2.1-5: Summary of independent laboratory validation data for determination of pydiflumetofen residues in plant matrices

Matrix	Analyte	LOQ (mg/kg)	Recovery fortification level (mg/kg)	Recoveries % range (mean)	Repeatability % RSD (n)	Linearity
High water group: Lettuce (head)	Pydiflumetofen Primary Transition *m/z* 426 → 193	0.01	0.01	68 - 75 (73)	4.2 (5)	0.05 - 10 ng/mL (0.0025 – 0.5 mg/kg) (n = 8) y = 5.64e+5x +4.2e+3 r = 0.9979
			5.0	76 - 79 (77)	1.8 (5)	
			Overall	68-79 (75)	4.1 (10)	
	Pydiflumetofen Confirmatory Transition *m/z* 426 → 171	0.01	0.01	67 - 76 (73)	5.1 (5)	As above y = 2.18e+5x +998 r = 0.9989
			5.0	76 - 80 (77)	2.0 (5)	
			Overall	67 - 80 (75)	4.8 (10)	
High oil group: Oilseed rape (seed) 1st ILV set*	Pydiflumetofen Primary Transition *m/z* 426 → 193	0.01	0.01	0 - 22 (16)	57.4 (5)	0.05 - 5 ng/mL (0.0025 – 0.25 mg/kg) (n = 7) y = 1.21e+6x +2.69e+4 r = 0.9992
			0.2	59 - 64 (61)	3.2 (5)	
			Overall	0 - 64 (38)	64.4 (10)	
	Pydiflumetofen Confirmatory Transition *m/z* 426 → 171	0.01	0.01	0 – 44 (17)	100 (5)	As above y = 4.76+5x +8.81e+3 r = 0.9996
			0.2	55 - 64 (59)	5.9 (5)	
			Overall	0 – 64 (38)	65.7 (10)	
High oil group: Oilseed rape (seed) 2nd ILV set*	Pydiflumetofen Primary Transition *m/z* 426 → 193	0.01	0.01	63 - 74 (70)	5.8 (5)	0.05 - 5 ng/mL (0.0025 – 0.25 mg/kg) (n = 7) y = 1.21e+6x +2.69e+4 r = 0.9992
			0.2	86 – 92 (90)	2.8 (5)	
			Overall	63 – 92 (80)	14.3 (10)	
	Pydiflumetofen Confirmatory Transition *m/z* 426 → 171	0.01	0.01	66 - 82 (75)	8.2 (5)	As above y = 4.76+5x +8.81e+3 r = 0.9996
			0.2	86 - 92 (90)	2.5 (5)	
			Overall	66 – 92 (83)	10.6 (10)	
High starch group: Wheat (grain)	Pydiflumetofen Primary Transition *m/z* 426 → 193	0.01	0.01	77 - 83 (81)	3.1 (5)	0.05 - 10 ng/mL (0.0025 – 0.5 mg/kg) (n = 8) y = 5.72e+5x +1.61e+4 r = 0.9994
			1.0	82 – 88** (86)	2.9 (4)	
			Overall	77 – 88 (83)	4.2 (9)	
	Pydiflumetofen Confirmatory Transition *m/z* 426 → 171	0.01	0.01	81 – 84 (82)	13 (5)	As above y = 2.2e+8x +7.38e+3 r = 0.9992
			1.0	83 – 87** (85)	2.1 (4)	
			Overall	81 – 87 (83)	2.5 (9)	

Matrix	Analyte	LOQ (mg/kg)	Recovery fortification level (mg/kg)	Recoveries % range (mean)	Repeatability % RSD (n)	Linearity
Difficult to analyse: Coffee bean	Pydiflumetofen Primary Transition *m/z* 426 → 193	0.01	0.01	78 - 87 (81)	4.6 (5)	0.05 - 10 ng/mL (0.0025 – 0.5 mg/kg) (n = 8) y = 9.98e+5x +1.17e+4 r = 0.9981
			0.2	84 - 89 (86)	2.2 (5)	
			Overall	78 - 89 (84)	4.6 (10)	
	Pydiflumetofen Confirmatory Transition *m/z* 426 → 171	0.01	0.01	78 - 86 (83)	4.7 (5)	As above y = 1.63e+5x +2.11e+3 r = 0.9997
			0.2	84 - 87 (86)	1.5 (5)	
			Overall	78 - 87 (84)	3.8 (10)	

*A second ILV set was determined with a minor modification to the method. The original method used 5.0 g of matrix, whereas a sample weight of 2 g was used for the 2nd ILV set. The volumes of solvent were kept the same as the primary method. This modification was necessary to avoid a high matrix influence during the extraction that led to unacceptable recoveries during the 1st ILV set for oilseed rape.

**For wheat grain for both the quantitative and confirmatory transition a percentage recovery of 58% was obtained at 1.0 mg/kg. This recovery has been confirmed as an outlier via the Dixons Q test with a confidence level of 95%. Therefore, it has not been included in the data set.

Specificity and Confirmation of Analyte Identity

LC-MS/MS with primary and confirmatory ion transitions is considered a highly specific technique. Therefore, additional methods to confirm the identity of the analytes are not considered necessary. Chromatograms have been provided showing a retention time match with standards. It is noted chromatograms have not been presented for the lowest calibrated level (0.05 ng/mL) for lettuce, wheat grain, oilseed rape and coffee bean but are available at the LOQ level of 0.01 mg/kg, which is acceptable. Furthermore, for oilseed rape and coffee beans chromatograms have been presented for samples fortified at 10 ng/mL (equivalent to 0.5 mg/kg) and not at the lowest fortification level. Nevertheless, no significant interference (>30% of the LOQ) between the peak pydiflumetofen and any of the plant commodity matrices was demonstrated. The ion transitions monitored are appropriate and the specificity of the method is considered acceptable.

Linearity

Linearity was demonstrated by the analysis of eight matrix matched standards, except for oilseed rape where seven matrix matched standards were used. The range of standard concentrations used was 0.05-10 ng/mL (equivalent to 0.0025-0.5 mg/kg) for all matrices except oilseed rape at 0.05-5 ng/mL (0.0025-0.25 mg/kg). According to the study report, the highest concentration calibration solution for the oilseed rape matrix was dropped to produce a better (visually linear) linearity. The lowest concentration injected was at 25% of the LOQ of the method and the highest concentration injected was equivalent to 50x LOQ, which is at least 20% above the highest analyte concentration level in a sample. The response was linear for both MS/MS transitions with a correlation coefficient ranging from 0.9979-0.9997.

It is noted for lettuce and wheat matrices the highest fortification levels are outside of the linear range (5 mg/kg and 1 mg/kg respectively). However, samples are diluted appropriately before analysis.

Accuracy and Precision

Fortified samples were analysed in quintuplet at the limit of quantification (LOQ: 0.01 mg/kg) and at a higher level (0.2 mg/kg for oilseed rape and coffee bean, 1 mg/kg for wheat grain and 5 mg/kg for lettuce). Mean recoveries were within the acceptable range (70-110%). The %RSD for all matrices at each level was acceptable (≤30% at 0.01 mg/kg, ≤15% at 0.2 mg/kg and 1.0 mg/kg, ≤10% at 5 mg/kg).

It should be noted that two sets of accuracy/precision data were provided for oilseed rape. The first set resulted in unacceptable recoveries for oilseed rape. According to the study report, the laboratory received advice from the sponsor to keep samples cool throughout the procedure to avoid any fat re-dissolving in to extract. The sample weight was also reduced from 5.0 g to 2.0 g. The volumes of solvents were kept to the original method and not

changed. This modification was necessary to avoid a high matrix influence during the extraction had previously resulted in the unacceptable results. Acceptable results were obtained when these changes were implemented.

Matrix effects

Significant matrix effects (suppression) were found in the oilseed rape matrix and significant matrix effects (enhancement) were found in lettuce and wheat grain matrices tested during the independent laboratory method validation. No significant matrix effects (>20%) were observed in the coffee bean matrix during the independent laboratory method validation. Matrix matched standards were used for all matrices in this study.

LOQ

The LOQ is defined as the lowest validated level. This corresponds to 0.01 mg/kg for pydiflumetofen in lettuce, wheat grain, oilseed rape and coffee bean.

Storage Stability of Extracts

Residues of pydiflumetofen were demonstrated to be stable in final extracts from lettuce, oilseed rape, wheat grain and coffee bean when stored at 4°C for at least 7 days. Samples were fortified at LOQ level (0.01 mg/kg) and the day 0 and day 7 recoveries were compared. At each interval and for each matrix, the mean recoveries were between 70-110% with a %RSD ≤20%, except for oilseed rape after 7 days (mean recovery: 68%). Overall, there was no significant difference between the day 0 and day 7 results. In the case of residues in oilseed rape extracts, the mean recovery is only slightly outside of the acceptable range, and the mean is close to the day 0 value (70%).

Stability of Standard Solutions

Stability of stored working standard solutions of pydiflumetofen was demonstrated to be stable after 25 days in a refrigerator at 4°C. The difference between the stored and freshly prepared standards was less than 10%. This covers the length of time standards were used for specimen analysis during this study.

Conclusion

Following independent laboratory validation, the method is acceptably validated in accordance with SANCO/825/00 rev. 8.1. for the determination of pydiflumetofen residues via LC-MS/MS in high water (lettuce), high oil (oilseed rape), high starch (wheat grain), and difficult to analyse (coffee beans) commodities, with an LOQ of 0.01 mg/kg. The stated deviations from the primary method are not considered to have a significant impact on the method performance or the study. An ILV for high acid and high protein commodities has not been conducted. However, this is acceptable as the primary method is identical for all matrix groups, therefore an ILV for every commodity group is not required.

B.5.2.2. Methods for residues in or on food and feed of animal origin

Summary Overview:

Two methods have been proposed as the enforcement method for residues in food and feed of animal origin. The QuEChERS method EN 15662 has been validated for the parent active substance only (pydiflumetofen). An additional method GRM061.07A has been validated for the metabolite 2,4,6- trichlorophenol (free and conjugated), this method was also used for data generation purposes (see B.5.1.2.5).

An independent laboratory validation of both methods was undertaken. Extraction efficiency was addressed for pydiflumetofen and the metabolite 2,4,6- trichlorophenol, including the hydrolysis step required to release conjugated 2,4,6- trichlorophenol.

Table B.5.2.2-1: Summary of analytical methods for monitoring residues of pydiflumetofen in or on food and feed of animal origin

Analyte	Limit of quantification (mg/kg)	Matrix	Method	Method Reference
pydiflumetofen	0.01	Whole milk Egg Liver Fat Blood Muscle	LC-MS/MS	QuEChERS
2,4,6-Trichlorophenol (Free and conjugated)	0.01	Whole milk Egg Liver Fat Blood Muscle Kidney	LC-MS/MS	GRM061.07A

Report:	**KCA1 4.2/13, [redacted] (2015)**
Title	SYN545974: Validation of the QuEChERS Method for the Determination of Residues of SYN545974 in Animal Matrices by LCMS/MS Report Number: P 3592 G
Guidelines:	SANCO/3029/99 rev. 4 (2000) and SANCO/825/00 rev. 8.1 (2010).
GLP:	Yes
Deviations	N/A
Previous evaluation:	None

The purpose of this study was the validation of QuEChERS method for the determination of pydiflumetofen in animal matrices (whole milk, egg, bovine liver, fat, blood, muscle) by LC-MS/MS.

Reference items:

pydiflumetofen, batch AMS 1432/1, purity 99.5 %w/w, CoA provided, expiry December 2017

Sample preparation:

*Samples (*10 g for milk, 5.0 g for egg, liver, and blood, 2.5g for fat) were extracted by manual shaking with acetonitrile, after the addition of a suitable volume of water if necessary (i.e., taking into account the natural water content of the samples). After the addition of a mixture of magnesium sulphate, sodium chloride, and buffering citrate salts (available pre-mixed commercially: dispersive SPE citrate extraction tube) the extracts were shaken and then centrifuged. In the case of whole egg and animal fat, the fat was frozen out. An aliquot of each extract for all matrices was cleaned up using a pre-mixed, commercially available dispersive SPE clean up tube. After centrifugation, extracts were diluted to within the calibration range with acetonitrile/water (20/80, v/v, containing 0.1% formic acid). Final determination was by HPLC-MS/MS under the conditions described below.

LC-MS/MS conditions:

Chromatographic system: Agilent Infinity 1290 HPLC system + oven & Autosampler
Column: Phenomenex Luna C8, 3.0 µm particle size, 50 mm length, 3.0 mm i.d.
Column oven temperature: 40°C
Injection volume: 20 µL
Mobile phase A: water + 0.1 % of formic acid
Mobile phase B: acetonitrile + 0.1 % of formic acid
Flow rate: 0.4 mL/minute
Mobile phase composition:

Time (minute)	% A	% B
0.00	80	20
1.00	80	20
2.50	10	90
5.00	10	90
5.10	80	20
6.50	80	20

Note: Under these conditions the retention time is 3.6 minutes for pydiflumetofen.

MS System: AB MDS Sciex API 5500 triple quadrupole LC-MS/MS
Ionisation: ESI
Polarity: Negative
Ions monitored: *m/z* 426 → 193 quantitative
m/z 428 → 195 confirmatory

A summary of the method validation data is given in Table B.5.2.2-2

Table B.5.2.2-2: Summary of method validation data for the determination of pydiflumetofen residues in animal matrices

Matrix	**Analyte**	**LOQ (mg/kg)**	**Recovery fortification level (mg/kg)**	**Recoveries % (mean)**	**Repeatability % RSD (n)**	**Linearity**
Milk	Pydiflumetofen Primary Transition *m/z* 426 → 193	0.01	0.01	87 – 113 (100)	9 (5)	0.025 – 2.5 ng/mL (n = 5) $y = 2.08 \times 10^6 x + 12300$ r = 0.9980
			0.1	93 – 106 (100)	5 (5)	
			Overall	87 – 113 (100)	7 (10)	
	Pydiflumetofen Confirmatory Transition *m/z* 428 → 195		0.01	87 – 114 (100)	9 (5)	as above $y = 2.0 \times 10^6 x + 11800$ r = 0.9979
			0.1	93 – 107 (100)	5 (5)	
			Overall	87 – 114 (100)	7 (10)	
Liver	Pydiflumetofen Primary Transition *m/z* 426 → 193	0.01	0.01	92 – 107 (101)	6 (5)	0.025 – 2.5 ng/mL (n = 5) $y = 2.02 \times 10^6 x + 24000$ r = 0.9990
			0.1	97 – 107 (101)	4 (5)	
			Overall	92 – 107 (101)	5 (10)	
	Pydiflumetofen Confirmatory Transition *m/z* 428 → 195		0.01	91 – 107 (101)	7 (5)	as above $y = 1.93 \times 10^6 x + 23900$ r = 0.9990
			0.1	97 – 108 (101)	4 (5)	
			Overall	91 – 107 (101)	5 (10)	
Blood	Pydiflumetofen Primary	0.01	0.01	95 – 98 (97)	1 (5)	0.025 – 2.5 ng/mL (n = 5)

Matrix	Analyte	LOQ (mg/kg)	Recovery fortification level (mg/kg)	Recoveries % (mean)	Repeatability % RSD (n)	Linearity
	Transition *m/z* 426 → 193		0.1	95 – 98 (96)	1(5)	$y = 1.75 \times 10^6 x + 11400$ r = 0.9994
			Overall	95 – 98 (97)	1 (10)	
	Pydiflumetofen Confirmatory Transition *m/z* 428 → 195		0.01	95 - 100 (98)	2 (5)	as above $y = 1.67 \times 10^6 x + 11000$ r = 0.9994
			0.1	95 - 98 (97)	2 (5)	
			Overall	95 – 100 (97)	2 (10)	
Egg	Pydiflumetofen Primary Transition *m/z* 426 → 193	0.01	0.01	93 – 98 (96)	2 (5)	0.025 – 2.5 ng/mL (n = 5) $y = 1.43 \times 10^6 x + 2720$ r = 0.9995
			0.1	95 - 102 (99)	2 (5)	
			Overall	93 – 102 (97)	3 (10)	
	Pydiflumetofen Confirmatory Transition *m/z* 428 → 195		0.01	91 – 98 (95)	3 (5)	as above $y = 1.38 \times 10^6 x + 2670$ r = 0.9995
			0.1	94 - 100 (98)	2 (5)	
			Overall	91 – 100 (96)	3 (10)	
Fat	Pydiflumetofen Primary Transition *m/z* 426 → 193	0.01	0.01	87 - 90 (88)	1 (5)	0.025 – 2.5 ng/mL (n = 5) $y = 1.48 \times 10^6 x + 5080$ r = 0.9996
			0.1	90 - 96 (92)	2 (5)	
			Overall	97 – 96 (90)	3 (10)	
	Pydiflumetofen Confirmatory Transition *m/z* 428 → 195		0.01	85 - 90 (87)	2 (5)	as above $y = 1.43 \times 10^6 x + 4620$ r = 0.9995
			0.1	89 - 96 (92)	3 (5)	
			Overall	85 – 96 (90)	4 (10)	

Specificity and Confirmation of Analyte Identity

LC-MS/MS with primary and confirmatory ion transitions is considered a highly specific technique. Therefore, additional methods to confirm the identity of the analytes are not considered necessary. Chromatograms have been provided showing a retention time match with standards. No significant interference (>30% of the LOQ) between the peak pydiflumetofen and any of the animal matrices was observed. The ion transitions monitored are appropriate.

Linearity

Linearity was demonstrated by the analysis of five standards of increasing concentration. The range of standard concentrations used was 0.025-2.5 ng/mL (0.002 mg/kg to 0.2 mg/kg). The lowest concentration injected was at 12.5% of the LOQ of the method and the highest concentration injected was equivalent to 12.5x LOQ. The response was linear for both MS/MS transitions with r values ranging from 0.9979-0.9996.

Accuracy and Precision

Fortified samples were analysed in quintuplet at the limit of quantification (LOQ, 0.01 mg/kg) and at 10x LOQ (0.1 mg/kg). Mean recoveries were within the acceptable range (70-110%) and the %RSD was ≤20% at each level.

Matrix effects

No significant matrix effects (>20%) were observed in matrices tested during method validation. Matrix matched linearity standards were used for quantification.

LOQ

The LOQ is defined as the lowest validated level. This corresponds to 0.01 mg/kg for pydiflumetofen in all matrices.

Storage Stability of extracts

Stability of sample extracts fortified with pydiflumetofen at the LOQ level was demonstrated to be stable for between 8 days in a refrigerator at 4°C. The mean recoveries were between 70-110% with a %RSD ≤20%.

Extraction Efficiency

Pydiflumetofen has been shown to be efficiently extracted from animal matrices using the conditions described in the multi-residue QuEChERS method in radiolabelled metabolism studies. The extraction system is based on procedures used in livestock metabolism studies (See Volume 3 B7.2.3).

For all matrices the same extraction solvent was used to extract residues in the animal metabolism studies as is used in the QuEChERS method (either acetonitrile or acetonitrile: water). The method allows for the addition of water to adjust the moisture content of the samples if needed. In line with SANTE/2017/10632, solvent mixtures are considered as being identical if their composition varies by not more than 20%. Hence, the extraction efficiency is considered acceptable for solvent extractions with ratio of acetonitrile: water from 100:0 to 80:20.

Residue extractabilities were generally high using the solvent extraction described in the metabolism studies. On this basis, the extraction efficiency of multi-residue QuEChERS method is considered satisfactorily addressed.

Conclusion

The QuEChERS method is acceptably validated in accordance with SANCO/825/00 rev. 8.1 for the determination of pydiflumetofen residues in animal matrices.

Report:	**KCA1 4.2/16, [redacted] (2017)**
Title	SYN545974 - Independent Laboratory Validation of QuEChERS Method for the Determination of Residues of SYN545974 in Egg and Muscle by LC-MS/MS Report Number: PASC-REP-1467
Guidelines:	SANCO/3029/99 rev. 4 (2000) and SANCO/825/00 rev. 8.1 (2010).
GLP:	No
Deviations	N/A
Previous evaluation:	None

The objective of this study was to perform an independent laboratory validation (ILV) of the QuEChERS analytical method for the determination of pydiflumetofen in egg and muscle. The ILV was carried out by Syngenta Crop Protection, LLC, Greensboro, NC 27419-8300 USA, a different test facility to the primary method.

No deviations from the primary method validation study were noted.

A summary of the method validation data is given in Table B.5.2.2-3

Table B.5.2.2-3: Summary of method validation data for the determination of pydiflumetofen residues in animal matrices

Matrix	Analyte	LOQ (mg/kg)	Recovery fortification level (mg/kg)	Recoveries % (mean)	Repeatability % RSD (n)	Linearity
Egg	Pydiflumetofen Primary Transition *m/z* 426 → 193	0.01	0.01	90-97 (95)	3 (5)	0.025 – 2.5 ng/mL (n = 5) $y = 2.37 \times 10^5 x + 1690$ $r^2 = 0.9962$
			0.1	77 – 86 (81)	4.5 (5)	
			Overall	77 -97 (88)	9.1 (10)	
	Pydiflumetofen Confirmatory Transition *m/z* 428 → 195		0.01	85 – 97 (93)	5.5 (5)	as above $y = 2.27 \times 10^5 x + 1660$ $r^2 = 0.9981$
			0.1	79 – 84 (81)	3.1 (5)	
			Overall	79 – 97 (87)	8.5 (10)	
Muscle	Pydiflumetofen Primary Transition *m/z* 426 → 193		0.01	83 – 88 (85)	2.8 (5)	0.025 – 2.5 ng/mL (n = 5) $y = 2.37 \times 10^5 x + 1690$ $r^2 = 0.9962$
			0.1	78 – 85 (80)	3.6 (5)	
			Overall	78 – 88 (80)	4.4 (10)	
	Pydiflumetofen Confirmatory Transition *m/z* 428 → 195		0.01	79 – 89 (85)	5.1 (5)	as above $y = 2.27 \times 10^5 x + 1660$ $r^2 = 0.9981$
			0.1	78 – 85 (80)	3.5 (5)	
			Overall	78 – 89 (82)	5.5 (10)	

Specificity and Confirmation of Analyte Identity

LC-MS/MS with primary and confirmatory ion transitions is considered a highly specific technique. Therefore, additional methods to confirm the identity of the analytes are not considered necessary. Chromatograms have been provided showing a retention time match with standards. No significant interference (>30% of the LOQ) between the peak pydiflumetofen and any of the animal matrices was observed. The ion transitions monitored are appropriate.

Linearity

Linearity was demonstrated by the analysis of eight standards of increasing concentration. The range of standard concentrations used was 0.025-2.5 ng/mL (0.04 – 0.4 mg/kg). The response was linear for both MS/MS transitions with r^2 values > 0.99.

Accuracy and Precision

Fortified samples were analysed in quintuplet at the limit of quantification (LOQ, 0.01 mg/kg) and at 10x LOQ (0.1 mg/kg). Mean recoveries were within the acceptable range (70-110%) and the %RSD was ≤20% at each level.

Matrix effects

No significant matrix effects (>20%) were observed in matrices tested during method validation. Solvent standards were used for calibration

LOQ

The LOQ is defined as the lowest validated level. This corresponds to 0.01 mg/kg for pydiflumetofen in all matrices.

Storage Stability

Stability of sample extracts fortified with pydiflumetofen at the LOQ level was demonstrated to be stable for between 8 days in a refrigerator at 4°C. The mean recoveries were between 70-110% with a %RSD ≤20%.

Extraction Efficiency

Not required for an ILV study

Conclusion

The QuEChERS method is acceptably independently validated in egg and muscle in accordance with SANCO/825/00 rev. 8.1 for the determination of pydiflumetofen residues

Report:	**KCA1 4.2/1, [redacted] (2015)**
Title	SYN545974 - Independent Laboratory Validation of the QuEChERS Method for the Determination of Residues of SYN545974 in Liver and Milk by LC-MS/MS Report Number: CEMR-7055
Guidelines:	SANCO/3029/99 rev. 4 (2000) and SANCO/825/00 rev. 8.1 (2010).
GLP:	Yes
Deviations	N/A
Previous evaluation:	None

The objective of this study was to perform an independent laboratory validation (ILV) of the QuEChERS analytical method for the determination of pydiflumetofen in liver and milk. The ILV was carried out in a different test facility to the primary method.

No deviations from the primary method validation study were noted.

A summary of the method validation data is given in Table B.5.2.2-4

Table B.5.2.2-4: Summary of method validation data for the determination of pydiflumetofen residues in animal matrices

Matrix	Analyte	LOQ (mg/kg)	Recovery fortification level (mg/kg)	Recoveries % (mean)	Repeatability % RSD (n)	Linearity
Liver	Pydiflumetofen Primary Transition *m/z* 426 → 193	0.01	0.01	74 – 84 (80)	4.6 (5)	0.025 – 2.5 ng/mL (n = 5) y = 123306.62x - 549.21 r^2 = 0.9988
			0.1	87 – 95 (92)	3.8 (5)	
			Overall	74 – 95 (86)	8.2 (10)	
	Pydiflumetofen Confirmatory Transition *m/z* 428 → 195		0.01	75 – 78 (77)	1.7 (5)	as above y = 127232.44x – 816.23 r^2 = 0.9987
			0.1	87 – 94 (91)	3.2 (5)	
			Overall	75 – 94 (84)	8.8 (10)	
Milk	Pydiflumetofen Primary Transition *m/z* 426 → 193	0.01	0.01	87-91 (88)	2 (5)	0.025 – 2.5 ng/mL (n = 5) y = 108320.54x + 161.23 r^2 = 0.9998
			0.1	90 – 95 (92)	2.2 (5)	
			Overall	87 – 95 (90)	3 (10)	
	Pydiflumetofen Confirmatory Transition *m/z* 428 → 195		0.01	81 – 91 (87)	4.5 (5)	as above y = 109126.99x + 378.23 r^2 = 0.9999
			0.1	91 – 99 (95)	3.2 (5)	
			Overall	81 – 99 (91)	5.6 (10)	

Specificity and Confirmation of Analyte Identity

LC-MS/ with primary and confirmatory ion transitions is considered a highly specific technique. Therefore, additional methods to confirm the identity of the analytes are not considered necessary. Chromatograms have been provided showing a retention time match with standards. No significant interference (>30% of the LOQ) between the peak pydiflumetofen and any of the animal matrices was observed. The ion transitions monitored are appropriate.

Linearity

Linearity was demonstrated by the analysis of seven standards of increasing concentration. The range of standard concentrations used was 0.025-2.5 ng/mL (0.04 – 0.4 mg/kg). The response was linear for both MS/MS transitions with r^2 values ranging from 0.9987-0.9999.

Accuracy and Precision

Fortified samples were analysed in quintuplet at the limit of quantification (LOQ, 0.01 mg/kg) and at 10x LOQ (0.1 mg/kg). Mean recoveries were within the acceptable range (70-110%) and the %RSD was ≤20% at each level.

Matrix effects

No significant matrix effects (>20%) were observed in matrices tested during method validation. Solvent standards were used for calibration.

LOQ

The LOQ is defined as the lowest validated level. This corresponds to 0.01 mg/kg for pydiflumetofen in all matrices.

Storage Stability

Stability of sample extracts fortified with pydiflumetofen at the LOQ level was demonstrated to be stable for between 8 days in a refrigerator at 4°C. The mean recoveries were between 70-110% with a %RSD ≤20%.

Extraction Efficiency

Not required for an ILV study.

Conclusion

The QuEChERS method is acceptably independently validated in liver and milk in accordance with SANCO/825/00 rev. 8.1 for the determination of pydiflumetofen residues.

Report:	**KCA1 4.2/10, [redacted] (2015)**
Title	SYN545974 - Analytical Method (GRM061.07A) for the Determination of Free and Conjugated 2,4,6-trichlorophenol in Bovine Milk, Liver, Kidney, Muscle, Fat, Blood, and Hen Eggs by LC-MS/MS Report Number GRM061.07A
Guidelines:	SANCO/3029/99 rev. 4 (2000) and SANCO/825/00 rev. 8.1 (2010).
GLP:	No
Deviations	N/A
Previous evaluation:	None

Report:	**KCA1 4.2/15, [redacted]. & [redacted]. (2015)**
Title	SYN545974 - Validation of the Analytical Method GRM 061.07A for the Determination of Residues of Conjugated 2,4,6-Trichlorophenol in Animal Matrices Report Number P 3613 G
Guidelines:	SANCO/3029/99 rev. 4 (2000) and SANCO/825/00 rev. 8.1 (2010).
GLP:	Yes
Deviations	N/A
Previous evaluation:	None

The purpose of this study was the validation of GRM 061.07A for the determination of residues of 2,4,6-trichlorophenol in animal matrices (whole milk, egg, bovine liver, fat, blood, muscle, kidney) by LC-MS/MS.

Reference items:

2,4,6-trichlorophenol, batch SZBB294XV/, purity 99.4 %, expiry 21 October 2016

Sample preparation:

Samples of bovine muscle, liver, kidney, milk, animal blood and chicken eggs (10g) were homogenized with acetonitrile: water (80/20 v/v), and centrifuged.

Samples of animal fat (10g) were dissolved into 50 mL n-hexane before liquid-liquid partitioning into acetonitrile: water (80/20, v/v).

An aliquot of each extract was evaporated to approximately 2.5 mL. Extracts were buffered with 0.4 M sodium acetate containing β-glucuronidase and diluted with water. Conjugates of 2,4,6-trichlorophenol were hydrolysed by incubation at 37 °C for 18 hrs to convert any conjugated 2,4,6-trichlorophenol present in the samples to free 2,4,6-trichlorophenol

After hydrolysis, samples were chilled at < -10°C in a freezer, diluted with water and submitted to SPE for additional clean-up (Oasis HLB 3cc, 60mg) prior to analysis of 2,4,6-trichlorophenol (free and conjugated) by LC-MS/MS using the conditions described below

LC-MS/MS conditions:

HPLC System: Agilent Infinity 1290 HPLC system and CTC Analytics HTC-Pal Autosampler
Column: ACE 3 C18, 3.0 µm particle size, 50 mm length, 3.0 mm i.d.
Column oven temperature: 40°C
Injection volume: 30 µL
Mobile phase A: water + 0.1 % of acetic acid
Mobile phase B: acetonitrile + 0.1 % of acetic acid
Stop time: 4 minutes
Mobile phase composition:

Time (minute)	% A	% B	Flow rate (mL/min)
0.00	80	20	0.6
1.50	10	90	0.6
2.50	10	90	0.6
2.60	80	20	0.6
4.00	80	20	0.6

Note: Under these conditions the retention time is approximately 2.0 minutes.

MS System: AB MDS Sciex API 5500 triple quadrupole LC-MS/MS
Ionisation: ESI
Polarity: Negative
Ions monitored: *m/z* 426 → 193 quantitative
m/z 428 → 195 confirmatory

The method was validated in milk, liver, blood, egg, fat kidney and muscle. A summary of the method validation data is given in Table B.5.2.2-5

Table B.5.2.2-5: Summary of method validation data for the determination of 2,4,6-Trichlorophenol residues in animal matrices

Matrix	**Analyte**	**LOQ (mg/kg)**	**Recovery fortification level (mg/kg)**	**Recoveries % (mean)**	**Repeatability % RSD (n)**	**Linearity**
Milk	2,4,6-trichlorophenol Primary Transition *m/z* 195 → 159	0.01	0.01	82,85,87,86,91 (86)	4 (5)	0.4 – 50 ng/mL (n = 7) $y = 1.18 \times 10^4 x - 796$ r = 0.9999
			0.1	86,92,91,90,94 (90)	3 (5)	
			Overall	(88)	4 (10)	
	2,4,6-trichlorophenol Confirmatory Transition *m/z* 197 → 161		0.01	82,83,90,85,87 (85)	4 (5)	as above $y = 7.5 \times 10^3 x - 506$ r = 1.0000
			0.1	86,92,90,89,91 (89)	3 (5)	
			Overall	(87)	4 (10)	
Liver	2,4,6-trichlorophenol Primary Transition *m/z* 195 → 159	0.01	0.01	78,80,86,81,81 (81)	4 (5)	as above $y = 1.23 \times 10^4 x + 176$ r = 0.9999
			0.1	85,88,88,86,86 (86)	2 (5)	
			Overall	(84)	4 (10)	
	2,4,6-trichlorophenol Confirmatory Transition *m/z* 197 → 161		0.01	81,84,85,84,89 (84)	4 (5)	0.4 – 50 ng/mL (n = 7) $y = 7.82 \times 10^3 x - 21.8$ r = 0.9999
			0.1	85,87,88,82,84 (85)	3 (5)	
			Overall	(85)	3 (10)	

Matrix	Analyte	LOQ (mg/kg)	Recovery fortification level (mg/kg)	Recoveries % (mean)	Repeatability % RSD (n)	Linearity
Blood	2,4,6-trichlorophenol Primary Transition *m/z* 195 → 159	0.01	0.01	83,85,88,89,90 (87)	3 (5)	0.4 – 50 ng/mL (n = 7) $y = 1.11 \times 10^4 x + 61.6$ r = 0.9998
			0.1	87,87,90,93,92 (90)	3 (5)	
			Overall	(88)	3 (10)	
	2,4,6-trichlorophenol Confirmatory Transition *m/z* 197 → 161		0.01	81,79,87,92,88 (85)	6 (5)	as above $y = 7.04 \times 10^3 x + 214$ r = 0.9998
			0.1	87,85,89,92,91 (89)	3 (5)	
			Overall	(87)	5 (10)	
Egg	2,4,6-trichlorophenol Primary Transition *m/z* 195 → 159	0.01	0.01	79,79,82,79,80 (80)	2 (5)	00.4 – 50 ng/mL (n = 7) $y = 1.25 \times 10^4 x + 149$ r = 0.9999
			0.1	85,82,83,84,82 (83)	2 (5)	
			Overall	(81)	3 (10)	
	2,4,6-trichlorophenol Confirmatory Transition *m/z* 197 → 161		0.01	78,81,84,81,79 (80)	3 (5)	as above $y = 7.95 \times 10^3 x - 77.9$ r = 0.9999
			0.1	84,82,83,83,81 (82)	1 (5)	
			Overall	(81)	3 (10)	
Fat	2,4,6-trichlorophenol Primary Transition *m/z* 195 → 159	0.01	0.01	85,85,86,88,86 (86)	1 (5)	0.4 – 50 ng/mL (n = 7) $y = 1.17 \times 10^4 x + 413$ r = 1.0000
			0.1	85,88,90,90,95 (89)	4 (5)	
			Overall	(87)	4 (10)	
	2,4,6-trichlorophenol Confirmatory Transition *m/z* 197 → 161		0.01	86,85,86,88,84 (85)	2 (5)	as above $y = 7.26 \times 10^3 x + 396$ r = 0.9999
			0.1	87,88,91,92,94 (90)	3 (5)	
			Overall	(88)	4 (10)	
Kidney	2,4,6-trichlorophenol Primary Transition *m/z* 195 → 159	0.01	0.01	89,87,84,84,83 (85)	3 (5)	0.4 – 50 ng/mL (n = 7) $y = 1.20 \times 10^4 x + 389$ r = 1.0000
			0.1	89,85,84,83,88 (86)	3 (5)	
			Overall	(85)	3 (10)	
	2,4,6-trichlorophenol Confirmatory Transition *m/z* 197 → 161		0.01	85,87,82,88,84 (85)	3 (5)	as above $y = 7.21 \times 10^3 x + 316$ r = 1.0000
			0.1	89,86,80,83,87 (85)	4 (5)	
			Overall	(85)	3 (10)	

Matrix	Analyte	LOQ (mg/kg)	Recovery fortification level (mg/kg)	Recoveries % (mean)	Repeatability % RSD (n)	Linearity
Muscle	2,4,6-trichlorophenol Primary Transition *m/z* 195 → 159	0.01	0.01	80,85,88,94,89 (87)	6 (5)	0.4 – 50 ng/mL (n = 7) $y = 1.06 \times 10^4 x + 69.5$ r = 0.9998
			0.1	86,82,86,91,82 (85)	4 (5)	
			Overall	(86)	5 (10)	
	2,4,6-trichlorophenol Confirmatory Transition *m/z* 197 → 161		0.01	81,84,86,90,87 (85)	4 (5)	as above $y = 6.7 \times 10^3 x + 53.1$ r = 0.9998
			0.1	87,82,87,88,81 (85)	4 (5)	
			Overall	(85)	4 (10)	

Specificity and Confirmation of Analyte Identity

LC-MS/MS is considered a specific technique as two different mass transitions were monitored. Therefore, additional methods to confirm the identity of the analytes are not considered necessary. Chromatograms have been provided showing a retention time match with standards. No significant interference (>30% of the LOQ) between the 2,4,6-Trichlorophenol peak and any of the matrices was observed. The ion transitions monitored are appropriate.

Linearity

Linearity was demonstrated by the analysis of eight matrix matched standards of increasing concentration. The range of standard concentrations used was 0.4 – 50 ng/mL, (equivalent to 0.002 – 0.25 mg/kg). The response was linear for both MS/MS transitions with r values ranging from 0.9979-0.9996.

Accuracy and Precision

Fortified samples of each matrix were analysed in quintuplet at the limit of quantification (LOQ, 0.01 mg/kg) and at 10x LOQ (0.1 mg/kg). Mean recoveries were within the acceptable range (70-110%) and the %RSD was ≤20% at each level.

Matrix effects

No significant matrix effects (>20%) were observed in the matrices tested. Matrix matched linearity standards were used for quantification.

LOQ

The LOQ is defined as the lowest validated level. This corresponds to 0.01 mg/kg for 2,4,6-trichlorophenol in all matrices.

Storage Stability

Stability of sample extracts fortified with 2,4,6-trichlorophenol at the LOQ level was demonstrated to be stable for between 10 to 26 days when stored at a target temperature of 4°C. The mean recoveries were between 70-110% with a %RSD ≤20%. The stability of stored working standard solutions of 2,4,6-trichlorophenol was demonstrated to be stable for 35 days when stored at a target temperature of 4°C.

Extraction Efficiency

Free and conjugated 2,4,6-trichlorophenol have been shown to be efficiently extracted using the conditions described in GRM061.07A in radiolabelled metabolism studies.

The metabolism of radiolabelled pydiflumetofen has been described in a study in lactating goats (See Volume 3 B.7.2.3). Liver and kidney samples were extracted by homogenizing with acetonitrile: water (80:20, v/v) twice followed by acetonitrile: water (1:1, v/v). Samples of fat were extracted two times with acetonitrile: water (4:1, v/v) + hexane and once with acetonitrile: water (1:1, v/v). Samples of milk were extracted with acetonitrile and hexane.

The extraction solvents used in the metabolism studies extracted >80% of the TRR from all samples except kidney. For kidney, the majority of the %TRR was extracted with the first acetonitrile: water (80:20, v/v) extraction. However, residues extractability was lower in liver (50.4 and 47.4% TRR for phenyl and pyrazole in ruminant liver, respectively).

Method GRM061.07A uses acetonitrile: water (80/20, v/v) for extraction of residues from samples. Hence, the same extraction solvent was used to extract residues in the animal metabolism studies as is used in Method GRM061.07A (acetonitrile: water (80/20, v/v)). On this basis, the extraction efficiency is considered satisfactorily addressed.

Hydrolysis step and release of conjugated residues

In the metabolism studies on goats, enzyme hydrolysis procedures were undertaken to release metabolites from their conjugated forms. Enough sodium acetate was weighed into the extract to produce a 0.2M solution, and the sample was then adjusted to pH using acetic acid before the addition of β-glucuronidase. The resulting mixture was incubated overnight in a shaking water bath at 37 °C (~18 hours).

As part of Method GRM061.07A, enzyme hydrolysis is performed using 2 mL of 5 mg/mL β-glucuronidase solution prepared in 0.4 M sodium acetate buffer (pH 5). Hence, similar hydrolysis conditions were used to release conjugated as part of Method GRM061.07A. As such, by implication the metabolism study fully validates the hydrolysis step used within the residue method.

Conclusion

The method is acceptably validated in accordance with SANCO/825/00 rev. 8.1 as well as SANCO3029/99 rev. 4 for the determination of 2,4,6-trichlorophenol (free and conjugated) residues in animal matrices. Although recovery determinations were only made using unconjugated 2,4,6-trichlorophenol the hydrolysis step in the method is considered validated for the release of conjugated metabolites.

Report:	**KCA1 4.2/14, [redacted] (2015)**
Title	SYN545974 - Independent Lab Validation of the Analytical Method for the Determination of Conjugated 2,4,6-Trichlorophenol in Animal Matrices Report number: RB5134
Guidelines:	SANCO/3029/99 rev. 4 (2000) and SANCO/825/00 rev. 8.1 (2010).
GLP:	Yes
Deviations	N/A
Previous evaluation:	None

The objective of this study was to perform an independent laboratory validation (ILV) of the analytical method for the determination of 2,4,6-Trichlorophenol in animal matrices. The ILV was carried out by ANADIAG in France, a different test facility to the primary method validation.

No deviations from the primary method validation study were noted.

A summary of the method validation data is given in Table B.5.2.2-6

Table B.5.2.2-6: Summary of method validation data for the determination of 2,4,6-Trichlorophenol residues in animal matrices

Matrix	Analyte	LOQ (mg/kg)	Recovery fortification level (mg/kg)	Recoveries % (mean)	Repeatability % RSD (n)	Linearity
Milk	2,4,6-trichlorophenol Primary Transition *m/z* 195 → 159	0.01	0.01	100, 106, 94, 95, 93 (98)	5 (5)	0.4 – 50.2 ng/mL (n = 7) $y = 5.3922 \times 10^{-4} X - 0.03$ r = 0.99997
			0.1	95, 96, 94, 95, 95 (95)	1 (5)	
			Overall	(96)	4 (10)	
	2,4,6-trichlorophenol Confirmatory Transition *m/z* 197 → 161		0.01	92, 95, 99, 98, 101 (97)	4 (5)	0.4 – 50.2 ng/mL (n = 7) $y = 8.4786 \times 10^{-4} X - 0.08$ r = 0.99998
			0.1	93, 94, 94, 97, 96 (96)	3 (5)	
			Overall	(96)	3 (10)	
Liver	2,4,6-trichlorophenol Primary Transition *m/z* 195 → 159	0.01	0.01	70, 76, 74, 76, 78 (75)	4 (5)	0.4 – 50.2 ng/mL (n = 7) $y = 5.4839 \times 10^{-4} X - 0.21$ r = 0.99969
			0.1	84, 82, 83, 82, 90 (84)	4 (5)	
			Overall	(80)	4 (10)	
	2,4,6-trichlorophenol Confirmatory Transition *m/z* 197 → 161		0.01	64, 72, 70, 76, 70 (71)	6 (5)	0.4 – 50.2 ng/mL (n = 7) $y = 8.5845 \times 10^{-3} X - 0.18$ r = 0.99969
			0.1	85, 81, 83, 81, 91 (84)	5 (5)	
			Overall	(77)	11 (10)	
Blood	2,4,6-trichlorophenol Primary Transition *m/z* 195 → 159	0.01	0.01	76, 80, 77, 79, 80 (78)	2 (5)	0.4 – 50.2 ng/mL (n = 7) $y = 5.2674 \times 10^{-4} X - 0.01$ r = 0.99992
			0.1	73, 71, 71, 82, 72 (74)	6 (5)	
			Overall	(76)	5 (10)	
	2,4,6-trichlorophenol Confirmatory Transition *m/z* 197 → 161		0.01	79, 79, 83, 83, 83 (81)	3 (5)	0.4 – 50.2 ng/mL (n = 7) $y = 8.2614 \times 10^{-4} X + 0.00$ r = 0.99987
			0.1	72, 74, 72, 82, 71 (74)	6 (5)	
			Overall	(78)	7 (10)	
Egg	2,4,6-trichlorophenol Primary Transition *m/z* 195 → 159	0.01	0.01	98, 92, 93, 94, 100 (95)	4 (5)	0.4 – 50.2 ng/mL (n = 7) $y = 4.914 \times 10^{-4} X - 0.02$ r = 0.99985
			0.1	101, 99, 96, 96, 99 (98)	2 (5)	
			Overall	(97)	3 (10)	
	2,4,6-trichlorophenol Confirmatory Transition *m/z* 197 → 161		0.01	101, 99, 102, 98, 101 (100)	2 (5)	0.4 – 50.2 ng/mL (n = 7) $y = 7.6801 \times 10^{-4} X + 0.05$ r = 0.99996
			0.1	101, 98, 97, 98, 98 (98)	2 (5)	
			Overall	(99)	2 (10)	

Matrix	Analyte	LOQ (mg/kg)	Recovery fortification level (mg/kg)	Recoveries % (mean)	Repeatability % RSD (n)	Linearity
Fat	2,4,6-trichlorophenol Primary Transition *m/z* 195 → 159	0.01	0.01	71, 70, 72, 74, 74 (73)	2 (5)	0.4 – 50.2 ng/mL (n = 7) $y = 5.3633 \times 10^{-4} X - 0.11$ r = 0.99977
			0.1	75, 74, 72, 73, 79 (74)	4 (5)	
			Overall	(73)	3 (10)	
	2,4,6-trichlorophenol Confirmatory Transition *m/z* 197 → 161		0.01	72, 71, 70, 77, 76 (73)	4 (5)	0.4 – 50.2 ng/mL (n = 7) y = 8.4119 X – 0.08 r = 0.99997
			0.1	74, 76, 74, 73, 79 (75)	3 (5)	
			Overall	(74)	4 (10)	
Kidney	2,4,6-trichlorophenol Primary Transition *m/z* 195 → 159	0.01	0.01	94, 99, 100, 96, 99 (98)	2 (5)	0.4 – 50.2 ng/mL (n = 7) $y = 6.9684 \times 10^{-4} X - 0.14$ r = 0.99998
			0.1	99, 100, 101, 101, 103 (101)	1 (5)	
			Overall	(99)	2 (10)	
	2,4,6-trichlorophenol Confirmatory Transition *m/z* 197 → 161		0.01	87, 89, 83, 89, 89 (88)	3 (5)	0.4 – 50.2 ng/mL (n = 7) $y = 1.0699 \times 10^{-3} X - 0.16$ r = 0.99996
			0.1	97, 99, 100, 99, 101 (99)	1 (5)	
			Overall	(93)	7 (10)	
Muscle	2,4,6-trichlorophenol Primary Transition *m/z* 195 → 159	0.01	0.01	96, 94, 98, 99, 95 (96)	2 (5)	0.4 – 50.2 ng/mL (n = 7) $y = 5.8702 \times 10^{-4} X - 0.04$ r = 0.99994
			0.1	98, 100, 99, 97, 97 (98)	1 (5)	
			Overall	(97)	2 (10)	
	2,4,6-trichlorophenol Confirmatory Transition *m/z* 197 → 161		0.01	96, 95, 97, 100, 96 (97)	2 (5)	0.4 – 50.2 ng/mL (n = 7) $y = 9.2666 \times 10^{-4} X - 0.04$ r = 0.99995
			0.1	100, 101, 98, 98, 99 (99)	1 (5)	
			Overall	(98)	2 (10)	

Specificity and Confirmation of Analyte Identity

LC-MS/MS is considered a specific technique as two different mass transitions were monitored. Therefore, additional methods to confirm the identity of the analytes are not considered necessary. Chromatograms have been provided showing a retention time match with standards. No significant interference (>30% of the LOQ) between the 2,4,6-Trichlorophenol peak and any of the matrices was observed. The ion transitions monitored are appropriate

Linearity

Linearity was demonstrated by the analysis of eight matrix matched standards of increasing concentration. The range of standard concentrations used was 0.4 – 50.2 ng/mL, (equivalent to 0.002 – 0.25 mg/kg). The response was linear for both MS/MS transitions with r values ranging from 0.9979-0.9996.

Accuracy and Precision

Fortified samples of each matrix were analysed in quintuplet at the limit of quantification (LOQ, 0.01 mg/kg) and at 10x LOQ (0.1 mg/kg). Mean recoveries were within the acceptable range (70-110%) and the %RSD was ≤20% at each level.

Matrix effects

No significant matrix effects (>20%) were observed in the matrices tested. Matrix matched linearity standards were used for quantification.

LOQ

The LOQ is defined as the lowest validated level. This corresponds to 0.01 mg/kg for 2,4,6-trichlorophenol in all matrices.

Storage Stability

Not considered as part of the ILV

Extraction Efficiency

Not required for an ILV study.

Conclusion

The method is acceptably independently validated in animal matrices in accordance with SANCO/825/00 rev. 8.1 for the determination of 2,4,6-trichlorophenol residues.

B.5.2.3. Methods for residues in soil and sediment

Table B.5.2.3-1: Summary of analytical methods for monitoring residues of pydiflumetofen in soil and sediment

Analyte	Limit of quantification (µg/kg)	Matrix	Method	Method Reference
pydiflumetofen	0.5	Soil	LC-MS/MS	GRM061.04A
pydiflumetofen SYN545547	0.5	Soil	LC-MS/MS	GRM061.02A

Report:	**KCA1 4.2/7, [redacted]. (2013)**
Title	SYN545974 – Analytical Method (GRM061.04A) for Determination of SYN545974 in Soil Report number: GRM061.04A (Document No.: VV-132594)
Guidelines:	SANCO/3029/99 rev. 4 (2000) and SANCO/825/00 rev. 8.1 (2010).
GLP:	No
Deviations	N/A
Previous evaluation:	None

Report:	**KCA1 4.2/9, [redacted] (2013)**
Title	SYN545974 – Independent Laboratory Validation of Residue Method (GRM061.02A) for the Determination of SYN545974 and SYN545547 in Soil Report number: 2387W (Document No.: VV-414595)
Guidelines:	SANCO/3029/99 rev. 4 (2000) and SANCO/825/00 rev. 8.1 (2010).
GLP:	Yes
Deviations	N/A
Previous evaluation:	None

Method GRM061.04A was developed and validated for the determination of pydiflumetofen in soil.

Reference items:

pydiflumetofen, batch AMS 1432/1, purity 99.5 %w/w, CoA provided, expiry December 2017

Sample preparation:

Soil samples (10g) are extracted with acetonitrile/0.1M ammonium acetate 80/20 (v/v) followed by two additional extractions with acetonitrile/0.1% acetic acid 80/20 (v/v). Extracts are combined and filtered. An aliquot is evaporated to remove acetonitrile and then acidified with 1 mL of 0.1% acetic acid in ultra-pure water. Samples are taken through a C18 solid phase extraction (SPE) procedure, eluting with methanol/0.1% acetic acid 60/40 (v/v) followed by 100 % methanol. Eluate are evaporated to remove the methanol and diluted with a mixture of methanol and 0.1% acetic acid prior to analysis by LC-MS/MS using the conditions described below.

LC-MS/MS conditions:

HPLC System: Waters Acquity UPLC® system (H Class)
Detector: Applied Biosystems Sciex API 4000 triple quadrupole mass spectrometer
Column: Agilent Varian Pursuit XRs 3 Diphenyl 100 x 4.6 mm i.d., 3.0 µm particle size or Kinetex 100 mm x 2.1 mm i.d. Phenyl-Hexyl 2.6 µm particle size
Column oven temperature: 40°C
Injection volume: 10-20 µL
Mobile phase A: 0.1% acetic acid in ultra-pure water
Mobile phase B: 0.1% acetic acid in MeOH
Mobile phase composition:

Time (minute)	% A	% B	Flow rate (mL/minute)
0.00	70	30	0.5
5.00	0	100	0.5
7.50	0	100	0.5

7.60	70	30	0.5
10.50	70	30	0.5

Note: Under these conditions the retention time is 5.4 minutes for pydiflumetofen.

Interface: TurboIonSpray
Polarity: Positive
Scan type: MRM
Ions monitored: *m/z* 426 → 193.2 quantitative
m/z 426 → 166.2 confirmatory

The method GRM061.04A was validated on a clay loam soil in the study [redacted] (2013). Note that although this study was described as an ILV study it contains the only validation data provided for the method. The method was named GRM061.02A in the study [redacted] (2013) as it also contained validation data for a minor soil metabolite (SYN545547).

As this metabolite was not included in the residue definition for monitoring for soil due to extremely low levels (< 3%) observed in laboratory metabolism studies, it is not discussed further. The extraction procedures and measurement of pydiflumetofen for method GRM061.02A are identical to those describe for method GRM061.04A

A summary of the method validation data is given in Table B.5.2.3-2.

Table B.5.2.3-2: Summary of method validation data for the determination of pydiflumetofen residues in soil

Matrix	**Analyte**	**LOQ (µg/kg)**	**Recovery fortification level (µg/kg)**	**Recoveries % (mean)**	**Repeatability % RSD (n)**	**Linearity**
Soil (Clay loam)	pydiflumetofen Primary Transition *m/z* 426 → 193	0.5	0.5	78, 74, 72, 86, 80 (78)	6 (5)	0.01 – 1 ng/mL (n = 6) y = 59539x + 680 r = 0.9996
			5	83, 79, 77, 83, 77 (80)	4 (5)	
			Overall	79	5 (10)	
	pydiflumetofen Confirmatory Transition *m/z* 428 → 195	0.5	0.5	98, 90, 74, 92, 78 (86)	12 (5)	as above y = 33983x + 278 r = 0.9977
			5	90, 82, 81, 85, 79 (83)	5 (5)	
			Overall	85	8 (10)	

Specificity and Confirmation of Analyte Identity

LC-MS/MS is considered a specific technique as two different mass transitions were monitored. Therefore, additional methods to confirm the identity of the analytes are not considered necessary. The ion transitions monitored are appropriate. Chromatograms have been provided showing a retention time match with standards. No significant interference (>30% of the LOQ) at the retention time of interest was observed for soil.

Linearity

Linearity was demonstrated by the analysis of six standards of increasing concentration. The range of standard concentrations used was 0.01-1 ng/L. The lowest concentration injected was equivalent to 2% of the LOQ of the method and the highest concentration injected was equivalent to 200% of the LOQ. The response was linear for both MS/MS transitions with a correlation coefficient ranging from 0.9977-0.9996.

Accuracy and Precision

Soil samples were fortified with pydiflumetofen and analysed in quintuplet at the limit of quantification (LOQ, 0.5 µg/kg and at 10x LOQ (5 µg/kg). Mean recoveries were within the acceptable range (70-110%) and the %RSD was ≤20% at each level.

Matrix effects

No significant matrix effects (>20%) were observed in soil. Therefore, matrix matched standards were not used.

LOQ

The LOQ is defined as the lowest validated level. This corresponds to 0.5 µg/kg for pydiflumetofen in soil.

Storage Stability

The stability of sample extracts fortified with pydiflumetofen at the LOQ and 10x LOQ level was checked after a storage period of 7 days in a refrigerator at approximately 4 °C against freshly prepared calibration standards. The results proved that the pydiflumetofen residues in the stored fortified samples were stable. The mean recovery values at the LOQ and 10x LOQ level were between 78-83% and within ±10% of the initial values when re-analysed.

Conclusion

Method GRM061.04A is acceptably validated in accordance with SANCO/825/00 rev. 8.1 and SANCO/3029/99 rev. 4 for the determination of pydiflumetofen residues in soil with an LOQ of 0.5 µg/kg.

B.5.2.4. Methods for residues in water

Summary Overview:

Method GRM061.01A has been proposed as the enforcement method for surface and ground (drinking) water. The method of analysis has been validated for the parent active substance only (pydiflumetofen). pydiflumetofen is the only component of the residue definition for enforcement in drinking water and surface water. Independent laboratory validation of the method for drinking water has been provided. It should be noted the ILV was the first validation to be completed and hence is the validation data cited in the analytical method (KCA1 4.2/4, [redacted]).

Method GRM061.01A has also been used for data generation purposes in support of ecotoxicology in the following studies:

- KCA1 8.2.4.2-01 – Study CEA.1644
- KCA1 8.2.4.2-02 – Study CEA.1666
- KCA1 8.2.4.2-03 – Study CEA.1667
- KCA1 8.2.4.2-09 – Study CEA.1664
- KCA1 8.2.4.2-04 – Study CEA.1661
- KCA1 8.2.4.2-05 – Study CEA.1662
- KCA1 8.2.4.2-07 – Study CEA.1642
- KCA1 8.2.4.2-06 – Study CEA.1645

Table B.5.2.4-1: Summary of analytical methods for monitoring residues of pydiflumetofen in water

Reference	Author	Study Scope	Method	Method Reference	Limit of quantification (µg/L)
KCA1 4.2/4	[redacted]	Residue method	LC-MS/MS	GRM061.01A	0.05
KCA1 4.2/8	[redacted]	Primary validation study in ground and surface water	LC-MS/MS	GRM061.01A	0.05
KCA1 4.2/8	[redacted]	ILV in ground and surface water	LC-MS/MS	GRM061.01A	0.05

Report:	**KCA1 4.2/4, [redacted] (2013)**
Title	SYN545974 – Residue method for the Determination of SYN545974 in Water Report number: GRM061.01A (Document no.: VV-132593) Syngenta task no.: TK0057867
Guidelines:	SANCO/825/00 rev.8.1
GLP:	No
Deviations	N/A
Previous evaluation:	None

Report:	**KCA1 4.2/8, [redacted] (2016)**
Title	SYN545974 – Validation of Residue Method (GRM061.01A) for the Determination of SYN545974 in Water Syngenta task no.: TK0290384 (Document no.: VV-415628)
Guidelines:	SANCO/825/00 rev.8.1
GLP:	No
Deviations	N/A
Previous evaluation:	None

Method GRM061.01A was developed and validated for the determination of pydiflumetofen in surface and ground (drinking) water. The primary method was conducted by Syngenta Crop Protection, LLC, Gereensboro, USA. Environmental water samples are directly injected into the system for analysis once acidic acetonitrile has been added. However, if the concentrations are too low, the water samples are concentrated using solid phase extraction (SPE) procedures prior to LC-MS/MS analysis.

Reference items:

pydiflumetofen, batch AMS 1432/1, purity 99.5 %w/w, CoA provided, expiry December 2017

Sample preparation:

A 4 mL aliquot of water sample was transferred to a 20 mL scintillation vial. The sample is fortified at this time, if required. A 1 mL portion of acidic acetonitrile (0.2% acetic acid; v/v) was added to each sample and the solution mixed. An aliquot of this sample was then transferred to an autosampler vial for LC-MS/MS analysis, monitoring for the primary transition (*m/z* 425.9→192.9) and the confirmatory transition (*m/z* 425.9→166.1). If SPE is required, the samples are eluted with acidic methanol (0.01% formic acid; v/v) from the SPE cartridges and collected. The collected methanol fractions are evaporated to dryness under a gentle stream on nitrogen in a water bath at approximately 35°C and re-constituted with acetonitrile/water (20/80 v/v) then subject to LC-MS/MS analysis.

LC-MS/MS conditions:

Chromatographic system:	Waters Acquity I Class
Analytical column:	Waters Acquity BEH C18, 2.1 x 50 mm, 1.7 µm
Target column temperature:	25°C
Injection volume:	50 µL
Mobile phase A:	0.1% formic acid in Optima water
Mobile phase B:	0.1% formic acid in acetonitrile
Flow rate:	0.35 mL/minute

Gradient:

Time (minute)	% A	% B
0.0	70	30
1.0	70	30
3.0	10	90
5.0	10	90
5.1	70	30
6.0	70	30

Stop time:	6.0 minutes
Sample tray temperature:	15°C
Detection system:	Sciex 5500 QTRAP with Analyst Software
Ionisation:	Positive
Scan type:	MRM
Retention time:	Pydiflumetofen (pydiflumetofen): approximately 3.3 minutes
Ions monitored:	*m/z* 425.9 → 192.9 quantitative *m/z* 425.9 → 166.1 confirmatory

Characterisation of water samples:

Source for surface water: Julian, NC water

Source for ground water: Summerfield, NC water

Sample	**pH**	**Calcium (ppm)**	**Magnesium (ppm)**	**Sodium (ppm)**	**Hardness (mg/L)**	**Conductivity (mmhos/cm)**	**Total Dissolved solids (ppm)**	**Turbidity (NDU)**
Surface	8.6	4.3	2.4	2.0	21	0.05	84	3.79
Ground	7.7	15	4.1	5.1	54	0.12	156	3.03

A summary of the method validation data is given in Table B.5.2.4-2.

Table B.5.2.4-2: Summary of method validation data for the determination of pydiflumetofen residues in ground and surface water by direct injection

Matrix	Analyte	LOQ (µg/L)	Recovery fortification level (µg/L)	Recoveries %* range (mean)	Repeatability % RSD (n)	Linearity
Surface water	pydiflumetofen Primary Transition *m/z* 425.9 → 192.9	0.05	0.05	81 - 95 (89)	6 (5)	0.01 – 5 µg/L (n = 5) y = 980x+480 r = 0.9993
			0.50	94 - 121 (107)	9 (5)	
			Overall	81 -121 (98)	12 (10)	
	pydiflumetofen Confirmatory Transition *m/z* 425.9 → 166.1	0.05	0.05	72 - 101 (86)	12 (5)	As above y = 997x+504 r = 0.9983
			0.50	102 - 111 (107)	4 (5)	
			Overall	72 - 111 (97)	14 (10)	
Ground water	pydiflumetofen Primary Transition *m/z* 425.9 → 192.9	0.05	0.05	82 - 112 (97)	13 (5)	0.01 – 5 µg/L (n = 5) y = 980x+480 r = 0.9993
			0.50	99 - 115 (106)	7 (5)	
			Overall	82 - 115 (102)	10 (10)	
	pydiflumetofen Confirmatory Transition *m/z* 425.9 → 166.1	0.05	0.05	98 – 111 (106)	5 (5)	As above y = 997x+504 r = 0.9983
			0.50	103 - 112 (108)	4 (5)	
			Overall	98 – 112 (107)	4 (10)	

* It is noted the recoveries reported are not true recoveries as for direct injection recovery cannot be determined.

Specificity and Confirmation of Analyte Identity

LC-MS/MS with primary and confirmatory ion transitions is considered a specific technique. Therefore, additional methods to confirm the identity of the analytes are not considered necessary. The ion transitions monitored are appropriate. Chromatograms have been provided showing a retention time match with standards. No significant interference (>30% of the LOQ) at the retention time of interest was observed for surface and ground water.

Linearity

Linearity was demonstrated by the analysis of five standards of increasing concentration. The range of standard concentrations used was 0.01-5 µg/L. The lowest concentration injected was equivalent to 20% of the LOQ of the method and the highest concentration injected was equivalent to 100 x LOQ. The response was linear for both MS/MS transitions with a correlation coefficient ranging from 0.9983-0.9993.

Accuracy and Precision

Accuracy and precision data has been provided for samples using direct aqueous injection only. Ground water and surface water samples were analysed in quintuplet at the limit of quantification (LOQ: 0.05 µg/L) and at 10 x LOQ (0.5 µg/L). Mean recoveries were within the acceptable range (70-120%) and the %RSD was ≤20% at each level. However, it is noted true recovery data is not possible for direct injection and in line with SANCO/825/00 rev. 8.1 only precision data is required. Accuracy and precision data is not required for the SPE procedure as this is only used if the concentration of the sample is too low. However, the method is validated at the LOQ, which is

considerably lower than the lowest effect concentrations. Therefore, it is not considered necessary to validate the method at a lower level.

Matrix effects

Matrix effects were investigated for samples analysed by direct injection and using the SPE procedure. No significant matrix effects (>20%) were observed in surface and ground water for both approaches. Therefore, matrix matched standards were not used.

LOQ

0.05 µg/L for pydiflumetofen in surface and ground water. In accordance with SANCO/825/00 rev 8., for drinking water and groundwater the LOQ must meet 0.1 µg/L. Therefore, this criterion has been met. The LOQ for surface water must also comply with the lowest effect concentration. The effect concentrations for pydiflumetofen are shown in the table below:

	Acute test (µg/L)	**Long-term test (µg/L)**
Fish	LC_{50} = 180	NOEC = 130
Daphnia	EC_{50} = 420	NOEC = 42
Chironomous sp	EC_{50} = 691	NOEC = 351
Algae	EC_{50} = 1600	-
Higher aquatic plants	EC_{50} = 6300*	-

*It is noted the EC_{50} value for higher aquatic plants is not suitable for use in risk assessment.

Therefore, the LOQ is sufficiently low.

Storage Stability of Extracts

The stability of pydiflumetofen in the injection solutions was assessed in the independent laboratory validation and not tested in this study. Residues of pydiflumetofen were demonstrated to be stable in surface and ground water when stored at 4°C for at least 7 days. Samples were fortified at the LOQ level (0.05 µg/L) and 10 x LOQ level (0.5 µg/L) and the method validation data (day 0) and day 7 recoveries were compared. After 7-days the mean recoveries were between 70-110% with a %RSD ≤20%. Overall, there was no significant difference between the day 0 and day 7 results. It should be noted storage stability data has only been provided for sample analysis by direct injection. The SPE procedure uses a different solvent (acidic methanol vs acetonitrile/water for direct injection). However, acidic methanol is dried off and the sample is reconstituted with acetonitrile/water before injection. Therefore, a separate storage stability consideration for analysis using the SPE procedure is not required.

Conclusion

GRM061.01A method is acceptably validated in accordance with SANCO/825/00 rev. 8.1 for the determination of pydiflumetofen residues via LC-MS/MS in surface water and ground water with an LOQ of 0.05 µg/L.

Report:	**KCA1 4.2/8 [redacted] (2013)**
Title	SYN545974: Independent Laboratory Validation of Residue Method (GRM061.01A) for the Determination of SYN545974 in Water Report number: 2386W (Document No.: VV-414597)
Guidelines:	SANCO/825/00 rev.8.1 and SANCO/3029/99 rev. 4
GLP:	Yes
Deviations	N/A
Previous evaluation:	None

The primary method (GRM061.01A) to determine residues of pydiflumetofen in surface and ground water was independently validated by PTRL West (a division of EAG Inc.), Hercules, USA. This is a different test facility to the primary method.

Quantification was performed using LC-MS/MS with two ion transitions (quantitative transition *m/z* 425.9 → 192.9 and confirmatory transition *m/z* 425.9 → 166.1). The LC-MS/MS conditions and sample preparation are identical to the primary method, except for the solvent used in the mobile phase. Due to analyst error, the LC-MS/MS analysis was conducted with acetic acid instead of formic acid. This deviation is expected to have no

negative impact on the study. Furthermore, the SPE procedure was not required for the independent laboratory validation.

Characterisation of water samples:

Source for surface water: Julian, NC water

Source for ground water: Summerfield, NC well water

Sample	**pH**	**Calcium (ppm)**	**Magnesium (ppm)**	**Sodium (ppm)**	**Hardness (mg/L)**	**Conductivity (mmhos/cm)**	**Total Dissolved solids (ppm)**	**Turbidity (NDU)**
Surface	7.3	6.0	2.9	2.3	27	0.08	58	10.4
Ground	7.5	16	4.5	6.4	59	0.15	122	0.28

A summary of the method validation data is given in Table B.5.2.4-3.

Table B.5.2.4-3: Summary of independent laboratory validation data for the determination of pydiflumetofen residues in ground and surface water by direct injection

Matrix	**Analyte**	**LOQ (µg/L)**	**Recovery fortification level (µg/L)**	**Recoveries % range (mean)**	**Repeatability % RSD (n)**	**Linearity**
Surface water	pydiflumetofen Primary Transition *m/z* 425.9 → 192.9	0.05	0.05	80 - 100 (92)	12 (5)	0.02 – 10 µg/L (n = 8) y = 242429x+729 r = 0.9994
			0.50	94 - 102 (96)	3 (5)	
			Overall	80 -102 (94)	8 (10)	
	pydiflumetofen Confirmatory Transition *m/z* 425.9 → 166.1	0.05	0.05	100 - 100 (100)	0 (5)	As above y = 136263x+689 r = 0.9992
			0.50	92 - 102 (97)	4 (5)	
			Overall	92 - 102 (98)	3 (10)	
Ground water	pydiflumetofen Primary Transition *m/z* 425.9 → 192.9	0.05	0.05	80 - 100 (96)	9 (5)	0.02 – 10 µg/L (n = 8) y = 242429x+729 r = 0.9994
			0.50	94 - 104 (100)	4 (5)	
			Overall	80 - 104 (98)	7 (10)	
	pydiflumetofen Confirmatory Transition *m/z* 425.9 → 166.1	0.05	0.05	80 – 120 (100)	14 (5)	As above y = 136263x+689 r = 0.9992
			0.50	96 - 104 (99)	4 (5)	
			Overall	80 – 120 (99)	10 (10)	

Specificity and Confirmation of Analyte Identity

LC-MS/MS with primary and confirmatory ion transitions is considered a highly specific technique. Therefore, additional methods to confirm the identity of the analytes are not considered necessary. The ion transitions monitored are appropriate. Chromatograms have been provided showing a retention time match with standards.

No significant interference (>30% of the LOQ) at the retention time of interest was observed for surface and ground water.

Linearity

Linearity was demonstrated by the analysis of eight standards of increasing concentration. The range of standard concentrations used was 0.02-10 µg/L. The lowest concentration injected was equivalent to 40% of the LOQ of the method and the highest concentration injected was equivalent to 200 x LOQ. However, in accordance with SANCO/825/00 rev. 8.1 the linear range should cover 30% of the LOQ to 20% above the highest level. The response was linear for both MS/MS transitions with a correlation coefficient ranging from 0.9992-0.9994.

Accuracy and Precision

Accuracy and precision data has been provided for samples analysed by direct injection. The SPE procedure mentioned in the primary method was not required in the ILV method. Ground water and surface water samples were analysed in quintuplet at the limit of quantification (LOQ: 0.05 µg/L) and at 10 x LOQ (0.5 µg/L). Mean recoveries were within the acceptable range (70-110%) and the %RSD was ≤20% at each level. However, it is noted true recovery data is not possible for direct injection and in line with SANCO/825/00 rev. 8.1 only precision data is required.

Matrix effects

No significant matrix effects (>20%) were observed in surface and ground water. Therefore, matrix matched standards were not used.

LOQ

0.05 µg/L for pydiflumetofen in surface and ground water. In accordance with SANCO/825/00 rev 8., for drinking water and groundwater the LOQ must meet 0.1 µg/L. Therefore, this criterion has been met. The LOQ for surface water must also comply with the lowest effect concentration. The effect concentrations for pydiflumetofen are shown in the table below:

	Acute test (µg/L)	**Long-term test (µg/L)**
Fish	LC_{50} = 180	NOEC = 130
Daphnia	EC_{50} = 420	NOEC = 42
Chironomous sp	EC_{50} = 691	NOEC = 351
Algae	EC_{50} = 1600	-
Higher aquatic plants	EC_{50} = 6300*	-

*It is noted the EC_{50} value for higher aquatic plants is not suitable for use in risk assessment.

Therefore, the LOQ is sufficiently low.

Storage Stability

Residues of pydiflumetofen were demonstrated to be stable in surface and ground water when stored at 4°C for at least 7 days. Samples were fortified at the LOQ level (0.05 µg/L) and 10 x LOQ level (0.5 µg/L) and the method validation data (day 0) and day 7 recoveries were compared. After 7 days the mean recoveries were between 70-110% with a %RSD ≤20%. Overall, there was no significant difference between the day 0 and day 7 results.

Conclusion

Method GRM061.01A is acceptably independently validated in accordance with SANCO/825/00 rev. 8.1 for the determination of pydiflumetofen residues in surface water and ground water with an LOQ of 0.05 µg/L. A minor deviation is noted for linearity in accordance with SANCO/825/00 rev. 8.1 as the linear range does not extend to 30% of the LOQ. However, the primary method validation data includes linearity data that covers 20% of the LOQ level. Therefore, it is considered that the method has been acceptably validated overall.

B.5.2.5. Methods for residues in air

Summary Overview:

Method GRM061.11A has been proposed as the enforcement method for residues in air. The method of analysis has been validated for the parent active substance (pydiflumetofen).

Table B.5.2.5-1: Summary of analytical methods for monitoring residues of pydiflumetofen in air

Reference	Author	Study Scope	Method	Method Reference	Limit of quantification ($\mu g/m^3$)
KCA1 4.2/3 KCA1 4.2/2	[redacted]	Analytical method and primary validation study in air	LC-MS/MS	GRM061.11A	30

Report:	**KCA1 4.2/3, [redacted] (2016)**
Title	SYN545974 – Analytical Method GRM.061.11A for the Determination of SYN545974 in Air Report number: GMR061.11A (Document no.: VV-132630)
Guidelines:	SANCO/825/00 rev.8.1
GLP:	No
Deviations	N/A
Previous evaluation:	None

Report:	**KCA1 4.2/2, [redacted] (2016a)**
Title	SYN545974 – Validation of an analytical Method for the Determination in Air Report number: S15-03698 (Document no.: VV-415636)
Guidelines:	SANCO/825/00 rev.8.1
GLP:	Yes
Deviations	N/A
Previous evaluation:	None

Method GRM061.11A was developed and validated for the determination of pydiflumetofen in air.

Reference items:

pydiflumetofen, batch AMS 1432/1, purity 99.5 %w/w, CoA provided, expiry December 2017

Sample preparation:

Air is drawn through an OVS-XAD-2 air sampling tube containing two layers of adsorbent. Ambient air is sucked from the climatized chamber at a rate of 0.5 L/minute at 35°C ± 2 °C and ≥80% relative humidity for a period of up to six hours (180 L of air sampled), using a pre-calibrated motorised pump. The front bed (including front plug) is transferred to an amber vial. The back-up bed is transferred to a second amber vial, if necessary. The adsorbent material is desorbed with 10 mL acetone by shaking on a flatbed shaker for 60 mins at 150 rpm and a temperature of around 20°C. Samples are diluted by a factor of 400 with acetonitrile/water (1/1, v/v). The samples are then analysed using high-performance liquid chromatography with MS/MS detection using two mass transitions (LC-MS/MS).

In the case of recoveries, method GRM061.11A was validated in air samples by fortifying the OVS-XAD-2 tubes with pydiflumetofen at the proposed limit of quantification (LOQ) of the method (30 $\mu g/m^3$, equivalent to 5.4 µg/tube) and at 10 x the LOQ (300 $\mu g/m^3$, equivalent to 54 µg/tube) and then drawing air (conditioned at 35 °C with a relative humidity ≥80%) through the tubes for six hours at a rate of 0.5 L/minute.

LC-MS/MS conditions:

Chromatographic system:	Shimadzu Nexera X2 UPLC System (LC-30 AD pumps, SIL 30 AC Autosampler, column oven and vacuum solvent degasser)
Pre-column	HPLC guard column (KJ0-4282, Phenomenex) with C18 cartridge (AJ0-4287, Phenomenex)
Analytical column:	Agilent Pursuit XRs Diphenyl, No. A6021100X046, 100 mm x 4.6 mm, 3 µm
Column oven temperature:	40°C
Injection volume:	2 µL
Mobile phase A:	Water + 0.1% v/v acetic acid
Mobile phase B:	Methanol + 0.1% v/v acetic acid
Flow rate:	500 µL/minute

Gradient:

Time (minute)	% A	% B
0.00	70	30
5.00	0	100
7.50	0	100
7.60	70	30
10.60	70	30

Divert valve:	0.0 min to 7.0 min to waste; 7.0 min to 8.5 min to MS; 8.5 min to 10.6 min to waste
Detection system:	API 5500™ LC/MS/MS System (AB Sciex)
Ionisation:	Electrospray, positive
Scan type:	MRM
Retention time:	Pydiflumetofen (SYN545974): approximately 7.5 minutes
Ions monitored:	*m/z* 425.9 → 192.9 quantitative *m/z* 425.9 → 166.1 confirmatory

A summary of the method validation data is given in Table B.5.2.5-2.

Table B.5.2.5-2: Summary of method validation data for the determination of pydiflumetofen residues in air

Matrix	**Analyte**	**LOQ (µg/m³)**	**Recovery fortification level (µg/m³)**	**Recoveries % range (mean)**	**Repeatability % RSD (n)**	**Linearity**
Air	pydiflumetofen Primary Transition *m/z* 425.9 → 192.9	30	30	100 - 108 (103)	3 (5)	0.2 – 25 ng/mL (equivalent to 4.44 -556 µg/m³) (n = 7) y = 4.25e+4x-313 r = 0.9999
			300	99 - 104 (101)	2 (5)	
			Overall	99 -108 (102)	3 (10)	
	pydiflumetofen Confirmatory Transition *m/z* 425.9 → 166.1	30	30	100 - 110 (105)	4 (5)	As above y = 2.74e+4x-772 r = 0.9999
			300	97 - 103 (101)	3 (5)	
			Overall	97 - 110 (103)	4 (10)	

Specificity and Confirmation of Analyte Identity

LC-MS/MS with primary and confirmatory ion transitions is considered a highly specific technique. Therefore, additional methods to confirm the identity of the analytes are not considered necessary. The ion transitions monitored are appropriate. Chromatograms have been provided showing a retention time match with standards. No significant interference (>30% of the LOQ) at the retention time of interest was observed for air.

Linearity

Linearity was demonstrated by the analysis of seven standards of increasing concentration. The range of standard concentrations used was 0.2-25 ng/mL, equivalent to 4.44-556 µg/m^3. This covers the range from no more than 30% of the LOQ to at least +20% of the highest analyte concentration (10 x LOQ). The response was linear for both MS/MS transitions with a correlation coefficient of 0.9999.

Accuracy and Precision

The tubes were fortified with pydiflumetofen at the proposed limit of quantification (LOQ, 30 µg/m^3) and at 10 x LOQ (300 µg/m^3) and then air was drawn through the tubes (conditioned at 35°C with a relative humidity ≥80%) for six hours at a rate of 0.5 L/minute. Five samples were prepared at each level. Mean recoveries were within the acceptable range (70-110%) and the %RSD was ≤20% at each level.

Matrix effects

Matrix effects were not investigated for air as this is not required. Nevertheless, the recoveries in the fortified samples were within the acceptable range and therefore matrix effects can be expected to be insignificant. Matrix matched standard were not used for quantification.

LOQ

The LOQ is 30 µg/m^3 for pydiflumetofen in air. No limit is established for Pydiflumetofen according to Council Directive 98/24/EC. Therefore, the LOQ should comply with the concentration calculated from the $AOEL_{inhalation}$.

Using the AOEL of 0.05 mg/kg bw/day, the concentration 'c' is calculated as 15 µg/m^3. Hence, the LOQ of 30 µg/m^3 is not acceptable. **Therefore, further method validation data is required to support the lower LOQ.**

Storage Stability

Stability was confirmed for pydiflumetofen in the following matrices:

- Calibration solutions when stored at 1-10°C for at least 9 days
- Stock solutions when stored at 1-10°C for at least 36 days
- Other working solutions when stored at 1-10°C for at least 35 days
- Final extracts when stored at 1-10°C for at least 8 days
- Tubes stored when stored at ambient temperature, in a refrigerator (1-10°C) and in a freezer (≤-18°C) for at least 8 days.

Extractability

Acceptable recoveries of pydiflumetofen demonstrate acceptable extractability of the analyte from the sorbent.

Determination of Breakthrough

For the determination of breakthrough, the front and back beds of the absorber tubes were analysed separately. No detectable residues of pydiflumetofen were found in the back sections of the cartridges for the 300 µg/m^3 fortification level at a flow rate of 180 L within 6 hours for both MS/MS transitions. This indicates that "break-through" did not occur under the analysis conditions demonstrating acceptable retention capacity.

Conclusion

Method GRM061.11A is not acceptably validated in accordance with SANCO/825/00 rev. 8.1 for the determination of pydiflumetofen residues via LC-MS/MS in air with an LOQ of 30 µg/m^3. The LOQ is not considered to be sufficiently low enough. The LOQ must comply with 15 µg/m^3. **Further validation data will be required to support a lower LOQ.**

B.5.2.6. Methods for residues in body fluids and tissues

No specific methods were submitted. The applicant has stated that methods for the analysis of pydiflumetofen and 2,4,6-trichlorphenol residues in milk and blood are available (methods QuEChERS and GRM061.07A, see B.5.2.2)

B.5.3. REFERENCES RELIED ON

Data Point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previous evaluation
KCA 4.1.1/2	[redacted]	2015	SYN545974 - Analytical Method SA-97/1 Report No. 300029020 Document No. VV-128116, SYN545974_10168 Test Facility Syngenta Crop Protection Not GLP Unpublished	N	Y	The study is necessary for this regulatory decision and is eligible for data protection	SYN	N
KCA 4.1.1/3	[redacted]	2015a	SYN545974 - Validation of Analytical Method SA-97/1 Report No. CHMU140778 Document No. VV-410836, SYN545974_10148 Test Facility Syngenta Crop Protection GLP Unpublished	N	Y	The study is necessary for this regulatory decision and is eligible for data protection	SYN	N
KCA1 7.1.2.2.16	[redacted]	2015	SYN545974 – Bare Soil Plot Soil Dissipation Study in Italy in 2013-2015 Report No. S13-02241-FINAL Document No. VV-413311, A19649B_10167 Test Facility Eurofins Agroscience Services GmbH GLP Unpublished	N	Y	The study is necessary for this regulatory decision and is eligible for data protection	SYN	N

Data Point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previous evaluation
KCA1 7.1.2.2.12	[redacted]	2015a	SYN545974 – Bare Soil Plot Dissipation Study in Northern France in 2013 - 2015 Report No. S13-02238-FINAL Document No. VV-413312, A19649B_10168 Test Facility Eurofins Agroscience Services GmbH GLP Unpublished	N	Y	The study is necessary for this regulatory decision and is eligible for data protection	SYN	N
KCA1 7.1.2.2.13	[redacted]	2015 b	SYN545974 – Bare Soil Plot Dissipation Study in Southern France in 2013 - 2015 Report No. S13-02239-FINAL Document No. VV-413238, A19649B_10170 Test Facility Eurofins Agroscience Services GmbH GLP Unpublished	N	Y	The study is necessary for this regulatory decision and is eligible for data protection	SYN	N
KCA1 7.1.2.2.14	[redacted]	2015c	SYN545974 – Bare Soil Plot Dissipation Study in Spain in 2013 - 2015 Report No. S13-02240-FINAL Document No. VV-413239, A19649B_10171 Test Facility Eurofins Agroscience Services GmbH GLP Unpublished	N	Y	The study is necessary for this regulatory decision and is eligible for data protection	SYN	N

Data Point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previous evaluation
KCA1 7.1.2.2.11	[redacted]	2015 d	SYN545974 – Bare Soil Plot Dissipation Study in Germany in 2013 - 2015 Report No. S13-02237-FINAL Document No. VV-413308, A19649B_10166 Test Facility Eurofins Agroscience Services GmbH GLP Unpublished	N	Y	The study is necessary for this regulatory decision and is eligible for data protection	SYN	N
KCA1 7.1.2.2.15	[redacted]	2015e	SYN545974 – Bare Soil Plot Dissipation Study in UK in 2013 - 2015 Report No. S13-02236-FINAL Document No. VV-413240, A19649B_10172 Test Facility Eurofins Agroscience Services GmbH GLP Unpublished	N	Y	The study is necessary for this regulatory decision and is eligible for data protection	SYN	N
KCA1 7.1.2.2.02	[redacted]	2019 b	SYN545974 – Soil Dissipation Study in Germany in 2016-2017 Report No. S16-01816 Document No. VV-719200 Test Facility Eurofins Agroscience Services GmbH GLP Unpublished	N	Y	The study is necessary for this regulatory decision and is eligible for data protection	SYN	N

Data Point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previous evaluation
KCA1 7.1.2.2.06	[redacted]	2020 b	SYN545974 – Soil Dissipation Study in Northern France in 2016-2017 Report No. S16-02708 Document No. VV-856218 Test Facility Eurofins Agroscience Services GmbH GLP Unpublished	N	Y	The study is necessary for this regulatory decision and is eligible for data protection	SYN	N
KCA1 7.1.2.2.08	[redacted]	2020 d	SYN545974 – Soil Dissipation Study in Southern France in 2016-2017 Report No. S16-02711 Document No. VV-856216 Test Facility Eurofins Agroscience Services GmbH GLP Unpublished	N	Y	The study is necessary for this regulatory decision and is eligible for data protection	SYN	N
KCA1 7.1.2.2.07	[redacted]	2020e	SYN545974 – Soil Dissipation Study in Portugal in 2016-2017 Report No. S16-02712 Document No. VV-856212 Test Facility Eurofins Agroscience Services GmbH GLP Unpublished	N	Y	The study is necessary for this regulatory decision and is eligible for data protection	SYN	N

Data Point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previous evaluation
KCA1 7.1.2.2.1-09	[redacted]	2020	SYN545974 - Additional Soil Sampling and Analysis at Five Historical Field Dissipation Sites in Northern Germany, Northern France, and UK in 2020. Report No. S20-06491, Document No. S20-06491 Test Facility Eurofins Agroscience Services GmbH GLP Unpublished	N	Y	The study is necessary for this regulatory decision and is eligible for data protection	SYN	N
KCA1 4.1.2/03	[redacted]	2014	SYN545974 - Supplementary Validation of the Assay for the determination of SYN545974 in VRF-1 Fine Ground Rodent Diet Report No. BFI0231 Document No.: TK0103654 Test facility Sequani Limited GLP Unpublished	N	Y	The study is necessary for this regulatory decision and is eligible for data protection	SYN	N
KCA1 4.1.2/04	[redacted]	2014a	SYN545974 - Validation of the Formulation Procedure for SYN545974 in VRF-1 Fine Ground Rodent Diet and Assessment of Formulation Stability Report No.: BFI0232 Document No.: VV-410268, SYN545974_10109 Test Facility Sequani Limited GLP Unpublished	Y	Y	The study is necessary for this regulatory decision and is eligible for data protection	SYN	N

Data Point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previous evaluation
KCA1 4.1.2/10	[redacted]	2013	SYN545974 – Validation of the Assay for the Determination of SYN545974 in R&M No. 3 Fine Ground Diet Report No. BFI0111 Document No. VV-404895, SYN545974_10079 Test Facility Sequani Limited GLP Unpublished	N	Y	The study is necessary for this regulatory decision and is eligible for data protection	SYN	N
KCA1 4.1.2/06	[redacted]	2012	SYN545974 - Validation of the Assay for the Determination of SYN545974 in 1 % w/v Aqueous Carboxymethylcellulose Report No. BFI0048 Document No. VV-402591, SYN545974_10019 Test Facility Sequani Limited GLP Unpublished	N	Y	The study is necessary for this regulatory decision and is eligible for data protection	SYN	N
KCA1 4.1.2/07	[redacted]	2012	SYN545974 - Validation of the Formulation Procedure of SYN545974 in 1 % w/v Aqueous Carboxymethylcellulose and Assessment of Formulation Stability Report No. BFI0049 Document No. VV-402593, SYN545974_10020 Test Facility Sequani Limited GLP Unpublished	N	Y	The study is necessary for this regulatory decision and is eligible for data protection	SYN	N

Data Point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previous evaluation
KCA1 4.1.2/08	[redacted]	~~2021~~ 2020	CA6519 - Validation of the Formulation Procedure for CA6519 in Corn Oil and Assessment of Formulation Stability Report No. BFI1026 Document No. VV-884148 Test Facility Sequani Limited GLP Unpublished	N	Y	The study is necessary for this regulatory decision and is eligible for data protection	SYN	N
KCA1 4.1.2/09	[redacted]	~~2021~~ 2020	CA6519 - Validation of the Assay for the Determination of CA6519 in Corn Oil Report No. BFI1024 Document No. VV-884147 Test Facility Sequani Limited GLP Unpublished	N	Y	The study is necessary for this regulatory decision and is eligible for data protection	SYN	N
KCA1 4.1.2/01	[redacted]	2021	CA6519 - Validation of an Analytical Method Using HPLC Report No. AG23LM.GTCHEM.BTL; tk0527779 Document No. AG23LM.GTCHEM.BTL Test facility BioReliance Corporation GLP Unpublished	N	Y	The study is necessary for this regulatory decision and is eligible for data protection	SYN	N

Data Point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previous evaluation
KCA1 4.1.2/22	[redacted]	2012	SYN545974 - Validation of Methodologies for the Analysis of SYN545974 in Dietary Formulations Report No. 32657 Document No. VV-400860, SYN545974_10006 Test Facility Charles River Laboratories GLP Unpublished	N	Y	The study is necessary for this regulatory decision and is eligible for data protection	SYN	N
KCA1 4.1.2/23	[redacted]	2013	SYN545974 - Validation of Methodologies for the Analysis of SYN545974 in RM1 Dietary Formulations Report No. 33720 Document No. VV-405904, SYN545974_10087 Test Facility Charles River Laboratories GLP Unpublished	N	Y	The study is necessary for this regulatory decision and is eligible for data protection	SYN	N
KCA1 4.1.2/14	[redacted]	2012	SYN545974 - Validation of an Analytical Method for the Determination of SYN545974 in Rat and Mouse Blood by LC-MS/MS Report No. 33236 Document No. VV-402650, SYN545974_10009 Test Facility Charles River Laboratories GLP Unpublished	Y	Y	The study is necessary for this regulatory decision and is eligible for data protection	SYN	N

Data Point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previous evaluation
KCA1 4.1.2/05	[redacted]	2013	SYN545974 - Partial Validation of a Bioanalytical Method for the Determination of SYN545974 in Rabbit Blood Water Report No.: BFI0127 Document No.: VV-415358, SYN545974_10372 Test Facility Sequani Limited GLP Unpublished	Y	Y	The study is necessary for this regulatory decision and is eligible for data protection	SYN	N
KCA1 4.1.2/33	[redacted]	2018	SYN508272 – Validation of a Bioanalytical Method for the Determination of SYN508272 in Rat Blood: Water [1:1 (v/v)] by LC-MS/MS Report No. 0057/002 Document No. VV-469573, SYN508272_10924 Test Facility Alderley Analytical, The BioHub, Alderley Edge, Cheshire, UK GLP Unpublished	N	Y	The study is necessary for this regulatory decision and is eligible for data protection	SYN	N
KCA1 4.1.2/18	[redacted]	2021	The Validation of a Bioanalytical Method for the Determination of 2,4,6 -trichlorophenol in Rat Whole Blood (K2EDTA) by LC-MS/MS Report No. 0029/027 Document No. VV-899602 Test Facility Alderley Analytical GLP Unpublished	N	Y	The study is necessary for this regulatory decision and is eligible for data protection	SYN	N

Data Point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previous evaluation
KCA1 4.1.2	[redacted]	2015	SYN545974 - Analytical Method for Determination of SYN545974 in Crops by LCMS/ MS with Validation Data Report No. GRM061.03A Document No. VV-618773, SYN545974_50054 Test Facility Syngenta Crop Protection Not GLP Unpublished	N	N	N/A	SYN	N
KCA1 4.1.2	[redacted]	2015	SYN545974 – Validation of the Syngenta Method GRM061.03A for the Determination of Residues of SYN545974 in Crop Matrices Report No. S14-05352 Document No. VV-412456, SYN545974_10180 Test Facility Eurofins Agroscience Services Chem GmbH GLP Unpublished	N	Y	The study is necessary for this regulatory decision and is eligible for data protection	SYN	N
KCA1 6.1	[redacted]	2015	SYN545974 - Storage Stability in Crops Stored Frozen for up to 23 months Report No. S13-02224 Document No. VV-414120, SYN545974_10278 Test Facility Eurofins Agroscience Services Chem GmbH GLP Unpublished	N	Y	The study is necessary for this regulatory decision and is eligible for data protection	SYN	N

Data Point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previous evaluation
KCA1 6.3.14	[redacted]	2017	SYN545974 - Residue Study on Barley in North France, Germany, Poland, Hungary and the UK in 2016 Report No. 38034 Document No. VV-467584, A21857B_10013 Test Facility Charles River Laboratories GLP Unpublished	N	Y	The study is necessary for this regulatory decision and is eligible for data protection	SYN	N
KCA1 6.5.3	[redacted]	2017	SYN545974 - Residue Study on Barley and Processed Specimens in Northern France, Germany and Poland in 2013 Report No. S13-02518 Document No. VV-463141, A17573A_10004 Test Facility Eurofins Agroscience Services Chem GmbH GLP Unpublished	N	Y	The study is necessary for this regulatory decision and is eligible for data protection	SYN	N
KCA1 6.3.13	[redacted]	2016	SYN545974 - Residue Study on Oilseed Rape in the United Kingdom and Northern France in 2013 Report No. S13-02259 Document No. VV-415279, A19649B_10230 Test Facility Eurofins Agroscience Services Chem GmbH GLP Unpublished	N	Y	The study is necessary for this regulatory decision and is eligible for data protection	SYN	N

Data Point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previous evaluation
KCA1 6.3.13	[redacted]	2017	SYN545974 - Residue Study on Oilseed Rape and Processed Products in Northern France, Germany and the United Kingdom in 2014 Report No. CEMR-6531 Document No. VV-468119, A19649B_10334 Test Facility CEM Analytical Services, Ltd. GLP Unpublished	N	Y	The study is necessary for this regulatory decision and is eligible for data protection	SYN	N
KCA1 6.3.13	[redacted]	2016	SYN545974 - Residue Study on Oilseed Rape in Southern France, Italy, and Spain in 2013 Report No. S13-02260 Document No. VV-415280, A19649B_10231 Test Facility Eurofins Agroscience Services Chem GmbH GLP Unpublished	N	Y	The study is necessary for this regulatory decision and is eligible for data protection	SYN	N
KCA1 6.3.13	[redacted]	2015	SYN545974 – Residue Study on Oilseed Rape in Southern France, Spain, and Italy in 2014 Report No. CEMR-6532 Document No. VV-412280, A19649B_10106 Test Facility CEM Analytical Services, Ltd. GLP Unpublished	N	Y	The study is necessary for this regulatory decision and is eligible for data protection	SYN	N

Data Point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previous evaluation
Appendix C 3.1.2.04-1	[redacted]	2017	SYN545974 – Residue Study on Carrot in Northern France, Germany, Poland, and the United Kingdom in 2016 Report No. CEMR-7597 Test Facility CEM Analytical Services, Ltd. GLP Unpublished	N	Y	The study is necessary for this regulatory decision and is eligible for data protection	SYN	N
Appendix C 3.1.2.04-2	[redacted]	2017	SYN545974 – Residue Study on Carrot in Southern France, Greece, Spain, and Italy in 2016 Report No. CEMR-7598 Test Facility CEM Analytical Services, Ltd. GLP Unpublished	N	Y	The study is necessary for this regulatory decision and is eligible for data protection	SYN	N
KCA1 6.6.2	[redacted]	2018	Adepydin - Residue Study on Rotational Crops in Northern France and Germany during 2016-2017 Report No. CEMR-7709 Document No. VV-469769, A19649B_10353 Test Facility CEM Analytical Services Ltd GLP Unpublished	N	Y	The study is necessary for this regulatory decision and is eligible for data protection	SYN	N

Data Point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previous evaluation
KCA1 6.6.2	[redacted]	2018	Pydiflumetofen - Residue Study on Rotational Crops in Southern France and Spain during 2016-2017 Report No. CEMR-7710 Document No. VV-470802, A19649B_10359 Test Facility CEM Analytical Services Ltd GLP Unpublished	N	Y	The study is necessary for this regulatory decision and is eligible for data protection	SYN	N
KCA1 6.6.2	[redacted]	2016	SYN545974 – Residue Study on Rotational Crops in Southern France and Italy during 2013 – 2015 Report No. S13-01023 Document No. VV-415410, A19649B_10235 Test Facility Eurofins Agroscience Services Chem GmbH GLP Unpublished	N	Y	The study is necessary for this regulatory decision and is eligible for data protection	SYN	N
KCA1 6.6.2	[redacted]	2016	SYN545974 – Residue Study on Rotational Crops in the United Kingdom and Germany During 2013 - 2014 Report No. S13-01022 Document No. VV-415357, A19649B_10234 Test Facility Eurofins Agroscience Services Chem GmbH GLP Unpublished	N	Y	The study is necessary for this regulatory decision and is eligible for data protection	SYN	N

Data Point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previous evaluation
KCA1 6.10.1	[redacted]	2017	SYN545974 and Fludioxonil – Residues in Honey Following Exposure of Bees to Treated Winter Oilseed Rape in Germany during 2016 Report No. S16-02006 Document No. VV-466889, A8240D_12181 Test Facility Eurofins Agroscience Services EcoChem GmbH GLP Unpublished	N	Y	The study is necessary for this regulatory decision and is eligible for data protection	SYN	N
KCA1 4.1.2	[redacted]	2015	SYN545974 - Analytical Method (GRM061.06A) for the Determination of SYN545974 in Bovine Milk, Liver, Kidney, Muscle, Fat, Blood, and Hen Eggs by LCMS/ MS Report No. GRM061.06A Document No. VV-132524, SYN545974_50123 Test Facility Syngenta Crop Protection Not GLP Unpublished	N	N	N/A	SYN	N
KCA1 4.1.2	[redacted]	2015	SYN545974 – Validation of an Analytical Method for the Determination of SYN545974 in Bovine Meat, Liver, Kidney, Fat, Milk and Chicken Eggs Report No. 36383 Document No. VV-413066, SYN545974_10247 Test Facility Charles River Laboratories GLP Unpublished	N	Y	The study is necessary for this regulatory decision and is eligible for data protection	SYN	N

Data Point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previous evaluation
KCA1 4.1.2	[redacted]	2015	SYN545974 - Analytical Method (GRM061.08A) for the Determination of SYN548264 and SYN508272 in Bovine Milk by LC-MS/MS Report No. GRM061.08A Document No. VV-132522, SYN548264_50000 Test Facility Syngenta Crop Protection Not GLP Unpublished	N	N	N/A	SYN	N
KCA1 4.1.2	[redacted]	2017	SYN545974 – Magnitude of Residues in Milk and Tissues of Dairy Cows Following Multiple Oral Administration of SYN545974 Report No. 35775 Amendment 2 Document No. VV-414196, SYN545974_10288 Test Facility [redacted] GLP Unpublished	Y	Y	The study is necessary for this regulatory decision and is eligible for data protection	SYN	N
KCA1 4.1.2	[redacted]	2015	SYN545974 - Analytical Method (GRM061.09A) for the Determination of Free and Conjugated SYN547897 and SYN548263 in Kidney and Liver by LC-MS/MS Report No. GRM061.09A Document No. VV-132523, SYN547897_50000 Test Facility Syngenta Crop Protection Not GLP Unpublished	N	N	N/A	SYN	N

Data Point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previous evaluation
KCA1 4.2	[redacted]	2015	SYN545974 - Analytical Method (GRM061.07A) for the Determination of Free and Conjugated 2,4,6-trichlorophenol in Bovine Milk, Liver, Kidney, Muscle, Fat, Blood and Hen Eggs by LC-MS/MS Report No. GRM061.07A Document No. VV-132521, SYN545974_50114 Test Facility Syngenta Crop Protection Not GLP Unpublished	N	N	N/A	SYN	N
KCA1 4.1.2	[redacted]	2015	SYN545974 - Validation of the Analytical Method GRM 061.07A for the Determination of Residues of Conjugated 2,4,6-Trichlorophenol in Animal Matrices Report No. PTRL Europe ID P 3613 G Document No. VV-412450, SYN545974_10178 Test Facility PTRL Europe GLP Unpublished	N	Y	The study is necessary for this regulatory decision and is eligible for data protection	SYN	N
KCA1 8.1.1.2-02	[redacted]	2013	SYN545974 – A Dietary LC50 Study with the Northern Bobwhite Report No. 528-391 Document No. VV-404461, SYN545974_10063 Test Facility [redacted] GLP Unpublished	Y	Y	The study is necessary for this regulatory decision and is eligible for data protection	SYN	N

Data Point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previous evaluation
KCA1 8.1.1.2-03	[redacted]	2013	SYN545974 – A Dietary LC50 Study with the Mallard Report No. 528-392 Document No. VV-404462, SYN545974_10064 Test Facility [redacted] GLP Unpublished	Y	Y	The study is necessary for this regulatory decision and is eligible for data protection	SYN	N
KCA1 8.1.1.3-02	[redacted]	2015	SYN545974 – A Reproduction Study with the Northern Bobwhite Report No. 528-396 Document No. VV-410869, SYN545974_10130 Test Facility [redacted] GLP Unpublished	Y	Y	The study is necessary for this regulatory decision and is eligible for data protection	SYN	N
KCA1 8.1.1.3-03	[redacted]	2014	SYN545974 – A Reproduction Study with the Mallard Report No. 528-397 Document No. VV-411097, SYN545974_10134 Test Facility [redacted] GLP Unpublished	Y	Y	The study is necessary for this regulatory decision and is eligible for data protection	SYN	N

Data Point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previous evaluation
KCA1 8.2.1-07	[redacted]	2012	SYN545974 – Acute Toxicity to Rainbow Trout (Oncorhynchus mykiss) Under Flow-Through Conditions Report No. 1781.6840 Document No. VV-402859, SYN545974_10014 Test Facility [redacted] GLP Unpublished	Y	Y	The study is necessary for this regulatory decision and is eligible for data protection	SYN	N
KCA1 8.2.1-05	[redacted]	2013	SYN545974 – Acute Toxicity to Fathead Minnow (Pimephales promelas) Under Flow–Through Conditions Report No. 1781.6883 Document No. VV-404422, SYN545974_10068 Test Facility [redacted] GLP Unpublished	Y	Y	The study is necessary for this regulatory decision and is eligible for data protection	SYN	N
KCA1 8.2.1-04	[redacted]	2013	SYN545974 – Acute Toxicity to Carp (Cyprinus carpio) Under Flow–Through Conditions Report No. 1781.6882 Document No. VV-404432, SYN545974_10066 Test Facility [redacted] GLP Unpublished	Y	Y	The study is necessary for this regulatory decision and is eligible for data protection	SYN	N

Data Point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previous evaluation
KCA1 8.2.1-06	[redacted]	2013	SYN545974 – Acute Toxicity to Sheepshead Minnow (Cyprinodon variegatus) Under Flow-Through Conditions Report No. 1781.6884 Document No. VV-404433, SYN545974_10067 Test Facility [redacted] GLP Unpublished	Y	Y	The study is necessary for this regulatory decision and is eligible for data protection	SYN	N
KCA1 8.2.1-03	[redacted]	2014	SYN545974 – Acute Toxicity to Bluegill Sunfish (Lepomis macrochirus) Under Flow – Through Conditions Report No. 1781.7025 Document No. VV-410863, SYN545974_10129 Test Facility [redacted] GLP Unpublished	N	Y	The study is necessary for this regulatory decision and is eligible for data protection	SYN	N
KCA1 8.2.2.1-03	[redacted]	2020	SYN545974 - Early Life-Stage Toxicity Test with Fathead Minnow (Pimephales promelas) Report No. 1781.6843 incl. amendments Document No. VV-405320, SYN545974_10080 Test Facility [redacted] GLP Unpublished	Y	Y	The study is necessary for this regulatory decision and is eligible for data protection	SYN	N

Data Point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previous evaluation
KCA1 8.2.2.1-04	[redacted]	2015	SYN545974 - Early Life-Stage Toxicity Test with Sheepshead Minnow, Cyprinodon variegatus Report No. 1781.6979 Document No. VV-414304, SYN545974_10293 Test Facility [redacted] GLP Unpublished	Y	Y	The study is necessary for this regulatory decision and is eligible for data protection	SYN	N
KCA1 8.2.4.1-03	[redacted]	2017	SYN545974 – Acute Toxicity to Water Fleas (Daphnia magna) Under Static Conditions Report No. 1781.6839 incl. amendment Document No. VV-402832, SYN545974_10016 Test Facility Smithers Viscient GLP Unpublished	N	Y	The study is necessary for this regulatory decision and is eligible for data protection	SYN	N
KCA1 8.2.4.2-10	[redacted]	2015	SYN545974 – A 48-Hour Static Acute Toxicity Test with the Freshwater Amphipod (Hyalella azteca) Report Number: 528A-287 Test facility Wildlife International GLP Unpublished	N	Y	The study is necessary for this regulatory decision and is eligible for data protection	SYN	N

Data Point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previous evaluation
KCA1 8.2.4.2-11	[redacted]	2016	SYN545974 – Acute toxicity to Mysid (Americamysis bahia), under static conditions Report No. 1781.6838 (Including Amendment 1) Document No. VV-402952, SYN545974_10015 Test Facility Smithers Viscient GLP Unpublished	N	Y	The study is necessary for this regulatory decision and is eligible for data protection	SYN	N
KCA1 8.2.4.2-12	[redacted]	2014	SYN545974 – Toxicity to Eastern Oyster (Crassostrea virginica) Under Flow-Through Conditions Report No. 1781.6885 Document No. VV-407528, SYN545974_10099 Test Facility Smithers Viscient GLP Unpublished	N	Y	The study is necessary for this regulatory decision and is eligible for data protection	SYN	N
KCA1 8.2.5.1-01	[redacted]	2016	SYN545974 – Full Life-Cycle Toxicity Test with Water Fleas, Daphnia magna, Under Static Renewal Conditions Report No. 1781.6842 (Including Amendments 5 and 6) Document No. VV-402673, SYN545974_10017 Test Facility Smithers Viscient GLP Unpublished	N	Y	The study is necessary for this regulatory decision and is eligible for data protection	SYN	N

Data Point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previous evaluation
KCA1 8.2.5.2-02	[redacted]	2015	SYN545974 - Life-Cycle Toxicity Test with Mysids (Americamysis bahia) Report No. 1781.6886 Document No. VV-411300, SYN545974_10167 Test Facility Smithers Viscient GLP Unpublished	N	Y	The study is necessary for this regulatory decision and is eligible for data protection	SYN	N
KCA1 8.2.5.3-01	[redacted]	2015	SYN545547 - A Prolonged Sediment Toxicity Test with the Midge (Chironomus riparius) Using Spiked Sediment Report Number: 528A-286 Test facility Wildlife International GLP Unpublished	N	Y	The study is necessary for this regulatory decision and is eligible for data protection	SYN	N
KCA1 8.2.5.4-03	[redacted]	2015	SYN545974 – 42-Day Toxicity Test Exposing Freshwater Amphipods (Hyalella azteca) to Spiked Sediment Report Number: 1781.6890 Test Facility Smithers Viscient GLP Unpublished	N	Y	The study is necessary for this regulatory decision and is eligible for data protection	SYN	N
KCA1 8.2.5.4-04	[redacted]	2015	SYN545974 - Life-Cycle Toxicity Test Exposing Midges (Chironomus dilutus) to Spiked Sediment Report Number: 1781.6889 Test Facility Smithers Viscient GLP Unpublished	N	Y	The study is necessary for this regulatory decision and is eligible for data protection	SYN	N

Data Point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previous evaluation
KCA1 8.2.5.4-05	[redacted]	2015	SYN545974 - 10-Day Toxicity Test Exposing Estuarine Amphipods (Leptocheirus plumulosus) to a Test Substance Applied to Sediment under Static Conditions Report Number: 1781.7069 Test Facility Smithers Viscient GLP Unpublished	N	Y	The study is necessary for this regulatory decision and is eligible for data protection	SYN	N
KCA1 8.2.6.1-03	[redacted]	2013	SYN545974 – 96-hour Toxicity Test with the Freshwater Green Alga, Pseudokirchneriella subcapitata Report No. 1781.6841 Document No. VV-402845, SYN545974_10013 Test Facility Smithers Viscient GLP Unpublished	N	Y	The study is necessary for this regulatory decision and is eligible for data protection	SYN	N
KCA1 8.2.6.2-01	[redacted]	2013	SYN545974 – Toxicity Test to the Freshwater Blue-Green Alga, Anabaena flos-aquae Report No. 1781.6881 Document No. VV-406480, SYN545974_10091 Test Facility Smithers Viscient GLP Unpublished	N	Y	The study is necessary for this regulatory decision and is eligible for data protection	SYN	N

Data Point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previous evaluation
KCA1 8.2.6.2-03	[redacted]	2015	SYN545974 – 96-Hour Toxicity Test with the Freshwater Diatom, Navicula pelliculosa Report No. 1781.6879 Document No. VV-407284, SYN545974_10097 Test Facility Smithers Viscient GLP Unpublished	N	Y	The study is necessary for this regulatory decision and is eligible for data protection	SYN	N
KCA1 8.2.6.2-02	[redacted]	2014	SYN545974 – 96-Hour Toxicity Test with the Marine Diatom, Skeletonema costatum Report No. 1781.6880 Document No. VV-409188, SYN545974_10105 Test Facility Smithers Viscient GLP Unpublished	N	Y	The study is necessary for this regulatory decision and is eligible for data protection	SYN	N
KCA1 8.2.7-01	[redacted]	2015	SYN545974 – 7-Day Toxicity Test with Duckweed (Lemna gibba) Report No. 1781.6878 Document No. VV-406021, SYN545974_10088 Test Facility Smithers Viscient GLP Unpublished	N	Y	The study is necessary for this regulatory decision and is eligible for data protection	SYN	N

Data Point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previous evaluation
KCA1 8.2.1-02	[redacted]	2015	SYN545547 - Acute Toxicity Test with Rainbow Trout (Oncorhynchus mykiss) under static conditions Report No. 1781.7096 Document No. VV-414084, SYN545547_10001 Test Facility [redacted] GLP Unpublished	Y	Y	The study is necessary for this regulatory decision and is eligible for data protection	SYN	N
KCA1 8.2.4.1-02	[redacted]	2015	SYN545547 - Acute Toxicity to Water Fleas (Daphnia magna) Under Static Conditions Report No. 1781.7095 Document No. VV-413198, SYN545547_10000 Test Facility Smithers Viscient GLP Unpublished	N	Y	The study is necessary for this regulatory decision and is eligible for data protection	SYN	N
KCA1 8.2.6.1-02	[redacted]	2015	SYN545547 – 96-Hour Toxicity Test with the Freshwater Green Alga, Pseudokirchnerierlla subcapitata Report No. 1781.7094 Document No. VV-413967, SYN545547_10002 Test Facility Smithers Viscient GLP Unpublished	N	Y	The study is necessary for this regulatory decision and is eligible for data protection	SYN	N

Data Point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previous evaluation
KCA1 8.2.1-08	[redacted]	2016	SYN548261 - Acute Toxicity to Oncorhynchus mykiss Report No. 3201085 Document No. VV-414937, SYN548261_10002 Test Facility [redacted] GLP Unpublished	Y	Y	The study is necessary for this regulatory decision and is eligible for data protection	SYN	N
KCA1 8.2.4.1-04	[redacted]	2016	SYN548261 - Acute Toxicity to Water Fleas, (Daphnia magna) under Static Conditions Report No. 3201086 Document No. VV-414931, SYN548261_10000 Test Facility Smithers Viscient (ESG) Ltd GLP Unpublished	N	Y	The study is necessary for this regulatory decision and is eligible for data protection	SYN	N
KCA1 8.2.6.1-04	[redacted]	2016	SYN548261 - Inhibition of Growth to the Alga Pseudokirchneriella subcapitata in a 96-hour test Report No. 3201084 Document No. VV-414932, SYN548261_10001 Test Facility Smithers Viscient (ESG) Ltd GLP Unpublished	N	Y	The study is necessary for this regulatory decision and is eligible for data protection	SYN	N

Data Point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previous evaluation
KCA1 8.2.1-01	[redacted]	2009	M700F001 (metabolite of BAS 700 F) - Acute toxicity for rainbow trout Report No. W/09/09|2009/1021591 Document No. VV-401998, CA4312_10909 | CA4312_50005 Test Facility [redacted] GLP Unpublished	Y	Y	The study is necessary for this regulatory decision and is eligible for data protection	SYN	N
KCA1 8.2.4.1-01	[redacted]	2009	M700F001 (metabolite of BAS 700 F) - Daphnia magna, acute immobilization test Report No. 2009/1021592|W/10/09 Document No. VV-401997, CA4312_50006 | CA4312_10908 Test Facility Institute of Industrial Organic Chemistry GLP Unpublished	N	Y	The study is necessary for this regulatory decision and is eligible for data protection	SYN	N
KCA1 8.2.6.1-01	[redacted]	2009	M700F001 (metabolite of BAS 700 F): Pseudokirchneriella subcapitata SAG.61.81 growth inhibition test Report No. 2009/1021593|W/11/09|2009/1102103 Document No. VV-401996, CA4312_10907 Test Facility Institute of Industrial Organic Chemistry GLP Unpublished	N	Y	The study is necessary for this regulatory decision and is eligible for data protection	SYN	N

Data Point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previous evaluation
KCA1 8.2.3-01	[redacted]	2020	Pydiflumetofen - Amphibian Metamorphosis Assay with African Clawed Frog (Xenopus laevis) Report No. 1781.7310 Document No. VV-858948 Test Facility [redacted] GLP Unpublished	Y	Y	The study is necessary for this regulatory decision and is eligible for data protection	SYN	N
KCA1 8.2.3-04	[redacted]	2020	Pydiflumetofen − Fish Short-Term Reproduction Assay with Fathead Minnow (Pimephales promelas) Report No. 1781.7303 Document No. VV-857838 Test Facility [redacted] GLP Unpublished	Y	Y	The study is necessary for this regulatory decision and is eligible for data protection	SYN	N
KCA1 8.2.4.2-01	[redacted]	2015	SYN545974 – Acute toxicity of SYN545974 to Asellus aquaticus Report No. CEA.1644 Document No. VV-414265, SYN545974_10305 Test Facility Cambridge Environmental Assessments GLP Unpublished	N	Y	The study is necessary for this regulatory decision and is eligible for data protection	SYN	N

Data Point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previous evaluation
KCA1 8.2.4.2-02	[redacted]	2015	SYN545974 – Acute toxicity of SYN545974 to Chaoborus crystallinius Report No. CEA.1666 Document No. VV-414780, SYN545974_10341 Test Facility Cambridge Environmental Assessments GLP Unpublished	N	Y	The study is necessary for this regulatory decision and is eligible for data protection	SYN	N
KCA1 8.2.4.2-03	[redacted]	2015	SYN545974 – Acute toxicity of SYN545974 to Chironomus riparius Report No. CEA.1667 Document No. VV-414602, SYN545974_10316 Test Facility Cambridge Environmental Assessments GLP Unpublished	N	Y	The study is necessary for this regulatory decision and is eligible for data protection	SYN	N
KCA1 8.2.4.2-09	[redacted]	2015	SYN545974 – Acute toxicity of SYN545974 to Cloeon dipterum Report No. CEA.1664 Document No. VV-414583, SYN545974_10315 Test Facility Cambridge Environmental Assessments GLP Unpublished	N	Y	The study is necessary for this regulatory decision and is eligible for data protection	SYN	N

Data Point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previous evaluation
KCA1 8.2.4.2-04	[redacted]	2015	SYN545974 – Acute Toxicity of SYN545974 to Crangonx pseudogracilis Report No. CEA.1661 Document No. VV-414266, SYN545974_10306 Test Facility Cambridge Environmental Assessments GLP Unpublished	N	Y	The study is necessary for this regulatory decision and is eligible for data protection	SYN	N
KCA1 8.2.4.2-05	[redacted]	2015	SYN545974 – Acute toxicity of SYN545974 to Cyclops agilis speratus Report No. CEA.1662 Document No. VV-414891, SYN545974_10347 Test Facility Cambridge Environmental Assessments GLP Unpublished	N	Y	The study is necessary for this regulatory decision and is eligible for data protection	SYN	N
KCA1 8.2.4.2-07	[redacted]	2015	SYN545974 – Acute toxicity of SYN545974 to Lumbriculus variegatus Report No. CEA.1642 Document No. VV-414260, SYN545974_10304 Test Facility Cambridge Environmental Assessments GLP Unpublished	N	Y	The study is necessary for this regulatory decision and is eligible for data protection	SYN	N

Data Point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previous evaluation
KCA1 8.2.4.2-06	[redacted]	2015	SYN545974 – Acute toxicity of SYN545974 to Lymnaea stagnalis Report No. CEA.1645 Document No. VV-414259, SYN545974_10303 Test Facility Cambridge Environmental Assessments GLP Unpublished	N	Y	The study is necessary for this regulatory decision and is eligible for data protection	SYN	N
KCA1 8.3.1.3-01	[redacted]	2015	SYN545974 SC (A19649B) – Chronic toxicity to the honeybee larvae Apis mellifera L. under laboratory conditions (in vitro) Report Number: 14 10 48 005 B Test facility BioChem agrar GLP Unpublished	N	Y	The study is necessary for this regulatory decision and is eligible for data protection	SYN	N
KCA1 8.3.1.3-02	[redacted]	2015	SYN545974 SC (A19649B) - A laboratory study to determine the chronic effects on the brood of the honeybee Apis mellifera L. (Hymenoptera: Apidae). Report Number: 037SRFR15C06 Test facility Syntech Research France SAS GLP Unpublished	N	Y	The study is necessary for this regulatory decision and is eligible for data protection	SYN	N

Data Point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previous evaluation
KCA1 8.3.1.3-05	[redacted]	2018	Pydiflumetofen - Effects on the honeybee brood Apis mellifera L. following chronic oral exposure under field conditions Report Number: 17 48 BFB 0001 Test facility BioChem agrar GLP Unpublished	N	Y	The study is necessary for this regulatory decision and is eligible for data protection	SYN	N
KCA1 8.3.1.3-06	[redacted]	2015	SYN545974 - A laboratory study to determine the chronic effects on the brood of the honeybee Apis Report Number: 037SRFR15C07 Test facility Syntech Research France SAS GLP Unpublished	N	Y	The study is necessary for this regulatory decision and is eligible for data protection	SYN	N
KCA1 8.6.2-01	[redacted]	2015	SYN545974 SC (A19649B) - Toxicity Effects on the Vegetative Vigour of Ten Species of Plants Report Number: 528P-116 Test facility Wildlife International GLP Unpublished	N	Y	The study is necessary for this regulatory decision and is eligible for data protection	SYN	N

Data Point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previous evaluation
KCA1 8.6.2-02	[redacted]	2015	SYN545974 SC (A19649B) - Toxicity Effects on the Seedling Emergence of Ten Species of Plants Report Number: 528P-115 Test facility Wildlife International GLP Unpublished	N	Y	The study is necessary for this regulatory decision and is eligible for data protection	SYN	N
KCA1 8.6.2-03	[redacted]	2015	SYN545974 SC (A19649B) - Toxicity Effects on the Seedling Emergence of Ten Species of Plants Report Number: 528P-124 Test facility Wildlife International GLP Unpublished	N	Y	The study is necessary for this regulatory decision and is eligible for data protection	SYN	N
KCA 4.1.2	[redacted]	2017	SYN545974 – Analytical Method ECO_066_03A and Validation for the Determination of SYN545974 in Pollinator Matrices (Pollen, Nectar, Foliage and Flowers) and in Feeding Solutions (Sucrose) from Honeybee Oral Laboratory Studies	N	Y	This study is necessary for this regulatory decision and is eligible for data protection	SYN	N

Data Point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previous evaluation
KCA1 2.2/1	[redacted]	2017	SYN545974 - Vapour Pressure Report No. SMG11739 + Amendments 1&2 Document No. VV-403324, SYN545974_10038 Test Facility Syngenta Biosciences Pvt. Ltd. GLP Unpublished	N	Y	The study is necessary for this regulatory decision and is eligible for data protection	SYN	N
KCA1 2.5/1	[redacted]	2012	SYN545974 - Solubility in water Report No. SMG11737 Document No. VV-402983, SYN545974_10031 Test Facility Syngenta Biosciences Pvt. Ltd. GLP Unpublished	N	Y	The study is necessary for this regulatory decision and is eligible for data protection	SYN	N
KCA 2.6/1	[redacted]	2012	SYN545974 - Solubility in Organic Solvents Report No. SMG11891 Document No. VV-402982, SYN545974_10030 Test Facility Syngenta Biosciences Pvt. Ltd. GLP Unpublished	N	Y	The study is necessary for this regulatory decision and is eligible for data protection	SYN	N

Data Point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previous evaluation
KCA 2.7/6	[redacted]	2012	SYN545974 - Octanol / Water Partition Coefficient Report No. SMG11738 Document No. VV-402984, SYN545974_10032 Test Facility Syngenta Biosciences Pvt. Ltd. GLP Unpublished	N	Y	The study is necessary for this regulatory decision and is eligible for data protection	SYN	N
KCA 2.7/7	[redacted]	2009	CA4312 - Octanol/water partition coefficient Report No. SMG10197 Document No. VV-385571, CA4312_10898 Test Facility Syngenta Biosciences Pvt. Ltd. GLP Unpublished	N	Y	The study is necessary for this regulatory decision and is eligible for data protection	SYN	N
KCA1 4.2	[redacted]	2015	SYN545974 - Validation of the QuEChERS Method for the Determination of Residues of SYN545974 in Crop Matrices by LC-MS/MS Report No. S14-05402 Document No. VV-412200, SYN545974_10174 Test Facility Eurofins Agroscience Services Chem SAS GLP Unpublished	N	Y	The study is necessary for this regulatory decision and is eligible for data protection	SYN	N

Data Point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previous evaluation
KCA1 4.2	[redacted]	2017	Pydiflumetofen - Evaluation of the Extraction Efficiency of two Analytical Methods Used for the Determination of Pydiflumetofen Residues in Crop Matrices Report No. CEMR-8368 Document No. VV-468712, SYN545974_10586 Test Facility CEM Analytical Services Ltd (CEMAS) - Berkshire, UK GLP Unpublished	N	Y	The study is necessary for this regulatory decision and is eligible for data protection	SYN	N
KCA1 4.2	[redacted]	2015	SYN545974 - Independent Laboratory Validation of the QuEChERS Method for the Determination of Residues of SYN545974 in Crop Matrices by LC-MS/MS Report No. S14-05729 Document No. VV-412466, SYN545974_10193 Test Facility Eurofins Agroscience Services Ltd GLP Unpublished	N	Y	The study is necessary for this regulatory decision and is eligible for data protection	SYN	N
KCA1 4.2	[redacted]	2013	SYN545974 - Residue Method for the Determination of SYN545974 in Water Report No. GRM061.01A Document No. VV-132593, SYN545974_50029 Test Facility Syngenta Crop Protection Not GLP Unpublished	N	N	n/a	SYN	N
KCA1 4.2	[redacted]	2016	SYN545974 - Validation of Residue Method (GRM061.01A) for the Determination of SYN545974 in Water Report No. GRM061.01A|TK0290384	N	N	n/a	SYN	N

Data Point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previous evaluation
			Document No. VV-415628, SYN545974_50462 Test Facility Syngenta Crop Protection Not GLP Unpublished					
KCA1 4.2	[redacted]	2013	SYN545974 - Independent Laboratory Validation of Residue Method (GRM061.01A) for the Determination of SYN545974 in Water Report No. 2386W|GRM061.01A Document No. VV-414594, SYN545974_50026 Test Facility PTRL West Inc. GLP Unpublished	N	Y	The study is necessary for this regulatory decision and is eligible for data protection	SYN	N
KCA1 4.2	[redacted]	2016	SYN545974 - Analytical Method GRM061.11A for the Determination of SYN545974 in Air Report No. GRM061.11A Document No. VV-132630, SYN545974_10366 Test Facility Eurofins Agroscience Services EcoChem GmbH Not GLP Unpublished	N	N	n/a	SYN	N
KCA1 4.2	[redacted]	2016	SYN545974 - Validation of an Analytical Method for the Determination in Air Report No. S15-03698 Document No. VV-415636, SYN545974_10365 Test Facility Eurofins Agroscience Services EcoChem GmbH GLP Unpublished	N	Y	The study is necessary for this regulatory decision and is eligible for data protection	SYN	N

Data Point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previous evaluation
KCA1 4.2	[redacted]	2015	SYN545974: Validation of the QuEChERS Method for the Determination of Residues of SYN545974 in Animal Matrices by LCMS/MS Report Number: PTRL Europe ID P 3592 G Test facility PTRL Europe GmbH GLP Unpublished	N	Y	The study is necessary for this regulatory decision and is eligible for data protection	SYN	N
KCA1 4.2	[redacted]	2017	SYN545974 - Independent Laboratory Validation of QuEChERS Method for the Determination of Residues of SYN545974 in Egg and Muscle by LC-MS/MS Report Number: PASC-REP-1467 Test Facility Primera Analytical Solutions Corp. GLP Unpublished	N	Y	The study is necessary for this regulatory decision and is eligible for data protection	SYN	N
KCA1 4.2	[redacted]	2015	SYN545974 - Independent Laboratory Validation of the QuEChERS Method for the Determination of Residues of SYN545974 in Liver and Milk by LC-MS/MS Report Number: CEMR-7055 Test facility CEM Analytical Services Ltd. (CEMAS) GLP Unpublished	N	Y	The study is necessary for this regulatory decision and is eligible for data protection	SYN	N
KCA1 4.2	[redacted]	2015	SYN545974 - Analytical Method (GRM061.07A) for the Determination of Free and Conjugated 2,4,6-trichlorophenol in Bovine Milk, Liver, Kidney, Muscle, Fat, Blood, and Hen Eggs by LC-MS/MS Report Number. GRM061.07A	N	Y	The study is necessary for this regulatory decision and is eligible for data protection	SYN	N

Data Point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previous evaluation
			Test facility Syngenta Crop Protection, LLC GLP Unpublished					
KCA1 4.2	[redacted]	2015	SYN545974 - Validation of the Analytical Method GRM 061.07A for the Determination of Residues of Conjugated 2,4,6-Trichlorophenol in Animal Matrices Report Number: PTRL Europe ID P 3613 G Test facility PTRL Europe GmbH GLP Unpublished	N	Y	The study is necessary for this regulatory decision and is eligible for data protection	SYN	N
KCNA1 4.2	[redacted]	2015	SYN545974 - Independent Lab Validation of the Analytical Method for the Determination of Conjugated 2,4,6-Trichlorophenol in Animal Matrices Report Number: R B5134 Test facility ANADIAG GLP Unpublished	N	Y	The study is necessary for this regulatory decision and is eligible for data protection	SYN	N
KCA1 4.2	[redacted]	2013	SYN545974 – Analytical Method (GRM061.04A) for Determination of SYN545974 in Soil Report Number: GRM061.04A Test facility Syngenta Crop Protection, LLC GLP Unpublished	N	Y	The study is necessary for this regulatory decision and is eligible for data protection	SYN	N
KCA1 4.2	[redacted]	2013	SYN545974 – Independent Laboratory Validation of Residue Method (GRM061.02A) for the Determination of SYN545974 and SYN545547 in Soil Report number: 2387W Test facility PTRL West	N	Y	The study is necessary for this regulatory decision and is eligible for data protection	SYN	N

Data Point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previous evaluation
			GLP Unpublished					