

# **Draft Assessment Report**

## **Evaluation of Active Substances**

Plant Protection Products

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

### **Pydiflumetofen**

#### **Volume 3 – B.5 (PPP) – Miravis Plus**

#### **Methods of Analysis**

Great Britain

June 2023

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## Version History

When	What
October 2022	Initial GB DAR
June 2023	Post Expert Committee on Pesticides (ECP) Independent Scientific Advice (ISA)

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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. Analysis of the plant protection product

##### *B.5.1.1.1. (a) Methods for the determination of the active substance and/or variant in the plant protection product*

<b>Reference:</b>	<b>KCP 5.1.1/01 (2016)</b>
<b>Title:</b>	A21857B - Validation of Analytical Method SF-861/1
<b>Report No.:</b>	SMG14011
<b>Guideline(s):</b>	SANCO/3030/99/rev.4
<b>GLP:</b>	Yes
<b>Previous evaluation:</b>	None

<b>Reference:</b>	<b>KCP 5.1.1/02 (2020)</b>
<b>Title:</b>	SF-861/1- Determination of SYN545974 in EC (062.5) by HPLC
<b>Report No.:</b>	090100b8810a3615
<b>Guideline(s):</b>	SANCO/3030/99/rev.4
<b>GLP:</b>	No
<b>Previous evaluation:</b>	None

#### *Reference items*

SYN545974 (pydiflumetofen) batch AMS1432/1 (99.5% (w/w))

A21857B ('Miravis Plus') batch JEA001-118-001 (Pydiflumetofen 62.5 g/l EC)

#### *Sample preparation*

Weigh 320 mg of the test substance to the nearest 0.1 mg into a 100 mL volumetric flask. Add acetonitrile (90 mL) and sonicate for 5 minutes. Allow to cool to room temperature and make up to volume with acetonitrile. Filter through a 0.45 µm syringe filter, discarding the first 1 mL.

#### *Analytical method*

Samples were analysed by HPLC-UV and quantified against external standards. The following conditions were noted:

<b>Instrument</b>		Agilent 1200 series HPLC system	
<b>Dwell volume</b>		1200 µL	
<b>Column description</b>		Kinetex C18 phase, 100 mm x 4.6 mm i.d, 2.6 µm particle size	
<b>Column temperature</b>		30 °C	
<b>Injection volume</b>		5 µL	
<b>Mobile phase</b>	<b>Time (min)</b>	0.1% (w/w) phosphoric acid in water	% Acetonitrile
	0.0	52	48
	10.0	52	48
	10.1	0	100
	12.0	0	100
	12.1	52	48
	15.0	52	48
<b>Flow rate</b>		1.5 mL/min	
<b>Detector wavelength</b>		230 nm	

**Specificity**

The chromatograms did not show any interference at the signal of pydiflumetofen or any other signals from the solvent, test item or reference item. Confirmation of identity is not required for the active substance in a preparation.

**Linearity**

Linearity was demonstrated by spiking a blank formulation at concentrations between 50–150% of the nominal active substance concentration. Six concentrations were tested in duplicate. The response was linear with a correlation coefficient (r) of 0.99997 ( $y = 0.995x - 0.565$ ).

**Precision (Repeatability)**

Six weighings of the formulation were analysed in duplicate. The mean value was 5.57% (w/w) with a %RSD of 0.34% (n = 6) which is below the acceptable RSD value of 2.07% calculated using the modified Horwitz equation.

**Table 5.1.1/01 Precision data**

Analyte	Mean value (% w/w)	% RSD (n)	Acceptable % RSD
Pydiflumetofen	5.57%	0.34% (6)	2.07%

**Accuracy (Recovery)**

Accuracy was tested by spiking a blank formulation at concentrations between 70–130% of the nominal active substance concentration. Two determinations were made at each level and the mean recovery at each level reported. Recovery values ranged from 99.0–99.6% with a mean overall recovery of 99.2%.

**Table 5.1.1/02 Recovery data**

Fortification level (% of nominal)	Recovery (mean, n = 2)
70	99.2
90	99.1
110	99.6
130	99.0
Mean	99.2

**Limit of Quantification (LOQ)**

Not required.

**Conclusion**

The analytical method is fully validated in accordance with SANCO/3030/99 rev. 4 for the determination of the active substance pydiflumetofen in the formulation A21857B using HPLC-UV.

**B.5.1.2. Methods for the determination of residues*****B.5.1.2.1. Methods in soil, water and any additional matrices used in support of environmental fate studies***

All relevant methods are reported in Volume 3 CA B.5.1.2 of this DAR.

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

All relevant methods are reported in Volume 3 CA B.5.1.2 of this DAR.

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

All relevant methods are reported in Volume 3 CA B.5.1.2 of this DAR.

***B.5.1.2.4. Methods in or on plants, plant products, processed food commodities, food of plant origin and animal origin, feed and any additional matrices used in support of residues studies***

All relevant methods are reported in Volume 3 CA B.5.1.2 of this DAR.

***B.5.1.2.5. Methods in soil, water, sediment, feed and any additional matrices used in support of ecotoxicology studies***

Study overview:

Data point	Author(s)	Year	Title Company Report No.	Conclusion
KCP 10.2.1-01	██████	2019	Pydiflumetofen EC (A21857B) – Toxicity to <i>pseudokirchneriella subcapitata</i> in a 96-Hour Algal Growth Inhibition Test Report number: 160713SF/SPO17425	Acceptable method  LOQ: 0.25 mg test item/L, corresponding to 0.014 mg a.s./L in fish dilution water.
KCP 10.2.1-02	██████	2019	Pydiflumetofen EC (A21857B) – Toxicity to the Water Flea <i>Daphnia magna</i> Straus under Laboratory Conditions (Acute Immobilization Test – Static) Report number: 160713SF/DAI17425	Acceptable method  LOQ: 0.25 mg test item/L, corresponding to 0.014 mg a.s./L in daphnia dilution water.
KCP 10.2.1-03	██████	2019	Pydiflumetofen EC (A21857B) – Acute Toxicity To Fish ( <i>Rainbow trout</i> ), Static, 96 Hours Report number: 160713SF/FAR17425	Acceptable method  LOQ: 0.25 mg test item/L, corresponding to 0.014 mg a.s./L in algae dilution water.  It should be noted a significant decline in the content of the test item is observed after 96 hours.
KCP 10.3.1.5-01	██████	2018	Pydiflumetfen SC (A19649B) – A Semi-Field Study to Evaluate the Side Effects on the Honey bee <i>Apis mellifera</i> L. in Germany in 2017 Report number: 17 48 BTB 0003	Method is validated in in Volume 3 CA B.5.1.2 of this DAR.

Data point	Author(s)	Year	Title Company Report No.	Conclusion
KCP 10.3.1.5-02	██████	2017	Pydiflumetofen SC (A19649B) – A Semi-Field Study to Evaluate Side Effects on Honeybees ( <i>Apis mellifera</i> L.) in <i>Phacelia tanacetifolia</i> in Germany 2016 Report number: S16-00293	Acceptable method  LOQ: 0.005 mg/kg in nectar, pollen, flowers and leaves.
KCP 10.3.1.5-03	██████ ██	2017	Pydiflumetofen SC (A19649B) – A Semi-Field Study to Evaluate Side Effects on Honeybees ( <i>Apis mellifera</i> L.) in <i>Phacelia tanacetifolia</i> in Germany 2016 Report number: S16-04919	See KCP 10.3.1.5-03

<b>Report:</b>	<b>KCP 10.2.1-01 █████ (2019)</b>
<b>Title</b>	Pydiflumetofen EC (A21857B) – Toxicity to <i>Pseudokirchneriella subcapitata</i> in a 96-Hour Algal Growth Inhibition Test Report number: 160713SF/ SPO17425
<b>Guidelines:</b>	SANCO/3029/9 rev. 4
<b>GLP:</b>	Yes
<b>Deviations</b>	N/A
<b>Previous evaluation:</b>	None

<b>Report:</b>	<b>KCP 10.2.1-02 █████ (2019)</b>
<b>Title</b>	Pydiflumetofen EC (A21857B) – Toxicity to the Water Flea <i>Daphnia magna</i> Straus under Laboratory Conditions (Acute Immobilization Test – Static) Report number: 160713SF/ DAI17425
<b>Guidelines:</b>	SANCO/3029/9 rev. 4
<b>GLP:</b>	Yes
<b>Deviations</b>	N/A
<b>Previous evaluation:</b>	None

<b>Report:</b>	<b>KCP 10.2.1-03 █████ (2019)</b>
<b>Title</b>	Pydiflumetofen EC (A21857B) – Acute toxicity to Fish ( <i>Rainbow Trout</i> ), Static, 96 hours Report number: 160713SF/ FAR17425
<b>Guidelines:</b>	SANCO/3029/9 rev. 4
<b>GLP:</b>	Yes
<b>Deviations</b>	N/A
<b>Previous evaluation:</b>	None

The studies that use the following LC-MS/MS method to determine the concentration of pydiflumetofen in the product A21857B are:

- KCP 10.2.1-01: Study 160713SF/ SPO17425
- KCP 10.2.1-02: Study 160713SF/DAI17425
- KCP 10.2.1-03: Study 160713SF/ FAR17425

*Sample preparation:*

The samples and control were diluted by a factor of two with acetonitrile to stabilize them. Further dilutions were made prior to analysis with acetonitrile: HPLC water (50:50).

Study Report	Nominal test item concentration (mg/L)	Dilution factor (including stabilization step (factor 2))
Study 160713SF/ SPO17425  KCP 10.2.1-01	100	200
	31.6	80
	10	20
	3.16	8
	1	2
	Control	2
Study 160713SF/DAI17425  KCP 10.2.1-02	10	20
	5	10
	2.5	4
	1.25	2
	0.625	2
	Control	2
Study 160713SF/ FAR17425  KCP 10.2.1-03	5	10
	2.5	4
	1.25	2
	0.625	2
	0.313	2
	Control	2

*LC-MS/MS conditions:*

Chromatographic system: Acquity UPLC, Waters  
 Analytical column: Acquity UPLC HSS C18 1.8 µm, 50 x 2.1 mm  
 Column temperature: 30°C  
 Injection volume: 10 µL  
 Mobile phase: A: HPLC water containing 1% formic acid  
 B: acetonitrile containing 1% formic acid

Gradient:	2% acetonitrile containing 1% formic acid		
	Time (minutes)	% solvent A	% solvent B
	0.00	70	30
	0.50	70	30
	1.50	5	95
	2.00	5	95
	2.10	70	30
	2.50	70	30

Flow rate: 0.5 mL/minute  
 Retention time: Approximately 1.79 minutes  
 Run time: 2.5 minutes  
 Detector: Mass selective detector, Xevo TQD MS, Acquity UPLC, Waters  
 Ionisation mode: Electrospray positive  
 Scan type: MRM  
 Mass transitions:  $m/z$  426 → 192.9 (primary)  
 $m/z$  426 → 186.1 (confirmatory)

A summary of the method validation data is given in Table 5.1.2.5-1.



Table 5.1.2.5-1: Summary of method validation data for determination of A21857B in fish medium and algae medium

Matrix	Analyte	LOQ (mg test item/L)	Recovery fortification level (mg test item/L)	Recoveries % range (mean)	Repeatability % RSD (n)	Linearity
Algae dilution water	A21857B (active: pydiflumetofen) Primary Transition $m/z$ 426 $\rightarrow$ 192.9	0.25	0.25 (corresponding to 0.014 mg a.s./L)	104 – 107 (105)	1.3 (5)	0.001 – 0.05 mg a.s./L (n = 8) $y = 181x + 99$ $r > 0.99$
			20 (corresponding to 1.12 mg a.s./L)	97 – 103 (101)	2.6 (5)	
			Overall	97 – 107 (103)	2.8 (10)	
	A21857B (active: pydiflumetofen) Confirmatory Transition $m/z$ 426 $\rightarrow$ 186.1	0.25	0.25 (corresponding to 0.014 mg a.s./L)	102 – 110 (106)	2.9 (5)	0.001 – 0.05 mg a.s./L (n = 8) $y = 101x + 44$ $r > 0.99$
			20 (corresponding to 1.12 mg a.s./L)	100 – 105 (103)	2.1 (5)	
			Overall	100 – 110 (104)	3.0 (10)	
Fish dilution water	A21857B (active: pydiflumetofen) Primary Transition $m/z$ 426 $\rightarrow$ 192.9	0.25	0.25 (corresponding to 0.014 mg a.s./L)	91 – 98 (94)	3.6 (3)	Refer to algae dilution water
	A21857B (active: pydiflumetofen) Confirmatory Transition $m/z$ 426 $\rightarrow$ 186.1	0.25	0.25 (corresponding to 0.014 mg a.s./L)	93 – 94 (93)	0.7 (3)	Refer to algae dilution water
Daphnia dilution water	A21857B (active: pydiflumetofen) Primary Transition $m/z$ 426 $\rightarrow$ 192.9	0.25	0.25 (corresponding to 0.014 mg a.s./L)	93 – 101 (97)	4.1 (3)	Refer to algae dilution water
	A21857B (active: pydiflumetofen) Confirmatory Transition $m/z$ 426 $\rightarrow$ 186.1	0.25	0.25 (corresponding to 0.014 mg a.s./L)	93 – 101 (97)	4.1 (3)	Refer to algae dilution water

Table 5.1.2.5-2: Procedural recoveries of A21857B from a toxicity test to *pseudokirchneriella subcapitata* in a 96-hour algal growth inhibition test

Study	Matrix	Analyte	Nominal concentration (mg test item/L)	Sample Interval	% Recovery
Study 160713SF/ SPO17425  KCP 10.2.1-01	Algae dilution water	A21857B (active: pydiflumetofen)	100	0 hours	71
				96 hours	53
			31.6	0 hours	77
				96 hours	79
			10	0 hours	73
				96 hours	78
			3.16	0 hours	82
				96 hours	84
			1	0 hours	82
				96 hours	78

Table 5.1.2.5-3: Procedural recoveries of A21857B from a toxicity test to the water flea under laboratory conditions

Study	Matrix	Analyte	Nominal concentration (mg test item/L)	Sample Interval	% Recovery
Study 160713SF/ DAI17425  KCP 10.2.1-02	Algae dilution water	A21857B (active: pydiflumetofen)	10	0 hours	95
				48 hours	75
			5	0 hours	93
				48 hours	78
			2.5	0 hours	96
				48 hours	74
			1.25	0 hours	99
				48 hours	74
			0.625	0 hours	111
				48 hours	88

Table 5.1.2.5-4: Procedural recoveries of A21857B from an acute toxicity test to fish (*Rainbow trout*)

Study	Matrix	Analyte	Nominal concentration (mg test item/L)	Sample Interval	% Recovery
Study 160713SF/ FAR17425  KCP 10.2.1-03	Fish dilution water	A21857B (active: pydiflumetofen)	5	0 hours	95
				96 hours	75
			2.5	0 hours	93
				96 hours	52
			1.25	0 hours	90
				96 hours	45
			0.625	0 hours	97
				96 hours	54
			0.313	0 hours	94
				96 hours	49

### Specificity

Chromatograms were presented for the lowest calibration standard (0.001 mg a.s./L), control medium at test initiation and termination and test medium at test initiation and termination for both mass transitions. No significant interference was observed at the retention time of interest. It is noted there is a discrepancy in the retention time of pydiflumetofen in study 160713F/DAI17425. The chromatograms for the lowest analytical standard and test item in fresh medium at 0 hours show a retention time of 1.03 minutes. However, the retention time at test termination for the test item in old medium is 1.78 minutes. Furthermore, in the other study reports with the same LC-MS/MS conditions the retention time is 1.78 minutes. The applicant was asked to provide an explanation for the change in the retention time. The applicant has stated it is not possible to state with certainty what led to the different retention times but it is likely the system was not fully equilibrated prior to analysis of the samples, leading to a shorter retention time of 1.03 minutes. Furthermore, the applicant has stated as the identity of the analyte was confirmed via MS/MS with both the quantifier and qualifier ions being monitored, this is not considered to have a negative impact on the study outcome. Therefore, specificity can be considered satisfactorily addressed.

### Linearity

Linearity was demonstrated by the analysis of eight standards of increasing concentration. The range of standard concentrations used was 0.001-0.05 mg a.s./L. The response was linear with a correlation coefficient ( $r$ ) > 0.99. It is noted samples are diluted to within the linear range.

### Accuracy

Fortified samples of algae dilution water were analysed at 0.25 mg test item/L and 20 mg test item/L, corresponding to 0.014 mg a.s./L and 1.12 mg a.s./L respectively. These are equivalent to the LOQ and 80 x LOQ. Fortified samples of fish and daphnia dilution water were also analysed at 0.25 mg test item/L, equivalent to 0.014 mg a.s./L. Five samples were prepared at each fortification level for the algae dilution water and three samples were prepared for the fish and daphnia dilution water. Mean recoveries were within the acceptable range (70-110%). The fortification levels are lower than some of the test concentrations (0.313-100 mg test item/L), however appropriate dilutions were made before analysis. Procedural recoveries at test initiation have been reported in the acceptable range (70-110%). However, after 96 hours in study 160713SF/ FAR17425 procedural recoveries are within the range 45-75%. Therefore, a significant decline in the content of the test item is observed at test termination.

### Precision

Precision was determined from the accuracy recovery data. The %RSD was  $\leq 20\%$  with five samples prepared at each fortification level for algae dilution water and three samples prepared for fish and daphnia dilution water. The applicant has stated the most complex medium in the aquatic toxicology studies is the algae dilution water and was therefore selected exemplarily for the method validation. The method was then checked by the analysis of three samples for the other dilution water. No further precision data is required.

### Matrix effects

Matrix matched standards were not specifically addressed. Solvent based standards have been used for the calibration. This is acceptable as the chromatograms do not seem to show significant interference at the retention time of interest.

### LOQ

The LOQ of the method is 0.25 mg test item/L, corresponding to 0.014 mg a.s./L for algae, fish and daphnia dilution water. 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 algae, fish and daphnia dilution water with an LOQ of 0.25 mg test item/L. Only three recovery determination have been reported for the fish and daphnia dilution water. However, five recovery determinations have been reported for the algae dilution water. The applicant has stated the algae dilution water is the most complex medium and was therefore selected exemplarily for the method validation. This is acceptable. Furthermore, it should be noted a significant decline in the content of the test item was observed after 96 hours in study 160713SF/FAR17425.

<b>Report:</b>	<b>KCP 10.3.1.5-02</b> (2017)
<b>Title</b>	Pydiflumetofen SC (A19649B) – A Semi-Field Study to Evaluate Side Effects on Honeybees ( <i>Apis mellifera</i> L.) in <i>Phacelia tanacetifolia</i> in Germany 2016 Report number: S16-00293
<b>Guidelines:</b>	SANCO/3029/9 rev. 4
<b>GLP:</b>	Yes
<b>Deviations</b>	N/A
<b>Previous evaluation:</b>	None

<b>Report:</b>	<b>KCP 10.3.1.5-03</b> (2017)
<b>Title</b>	Pydiflumetofen SC (A19649B) – A Semi-Field Study to Evaluate Side Effects on Honeybees ( <i>Apis mellifera</i> L.) in <i>Phacelia tanacetifolia</i> in Germany 2016 Report number: S16-04919
<b>Guidelines:</b>	SANCO/3029/9 rev. 4
<b>GLP:</b>	Yes
<b>Deviations</b>	N/A
<b>Previous evaluation:</b>	None

GRM061.12A was used to determine residues of pydiflumetofen in product A19649B in flowers, leaves, nectar and pollen samples.

*Sample preparation:*

Pollen and nectar:

Samples (~100 mg) were weighed into a 2 mL centrifuge tube with silica spheres and 1 mL of acetonitrile was added. The samples were processed in parallel using a Fast-Prep tissue homogeniser or equivalent for 90 seconds at 6.0 m/sec and then centrifuged for 2 minutes at 10,000 rpm. A volume of extract was filtered through an SPE cartridge. 50 µL of internal standard (SYN550022, 0.05 µg/mL) was added to 0.5 mL of sample extract. Furthermore, 450 µL of water were added, giving a final volume of 1 mL. The vial was capped and mixed thoroughly using a vortex mixer. Samples were determined using LC-MS/MS using internal standardisation and matrix matched calibration.

Flowers and leaves:

Samples (~1 g) were weighed into a 15 mL centrifuge tube and a large ceramic bead was added. 10 mL of acetonitrile was added, the samples were processed in parallel using a Fast-Prep tissue homogeniser or equivalent for 90 seconds at 6.0 m/sec and then centrifuged for 2 minutes at 10,000 rpm. 50 µL of internal standard (SYN550022, 0.05 µg/mL) was added to 0.5 mL of sample extract. Furthermore, 450 µL of water were added, giving a final volume of 1 mL. The vial was capped and mixed thoroughly using a vortex mixer. Samples were determined using LC-MS/MS using internal standardisation and matrix matched calibration.

*LC-MS/MS conditions:*

Chromatographic system: HPLC Agilent 1290 Infinity/ Shimadzu HPLC  
Analytical column: Phenomenex Luna C18 (2) 100A, 50 mm x 3.0 mm, 3 µm + C18 guard column  
Column temperature: 25°C  
Injection volume: 30 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	70	30
	0.50	70	30
	1.00	10	90
	1.80	10	90
	2.00	70	30
	3.00	70	30

Flow rate: 1 mL/minute

Internal standard: SYN550022 (stable isotope labelled pydiflumetofen)  
Retention time: Approximately 1.6 minutes  
Detector: MS API 5000/5500/6500  
Ionisation mode: Turbospray positive  
Scan type: MRM  
Mass transitions:

Analyte	MS/MS transition ( <i>m/z</i> )
pydiflumetofen Quantifier	426 → 193
pydiflumetofen Confirmatory	426 → 166
SYN550022 (Internal standard) Primary transition	435 → 199
SYN550022 (Internal standard) Confirmatory	435 → 169

A summary of the method validation data is given in Table 5.1.2.5-7 and 5.1.2.5-8.

Table 5.1.2.5-7: Study S16-00293 - summary of method validation data for determination of pydiflumetofen in product A19649B in flowers, leaves, nectar and pollen matrices

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 <i>m/z</i> 426 → 193	0.005	0.005	75 – 103 (91)	12 (5)	0.075 – 10 ng a.s./mL (equivalent to 0.0015 – 0.2 mg a.s./kg)  (n = 7)  $y = 0.86x - 0.00127$  $r = 0.9996$
			0.1	99 – 107 (104)	4 (3)	
			0.2	84 – 95 (89)	5 (5)	
			Overall	75 – 107 (93)	10 (13)	
Pollen	pydiflumetofen Primary Transition <i>m/z</i> 426 → 193	0.005	0.005	96 – 100 (98)	2 (5)	0.075 – 10 ng a.s./mL (equivalent to 0.0015 – 0.2 mg a.s./kg)  (n = 7)  $y = 0.993x + 0.0144$  $r = 0.9997$
			0.1	98 – 102 (100)	2 (3)	
			50	86 – 93 (90)	3 (5)	
			Overall	86 – 102 (95)	5 (13)	
Flowers	pydiflumetofen Primary Transition <i>m/z</i> 426 → 193	0.005	0.005	84 – 95 (89)	5 (5)	0.075 – 10 ng a.s./mL (equivalent to 0.0015-2 mg a.s./kg)  (n = 7)  $y = 1.1x + 0.00507$  $r = 0.9999$
			0.1	90 – 95 (93)	3 (3)	
			50	84 – 94 (89)	4 (5)	
			Overall	84 – 95 (90)	4 (13)	
Leaves	pydiflumetofen Primary Transition <i>m/z</i> 426 → 193	0.005	0.005	84 – 99 (89)	7 (5)	0.075 – 10 ng a.s./mL (equivalent to 0.0015-2 mg a.s./kg)  (n = 7)  $y = 1.03x + 0.0168$  $r = 0.9999$
			0.1	95 – 98 (96)	2 (3)	
			50	92 – 94 (93)	1 (5)	
			Overall	84 – 99 (92)	5 (13)	

Table 5.1.2.5-8: Study S16-04919 - summary of method validation data for determination of pydiflumetofen in product A19649B in flowers, leaves, nectar and pollen matrices

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 $\rightarrow$ 193	0.005	0.005	95 – 102 (99)	3 (5)	0.075 – 10 ng a.s./mL (equivalent to 0.0015 – 0.2 mg a.s./kg)  (n = 7) $y = 1x + 0.00641$ $r = 0.9996$
			0.5	94 – 101 (98)	3 (5)	
			Overall	94 – 102 (98)	3 (10)	
Pollen	pydiflumetofen Primary Transition $m/z$ 426 $\rightarrow$ 193	0.005	0.005	94 – 101 (96)	3 (5)	0.075 – 10 ng a.s./mL (equivalent to 0.0015 – 0.2 mg a.s./kg)  (n = 7) $y = 1.41x + 0.00959$ $r = 0.9999$
			50	75 – 86 (82)	5 (5)	
			Overall	75 – 101 (89)	9 (10)	
Flowers	pydiflumetofen Primary Transition $m/z$ 426 $\rightarrow$ 193	0.005	0.005	96 – 103 (100)	3 (5)	0.075 – 10 ng a.s./mL (equivalent to 0.0015-2 mg a.s./kg)  (n = 7) $y = 0.938x + 0.0124$ $r = 0.9999$
			50	78 – 97 (90)	9 (5)	
			Overall	78 – 103 (95)	8 (10)	
Leaves	pydiflumetofen Primary Transition $m/z$ 426 $\rightarrow$ 193	0.005	0.005	81 – 92 (87)	5 (5)	0.075 – 10 ng a.s./mL (equivalent to 0.0015-2 mg a.s./kg)  (n = 7) $y = 0.996x + 0.00917$ $r = 0.9999$
			50	79 – 91 (85)	6 (5)	
			Overall	79 – 92 (86)	6 (10)	

### Specificity

Chromatograms were presented for the calibration standard at 0.25 ng a.s./mL (equivalent to 0.005 mg a.s./kg), control, internal standard, fortified samples at the LOQ (0.005 mg a.s./kg) and treated samples for each matrix for the quantitative transition. No significant interference was observed at the retention time of interest. It is noted no chromatograms have been presented for the qualitative transition. This is acceptable as confirmation of analyte identity is not required for pre-registration methods.

### Linearity

Linearity was demonstrated by the analysis of seven standards of increasing concentration. The range of standard concentrations used was 0.075-10 ng/mL, equivalent to 0.0015-2 mg a.s./kg. The response is linear with a correlation coefficient ( $r$ ) > 0.999. It is noted samples are diluted to within the linear range.

### Accuracy

Study S16-00293:

Samples of pollen, flower and leaves were fortified at 0.005 (LOQ), 0.1 (20x LOQ) and 50 mg a.s./kg (10,000 x LOQ). Samples of nectar were fortified at 0.005 (LOQ), 0.1 (20x LOQ) and 0.2 mg a.s./kg (40 x LOQ). Five samples were prepared at each fortification level, except for 0.1 mg/kg where three samples were prepared. Mean recoveries were within the acceptable range (70-110%). The fortification levels are appropriate to the levels of residues of pydiflumetofen detected in the study.

Study S16-04919:

Samples of pollen, flower and leaves were fortified at 0.005 (LOQ) and 50 mg a.s./kg (10,000 x LOQ). Samples of nectar were fortified at 0.005 (LOQ) and 0.5 mg a.s./kg (100x LOQ). Five samples were prepared at each fortification level. Mean recoveries were within the acceptable range (70-110%). The fortification levels are appropriate to the levels of residues of pydiflumetofen detected in the semi-field study.

### Precision

Precision was determined from the accuracy recovery data for pydiflumetofen. The %RSD was  $\leq 20\%$  for each matrix. For study S16-00293, five determinations were prepared at the lowest and highest fortification level and three determinations were made at the middle level (0.1 mg a.s./kg). However, in accordance with SANCO/3029/99 rev. 4 five determination should be made at every fortification level. For study S16-04919, five determination were prepared at each fortification level. Overall, the precision data is sufficient.

### Matrix effects

Two tests were undertaken to investigate matrix effects in both study reports. The first test compared the mean response area of pydiflumetofen to standards in acetonitrile (50/50, v/v). For the second test, the mean peak area ratios of pydiflumetofen to the internal standard were compared with standards in acetonitrile/water (50/50, v/v).

Study S16-00293: significant matrix effects were observed in the first test for flowers, leaves and pollen. No significant matrix effects were observed in the second test for all matrices. Nevertheless, matrix matched standards were used for quantification.

Study S16-004919: significant matrix effects were observed in the first test for nectar and pollen. No significant matrix effects were observed in the second test for all matrices. Nevertheless, matrix matched standards were used for quantification.

### LOQ

The LOQ of the method is 0.005 mg a.s./kg for nectar, pollen, flowers and leaves. 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 product A19649B in nectar, pollen, flower and leaf samples with an LOQ of 0.005 mg a.s./kg.

#### ***B.5.1.2.6. Methods in water, buffer solutions, organic solvents and any additional matrices resulting from the physical and chemical properties tests.***

No method validation required from the physical and chemical properties tests.

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

Methods for post-approval control and monitoring purposes are reported in Volume 3 CA B.5.2 of this DAR.

**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
KCP 5.1.1/01	██████	2016	A21857B – Validation of Analytical Method SF-861/1 Report No. SMG14011 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
KCP 5.1.1/02	██████	2020	SF-861/1 – Determination of SYN545974 in EC (062.5) by HPLC Report No. 090100b8810a3615 Not GLP Unpublished	N	Y	The study is necessary for this regulatory decision and is eligible for data protection	SYN	N
KCP 10.2.1-01	██████	2019	Pydiflumetofen EC (A21857B) – Acute Toxicity to <i>Pseudokirchneriella subcapitata</i> in a 96-Hour Algal Growth Inhibition Test Report No. 160713SF/ SPO17425 Test Facility Noack Laboratorien GmbH GLP Unpublished	N	Y	The study is necessary for this regulatory decision and is eligible for data protection	SYN	N
KCP 10.2.1-02	██████	2019	Pydiflumetofen EC (A21857B) – Acute Toxicity to the Water Flea <i>Daphnia magna</i> Straus under Laboratory Conditions (Acute Immobilization Test – Static) Report No. 160713SF/ SAI17425 Test Facility Noack Laboratorien 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
KCP 10.2.1-03	██████	2019	Pydiflumetofen EC (A21857B) – Acute Toxicity to Fish (Rainbow trout), Static, 96 Hours Report No. 160713SF/ FAR17425 Test Facility ██████████ GLP Unpublished	N	Y	The study is necessary for this regulatory decision and is eligible for data protection	SYN	N
KCP 10.3.1.5-03	██████	2018	Pydiflumetofen SC (A19649B) - A semi-field study to evaluate the side effects on the honey bee <i>Apis mellifera</i> L. in Germany in 2017 Syngenta - Jealott's Hill, Bracknell, United Kingdom BioChem Agrar, Gerichshain, Germany, 17 48 BTB 0003 GLP Unpublished	N	Y	The study is necessary for this regulatory decision and is eligible for data protection	SYN	N
KCP 10.3.1.5-02	██████	2017	Pydiflumetofen SC (A19649B) - A Semi-Field Study to Evaluate Side Effects on Honeybees ( <i>Apis mellifera</i> L.) in <i>Phacelia tanacetifolia</i> in Germany 2016 Syngenta Eurofins Agrosience Services EcoChem GmbH, N-Osch., Germany, S16-00293 (interim report Report Amendment 1) GLP not published	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
KCP 10.3.1.5-03	■■■■■ ■■■	2017	Pydiflumetofen SC (A19649B) - A Semi-Field Study to Evaluate Side Effects on Honeybees ( <i>Apis mellifera</i> L.) in <i>Phacelia tanacetifolia</i> in Germany 2016 (interim report) Syngenta Eurofins Agrosience Services EcoChem GmbH, N-Osch., Germany, S16-04919(interim report Final Report) GLP not published	N	Y	The study is necessary for this regulatory decision and is eligible for data protection	SYN	N