Analytical method for fluindapyr [IR9792 (F9990)] and its metabolites cis-1-carboxy-fluindapyr, trans-1-carboxy-fluindapyr and 3-hydroxy-fluindapyr in water

Reports:	ECM: EPA MRID No.: 50518216. Soddu, R., and F. Sicbaldi. 2014. Set up and Validation of the Analytical Method for Determination of IR9792 (F9990) Residue in Drinking Water and in Surface Water. Study No.: RA.14.13. Tracking No.: 2014RES-IFP1560. Report prepared ISAGRO - GLP Test Facility, Novara, Italy; and sponsored and submitted by ISAGRO SpA, Milano, Italy, and FMC Corporation, Ewing, New Jersey; 72 pages. Final report issued December 12, 2014. ILV: EPA MRID No. 50676901. Schmiedt, S. 2018. Independent Laboratory Validation (ILV) of an Analytical Method for the Determination of Fluindapyr and Metabolites in Drinking Water. EAG Laboratories ID: P 4865 G. FMC Tracking No.: 2018AMT-IFP4416. Report prepared by EAG Laboratories GmbH, Ulm, Germany, sponsored and submitted by ISAGRO S.p.A., Milano, Italy, and FMC Corporation, Ewing, New Jersey; 65 pages. Final report issued July 27, 2018.
Document No.:	MRIDs 50518216 & 50676901
Guideline:	850.6100
Statements:	ECM: The study was conducted in accordance with OECD Good Laboratory Practice (GLP) standards and European Community Directives 2004/09/EC and 2004/10/EC (p. 5; Enclosure D, pp. 71-72 of MRID 50518216). Signed and dated Data Confidentiality, GLP, Authenticity, and Quality Assurance statements were provided (pp. 3, 5, 7; Enclosure D, pp. 71-72). ILV: The study was conducted in accordance with German GLP standards, which are based on OECD, EPA FIFRA and FDA, and Japanese GLP, and EC guidance document on residue analytical methods (p. 3; Appendix 1, p. 59 of MRID 50676901). Signed and dated No Data Confidentiality, GLP, Quality Assurance, Authenticity statements were provided (pp. 2-5; Appendix 1, p. 59).
Classification:	This analytical method is classified as Supplemental. For metabolites cis-1-carboxy-fluindapyr and trans-1-carboxy-fluindapyr, and 3-hydroxy-fluindapyr, only one set of performance data was submitted, an ILV. ECM linearity was not satisfactory for fluindapyr. The number of ILV trials required to successfully validate the ECM was not reported.
PC Code:	138008
EFED Final Reviewer:	Patricia Engel Physical Scientist Signature: Patricia Engel Digitally signed by PATRICIA ENGEL Date: 2020.04.22 10:51:43 -04'00' Date: 4/21/2020
CDM/CSS-Dynamac JV Reviewers:	Lisa Muto, Environmental Scientist Signature: Lisa Muto Date: 01/14/2019

Mary Samuel, M.S.,
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Date: 01/14/2019

This Data Evaluation Record may have been altered by the Environmental Fate and Effects Division subsequent to signing by CDM/CSS-Dynamac JV personnel. The CDM/CSS-Dynamac Joint Venture role does not include establishing Agency policies.

Executive Summary

The analytical method, ISAGRO Study No. RA.14.13 and Tracking No. 2014RES-IFP1560 (ECM MRID 50518216), is designed for the quantitative determination of fluindapyr [IR9792 (F9990)] in water at the LOQ of 0.1 µg/L using LC/MS/MS. The LOQ is less than the lowest toxicological level of concern in water for fluindapyr. The analytical method, ISAGRO Study No. RA.18.09 and Tracking Number 2018RES-IFP4319, is designed for the quantitative determination of fluindapyr metabolites cis-1-carboxy-fluindapyr, trans-1-carboxy-fluindapyr, and 3-hydroxy-fluindapyr in water at the LOQ of 0.1 µg/L using LC/MS/MS. The relative magnitude of the LOQ to the lowest toxicological level of concern in water for each fluindapyr metabolite is unknown. [1] The ECM MRID 50518216 validated its method using two characterized water matrices, drinking and surface water. The ILV validated both methods using one characterized water matrix, drinking water. The number of trials was not reported in the ILV; however, the reviewer believed that the ILV successfully validated the ECM in the first trial since the method was performed as written with only minor LC/MS instrument and parameter modifications. However, **an initial laboratory validation of the ECM for fluindapyr metabolites cis-1-carboxy-fluindapyr, trans-1-carboxy-fluindapyr, and 3-hydroxy-fluindapyr should be submitted to determine the reproducibility of the method for these analytes**. All submitted ECM and ILV data pertaining to precision, repeatability, reproducibility, linearity, and specificity was acceptable, except ECM linearity was not satisfactory for fluindapyr. Additionally, the reviewer noted elevated baseline around fluindapyr in ECM and ILV representative chromatograms of all calibration standards and fortified samples which interfered with analyte integration and attenuation.

[1] Toxicological levels of concern have not been established for metabolites.

Table 1. Analytical Method Summary

Analyte(s) by Pesticide	MRID		EPA Review	Matrix	Method Date (dd/mm/yyyy)	Registrant	Analysis	Limit of Quantitation (LOQ)
	Environmental Chemistry Method	Independent Laboratory Validation						
Fluindapyr [IR9792 (F9990)]	50518216[1]	50676901[3]		Water	12/12/2014	ISAGRO SpA and FMC Corporation	LC/MS/MS	0.1 µg/L
cis-1-Carboxy-fluindapyr	None submitted[2]							
trans-1-Carboxy-fluindapyr								
3-Hydroxy-fluindapyr								

1 In ECM, the drinking water (pH 7.9 ± 0.05, hardness 9.82 ± 0.2°F, dissolved organic carbon 0.928 ± 0.093, conductivity 256 ± 8 µS/cm), obtained from the municipal aqueduct, and the surface (river) water (pH 7.73 ± 0.05, hardness 5.78 ± 0.2°F, dissolved organic carbon 1.71 ± 0.17, conductivity 170 ± 5 µS/cm), obtained from Ticino river at Cameri (NO) in the Northern Italy geographical area, were used in the study (pp. 16-17; Table 1, p. 31; Enclosure C, p. 70 of MRID 50518216). The characterization was performed in the external laboratory "THEOLAB S.p.A".

2 In the ILV, the drinking water (pH 7.54, total hardness 3.22 mmol/L, total organic carbon 0.31 mg/L, electrical conductivity at 25°C: 696 µS/cm) obtained as tap water from the rooms in EAG Laboratories GmbH was used in the study and characterized by Institut Alpha, Ulm, Germany (p. 12; Appendix 3, pp. 64-65 of MRID 50676901).

3 Metabolites cis-1-carboxy-fluindapyr, trans-1-carboxy-fluindapyr, and 3-hydroxy-fluindapyr were not included in the ECM.

I. Principle of the Method

Water samples (100 mL), prefiltered on microglass fibers, in 100-mL volumetric flasks were fortified (1 mL of 10.05/10.08 µg/L or 100.5/100.8 µg/L fluindapyr fortification solution in methanol and water), as necessary (pp. 16-19 of MRID 50518216). The samples were analyzed by LC/MS/MS via direct injection.

Fluindapyr was identified and quantified by LC/MS/MS using an Agilent 1200 HPLC coupled with 6410 Mass detector (pp. 19-21 of MRID 50518216). The following conditions were employed: Phenomenex Kinetex 2.6 µ C18 100A analytical column (50 mm x 4.6 mm; column temperature 30 ± 0.8°C) with Phenomenex Security Guard Ultra Cartridge UHPLC C18 for 4.6 mm pre-column eluted with a gradient mobile phase of (A) aqueous 10mM ammonium acetate + 0.2% formic acid and (B) methanol + 0.2% formic acid [time, percent A:B; time 0 min. 40:60, 3 min. 20:80, 3.5 min. 40:60] using an injection volume of 10 µL and positive ESI ionization MRM scan mode. Fluindapyr was identified using two ion transitions (quantitation and confirmation, respectively): *m/z* 352→256.1 and *m/z* 352→312.2. Expected retention time was *ca*. 2.9 minutes for fluindapyr.

The ILV was performed to independently validate two ECMs: ECM MRID 50518216 (for the parent) and an ECM for fluindapyr metabolites [S. Riccelli, 04-Jun-2018: "Method validation for the determination of cis-1-carboxy-IR9792/F9990 (Code#510170), trans-1-carboxy-IR9792/F9990 (Code#510169) and 3-Hydroxy-IR9792/F9990 (Code#510152) in drinking water." Isagro – Centro di Saggio BPL., Novara, Italy, RA.18.09. Tracking Number 2018RES-

IFP4319; p. 9 of MRID 50676901]. The ECM for fluindapyr metabolites was not submitted for review.

In the ILV, ECM MRID 50518216 was performed as written, except for minor LC/MS instrument and parameter modifications; however, the parent fluindapyr and its three metabolites, cis-1-carboxy-fluindapyr, trans-1-carboxy-fluindapyr, and 3-hydroxy-fluindapyr, were included as analytes (pp. 14-19, 24 of MRID 50676901). Fluindapyr was identified and quantified by LC/MS/MS using an Agilent 1290 HPLC coupled with an AB Sciex API 6500+ Triple Quadrupole MS. The following conditions were employed: Waters XTerra C_{18} analytical column (50 mm x 4.6 mm, 3.5 µm particle size; column temperature 40°C) with Phenomenex C_{18} pre-column (4 x 3 mm). The other LC/MS conditions were the same as the ECM; however, the MS temperature was reported as 550°C. Fluindapyr was identified using two ion transitions (quantitation and confirmation, respectively): *m/z* 352→256 and *m/z* 352→312; the MS transitions were similar to those of ECM. Expected retention time was *ca*. 3.1 minutes for fluindapyr. Fluindapyr metabolites were identified and quantified by LC/MS/MS using an Agilent 1290 Series HPLC coupled with an AB Sciex API 5500 Triple Quadrupole MS. The following conditions were employed: Phenomenex Kinetex C_{18} analytical column (50 mm x 2.1 mm, 2.6 µm particle size; column temperature 25°C) with Phenomenex C_{18} pre-column eluted with a gradient mobile phase of (A) water + 0.1% formic acid and (B) methanol + 0.1% formic acid [time, percent A:B; time 0.0-3.5 min. 90:10, 6.0 min. 60:40, 15.0 min. 37.5:62.5, 15.1-18.0 min. 5.0:95, 18.1-22.0 min. 90:10] using an injection volume of 40 µL and Turbo IonSpray ESI ionization MRM scan mode (source temperature 550°C). Polarity was positive for cis-1-carboxy-fluindapyr and trans-1-carboxy-fluindapyr and negative for 3-hydroxy-fluindapyr. Analytes were identified using two ion transitions (quantitation and confirmation, respectively): *m/z* 382→296 and *m/z* 382→336 for cis-1-carboxy-fluindapyr and trans-1-carboxy-fluindapyr, and *m/z* 366→131 and *m/z* 366→175 for 3-hydroxy-fluindapyr. Expected retention times were *ca*. 8.3, 7.7, and 12.0 minutes for cis-1-carboxy-fluindapyr, trans-1-carboxy-fluindapyr, and 3-hydroxy-fluindapyr, respectively. All ILV modifications were minor LC/MS instrument and parameter modifications (p. 24).

The method Limit of Quantification (LOQ) and Limit of Determination (LOD) for fluindapyr in water were 0.1 µg/L and 0.05 µg/L in the ECM, respectively (pp. 23, 26-27 of MRID 50518216). Calculated LOQ and LOD values supported the method LOQ and LOD. The LOQ and LOD for fluindapyr in water were 0.1 µg/L and 0.02 µg/L in the ILV, respectively (pp. 21-22, 24; Table 1, p. 25; Table 4, p. 28 of MRID 50676901). The LOQ and LOD for fluindapyr metabolites [cis-1-carboxy-fluindapyr, trans-1-carboxy-fluindapyr, and 3-hydroxy-fluindapyr] in water were 0.1 µg/L and 0.03 µg/L in the ILV, respectively.

II. Recovery Findings

ECM (MRID 50518216): Mean recoveries and RSDs were within guideline requirements [means between 70% and 120% and relative standard deviations (RSD) ≤20%] for analysis of fluindapyr [IR9792 (F9990)] at fortification levels of 0.1 µg/L (LOQ) and 1.0 µg/L (10×LOQ) in two water matrices (Tables 3-6, pp. 33-36). Fluindapyr was identified using two ion transitions; performance data (recovery results) for the quantitation and confirmation ion analyses were comparable. The drinking water (pH 7.9 ± 0.05, hardness 9.82 ± 0.2°F, dissolved organic carbon 0.928 ± 0.093, conductivity 256 ± 8 µS/cm), obtained from the municipal aqueduct, and the surface (river) water (pH 7.73 ± 0.05, hardness 5.78 ± 0.2°F, dissolved organic carbon 1.71 ± 0.17, conductivity 170 ± 5 µS/cm), obtained from Ticino river at Cameri (NO) in the Northern Italy geographical area, were used in the study (pp. 16-17; Table 1, p. 31; Enclosure C, p. 70). The characterization was performed in the external laboratory "THEOLAB S.p.A".

ILV (MRID 50676901): Mean recoveries and RSDs were within guideline requirements for analysis of fluindapyr [IR9792 (F9990)] and its metabolites cis-1-carboxy-fluindapyr, trans-1-carboxy-fluindapyr, and 3-hydroxy-fluindapyr at fortification levels of 0.1 µg/L (LOQ) and 1.0 µg/L (10×LOQ) in one water matrix (Table 1, p. 25; Table 4, p. 28; DER Attachment 2). All analytes were identified using two ion transitions; performance data (recovery results) for the quantitation and confirmation ion analyses were comparable. The drinking water (pH 7.54, total hardness 3.22 mmol/L, total organic carbon 0.31 mg/L, electrical conductivity at 25°C: 696 µS/cm) obtained as tap water from the rooms in EAG Laboratories GmbH was used in the study and characterized by Institut Alpha, Ulm, Germany (p. 12; Appendix 3, pp. 64-65). The ILV was performed to independently validate two ECMs: ECM MRID 50518216 (for the parent) and the ECM for fluindapyr metabolites. The number of trials was not reported in the ILV; however, the reviewer believed that the ILV successfully validated the ECM MRID 50518216 (for the parent) and the ECM for fluindapyr metabolites in the first trial since the method was performed as written with only minor LC/MS instrument and parameter modifications (pp. 10-11, 24). However, only the parent fluindapyr was included as an analyte in the submitted ECM; only one of the two ECMs was submitted for review.

Table 2. Initial Validation Method Recoveries for Fluindapyr [IR9792 (F9990)] in Water[1,2,3]

Analyte	Fortification Level (µg/L)[4]	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
	Drinking Water					
	Quantitation Ion Transition					
Fluindapyr [IR9792 (F9990)]	0.1 (LOQ)	7	82.0-107.3	93.1	9.2	9.9
	1.0	5	83.0-108.8	102.6	11.0	10.8
	Confirmation Ion Transition					
Fluindapyr [IR9792 (F9990)]	0.1 (LOQ)	7	86.2-105.7	94.9	8.4	8.9
	1.0	5	77.3-115.7	102.1	14.6	14.3
	Surface Water					
	Quantitation Ion Transition					
Fluindapyr [IR9792 (F9990)]	0.1 (LOQ)	7	81.4-109.5	93.6	10.4	11.1
	1.0	5	70.8-97.7	82.8	10.4	12.5
	Confirmation Ion Transition					
Fluindapyr [IR9792 (F9990)]	0.1 (LOQ)	7	82.4-107.5	93.2	10.2	11.0
	1.0	5	71.3-85.1	77.0	6.3	8.2

Data (uncorrected recovery results, pp. 21-22) were obtained from Tables 3-6, pp. 33-36 of MRID 50518216.

1 The drinking water (pH 7.9 ± 0.05, hardness 9.82 ± 0.2°F, dissolved organic carbon 0.928 ± 0.093, conductivity 256 ± 8 µS/cm), obtained from the municipal aqueduct, and the surface (river) water (pH 7.73 ± 0.05, hardness 5.78 ± 0.2°F, dissolved organic carbon 1.71 ± 0.17, conductivity 170 ± 5 µS/cm), obtained from Ticino river at Cameri (NO) in the Northern Italy geographical area, were used in the study (pp. 16-17; Table 1, p. 31; Enclosure C, p. 70). The characterization was performed in the external laboratory "THEOLAB S.p.A".

2 Fluindapyr was identified using two ion transitions (quantitation and confirmation, respectively): *m/z* 352→256.1 and *m/z* 352→312.2.

3 Fluindapyr metabolites cis-1-carboxy-fluindapyr and trans-1-carboxy-fluindapyr, and 3-hydroxy-fluindapyr were not included in the ECM.

4 Nominal fortifications reported.

Table 3. Independent Validation Method Recoveries for Fluindapyr [IR9792 (F9990)] and its Metabolites cis-1-Carboxy-fluindapyr, trans-1-Carboxy-fluindapyr, and 3-Hydroxy-fluindapyr in Water[1,2]

Analyte	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)[3]	Relative Standard Deviation (%)
	Drinking Water					
	Quantitation Ion Transition					
Fluindapyr [IR9792 (F9990)]	0.1 (LOQ)	5	86-109	98	10	10
	1.0	5	106-115	109	4	3
cis-1-Carboxy-fluindapyr	0.1 (LOQ)	5	95-109	104	6	6
	1.0	5	102-107	104	2	2
trans-1-Carboxy-fluindapyr	0.1 (LOQ)	5	93-99	96	3	3
	1.0	5	99-104	102	2	2
3-Hydroxy-fluindapyr	0.1 (LOQ)	5	104-108	107	2	2
	1.0	5	107-110	108	1	1
	Confirmation Ion Transition					
Fluindapyr [IR9792 (F9990)]	0.1 (LOQ)	5	97-107	100	4	4
	1.0	5	104-117	109	5	5
cis-1-Carboxy-fluindapyr	0.1 (LOQ)	5	101-107	104	2	3
	1.0	5	100-106	103	2	2
trans-1-Carboxy-fluindapyr	0.1 (LOQ)	5	95-111	102	5	6
	1.0	5	99-104	102	2	2
3-Hydroxy-fluindapyr	0.1 (LOQ)	5	104-115	109	4	4
	1.0	5	108-112	110	2	2

Data (uncorrected recovery results, p. 20) were obtained from Table 1, p. 25 and Table 4, p. 28 of MRID 50676901 and DER Attachment 2.

1 The drinking water (pH 7.54, total hardness 3.22 mmol/L, total organic carbon 0.31 mg/L, electrical conductivity at 25°C: 696 µS/cm) obtained as tap water from the rooms in EAG Laboratories GmbH was used in the study and characterized by Institut Alpha, Ulm, Germany (p. 12; Appendix 3, pp. 64-65).

2 Analytes were identified using two ion transitions (quantitation and confirmation, respectively): *m/z* 352→256 and *m/z* 352→312 for fluindapyr, *m/z* 382→296 and *m/z* 382→336 for cis-1-carboxy-fluindapyr and trans-1-carboxy-fluindapyr, and *m/z* 366→131 and *m/z* 366→175 for 3-hydroxy-fluindapyr. The fluindapyr MS transitions were similar to those of ECM.

3 Standard deviations were reviewer-calculated since these values were not reported in the study report. The rules of significant figures were followed.

III. Method Characteristics

The method Limit of Quantification (LOQ) and Limit of Determination (LOD) for fluindapyr in water were 0.1 µg/L and 0.05 µg/L in the ECM, respectively (pp. 23-24, 26-27 of MRID 50518216). The LOQ and LOD were calculated for each matrix using the following equations:

$LOD = (t_{0.99} \times SD)$

$LOQ = 3 \times LOD$

Where, $t_{0.99}$ is the t value for n-1 replicates at the 99% confidence level and SD is the standard deviation of the analyte recovery measurements at the target LOQ. Calculated LOQ and LOD values supported the method LOQ and LOD (see **Table 4** below).

The LOQ and LOD for fluindapyr in water were 0.1 µg/L and 0.02 µg/L in the ILV, respectively (pp. 21-22, 24; Table 1, p. 25; Table 4, p. 28 of MRID 50676901). The LOQ and LOD for fluindapyr metabolites [cis-1-carboxy-fluindapyr, trans-1-carboxy-fluindapyr, and 3-hydroxy-fluindapyr] in water were 0.1 µg/L and 0.03 µg/L in the ILV, respectively. The LODs were based on the lowest calibration standard. No calculations for the LOQ and LOD were provided in the ILV.

Table 4. Method Characteristics

Analyte		Fluindapyr [IR9792 (F9990)]	cis-1-Carboxy-fluindapyr	trans-1-Carboxy-fluindapyr	3-Hydroxy-fluindapyr
Limit of Quantitation (LOQ)	ECM (Method)	0.1 µg/L	**Not performed**		
	ECM (Calculated)	0.0792-0.0975 µg/L			
	ILV	0.1 µg/L			
Limit of Detection (LOD)	ECM (Method)	0.05 µg/L	**Not performed**		
	ECM (Calculated)	0.0264-0.0325 µg/L			
	ILV	0.02 µg/L	0.03 µg/L		
Linearity (calibration curve r^2 and concentration range)[1]	ECM	r^2 = **0.99302565** (Q) r^2 = 0.99638835 (C)	**Not performed**		
		0.029998-1.2096 µg/L			
	ILV	r^2 = 0.9984 (Q) r^2 = 0.9996 (C)	r^2 = 0.9998 (Q) r^2 = 0.9996 (C)	r^2 = 0.9996 (Q & C)	r^2 = 0.9992 (Q) r^2 = 0.9998 (C)
		0.02-1.20 ng/mL	0.03-50 ng/mL		
Repeatable	ECM[2]	Yes at LOQ and 10×LOQ (two characterized waters).	**Not performed**		
	ILV[3,4]	Yes at LOQ and 10×LOQ (one characterized water)			
Reproducible		Yes at LOQ and 10×LOQ.	**Could not be determined**; only one set of performance data was submitted.		
Specificity	ECM/ILV	Elevated baseline was noted around the analyte peak which interfered with analyte integration and attenuation. Interference was observed in all calibration standards and fortified samples. Also, interference was greater in drinking water versus surface water.[5]			
	ECM	Yes, no matrix interferences were observed.	**Not performed**		
	ILV	Yes, matrix interferences were <5% of the LOQ (based on peak area).	Yes, matrix interferences were <1% of the LOQ (based on peak area).	Yes, matrix interferences were <3% of the LOQ (based on peak area).	Yes, matrix interferences were <2% of the LOQ (based on peak area).

Data were obtained from pp. 23-24, 26-27 (LOQ/LOD); Tables 3-6, pp. 33-36 (recovery data); pp. 41-42 (calibration curve); Panels 1-10, pp. 44-53 (chromatograms) of MRID 50518216; pp. 21-22, 24; Table 1, p. 25; Table 4, p. 28 (LOQ/LOD); Table 1, p. 25; Table 4, p. 28 (recovery data); Figure 1, p. 31; Figure 7, p. 38; Figure 13, p. 45; Figure 19, p. 52 (calibration curves); Figures 2-6, pp. 33-37; Figures 8-12, pp. 40-44; Figures 14-18, pp. 47-51; Figures 20-24, pp. 54-58 (chromatograms) of MRID 50676901; DER Attachment 2. Q = Quantitation ion transition; C = Confirmatory ion transition.

1 Reported correlation coefficients were reviewer-calculated from r values reported in the study report (p. 16; Figure 1, p. 31; Figure 7, p. 38; Figure 13, p. 45; Figure 19, p. 52 of MRID 50676901; DER Attachment 2). Significant figures of r^2 were limited to four.

2 In ECM, the drinking water (pH 7.9 ± 0.05, hardness 9.82 ± 0.2°F, dissolved organic carbon 0.928 ± 0.093, conductivity 256 ± 8 µS/cm), obtained from the municipal aqueduct, and the surface (river) water (pH 7.73 ± 0.05, hardness 5.78 ± 0.2°F, dissolved organic carbon 1.71 ± 0.17, conductivity 170 ± 5 µS/cm), obtained from Ticino river at Cameri (NO) in the Northern Italy geographical area, were used in the study (pp. 16-17; Table 1, p. 31; Enclosure C, p. 70 of MRID 50518216). The characterization was performed in the external laboratory "THEOLAB S.p.A".

3 In the ILV, the drinking water (pH 7.54, total hardness 3.22 mmol/L, total organic carbon 0.31 mg/L, electrical conductivity at 25°C: 696 µS/cm) obtained as tap water from the rooms in EAG Laboratories GmbH was used in the study and characterized by Institut Alpha, Ulm, Germany (p. 12; Appendix 3, pp. 64-65 of MRID 50676901).

4 The ILV was performed to independently validate two ECMs: ECM MRID 50518216 (for the parent) and the ECM for fluindapyr metabolites. The number of trials was not reported in the ILV; however, the reviewer believed that the ILV successfully validated the ECM MRID 50518216 (for the parent) and the ECM for fluindapyr metabolites in the first trial since the method was performed as written with only minor LC/MS instrument and parameter modifications (pp. 10-11, 24 of MRID 50676901). However, only the parent fluindapyr was included as an analyte in the submitted ECM; only one of the two ECMs was submitted for review.

5 Based on Panels 1-10, pp. 44-53 of MRID 50518216 and Figures 2-6, pp. 33-37 of MRID 50676901. Linearity is satisfactory when $r^2 \geq 0.995$.

IV. Method Deficiencies and Reviewer's Comments

1. The ILV was performed to independently validate two ECMs: ECM MRID 50518216 (for the parent) and the ECM for fluindapyr metabolites [S. Riccelli, 04-Jun-2018: "Method validation for the determination of cis-1-carboxy-IR9792/F9990 (Code#510170), trans-1-carboxy-IR9792/F9990 (Code#510169) and 3-Hydroxy-IR9792/F9990 (Code#510152) in drinking water." Isagro – Centro di Saggio BPL., Novara, Italy, RA.18.09. Tracking Number 2018RES-IFP4319; p. 9 of MRID 50676901]. The ECM for fluindapyr metabolites was not submitted for review. OCSPP guidelines state that two sets of performance data should be submitted, one for the initial or other internal validation and one for the ILV. An initial laboratory validation of the ECM for fluindapyr metabolites including fluindapyr metabolites cis-1-carboxy-IR9792/F9990 (Code#510170), trans-1-carboxy-IR9792/F9990 (Code#510169) and 3-hydroxy-IR9792/F9990 should be submitted.

2. In the ECM, the linearity was not satisfactory for fluindapyr, $r^2 = 0.99302565$ (Q; pp. 41-42 of MRID 50518216). Linearity is satisfactory when $r^2 \geq 0.995$.

3. The number of ILV trials required to successfully validate the ECM was not reported in the ILV study report (pp. 10-11, 24 of MRID 50676901). OCSPP guidelines state that maximum of three sample sets should be used to validate the ECM.

4. In the ECM and ILV representative chromatograms of fluindapyr, the reviewer noted elevated baseline around the analyte peak which interfered with analyte integration and attenuation. Interference was observed in all calibration standards and fortified samples (Panels 1-10, pp. 44-53 of MRID 50518216 and Figures 2-6, pp. 33-37 of MRID

50676901). Also, interference was greater in drinking water versus surface water. The reviewer believed that additional sample processing may be required to enhance the specificity of the method for this analyte; however, since the interference was persistent in calibration standards, it seemed that additional sample processing may not affect the LC spectra. Additionally, the reviewer noted that the chemical purities of fluindapyr were reported as 99.72% in the ECM and 98.41% in the ILV (p. 17 of MRID 50518216; Appendix 2, p. 60 of MRID 50676901).

5. Matrix effects were studied in the ILV and determined to be insignificant for fluindapyr (p. 22; Table 2, p. 26 of MRID 50676901). The ILV reported that matrix effects were determined to be insignificant for fluindapyr metabolites in the corresponding ECM (p. 23). Solvent-based calibration standards were used for all analyses.

6. Storage stability was studied in the ILV, and it was determined that all fortification and calibration solutions were stable for up to 7 days (fluindapyr) or 13 days (fluindapyr metabolites) of refrigerated storage (storage conditions not reported; pp. 21-23 of MRID 50676901). Sample extracts were were stable for up to 12 days (fluindapyr) or 13 days (fluindapyr metabolites) of refrigerated storage.

7. The ILV reported that no communication between the ILV and method developer occurred (p. 23 of MRID 50676901).

8. The estimation of LOQ and LOD in the ECM and ILV was not based on scientifically acceptable procedures as defined in 40 CFR Part 136 (pp. 23-24, 26-27 of MRID 50518216). In the ECM, the LOQ and LOD were calculated for each matrix using the following equations: LOD = ($t_{0.99}$ x SD) and LOQ = 3 × LOD, where, $t_{0.99}$ is the t value for n-1 replicates at the 99% confidence level and SD is the standard deviation of the analyte recovery measurements at the target LOQ. Calculated LOQ and LOD values supported the method LOQ and LOD. In the ILV, the LODs were based on the lowest calibration standard. No calculations for the LOQ and LOD were provided in the ILV. Further work could have been done to explore the actual LOQ. This means that concentrations can be reliably quantified at the LOQ (i.e., LLMV), but whether lower concentrations may also be reliably quantified is uncertain.

9. In the ILV, the time required to complete the extraction of one set of 12 samples required *ca*. 1 calendar day of work, including calculation of results (preparation, 4 hours; LC/MS/MS analysis, 4-6 hours; pp. 22-23 of 50676901).

V. References

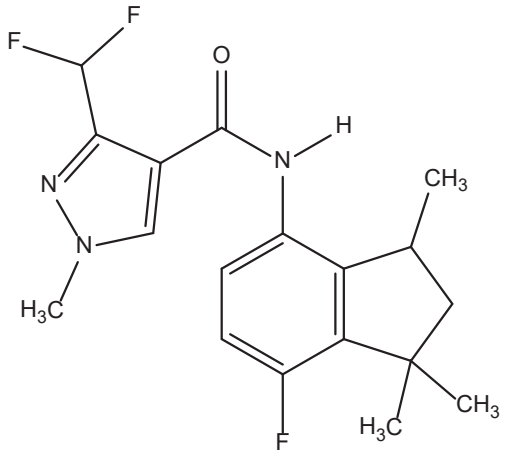
U.S. Environmental Protection Agency. 2012. Ecological Effects Test Guidelines, OCSPP 850.6100, Environmental Chemistry Methods and Associated Independent Laboratory Validation. Office of Chemical Safety and Pollution Prevention, Washington, DC. EPA 712-C-001.

40 CFR Part 136. Appendix B. Definition and Procedure for the Determination of the Method Detection Limit-Revision 1.11, pp. 317-319.

Attachment 1: Chemical Names and Structures

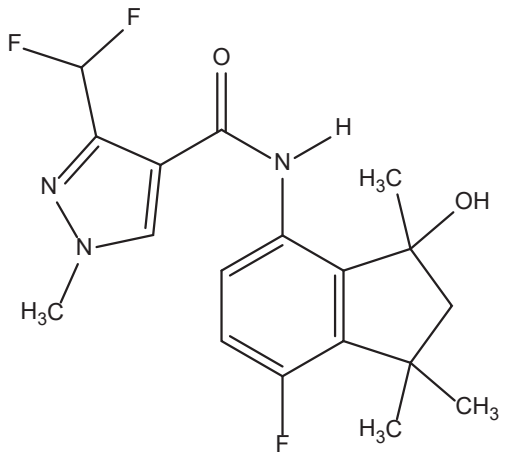
Fluindapyr (IR9792; F9990)

IUPAC Name:	3-(Difluoromethyl)-N-[(3RS)-7-fluoro-2,3-dihydro-1,1,3-trimethyl-1H-inden-4-yl]-1-methyl-1H-pyrazole-4-carboxamide
CAS Name:	3-(Difluoromethyl)-N-(7-fluoro-2,3-dihydro-1,1,3-trimethyl-1H-inden-4-yl)-1-methyl-1H-pyrazole-4-carboxamide
CAS Number:	1383809-87-7
SMILES String:	FC1=CC=C(N([H])C(C2=CN(C)N=C2C(F)F)=O)C3=C1C(C)(C)CC3C



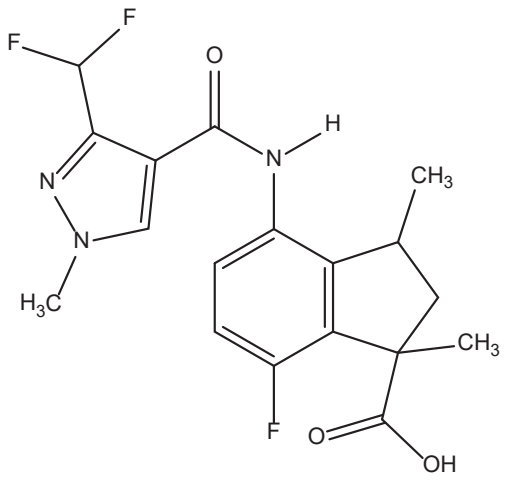
3-Hydroxy-IR9792/F9990

IUPAC Name:	3-(Difluoromethyl)-N-(7-fluoro-3-hydroxy-1,1,3-trimethyl-2,3-dihydro-1H-inden-4-yl)-1-methyl-1H-pyrazole-4-carboxamide
CAS Name:	Not reported
CAS Number:	Not reported
SMILES String:	FC1=CC=C(N([H])C(C2=CN(C)N=C2C(F)F)=O)C3=C1C(C)(C)CC3(O)C



trans-1-Carboxy-IR9792/F9990

IUPAC Name:	(1R,3S)-4-(3-(Difluoromethyl)-1-methyl-1H-pyrazole-4-carboxamido)-7-fluoro-1,3-dimethyl-2,3-dihydro-1H-indene-1-carboxylic acid
CAS Name:	Not reported
CAS Number:	Not reported
SMILES String:	FC1=CC=C(N([H])C(C2=CN(C)N=C2C(F)F)=O)C3=C1C(C(O)=O)(C)CC3C



cis-1-Carboxy-IR9792/F9990

IUPAC Name:	(1R,3R)-4-(3-(Difluoromethyl)-1-methyl-1H-pyrazole-4-carboxamido)-7-fluoro-1,3-dimethyl-2,3-dihydro-1H-indene-1-carboxylic acid
CAS Name:	Not reported
CAS Number:	Not reported
SMILES String:	FC1=CC=C(N([H])C(C2=CN(C)N=C2C(F)F)=O)C3=C1C(C(O)=O)(C)CC3C