

REGISTRATION REPORT

Part B

Section 5

Analytical Methods

Detailed summary of the risk assessment

Product code: GF-3969

Chemical active substances:

Rimsulfuron, 148.15 g/kg

Thifensulfuron methyl, 92.60 g/kg

Isoxadifen-ethyl, 111.1 g/kg

Central Zone

Zonal Rapporteur Member State: Poland

CORE ASSESSMENT

(authorization)

Applicant: Corteva/DuPont/DowAgroScience/Pioneer*

Submission date: 07/December/2020

MS Finalisation date: December 2021(initial Core Assessment)

May 2022 (final Core Assessment)

*Corteva Agriscience is new Legal Entity in most of EU countries and should be treated as an Applicant for GF-3969 registration. Information about Applicant for each country is provided in dRR Part A.

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Version history

When	What
December 2021	Applicant initial dRR
November 2021	Additional data provided by Applicant
December 2021	Additional data provided by Applicant
December 2021	Initial assessment by the zRMS The report in the dRR format has been prepared by the Applicant, therefore all comments, additional evaluations and conclusions of the zRMS are presented in grey commenting boxes. Minor changes are introduced directly in the text and highlighted in grey . Not agreed or not relevant information are struck through and shaded for transparency .
May 2022	Final report (Core Assessment updated following the commenting period) Additional information/assessments included by the zRMS in the report in response to comments recieved from the CMS and the Applicant are highlighted in yellow . Information no longer relevant is struck through and shaded .

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Thifensulfuron methyl information belongs to FMC, and Corteva has a letter of access. Unless otherwise specified, endpoints used in this section for isoxadifen-ethyl originate from Bayer CropScience and Corteva has a letter of access.

5 Analytical methods

Details on analytical methods for the active substances in GF-3969, rimsulfuron, thifensulfuron methyl and isoxadifen-ethyl, are derived from the respective EFSA conclusions for these actives as indicated below.

For rimsulfuron: EFSA Scientific Report (2005) 45, 1-61. Conclusion regarding the peer review of the pesticide risk assessment of the active substance rimsulfuron; EFSA Journal 2018;16(5):5258. Peer review of the pesticide risk assessment of the active substance rimsulfuron.

For thifensulfuron methyl: EFSA Journal 2015;13(7):4201. Conclusion on the peer review of the pesticide risk assessment of the active substance thifensulfuron-methyl.

For isoxadifen-ethyl: Summary of the German national evaluation of the safener isoxadifen-ethyl (2002) and Austrian (AGES) evaluation on product Laudis in 2006.

5.1 Conclusion and summary of assessment

zRMS conclusions:

Rimsulfuron

The residue definition for primary crops both for risk assessment and monitoring is set as rimsulfuron. The current residue definition set in Regulation (EC) No 396/2005 (Reg. (EU) No 617/2014) is identical to the residue definition for enforcement derived in the peer review.

In EFSA Scientific Report (2005) 45, 1-61, Conclusion on the peer review of rimsulfuron it is stated that *“Adequate methods are available to monitor all compounds given in the respective residue definition, i.e. rimsulfuron in food of plant origin, soil, water and air.*

The methodology used is HPLC with UV or MS/MS detection. A multi-residue method like the Dutch MM1 or the German S19 is not applicable to due the nature of the residues.

An analytical method for food of animal origin is not required due to the fact that no residue definition is proposed.”

Residue definitions

Soil

Definitions for risk assessment: rimsulfuron, IN-70941;IN-70942; IN-E9260

Definitions for monitoring: rimsulfuron

Water

Ground water

Definitions for exposure assessment: rimsulfuron, IN-70941, IN-E9260, IN-70942, INJ-290

Definitions for monitoring: rimsulfuron

Surface water

Definitions for risk assessment:

surface water and sediment: rimsulfuron, IN-70941, IN-70942

surface water only: IN-E9260 (where surface water is fed by groundwater)

sediment only: IN-JF999

Definitions for monitoring: rimsulfuron

Air

Definitions for risk assessment: rimsulfuron

Definitions for monitoring: rimsulfuron

Food of plant origin

Definitions for risk assessment: rimsulfuron

Definitions for monitoring: rimsulfuron

Food of animal origin

Definitions for risk assessment: no residue definition needed
Definitions for monitoring: no residue definition needed

Analytical methods for residues (Annex IIA, point 4.2)

Food/feed of plant origin (analytical technique and LOQ for methods for monitoring purposes)	HPLC-UV 0.05 mg/kg (maize, potato, tomato) LC-MS/MS 0.01 mg/kg (maize, potato, tomato)
Food/feed of animal origin (analytical technique and LOQ for methods for monitoring purposes)	Not relevant, no residue definition is proposed
Soil (analytical technique and LOQ)	LC-MS/MS 0.2 µg/kg
Water (analytical technique and LOQ)	HPLC-UV 0.1 µg/L LC-MS/MS 0.05 µg/L (drinking- and surface water)
Air (analytical technique and LOQ)	LC-MS/MS 3 µg/m ³
Body fluids and tissues (analytical technique and LOQ)	Not relevant, the active substance is not classified as toxic or very toxic

According to the EFSA Journal 2012;10(10):2911 “During the peer review under Directive 91/414/EEC, an analytical method using HPLC-MS/MS was submitted and validated with an LOQ of 0.01 mg/kg in dry (maize grain) and high water content (potato, tomato) commodities and 0.05 mg/kg for maize forage and stover (Germany, 2003). This method was taken into account by the RMS, but an ILV fully validated with an LOQ of 0.01 mg/kg is missing.

In addition, after Annex I inclusion, France evaluated an LC-MS/MS method and its ILV which were validated for the determination of rimsulfuron with an LOQ of 0.01 mg/kg in high water content (apple, cherry and plum), acidic (grape, lemon and lime) and dry (corn grain) commodities (France, 2012). The HPLC-MS/MS method from the DAR reported above can be used as confirmatory method for dry and high water content commodities. Hence, it is concluded that parent rimsulfuron can be enforced in food of plant origin with an LOQ of 0.01 mg/kg in dry and high water content commodities.

No analytical method is available for food of animal origin. As there is no significant intake of residues by livestock, no residue definition and no MRL were proposed for commodities of animal origin. Therefore, an analytical method for enforcement of residues in food of animal origin is not necessary.”

The summary and evaluation of new methods for the determination of rimsulfuron in food of plant origin, soil, water, air and in body fluids provided for renewal of active substance were presented in Renewal Assessment Report for Review of Annex I Inclusion of Rimsulfuron, in B.5 – Methods of Analysis, October 2017. The conclusions were published in EFSA Journal 2018;16(5):5258. The available methods are acceptable and sufficient to support the proposed use.

In EFSA Journal 2018;16(5):5258 it is stated that “Rimsulfuron residue can be monitored in food and feed of plant origin by high-performance liquid chromatography with tandem mass spectrometry (HPLC–MS/MS) with a limit of quantification (LOQ) of 0.01 mg/kg in all commodity groups. Rimsulfuron residue in dry and high water content commodities can be determined also by the quick, easy, cheap, effective and safe method (QuEChERS) using HPLC–MS/MS with a LOQ of 0.01 mg/kg. An analytical method for food of animal origin is not required due to the fact that no residue definition is proposed.

Rimsulfuron residue in soil can be monitored by HPLC–MS/MS with a LOQ 0.05 µg/kg. Rimsulfuron residue in water can be monitored by QuEChERS HPLC–MS/MS or single HPLC–MS/MS with LOQs 0.05 µg/L and 0.1 µg/L, respectively. An appropriate HPLC–MS/MS method exists for monitoring of rimsulfuron residue in air with a LOQ of 3.0 µg/m³.

The HPLC-MS/MS method can be used for monitoring of rimsulfuron in body fluids (urine and plasma) with LOQ of 0.01 mg/kg. Rimsulfuron residue in body tissues can be determined by HPLC-MS/MS with LOQ of 0.01 mg/kg.”

Furthermore the Applicant submitted a number of methods for analysis of residues of rimsulfuron for the generation of pre-authorization data. The details of the evaluation of new and additional studies are referred in Appendix 2.

Thifensulfuron methyl

In EFSA Journal 2015;13(7):4201 it is stated that “For plants, soil, water and air LC-MS/MS methods are available. A method of analysis for products of animal origin is not required as no MRLs are proposed. A method of analysis for body fluids and tissues is not required.”

Residue definitions for monitoring purposes

Food/feed of plant origin	For oilseeds and cereals (weed-control use): Thifensulfuron-methyl (parent only) Although currently no EU MRLs are set for feed commodities, for possible future applicability it is proposed: For Animal feed items (grass / alfalfa): Sum of thifensulfuron-methyl and thifensulfuron acid (IN-L9225), expressed as thifensulfuron-methyl
Food/feed of animal origin	Thifensulfuron-methyl (parent only)
Soil	Thifensulfuron-methyl
Water (surface, drinking/ground)	Thifensulfuron-methyl
Air	Thifensulfuron-methyl
Body fluids and tissues	Thifensulfuron-methyl

Monitoring/Enforcement methods

Food/feed of plant origin (analytical technique and LOQ for methods for monitoring purposes)	DuPont: LC-MS/MS – LOQ = 0.01 mg/kg for soybean seed, olives, corn grain, oranges and lettuce.
Food/feed of animal origin (analytical technique and LOQ for methods for monitoring purposes)	Not required as no MRLs are proposed
Soil (analytical technique and LOQ)	DuPont: LC-MS/MS – LOQ = 0.05 µg /kg for soil
Water (analytical technique and LOQ)	DuPont: LC-MS/MS – LOQ = 0.05 µg/L for both drinking and surface water
Air (analytical technique and LOQ)	DuPont: LC-MS/MS – LOQ = 2.8 µg/m ³ for air
Body fluids and tissues (analytical technique and LOQ)	Not required.

Excerpt from EFSA Journal 2015;13(7):4201:

Plant residue definition for monitoring - Thifensulfuron-methyl (parent only) (for oilseeds and cereals),
Plant residue definition for risk assessment - Thifensulfuron-methyl and provisionally triazine amine (IN-A4098) (for oilseeds and cereals).

Remark: The risk assessment definition is not finalised with regard to metabolites IN-A4098 and IN-B5528. The consumer exposure assessment is moreover pending further clarification on the toxicological properties of IN-W8268 and IN-A5546.

Furthermore the Applicant submitted a number of methods for analysis of residues of thifensulfuron-methyl for the generation of pre-authorization data and for post-authorization control and monitoring purposes. The details of the evaluation of new and additional studies are referred in Appendix 2.

According to the EFSA Journal 2015;13(7):4201 a method of analysis for body fluids and tissues is not required. However in Reg (EU) No 283/2013 it is stated that “*methods, with a full description, shall be submitted for the analysis in body fluids and tissues for active substance and relevant metabolites*”.

Applicant provided analytical method for the determination of thifensulfuron methyl in plasma and urine (R. M. Henze, J. J. Stry, 2016, Dupont-47394).

The analytical method was developed and validated for the detection, quantitative analysis and confirmation of residues of thifensulfuron methyl (DPX-M6316) in plasma and urine. The determined limit of quantitation (LOQ) was 1.0 µg/kg (ppb) for plasma and 3.0 µg/kg for urine. The study is acceptable. The details of the evaluation of additional study is referred in Appendix 2.

Additionally Applicant provided analytical method for the determination of thifensulfuron methyl in drinking, ground and surface water (R. M. Henze, J. J. Stry, 2013, DuPont-35704) and independent laboratory validation of DuPont-35704 (Mason, B., 2013 (DuPont-36531)).

The analytical method (DuPont-35704) was developed and validated for the detection, quantitative analysis and confirmation of residues of thifensulfuron methyl (DPX-M6316) in water using LC/MS/MS. The determined limit of quantitation (LOQ) was 0.1 µg/kg (ppb) for water. The DuPont-35704 analytical method was successfully independently validated for the determination of residues of thifensulfuron methyl in drinking, ground and surface water with a LOQ of 0.10 µg/kg using LC-MS/MS. The studies are acceptable. The details of the evaluation of additional studies are referred in Appendix 2.

Isoxadifen-ethyl

It should be pointed out that formulation GF-3969 contains 111.1 g/kg of safener, isoxadifen-ethyl. Isoxadifen-ethyl is not considered as an active substance and at present MRLs are not set in the EU for safeners. The Applicant provided the data for safener reviewed by Germany. According to Regulation 1107/2009, data for safener should be evaluated in line with requirements relevant for active substances and EU agreed and peer-reviewed endpoints should be generated. Such evaluation, however, is outside the scope of the product registration and should be carried out at the EU level in order to derive uniform endpoints that may be used in evaluation of various formulations. For this reason studies provided for isoxadifen-ethyl were not validated by the zRMS.

Sufficiently sensitive and selective analytical methods are available for the active substances and relevant impurities in the plant protection product.

Noticed data gaps are: none

Sufficiently sensitive and selective analytical methods are available for all analytes included in the residue definitions.

Noticed data gaps are: none

Commodity/crop	Supported/ Not supported
Maize (grain and silage)	Supported

5.2 Methods used for the generation of pre-authorization data (KCP 5.1)

5.2.1 Analysis of the plant protection product (KCP 5.1.1)

5.2.1.1 Determination of active substance and/or variant in the plant protection product (KCP 5.1.1)

An overview on the acceptable methods and possible data gaps for analysis of rimsulfuron and thifensulfuron methyl and safener isoxadifen-ethyl in plant protection product is provided as follows:

Comments of zRMS:	The proposed method is acceptable and was successfully validated for the determination of the content of rimsulfuron and thifensulfuron methyl and safener isoxadifen-ethyl in GF-3969 (DPX-V4B07 WG) formulation according to the requirements laid down by SANCO/3030/99 rev.4.
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Reference:	KCP 5.1.1/01
Report:	Robson, D.D., (2017 ^a); Validation of the analytical method for determination of thifensulfuron methyl (DPX-M6316), dicamba (DPX-Y0727), nicosulfuron (DPX-V9360), rimsulfuron (DPX-E9636) and isoxadifen ethyl (DPX-X4145) in DPX-V4B07 24.08WG and DPX-VRF36 60.42 blends of paste-extruded products
DuPont Report No.:	DuPont-44927
Testing Facility Report No.:	DuPont-44927
Guidelines	OCSPP 830.1800 (1996), EC SANCO/3030/99 rev. 5 (2019 2000), Directive Dir98-04 Appendix I, Subsection 2. 13 (1998), APVMA Guidelines for the Validation of Analytical Methods for the Active Constituent, Agricultural and Veterinary Chemical Products (2004)
Deviations:	None
GLP:	Yes
Acceptability:	Yes

Reference:	KCP 5.1.1/02
Report:	Robson, D.D., (2017b); Determination of thifensulfuron methyl (DPX-M6316), dicamba (DPX-Y0727), nicosulfuron (DPX-V9360), rimsulfuron (DPX-E9636) and isoxadifen ethyl (DPX-X4145) in DPX-V4B07 24.08WG and DPX-VRF36 60.42WG blends of paste-extruded products
DuPont Report No.:	DuPont-50247
Testing Facility Report No.:	DuPont-50247
Guidelines	OPPTS 830.1700 (1996), OPPTS 830.1800 (1996), European Economic Community Council, Directive 94/37/EC: Annex I, Subsections 1.1 – 1.7, 1.10 – 1.11 (1994); SANCO/3029/99 rev. 4 (2000), Directive Dir98-04 Appendix I, Subsection 2.13, May 8, 1998, APVMA (Jul 2004), APVMA (Oct 2004) OCSPP 830.1800 (1996), EC SANCO/3030/99 rev. 5 4 (2019 2000), Directive Dir98-04 Appendix I, Subsection 2. 13 (1998), APVMA Guidelines for the Validation of Analytical Methods for the Active Constituent, Agricultural and Veterinary Chemical Products (2004)
Deviations:	None
GLP:	No
Acceptability:	Yes

Materials and methods

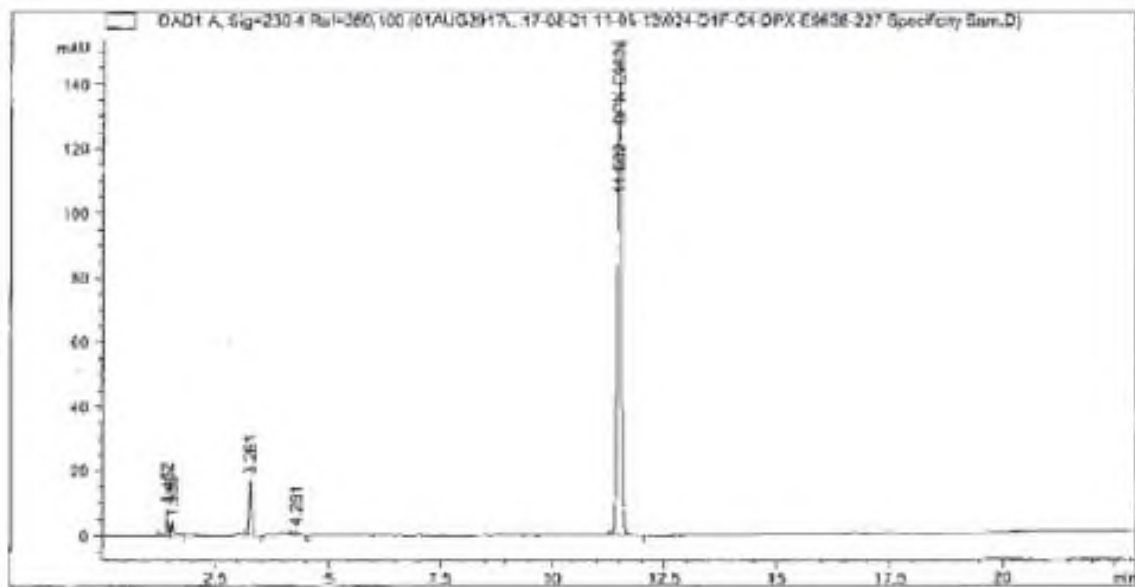
The sample, and standards are dissolved in acetonitrile and analysed by reversed-phase liquid chromatography using a 4.6 mm x 150 mm Zorbax® SB-Phenyl column (3.5-µm particles) and UV detection at 230 nm. Internal standard technique is used for method calibration with diphenyl sulfone used as the internal standard. The weight percent of each active ingredient, rimsulfuron, thifensulfuron methyl, and isoxadifen-ethyl (safener) is determined by comparison to the calibration curves.

Validation - Results and discussions

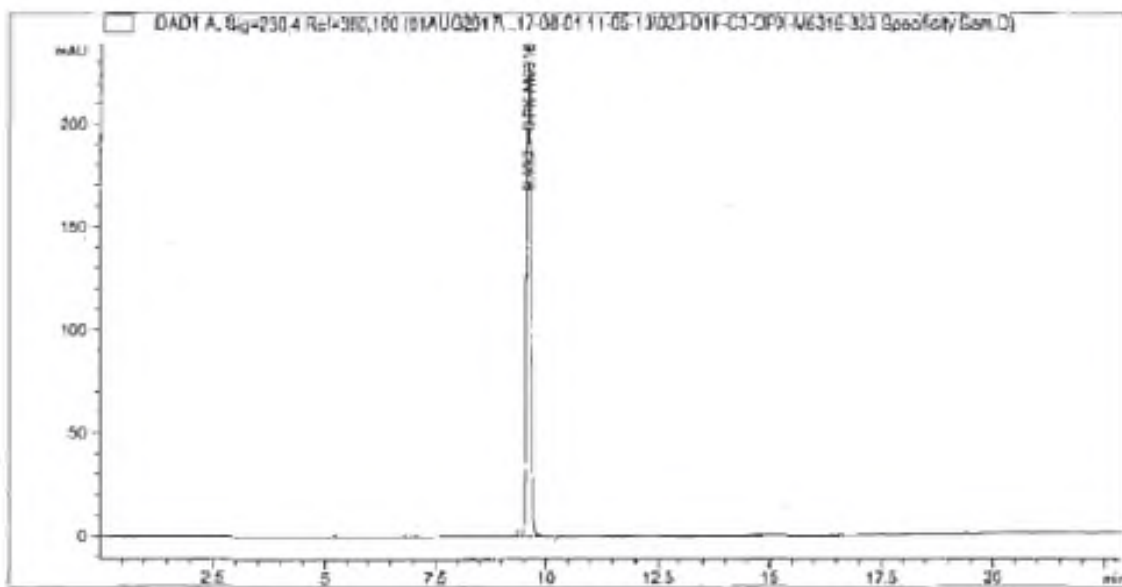
Table 5.2-1: Methods suitable for the determination of active substances rimsulfuron and thifensulfuron methyl and safener isoxadifen-ethyl in plant protection product GF-3969

	Rimsulfuron	Thifensulfuron methyl	Isoxadifen-ethyl
Author(s), year	Robson, D.D., 2017 ^a (DuPont-44927)	Robson, D.D., 2017 ^a (DuPont-44927)	Robson, D.D., 2017 ^a (DuPont-44927)
Principle of method	Reverse Phase HPLC	Reverse Phase HPLC	Reverse Phase HPLC
Linearity (linear between mg/L/ % range of the declared content) (correlation coefficient, expressed as r)	Linear 0.12-1.00 mg/mL (2.4 – 20% wt, nominal is 14.82%); 16.2 – 135% of declared content) Correlation Coefficient = 0.99996	Linear between 0.07-0.62 mg/mL (1.4 – 12.4% wt, nominal is 9.26%); (15.1 – 134% of declared content) Correlation Coefficient = 0.99994	Linear between 0.08-0.70 mg/mL (1.6 – 14% wt, nominal is 11.11%); (14.4 – 126% of declared content) Correlation Coefficient = 0.99993
Precision – Repeatability Mean n = 8 (% RSD)	Mean=14.79 % RSD = 1.51 Horrat = 1.51/1.79 = 0.84; ≤ 1	Mean=8.89 % RSD=1.52 Horrat = 1.52/1.93 = 0.79; ≤ 1	Mean= 11.47 % RSD= 1.21 Horrat = 1.21/1.86 = 0.65; ≤ 1
Accuracy n = 6 (% Recovery)	Percent Recovery = 98.48	Percent Recovery = 99.52	Percent Recovery = 102.46 100.24
Interference/ Specificity	No interferences were observed for each separation.	No interferences were observed for each separation	No interferences were observed for each separation
Comment	Rimsulfuron meets acceptance criteria.	Thifensulfuron methyl meets acceptance criteria.	Isoxadifen-ethyl meets acceptance criteria.

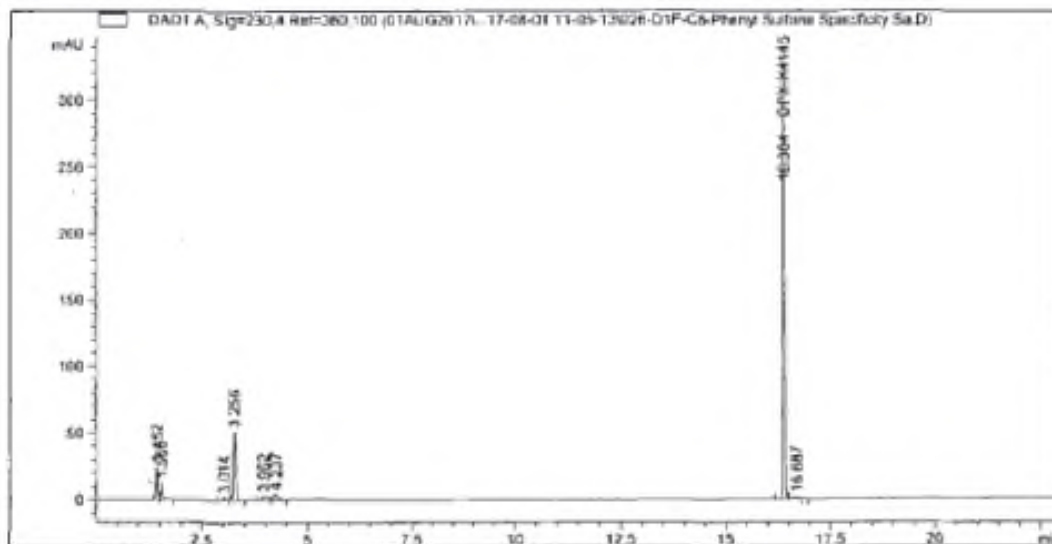
Example Chromatogram for GF-3866 -Rimsulfuron



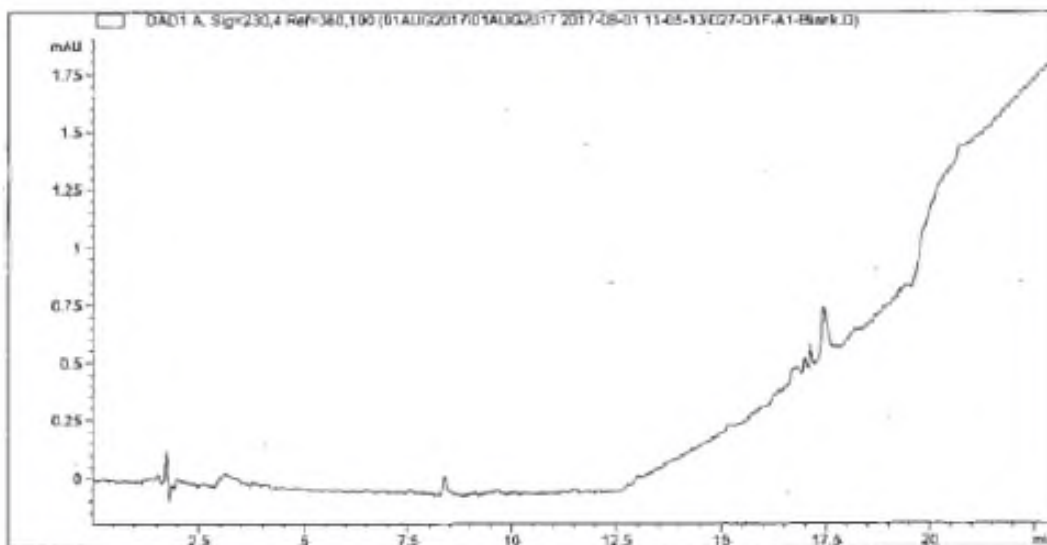
Example Chromatogram for GF-3868 - Thifensulfuron methyl



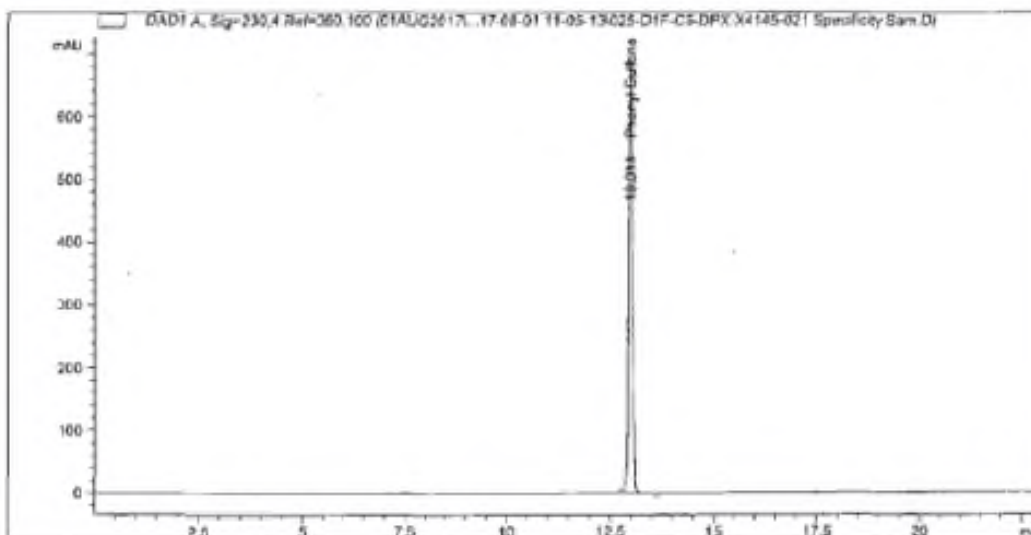
Example Chromatogram for GF-3866 - Isoxadifen-ethyl



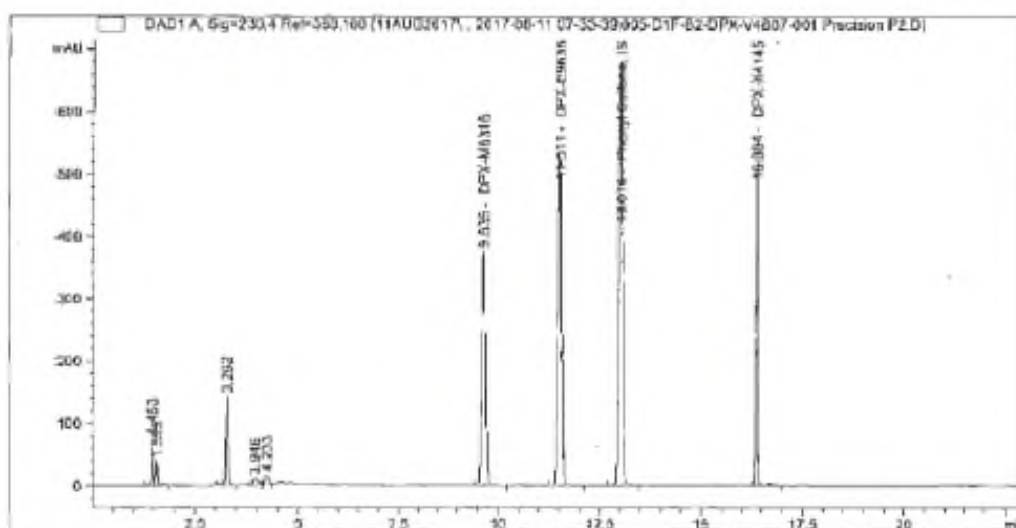
Acetonitrile Blank Chromatogram



Example Chromatogram of Phenyl Sulfone (Internal Standard)



Example Chromatogram of GF-3969 (DPX-V4B07)



Conclusion

The validation results for the analytical method to test for rimsulfuron, thifensulfuron methyl, and isoxadifen-ethyl, DuPont Method No. X4145.220.03.ST, contained in DuPont-50247, meet the following test and reporting guidelines: (1) U.S. Environmental Protection Agency (EPA), (2) European Union (EU), (3) Health Canada Pest Management Regulatory Agency (PMRA), and (4) Australian Pesticides and Veterinary Medicines Authority (APVMA) for selectivity (interferences), linearity, accuracy (recovery) and repeatability (precision). The method can be used to support the registration of GF-3969.

5.2.1.2 Description of analytical methods for the determination of relevant impurities (KCP 5.1.1)

No relevant impurities.

5.2.1.3 Description of analytical methods for the determination of formulants (KCP 5.1.1)

Not relevant.

5.2.1.4 Applicability of existing CIPAC methods (KCP 5.1.1)

There are no CIPAC methods for this formulation.

5.2.2 Methods for the determination of rimsulfuron residues (KCP 5.1.2)

An overview on the acceptable methods and possible data gaps for analysis of residues of rimsulfuron for the generation of pre-authorization data is given in the following table. For the detailed evaluation of new studies, refer to Appendix 2.

The residue definition for food of plant origin is rimsulfuron (risk assessment and monitoring). The crop method used to analyse for rimsulfuron residues in maize in study DuPont-49732 was DuPont-13412, Revision No. 1/Supplement No. 1. The extraction solvent used in this method (3/1 (v/v) acetonitrile/water (buffered)) differs in composition by no more than 20 vol% compared to the solvent used in the maize metabolism study AMR 1233-88 (2/1 (v/v) acetonitrile/water (buffered)). Extraction efficiency has therefore been demonstrated.

Table 5.2-2: Validated methods for the generation of pre-authorization data

Component of residue definition: rimsulfuron				
Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year/ missing/ EU agreed
Maize (whole plant, stover and grain)	Primary	0.01 mg/kg	LC-MS/MS	Spence, C., 2020 (DuPont-49732) EU agreed: No
	Confirmatory	—	—	—
Air (Exposure)	Primary	3 µg/m ³	LC-MS/MS	Bacher, R., 2001 (DuPont-4560 Amended) EU agreed: Yes
	Confirmatory	—	—	—
Honey bee diet (ecotoxicology)	Primary	0.0030 g a.s./L	HPLC-MS/MS	Cornement, M., 2018 (20170301) EU agreed: No
	Confirmatory	—	—	—
Soil, water (Ecotoxicology)	Primary	0.0000148 mg a.s./L	LC-MS/MS	Bergfield, A., 2019 (DuPont-49944) EU agreed: No
	Primary	0.0000148 mg a.s./L	LC-MS/MS	Goudie, O.J., 2019 (DuPont-49978) EU agreed: No
	Primary	0.0743 mg a.s./L	LC-MS/MS	Dinehart, S., 2019 (DuPont-49948, Revision No. 1) EU agreed: No
	Primary	0.743 mg a.s./L	LC-MS/MS	Goudie, O.J., 2019 (DuPont-49949, Revision No. 1) EU agreed: No
	Primary	14.8 mg a.s./kg	HPLC-MS/MS	Verge, E., 2019 (DuPont-48899, Revision No. 1) EU agreed: No
	Primary	14.8 mg a.s./kg	HPLC-MS/MS	Verge, E., 2018 (DuPont-48951) EU agreed: No
	Primary	0.5 mg a.s./kg	HPLC-UV	Arnie, J.R., McKelvey, R.A., Aufderheide, J.A., Lockard, L.A., Zhang, L., 2020 (49942) EU agreed: No

Component of residue definition: rimsulfuron				
Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year/ missing/ EU agreed
	Primary	0.00297 mg a.s./L	LC-MS/MS	Hoover, E., 2019 (DuPont-49943) EU agreed: No
Water, buffer solutions (Properties)	Primary	10 µg/mL	HPLC	Siripriya, G., 2014 (DuPont-36445 ^a) EU agreed: No Yes
	Confirmatory	Confirmatory	—	—

a Summarized in Rimsulfuron RAR, 2017. Renewal Assessment Report for Review of Annex I Inclusion of Rimsulfuron. Annex B: RMS summary and evaluation of the data and information. Annex B.5 – Methods of Analysis. Notifier - DuPont de Nemours (Deutschland) GmbH. Rapporteur Member State: Slovenia; Co-Rapporteur Member State: Finland. Republic of Slovenia Ministry of Agriculture, Forestry, and Food. October 2017.

5.2.3 Methods for the determination of thifensulfuron methyl residues (KCP 5.1.2)

An overview on the acceptable methods and possible data gaps for analysis of residues of thifensulfuron methyl for the generation of pre-authorization data is given in the following table. For the detailed evaluation of new studies, refer to Appendix 2.

The residue definition for food of plant origin is thifensulfuron methyl (risk assessment and monitoring). The crop method used to analyse for thifensulfuron methyl residues in maize in study DuPont-49732 was DuPont-28527. The extraction solvent used in this method (80:20 acetone:water) is identical to the extraction solvent used in the soybean and corn metabolism studies, AMR 572-86 and AMR-532-86, respectively. Extraction efficiency has therefore been demonstrated.

Table 5.2-3: Validated methods for the generation of pre-authorization data

Component of residue definition: thifensulfuron methyl				
Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year/ missing/ EU agreed
Maize (whole plant, stover and grain)	Primary	0.01 mg/kg	LC-MS/MS	Spence, C., 2020 (DuPont-49732) EU agreed: No
	Confirmatory	—	—	—
Soil, water (Ecotoxicology)	Primary	0.00000925 mg a.s./L	LC-MS/MS	Bergfield, A., 2019 (DuPont-49944) EU agreed: No
	Primary	0.00000925 mg a.s./L	LC-MS/MS	Goudie, O.J., 2019 (DuPont-49978) EU agreed: No
	Primary	0.0464 mg a.s./L	LC-MS/MS	Dinehart, S., 2019 (DuPont-49948, Revision No. 1) EU agreed: No
	Primary	0.464 mg a.s./L	LC-MS/MS	Goudie, O.J., 2019 (DuPont-49949, Revision No. 1) EU agreed: No
	Primary	9.26 mg a.s./kg	HPLC-MS/MS	Verge, E., 2019 (DuPont-48899, Revision No. 1) EU agreed: No
	Primary	9.26 mg a.s./kg	HPLC-MS/MS	Verge, E., 2018 (DuPont-48951) EU agreed: No

Component of residue definition: thifensulfuron methyl				
Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year/ missing/ EU agreed
	Primary	0.5 mg a.s./kg	HPLC-UV	Arnie, J.R., McKelvey, R.A., Aufderheide, J.A., Lockard, L.A., Zhang, L., 2020 (49942) EU agreed: No
	Primary	0.00186 mg a.s./L	LC-MS/MS	Hoover, E., 2019 (DuPont-49943) EU agreed: No
	Confirmatory	—	—	—

5.2.4 Methods for the determination of isoxadifen-ethyl (safener) residues (KCP 5.1.2)

An overview on the acceptable methods and possible data gaps for analysis of residues of safener isoxadifen-ethyl for the generation of pre-authorization data is given in the following table. For the detailed evaluation of new/ additional studies, refer to Appendix 3.

Table 5.2-4: Validated methods for the generation of pre-authorization data

Component of residue definition: isoxadifen-ethyl (AE F122006)				
Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year/ missing/ EU agreed
Maize grain	Primary Method DFG S19	0.01 mg/kg	GC-MSD	EU peer-reviewed Germany, 2002*
Maize grain	ILV Method DFG S19	0.01 mg/kg	GC-MSD	EU peer-reviewed Germany, 2002*
Animal products, food of animal origin	Not relevant			
Component of residue definition: isoxadifen-ethyl (AE F122006), isoxadifen free acid (AE F129431)				
Dry commodities: rice (grain, shoot, straw)	Primary DGM F02/98-0	0.01 mg/kg (grain) 0.05 mg/kg (shoots, straw)	HPLC-MS/MS	EU peer-reviewed Germany, 2002*
Maize (grain forage, hay)	Primary Method RAM CA/01/01	0.05 mg/kg for each residue compound in forage and hay 0.02 mg/kg for each residue compound in grain	GC-MS/MS	Dacus, S.C., Neal, J. L., Cole, M., 2001/ Not EU Peer Reviewed Appendix 3 M-238876-02-1 (B003344)
Component of residue definition: isoxadifen-ethyl (AE F122006), isoxadifen (AE F129431), AE F162241				
Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year/ missing/ EU agreed
Rice (shoot, straw)	Primary Method DGM F02/98-0	0.05 mg/kg for each residue compound in shoot and straw	LC-MS/MS	EU peer-reviewed Germany, 2002*
Maize (grain, forage, hay)	Primary Method RAM	0.05 mg/kg for each residue compound in	GC-MS/MS	Dacus, S.C., Neal, J. L., 2000/ Not EU Peer Reviewed

Component of residue definition: isoxadifen-ethyl (AE F122006)				
Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year/ missing/ EU agreed
	CA/01/00	forage and hay 0.02 mg/kg for each residue compound in grain		Appendix 3 M-238556-01-1 (B002825)
Maize (shoot, cob, grain)	Primary Method 00905 (AM 01/08)	0.05 mg/kg for each residue compound in shoot and cob 0.01 mg/kg for each residue compound in grain	HPLC-MS/MS	Kaune, A., 2002, amended by Freitag, Th., 2016/ Not EU Peer Reviewed Appendix 3 M-206994-01-1 (C018951) M-206993-02-1 (C018950)
Component of residue definition: isoxadifen-ethyl, AE F129431, AE C637375				
Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year/ missing/ EU agreed
Rice grain	Primary Method DGM F02/98-0	0.01 mg/kg for each compound in grain	LC-MS/MS	EU peer-reviewed Germany, 2002*
Rice (grain, straw)	Primary Method RAM CA/01/01	0.05 mg/kg for each residue compound in straw 0.02 mg/kg for each residue compound in grain	GC-MS/MS	Dacus, S.C., Neal, J. L., Cole, M., 2001/ Not EU Peer Reviewed Appendix 3 M-238876-02-1 (B003344)
Component of residue definition: isoxadifen-ethyl (AE F122006)				
Soil, water, sediment (Environmental fate)	Not relevant as no studies submitted.			
Soil, water (Efficacy)	Not relevant as no studies submitted.			
Feed, body fluids (Toxicology)	Not relevant as no studies submitted.			
Body fluids, air (Exposure)	Not relevant as no studies submitted.			
Water (Ecotoxicology)	Not relevant as no studies submitted.			
Water, buffer solutions (Properties)	Not relevant as no studies submitted.			

(*) EU peer-reviewed, Germany, 2002 (Summary of the German national evaluation of the safener - isoxadifen-ethyl M-263999-01-1)

5.3 Methods for post-authorization control and monitoring purposes (KCP 5.2)

DuPont is not the notifier for thifensulfuron methyl and does not have direct access to the method reports evaluated in the Renewal Assessment Report (RAR). A letter of access to thifensulfuron methyl data is provided to the Applicant by FMC.

DuPont is not the notifier for the safener isoxadifen-ethyl and does not have direct access to the method reports evaluated in the German national evaluation. A letter of access to isoxadifen-ethyl data is provided to the Applicant by Bayer CropScience.

5.3.1 Analysis of the plant protection product (KCP 5.2)

Please refer to Section 5.2.1.

5.3.2 Description of analytical methods for the determination of residues of rimsulfuron (KCP 5.2)

5.3.2.1 Overview of residue definitions and levels for which compliance is required

Compared to the residue definition proposed in the Draft Assessment Report (incl. its addenda) the current legal residue definition is identical.

Table 5.3-1: Relevant residue definitions for monitoring/enforcement and levels for which compliance is required

Matrix	Residue definition	MRL/ limit	Reference for MRL/level Remarks
Plant, high water content	Rimsulfuron	0.01 mg/kg	Reg. (EU) No 617/2014
Plant, high acid content			
Plant, high protein/high starch content (dry commodities)			
Plant, high oil content			
Plant, difficult matrices (hops, spikes, tea)		0.05 mg/kg	Reg. (EU) No 617/2014
Muscle, Milk, Eggs, Fat, Liver, Kidney	None (not required as intake by animals not significant)	0.02 mg/kg* 0.05 mg/kg* (liver)	Reg. (EU) No 617/2014
Soil (Ecotoxicology)	Rimsulfuron	0.05 mg/kg	Common Limit EFSA Journal 2018;16(5):5258 NOEC = 100 mg a.s./kg dsw, <i>E. fetida</i>
Drinking Water (Human toxicology)	Rimsulfuron	0.1 µg/L	Common Limit, Directive 2006/118/EC
Surface Water (Ecotoxicology)	Rimsulfuron	EC ₅₀ (fronds) = 0.0046 mg a.s./L, <i>L. minor</i>	EFSA Scientific Report (2005) 45, 1-61
Air	Rimsulfuron	21 µg/m ³	EFSA Scientific Report (2005) 45, 1-61 AOEL: 0.07 mg/kg bw/d
Body fluids and tissues	Rimsulfuron	0.05 mg/L* (fluids) 0.1 mg/kg* (tissues)	SANCO/825/00 rev. 8.1 Reg 283/2013

* Default MRL set for animal matrices.

5.3.2.2 Description of analytical methods for the determination of residues in plant matrices (KCP 5.2)

An overview on the acceptable methods and possible data gaps for analysis of rimsulfuron in plant matrices is given in the following tables. For the detailed evaluation of new/additional studies, refer to the Rimsulfuron RAR, Slovenia, Volume 3, CA, Annex B.5, 2017.

Table 5.3-2: Validated methods for food and feed of plant origin (required for all matrix types, “difficult” matrix only when indicated by intended GAP)

Component of residue definition: Rimsulfuron				
Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year/ missing/ EU agreed
High water content	Primary	0.010 mg/kg	HPLC-MS/MS	Cabusas, M.E.Y., Rodgers, C., 2012 (DuPont-32277 ^a) EU agreed: No Yes
	ILV	0.010 mg/kg	HPLC-MS/MS	Rogers, P., 2012 (DuPont-32278 ^a) EU agreed: No Yes
	Confirmatory	0.010 mg/kg	HPLC-MS/MS	Cabusas, M.E.Y., Rodgers, C., 2012 (DuPont-32277 ^a) EU agreed: No Yes
High acid content	Primary	0.010 mg/kg	HPLC-MS/MS	Cabusas, M.E.Y., 2012 (DuPont-15033, Revision No. 2 ^a) EU agreed: No Yes
	ILV	0.010 mg/kg	HPLC-MS/MS	Connolly, P., 2005 (DuPont-15029, Revision No. 1 ^a) EU agreed: No Yes
	Confirmatory	0.010 mg/kg	HPLC-MS/MS	Cabusas, M.E.Y., 2012 (DuPont-15033, Revision No. 2 ^a) EU agreed: No Yes
High oil content	Primary	0.010 mg/kg	HPLC-MS/MS	Cabusas, M.E.Y., 2012 (DuPont-15027, Revision No. 2 ^a) EU agreed: No Yes
	ILV	0.010 mg/kg	HPLC-MS/MS	Platridge, B., 2005 (DuPont-15030 ^a) EU agreed: No Yes
	Confirmatory	0.010 mg/kg	HPLC-MS/MS	Cabusas, M.E.Y., 2012 (DuPont-15027, Revision No. 2 ^a) EU agreed: No Yes
High protein/high starch content (dry)	Primary	0.010 mg/kg	HPLC-MS/MS	Cabusas, M.E.Y., 2012 (DuPont-15033, Revision No. 2 ^a) EU agreed: No Yes
	ILV	0.010 mg/kg	HPLC-MS/MS	Connolly, P., 2005 (DuPont-15029, Revision No. 1 ^a) EU agreed: No Yes
	Confirmatory	0.010 mg/kg	HPLC-MS/MS	Cabusas, M.E.Y., 2012 (DuPont-15033, Revision No. 2 ^a) EU agreed: No Yes

^a Summarized in Rimsulfuron RAR, 2017. Renewal Assessment Report for Review of Annex I Inclusion of Rimsulfuron. Annex B: RMS summary and evaluation of the data and information. Annex B.5 – Methods of Analysis. Notifier - DuPont de Nemours (Deutschland) GmbH. Rapporteur Member State: Slovenia; Co-Rapporteur Member State: Finland. Republic of Slovenia Ministry of Agriculture, Forestry, and Food. October 2017.

Table 5.3-3: Statement on extraction efficiency

	Method for products of plant origin
Required, available from:	AMR 1241-88 and AMR 1222-88 (Rimsulfuron DAR, Germany, Annex B5, 2005)

Extraction efficiency for watery, acidic, and dry crops was evaluated by La Rochelle *et al.* (AMR 1241-88, 1989) using field treated corn plant samples with radiolabelled rimsulfuron. The extraction efficiency of pyridine-labelled rimsulfuron from treated corn plant samples was estimated to range from 89-93%. The extraction solvent used in methods DuPont-32277 and DuPont-15033, Revision No. 2 is identical to the solvent used in the maize metabolism study AMR 1241-88: 4/1 (v/v) methanol/water (buffered).

Extraction efficiency of the extraction procedure used for oily crops was evaluated by Brown, A.M. and Young, G.A. (AMR 1222-88, 1989) using field treated corn plant samples with radiolabelled rimsulfuron. The extraction efficiency of radiolabelled rimsulfuron from treated corn plant samples was estimated to range from 92.3-99.7%. The extraction solvent used in method DuPont-15027, Revision No. 2 (3/1 (v/v) acetonitrile/water (buffered)) differs in composition by no more than 20 vol% compared to the solvent used in the maize metabolism study AMR 1222-88 (2/1 (v/v) acetonitrile/water (buffered)).

5.3.2.3 Description of analytical methods for the determination of residues in animal matrices (KCP 5.2)

An overview on the acceptable methods and possible data gaps for analysis of rimsulfuron in animal matrices is given in the following tables. For the detailed evaluation of new/additional studies, refer to the Rimsulfuron RAR, Slovenia, Volume 3, CA, Annex B.5, 2017. While an enforcement method for animal matrices is not required as the intake by animals has been deemed insignificant and therefore no MRLs proposed, one is provided anyway for good product stewardship.

Table 5.3-4: Validated methods for food and feed of animal origin (if appropriate)

Component of residue definition: Rimsulfuron				
Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year/ missing
Milk, eggs, muscle, fat, kidney, liver	Primary	0.01 mg/kg	HPLC-MS/MS	Pentz, A.M., Cabusas, M.E.Y. 2012 (DuPont-30449 ^a) EU agreed: No Yes
	ILV	0.01 mg/kg	HPLC-MS/MS	Gant, A.G., 2012 (DuPont-30450 ^a) EU agreed: No Yes
	Confirmatory	0.01 mg/kg	HPLC-MS/MS	Pentz, A.M., Cabusas, M.E.Y. 2014 (DuPont-30449, Supplement No. 1 ^a) EU agreed: No Yes

^a Summarized in Rimsulfuron RAR, 2017. Renewal Assessment Report for Review of Annex I Inclusion of Rimsulfuron. Annex B: RMS summary and evaluation of the data and information. Annex B.5 – Methods of Analysis. Notifier - DuPont de Nemours (Deutschland) GmbH. Rapporteur Member State: Slovenia; Co-Rapporteur Member State: Finland. Republic of Slovenia Ministry of Agriculture, Forestry, and Food. October 2017.

Table 5.3-5: Statement on extraction efficiency

	Method for products of animal origin
Required, available from:	AMR 1808-90 (summarized in Rimsulfuron DAR, Germany, Annex B5, 2005)

The method extraction procedure used in DuPont-30449 is similar to the ones used in metabolism studies of radiolabelled compounds of rimsulfuron in egg-laying hens. The extraction solvent used in the radiolabelled study was 2:1 (v/v) acetonitrile:water, while the extraction solvent used in method DuPont-30449 is 9:1 (v/v) acetonitrile:water. While this exceeds the composition difference allowed in SANTE 2017/10632 Rev. 3 (no more than 20 vol% compared), the current animal method is considered fit for purpose. Livestock exposure is low and no MRLs have been set, thus an animal matrix enforcement method is only needed for due diligence. Additionally, literature on sulfonylureas indicates good extraction efficiency in organic solvents (50-90%) and the extraction solvent used in the proposed enforcement method has a higher organic ratio than the one in the metabolism studies. If livestock exposure was deemed significant at a later date, a new animal enforcement method could be generated at that time. A new metabolism study using the 9:1 (v/v) acetonitrile:water extraction solvent would not be conducted as per SANTE 2017/10632 Rev. 3 “it is not expected that new animal metabolism studies or new animal feeding studies should be set up only in order to evaluate aspects of analytical methods and extraction efficiency”.

5.3.2.4 Description of methods for the analysis of soil (KCP 5.2)

An overview on the acceptable methods and possible data gaps for analysis of rimsulfuron in soil is given in the following tables. For the detailed evaluation of new/additional studies, refer to the Rimsulfuron RAR, Slovenia, Volume 3, CA, Annex B.5, 2017.

Table 5.3-6: Validated methods for soil (if appropriate)

Component of residue definition: Rimsulfuron			
Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year/ missing
Primary	0.2 µg/kg	HPLC/MS/MS	Pentz, A., Cabusas, M.E., 2014 (DuPont-38604 ^a) EU agreed: No Yes
Confirmatory	0.2 µg/kg	HPLC/MS/MS	Pentz, A., Cabusas, M.E., 2014 (DuPont-38604 ^a) EU agreed: No Yes
ILV	0.2 µg/kg	HPLC/MS/MS	Fiorito, B., 2014 (DuPont-38605 ^a) EU agreed: No Yes

^a Summarized in Rimsulfuron RAR, 2017. Renewal Assessment Report for Review of Annex I Inclusion of Rimsulfuron. Annex B: RMS summary and evaluation of the data and information. Annex B.5 – Methods of Analysis. Notifier - DuPont de Nemours (Deutschland) GmbH. Rapporteur Member State: Slovenia; Co-Rapporteur Member State: Finland. Republic of Slovenia Ministry of Agriculture, Forestry, and Food. October 2017.

5.3.2.5 Description of methods for the analysis of water (KCP 5.2)

An overview on the acceptable methods and possible data gaps for analysis of rimsulfuron in surface and drinking water is given in the following tables. For the detailed evaluation of new/additional studies, refer to the Rimsulfuron RAR, Slovenia, Volume 3, CA, Annex B.5, 2017.

Table 5.3-7: Validated methods for water (if appropriate)

Component of residue definition: Rimsulfuron				
Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year/ missing
Drinking water Surface water	Primary	0.05 µg/L	HPLC-MS/MS	Taoudi, M., 2015 (Amendment 1, 2017) (Battelle UK Ltd. Report No. FH/14/012 ^a) EU agreed: No Yes
	Confirmatory	0.05 µg/L	HPLC-MS/MS	Taoudi, M., 2015 (Amendment 1, 2017) (Battelle UK Ltd. Report No. FH/14/012 ^a) EU agreed: No
	ILV	0.05 µg/L	HPLC-MS/MS	Benotti, M.J., 2015 (Battelle, USA Report No. 100060226B ^a) EU agreed: No

^a Summarized in Rimsulfuron RAR, 2017. Renewal Assessment Report for Review of Annex I Inclusion of Rimsulfuron. Annex B: RMS summary and evaluation of the data and information. Annex B.5 – Methods of Analysis. Notifier - Task Force Rimsulfuron. Rapporteur Member State: Slovenia; Co-Rapporteur Member State: Finland. Republic of Slovenia Ministry of Agriculture, Forestry, and Food. October 2017.

5.3.2.6 Description of methods for the analysis of air (KCP 5.2)

An overview on the acceptable methods and possible data gaps for analysis of rimsulfuron in air is given in the following table.

Table 5.3-8: Validated methods for air (if appropriate)

Component of residue definition: Rimsulfuron			
Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year/ missing
Primary	3.0 µg/m ³	LC/MS/MS	Bacher, R., 2001 (DuPont-4560 Amended) EU agreed: Yes
Confirmatory	—	—	—

5.3.2.7 Description of methods for the analysis of body fluids and tissues (KCP 5.2)

An overview on the acceptable methods and possible data gaps for analysis of rimsulfuron in body fluids and tissues is given in the following table. For the detailed evaluation of new/additional studies, refer to the Rimsulfuron RAR, Slovenia, Volume 3, CA, Annex B.5, 2017.

Table 5.3-9: Validated methods for body fluids

Method type	Method LOQ	Method Principle	Author(s), year/ missing
Primary	0.010 mg/kg	LC-MS/MS	Pentz, A.M., Cabusas, M.E.Y. 2017 (DuPont-48528 ^a) EU agreed: No Yes

^a Summarized in Rimsulfuron RAR, 2017. Renewal Assessment Report for Review of Annex I Inclusion of Rimsulfuron. Annex B: RMS summary and evaluation of the data and information. Annex B.5 – Methods of Analysis. Notifier - DuPont de Nemours (Deutschland) GmbH. Rapporteur Member State: Slovenia; Co-Rapporteur Member State: Finland. Republic of Slovenia Ministry of Agriculture, Forestry, and Food. October 2017.

Table 5.3-10: Validated methods for body tissues

Method type	Method LOQ	Method Principle	Author(s), year/ missing
Primary	0.01 mg/kg	LC-MS/MS	Pentz, A.M., Cabusas, M.E.Y., 2014 (DuPont-30449, Supplement No. 1 ^a) EU agreed: No Yes

- a Summarized in Rimsulfuron RAR, 2017. Renewal Assessment Report for Review of Annex I Inclusion of Rimsulfuron. Annex B: RMS summary and evaluation of the data and information. Annex B.5 – Methods of Analysis. Notifier - DuPont de Nemours (Deutschland) GmbH. Rapporteur Member State: Slovenia; Co-Rapporteur Member State: Finland. Republic of Slovenia Ministry of Agriculture, Forestry, and Food. October 2017.

5.3.2.8 Other studies/ information

None.

5.3.3 Description of analytical methods for the determination of residues of thifensulfuron methyl (KCP 5.2)

5.3.3.1 Overview of residue definitions and levels for which compliance is required

Compared to the residue definition proposed in the Draft Assessment Report (incl. its addenda) the current legal residue definition is identical.

Table 5.3-11: Relevant residue definitions for monitoring/enforcement and levels for which compliance is required

Matrix	Residue definition	MRL/ limit	Reference for MRL/level Remarks
Plant, high water content	thifensulfuron methyl	0.01 mg/kg	Reg. (EU) No 617/2014
Plant, high acid content			
Plant, high protein/high starch content (dry commodities)			
Plant, high oil content			
Plant, difficult matrices (hops, spices, tea)		0.05 mg/kg	Reg. (EU) No 617/2014
Muscle	thifensulfuron methyl	0.01 mg/kg*	Reg. (EU) No 617/2014
Milk			
Eggs			
Fat			
Liver, kidney			
Soil (Ecotoxicology)	thifensulfuron methyl	0.05 mg /kg	Common Limit EFSA Journal 2015;13(7):4201 LC ₅₀ >2000 mg/kg dw soil, <i>E. fetida</i>
Drinking water (Human toxicology)	thifensulfuron methyl	0.1 µg/L	Common Limit Directive 2006/118/EC
Surface water (Ecotoxicology)	thifensulfuron methyl	EC ₅₀ >100 mg/L (nom), <i>C. riparius</i> ErC ₅₀ = 0.00023 mg/L (mm), <i>V. americana</i> NOEC = 0.00011 mg/L (mm), <i>V. americana</i>	EFSA Journal 2015;13(7):4201
Air	thifensulfuron methyl	21 µg/m ³	EFSA Journal 2015;13(7):4201 AOEL = 0.07 mg/kg bw/d

Matrix	Residue definition	MRL/ limit	Reference for MRL/level Remarks
Body fluids and tissues	thifensulfuron methyl	0.05 mg/L (fluids) 0.1 mg/kg (tissues)	SANCO/825/00 rev. 8.1 Reg 283/2013

* No MRLs proposed for animal matrices, default MRL set.

5.3.3.2 Description of analytical methods for the determination of residues in plant matrices (KCP 5.2)

An overview on the acceptable methods and possible data gaps for analysis of thifensulfuron methyl in plant matrices is given in the following tables.

Table 5.3-12: Validated methods for food and feed of plant origin (required for all matrix types, “difficult” matrix only when indicated by intended GAP)

Component of residue definition: Thifensulfuron methyl ^a				
Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year/ missing/ EU agreed
High water content	Primary	0.01 mg/kg	LC-MS/MS	Henze and Stry, 2014 (DuPont-13412, Supplement No. 4, Revision No. 1) EU agreed: Yes F. M. Brookey, G. L. Westberg, 2007, Report No. DuPont-5367, supplement No. 1 RAR, UK, 2014 EU Agreed
	ILV	0.01 mg/kg	LC-MS/MS	Charles, E., Doran, A. M., Klems, J. P., 2017 (DuPont-13398, Supplement No. 1) EU agreed: No Pentz and Bramble, 2002 (DuPont-8054) EU agreed: Yes
	Confirmatory (if required)	0.01 mg/kg	LC-MS/MS	Henze and Stry, 2014 (DuPont-13412, Supplement No. 4, Revision No. 1) EU agreed: Yes F. M. Brookey, G. L. Westberg, 2007, Report No. DuPont-5367, supplement No. 1 RAR, UK, 2014 EU Agreed
High acid content	Primary	0.01 mg/kg	LC-MS/MS	Devine and Nanita, 2007 (DuPont-13412, Supplement No. 1) EU agreed: Yes
	ILV	0.01 mg/kg	LC-MS/MS	Charles, E., Doran, A. M., Klems, J. P., 2017 (DuPont-13398, Supplement No. 1) EU agreed: No Platridge, 2006 (DuPont-17207, Revision No. 1) EU agreed: Yes
	Confirmatory (if required)	0.01 mg/kg	LC-MS/MS	Henze and Stry, 2014 (DuPont-13412, Supplement No. 4, Revision No. 1) EU agreed: Yes
High oil content and High protein/high starch	Primary	0.01 mg/kg	LC-MS/MS	Pentz and Bramble, 2005 (DuPont-13412, Revision No. 1) EU agreed: Yes

Component of residue definition: Thifensulfuron methyl ^a				
Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year/ missing/ EU agreed
content (dry)	ILV	0.01 mg/kg	LC-MS/MS	Plastridge, 2006 (DuPont-17207, Revision No. 1) EU agreed: Yes
	Confirmatory (if required)	0.01 mg/kg	LC-MS/MS	Henz and Stry, 2014 (DuPont-13412, Supplement No. 4, Revision No. 1) EU agreed: Yes
High protein/high starch content (dry)	Primary	0.01 mg/kg	LC-MS/MS	Amoo and Jones, 2001 (DuPont-5367); Brookey and Westberg, 2007 (DuPont-5367 Supplement No 1) EU agreed: Yes
	ILV	0.01 mg/kg	LC-MS/MS	Pentz and Bramble, 2002 (DuPont-8054) EU agreed: Yes
	Confirmatory (if required)	0.01 mg/kg	LC-MS/MS	Amoo and Jones, 2001 (DuPont-5367); Brookey and Westberg, 2007 (DuPont-5367 Supplement No 1) EU agreed: Yes

a Residue definition for oilseeds and cereals (weed-control use) is thifensulfuron methyl (parent only). Although currently no EU MRLs are set for feed commodities, for possible future applicability it is proposed for animal feed items (grass / alfalfa): Sum of thifensulfuron methyl and thifensulfuron acid (IN-L9225), expressed as thifensulfuron methyl.

Table 5.3-13: Statement on extraction efficiency

	Method for products of plant origin
Required, available from:	Numerous metabolism studies on sulfonylurea

The extraction solvent used in the soybean and corn thifensulfuron methyl radiolabelled studies (AMR 572-86, AMR 532-86) was 80:20 (v/v) acetone:water, while the extraction solvent used in DuPont-13412 (all revisions and supplements) is 3/1 (v/v) acetonitrile/water (buffered). While this exceeds the composition difference allowed in SANTE 2017/10632 Rev. 3 (no more than 20 vol% compared), the current enforcement method is considered fit for purpose. Previous crop metabolism studies of radiolabelled sulfonylurea compounds have demonstrated sufficient extraction efficiency is achieved in a mostly organic solvent (acetone, acetonitrile, and methanol have been used) with an aqueous component buffered for analyte stability. Given the similar structures, polarities, and solubilities of sulfonylurea herbicide actives, it is reasonable to conclude that DuPont-13412 adequately extracts any compound belonging to the sulfonylurea class of chemistry, including thifensulfuron methyl.

5.3.3.3 Description of analytical methods for the determination of residues in animal matrices (KCP 5.2)

An overview on the acceptable methods and possible data gaps for analysis of thifensulfuron methyl in animal matrices is given in the following tables. Methods for the determination of thifensulfuron methyl in foodstuffs of animal origin are not needed, because due to the absence of residues in feedingstuffs, residues cannot occur in products of animal origin. Consistently, the Reasoned Opinion on the review of the existing maximum residue levels (MRLs) for thifensulfuron methyl according to Article 12 of Regulation (EC) No 396/2005 (EFSA Journal 2012;10(8):2863); Thifensulfuron methyl RAR, Volume 3, Annex B.7, March 2015; and EFSA Conclusion on the Peer Review of the Pesticide Risk Assessment of thifensulfuron methyl (EFSA Journal 2015; 13(7):4201)), indicate that MRLs for all groups of livestock products are not required given the use pattern specifications proposed herein. However, it is

worthwhile to note that the MRL regulation published on 3.6.2014 (Regulation No. 617/2014) set an MRL of 0.01 mg/kg (as a default) for tissues, milk and birds eggs for products of animal origin. An enforcement method for animal matrices is therefore provided for good product stewardship.

Table 5.3-14: Validated methods for food and feed of animal origin (if appropriate)

Component of residue definition: Thifensulfuron methyl				
Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year/ missing
Milk, cream, fat, kidney, liver, muscle, eggs	Primary	0.01 mg/kg	Reverse-phase HPLC-MS/MS	Pentz and Cabusas, 2012 (DuPont-30449)/ EU agreed: No
	ILV (milk, liver, eggs)	0.01 mg/kg	Reverse-phase HPLC-MS/MS	Gant, 2012 (DuPont-30450)/ EU agreed: No
	Confirmatory (if required)	0.01 mg/kg	Reverse-phase HPLC-MS/MS	Pentz and Cabusas, 2014 (DuPont-30449, Supplement No. 1)/ EU agreed: No

Table 5.3-15: Statement on extraction efficiency

	Method for products of animal origin
Required, available from:	Numerous metabolism studies on sulfonylurea.

An extraction efficiency study was not conducted since incurred residues of thifensulfuron methyl in animal tissues are not available. New animal metabolism studies should not be conducted per SANTE 2017/10632 Rev. 3 “it is not expected that new animal metabolism studies or new animal feeding studies should be set up only in order to evaluate aspects of analytical methods and extraction efficiency”. However, the residue method extraction procedure is similar to the extraction procedures used in radiolabelled metabolism studies of flupyrsulfuron methyl, nicosulfuron, tribenuron methyl, rimsulfuron, and triflurosulfuron methyl. In these radiolabelled studies flupyrsulfuron methyl, nicosulfuron, tribenuron methyl, rimsulfuron, triflurosulfuron methyl and thifensulfuron methyl were sufficiently extracted from animal tissue samples. Flupyrsulfuron methyl, nicosulfuron, tribenuron methyl, rimsulfuron, triflurosulfuron methyl and thifensulfuron methyl are all sulfonylurea herbicides and have similar polarities and solubility. Therefore since the residue and the metabolism extraction methods are similar and the metabolism studies demonstrated adequate extraction of sulfonylurea herbicides extraction efficiency for animal methods can be assumed to be acceptable.

5.3.3.4 Description of methods for the analysis of soil (KCP 5.2)

An overview on the acceptable methods and possible data gaps for analysis of thifensulfuron methyl in soil is given in the following tables.

Table 5.3-16: Validated methods for soil (if appropriate)

Component of residue definition: Thifensulfuron methyl			
Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year/ missing
Primary	0.05 µg/kg	LC-MS/MS	Hill and Stry, 2001 (DuPont-5082, Revision No. 1)/ EU agreed: Yes
Confirmatory	0.05 µg/kg	LC-MS/MS	Hill and Stry, 2001 (DuPont-5082, Revision No. 1)/ EU agreed: Yes

5.3.3.5 Description of methods for the analysis of water (KCP 5.2)

An overview on the acceptable methods and possible data gaps for analysis of thifensulfuron methyl in surface and drinking water is given in the following tables.

Table 5.3-17: Validated methods for water (if appropriate)

Component of residue definition: Thifensulfuron methyl				
Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year/ missing
Drinking water and surface water	Primary	0.1 ppb (µg/kg)	LC-MS/MS	Henze, R.M., Stry, J., 2013 (DuPont-35704)/EU agreed: Yes No
	ILV	0.1 ppb (µg/kg)	-	Mason, B., 2013 (DuPont-36531)/EU agreed: Yes No
	Confirmatory	0.1 ppb (µg/kg)	-	Henze, R.M., Stry, J., 2013 (DuPont-35704)/EU agreed: Yes No

Evaluator remark:

According to the Thifensulfuron-methyl – Volume 1 List of endpoints, after commenting on the evaluation of confirmatory data (March 2019):

Water (analytical technique and LOQ)

DuPont:
LC-MS/MS – LOQ = 0.05 µg/L for both drinking and surface water
Task Force:
LC-MS/MS – LOQ = 0.1 µg/L for both drinking and surface water.

Additionally in Volume 3, B.5 for Thifensulfuron-methyl (March 2019) it is stated that “*The method DuPont-5491, Revision No. 1 (Analytical method for the determination and confirmation of 13 DuPont sulfonylurea herbicides in water using LC/MS/MS Devine, T.J., Jin, L. (2004) is the proposed enforcement procedure for the analysis of Thifensulfuron-methyl in water sources. The method uses LC/MS/MS detection and has a limit of quantification of 0.05 µg/L. Further validation for water sources were provided in the report DuPont-5491, Supplement No. 1, Revision No. 1. These additional data were also validated down to a level of 0.05 µg/L. (...) Due to the selective nature of the LC-MS/MS method, a separate confirmation method was not necessary.*” ILV method for drinking water was not provided during EU review.

Applicant provided analytical method for the determination of thifensulfuron methyl in drinking, ground and surface water (R. M. Henze, J. J. Stry, 2013, DuPont-35704) and independent laboratory validation of DuPont-35704 (Mason, B., 2013 (DuPont-36531)).

The analytical method (DuPont-35704) was developed and validated for the detection, quantitative analysis and confirmation of residues of thifensulfuron methyl (DPX-M6316) in water using LC/MS/MS. The determined limit of quantitation (LOQ) was 0.1 µg/kg (ppb) for water. The DuPont-35704 analytical method was successfully independently validated for the determination of residues of thifensulfuron methyl in drinking, ground and surface water with a LOQ of 0.10 µg/kg using LC-MS/MS. The studies are acceptable. The details of the evaluation of additional studies are referred in Appendix 2.

5.3.3.6 Description of methods for the analysis of air (KCP 5.2)

An overview on the acceptable methods and possible data gaps for analysis of thifensulfuron methyl in air is given in the following tables.

Table 5.3-18: Validated methods for air (if appropriate)

Component of residue definition: Thifensulfuron methyl			
Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year/ missing
Primary	2.8 µg/m ³	LC-MS/MS	Bacher, R., 2001 (DuPont-4560 Amended) / EU agreed: Yes
Confirmatory	-	-	-

5.3.3.7 Description of methods for the analysis of body fluids and tissues (KCP 5.2)

A method for the analysis of thifensulfuron methyl residues in body fluids and tissues is not required.

Evaluator remark:

According to the EFSA Journal 2015;13(7):4201 a method of analysis for body fluids and tissues is not required. However in Reg (EU) No 283/2013 it is stated that “*methods, with a full description, shall be submitted for the analysis in body fluids and tissues for active substance and relevant metabolites*”.

Applicant provided analytical method for the determination of thifensulfuron methyl in plasma and urine (R. M. Henze, J. J. Stry, 2016, Dupont-47394).

The analytical method was developed and validated for the detection, quantitative analysis and confirmation of residues of thifensulfuron methyl (DPX-M6316) in plasma and urine. The determined limit of quantitation (LOQ) was 1.0 µg/kg (ppb) for plasma and 3.0 µg/kg for urine. The study is acceptable. The details of the evaluation of additional study is referred in Appendix 2.

5.3.3.8 Other studies/ information

None.

5.3.4 Description of analytical methods for the determination of residues of isoxadifen-ethyl (KCP 5.2)

Evaluator remark:

It should be pointed out that formulation GF-3969 contains 111.1 g/kg of safener, isoxadifen-ethyl. Isoxadifen-ethyl is not considered as an active substance and at present MRLs are not set in the EU for safeners.

The Applicant provided the data for safener reviewed by Germany. According to Regulation 1107/2009, data for safener should be evaluated in line with requirements relevant for active substances and EU agreed and peer-reviewed endpoints should be generated. Such evaluation, however, is outside the scope of the product registration and should be carried out at the EU level in order to derive uniform endpoints that may be used in evaluation of various formulations. For this reason studies provided for isoxadifen-ethyl were not validated by the zRMS.

5.3.4.1 Overview of residue definitions and levels for which compliance is required

Isoxadifen-ethyl (a crop safener) is not considered as an active substance and at present MRLs are not set in the EU for safeners. Nevertheless, an Annex II dossier was prepared by BCS for isoxadifen-ethyl and was submitted for evaluation at Member State level. The data has been reviewed by Germany (M-263999-01-1) and a comprehensive evaluation report generated.

The following table summarises the proposed residue definitions for isoxadifen-ethyl. In the 2002 German evaluation (M-263999-01-1) a preliminary plant residue definition for risk assessment and monitoring was proposed as the parent compound only. In the Austrian evaluation in 2006 (Document AGES 2127/06), the parent compound was accordingly proposed to be included in the residue definitions for plants.

Isoxadifen-ethyl is rapidly hydrolysed to form isoxadifen free acid (AE F129431) in plants, the latter

representing the major portion of the residue. Also under deep-freezing storage conditions, isoxadifen-ethyl tends to degrade to isoxadifen (AE F129431) overtime. Thus, the Bayer proposal for the residue definition in plants for risk assessment and monitoring includes the active ingredient and isoxadifen free acid (AE F129431).

Table 5.3-19: Relevant residue definitions for monitoring/enforcement and levels for which compliance is required

Matrix	Residue definition	MRL/ limit	Reference for MRL/level Remarks
Plant, high water content	Isoxadifen-ethyl and isoxadifen free acid (AE F129431), expressed as isoxadifen-ethyl	0.02 mg/kg (LOQ)	Austria, 2006 (Document AGES 2127/06)
Plant, high protein/high starch content (dry commodities)			
Muscle	Not required	-	EU peer-reviewed, Germany, 2002 (M-263999-01-1)*
Milk			
Eggs			
Fat			
Liver, kidney			
Soil (Ecotoxicology)	Isoxadifen-ethyl	0.05 mg/kg	Common limit EU peer-reviewed, Germany, 2002 (M-263999-01-1)* LC ₅₀ >1000 mg/kg dw, <i>E. fetida</i>
Drinking water (Human toxicology)	Isoxadifen-ethyl	0.1 µg/L	General limit for drinking water (0.1 µg/L) EU peer-reviewed, Germany, 2002 (M-263999-01-1)*
Surface water (Ecotoxicology)	Isoxadifen-ethyl	220 µg/L	ErC ₅₀ (fish) = 220 µg/L EU peer-reviewed, Germany, 2002 (M-263999-01-1)*
Air	Isoxadifen-ethyl	6 µg/m ³	AOEL sys: 0.2 mg/kg bw/d Concentration calculated from AOEL: 60 µg/m ³ ** EU peer-review, Germany, 2002 (M-263999-01-1)*
Tissue (meat or liver)	Not relevant	Not relevant	EU peer-reviewed, Germany, 2002 (M-263999-01-1)*
Body fluids	Not relevant	Not relevant	EU peer-reviewed, Germany, 2002 (M-263999-01-1)*

CR: Commission Regulation

* EU peer-reviewed, Germany, 2002 (Summary of the German national evaluation of the safener - isoxadifen-ethyl M-263999-01-1)

** According to SANCO/825/00 rev. 8.1 for monitoring method in air, the concentration calculated from the AOEL is included

5.3.4.2 Description of analytical methods for the determination of residues in plant matrices (KCP 5.2)

An overview on the acceptable methods and possible data gaps for analysis of isoxadifen-ethyl in plant matrices is given in the following tables. For the detailed evaluation of new/ additional studies refer to Appendix 3.

There is no EU agreed residue definition for enforcement in plant and therefore no method is needed. Nevertheless, the analytical methods used for generating the residue data of isoxadifen-ethyl in plant were already provided in the Annex II dossier for isoxadifen-ethyl, evaluated by Germany and later by Greece (as a zRMS) in 2016. All these methods may be used in order to determine and monitor the residues resulting from the use of the formulation GF-3969.

Nevertheless, for the sake of clarity, all analytical methods are reported below.

Table 5.3-20: Validated methods for food and feed of plant origin (required for all matrix types, “difficult” matrix only when indicated by intended GAP)

Component of residue definition: isoxadifen-ethyl				
Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year/ missing/ EU agreed
High protein/high starch content (cereal grain)	Primary EM Method DFG S19	0.01 mg/kg	GC-MSD	EU peer-reviewed Germany, 2002*
	ILV	0.01 mg/kg	GC-MSD	EU peer-reviewed Germany, 2002*
Component of residue definition: isoxadifen-ethyl, isoxadifen (AE F129431)				
Dry commodities: cereal grain**	Primary F02/98-0	0.01 mg/kg	LC-MS/MS	EU peer reviewed Germany, 2002*
Dry commodities: cereal grain: wheat (grain)***	Primary 01300/M029	0.01 mg/kg	HPLC-MS/MS	Winter, O., Amann, S., 2014, M-573745-01-1 (S16-03605) Not EU peer-reviewed Appendix 3
Maize (grain)	ILV (01300/M029)	0.01 mg/kg	HPLC-MS/MS	Meseguer, C., 2017, M-590984-01-1 (S16-04195) Not EU peer-reviewed Appendix 3

* EU peer-reviewed, Germany, 2002 (Summary of the German national evaluation of the safener - isoxadifen-ethyl M-263999-01-1)

** Method validated crop groups: high protein/high starch content (rice straw), High water content (rice shoot)

*** Method validated for various crop groups (e.g. high acid content, high oil content, high water content...).

For any special comments or remarkable points concerning the analytical methods for the determination of residues in plant matrices, please refer to Appendix 3.

Table 5.3-21: Statement on extraction efficiency

	Method for products of plant origin
Required, available from:	-
Not required, because:	Isoxadifen-ethyl is a crop safener, not at active ingredient. Additionally, residues are not expected to be \geq LOQ in maize grain.

5.3.4.3 Description of analytical methods for the determination of residues in animal matrices (KCP 5.2)

Residues of isoxadifen-ethyl and its metabolites are <0.1 mg/kg in corn grain (which may be fed to chicken, dairy cattle, beef cattle and pig) and in corn shoot, which is used for silage and fed to dairy cattle, beef cattle and pig. Thus, there are no significant residues in livestock feed (total animal diet) and no metabolism study is required. Therefore, no analytical method for the determination of residues in food of animal origin is required. Furthermore, no significant residues are likely to occur in any edible animal tissue, and residues of isoxadifen-ethyl do not accumulate in animals. Therefore, no MRLs need to be established for animal tissues. No analytical method for the determination of residues in food of

animal origin is required.

Table 5.3-22: Validated methods for food and feed of animal origin (if appropriate)

Component of residue definition: -				
Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing
Milk	Not relevant			
Eggs				
Muscle				
Liver				
Kidney				

Table 5.3-23: Statement on extraction efficiency

	Method for products of animal origin
Required, available from:	-
Not required, because:	Method for animal matrices not required

5.3.4.4 Description of methods for the analysis of soil (KCP 5.2)

An overview on the acceptable methods and possible data gaps for analysis of isoxadifen-ethyl in soil is given in the following tables. For the detailed evaluation of new/ additional studies refer to Appendix 3.

Table 5.3-24: Validated methods for soil (if appropriate)

Component of residue definition: Isoxadifen-ethyl (AE F122006)			
Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year/ missing
Primary CA/02/069	0.002 µg/kg	GC-NPD	EU peer-reviewed Germany, 2002*
Validation CA/02/99	0.002 µg/kg	GC-NPD	Cole, M. G., Neal, J. L., Dacus, S. C., 2001/ Not EU Peer Reviewed M-185178-02-1 (B003389)
Confirmatory	Method CA/02/99 was initially developed using only GC-NPD as the means of detection and analysis. Subsequent to the development of this method, a GC/MS/MS method was developed as an alternative means of detection. Consequently, GC/MS/MS may be used as a confirmatory technique or as the primary means of analysis for these compounds.		

* EU peer-reviewed, Germany, 2002 (Summary of the German national evaluation of the safener - isoxadifen-ethyl M-263999-01-1)

For any special comments or remarkable points concerning the analytical methods for soil please refer to Appendix 3.

5.3.4.5 Description of methods for the analysis of water (KCP 5.2)

An overview on the acceptable methods and possible data gaps for analysis of isoxadifen-ethyl in surface and drinking water is given in the following tables.

Table 5.3-25: Validated methods for water (if appropriate)

Component of residue definition: Isoxadifen-ethyl (AE F122006)				
Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year/ missing
Drinking water Surface water	Primary/Validation CR99/005	0.05 µg/L	GC-MSD	EU peer-reviewed Germany, 2002*
	Confirmatory	Detection of the substances within this analytical method is based on 2 mass to charge relationships. Additional 3 confirmatory mass to charge relationships are given. The relationship of peak intensities of the different mass to charge relationships can be also be used as additional information		

* EU peer-reviewed, Germany, 2002 (Summary of the German national evaluation of the safener - isoxadifen-ethyl M-263999-01-1)

5.3.4.6 Description of methods for the analysis of air (KCP 5.2)

An overview on the acceptable methods and possible data gaps for analysis of isoxadifen-ethyl in air is given in the following tables. For the detailed evaluation of new/ additional studies please refer to Appendix 3.

Table 5.3-26: Validated methods for air (if appropriate)

Component of residue definition: Isoxadifen-ethyl (AE F122006)			
Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year/ missing
Primary EM C01/99-0	0.6 µg/m ³	GC-MSDS	EU peer-reviewed Germany, 2002*
Validation P 658 G	0.6 µg/m ³	GC-MSDS	Bacher, R., 2003/ M-217537-01-1 (C029624) Not EU Peer Reviewed
Confirmatory	The method uses three ions >100 m/z for the detection of isoxadifen-ethyl residues. A high level of specificity is ensured. Thus no additional confirmatory method is required.		

* EU peer-reviewed, Germany, 2002 (Summary of the German national evaluation of the safener - isoxadifen-ethyl M-263999-01-1)

For any special comments or remarkable points concerning the analytical methods for air it is referred to Appendix 3.

5.3.4.7 Description of methods for the analysis of body fluids and tissues (KCP 5.2)

Not required as isoxadifen-ethyl is as crop safener, not an active substance.

Table 5.3-27: Methods for body fluids and tissues (if appropriate)

Component of residue definition:			
Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing
Primary	Not relevant		
Confirmatory	-		

5.3.4.8 Other studies/ information

None.

Appendix 1 Lists of data considered in support of the evaluation

List of data submitted by the applicant and relied on – all documents

Data point	Author(s)	Year	Title Company Report No. Source GLP or GEP Status Published or not	Vertebrate study Y/N	Owner	Relied upon Y/N
KCP, 5.1.1/01	Robson, D.D.	2017 ^a	Validation of the analytical method for determination of thifensulfuron methyl (DPX-M6316), dicamba (DPX-Y0727), nicosulfuron (DPX-V9360), rimsulfuron (DPX-E9636) and isoxadifen ethyl (DPX-X4145) in DPX-V4B07 24.08WG and DPX-VRF36 60.42 blends of paste-extruded products DuPont-44927 DuPont Stine-Haskell Research Center GLP: Yes Published: No	N	DuPont	Y
KCP, 5.1.1/02	Robson, D.D.	2017 ^b	Determination of thifensulfuron methyl (DPX-M6316), dicamba (DPX-Y0727), nicosulfuron (DPX-V9360), rimsulfuron (DPX-E9636) and isoxadifen ethyl (DPX-X4145) in DPX-V4B07 24.08WG and DPX-VRF36 60.42WG blends of paste-extruded products DuPont-50247 DuPont Stine-Haskell Research Center GLP: No Published: No	N	DuPont	Y
KCP, 5.1.1/03	Baker L.	2022	GF-3969 (DPX-V4B07) - Example Chromatograms E. I. du Pont de Nemours and Company GLP: No Published: No	N	DuPont	Y
KCP, 5.1.2/01	Arnie, J.R., Aufderheidie, J, Lockard, L., Zhang, L.	2020	Isoxadifen ethyl 50WG/Rimsulfuron 25SG/Thifensulfuron methyl 50SG (DPX-V4B07), A blend of paste extruded granules plus isodecylalcohol ethoxylated (DPX-KG691) surfactant: A greenhouse study to investigate the effects on vegetative vigor of ten terrestrial plants following foliar exposure 49942 Eurofins EAG Agrosience LLC GLP: Yes Published: No	N	DuPont	Y
KCP, 5.1.2/02	Bergfield, A.	2019	DPX-V4B07 24 WG (Rimsulfuron 25 SG + Thifensulfuron 50 SG + Isoxadifen 50 WG) A blend of paste extruded granules plus isodecylalcohol ethoxylated (DPX-KG691) surfactant: 7-Day growth inhibition test with the freshwater aquatic plant, duckweed, <i>Lemna gibba</i> DuPont-49944 Eurofins EAG Agrosience, LLC GLP: Yes Published: No	N	DuPont	Y

Data point	Author(s)	Year	Title Company Report No. Source GLP or GEP Status Published or not	Vertebrate study Y/N	Owner	Relied upon Y/N
KCP, 5.1.2/03	Cornement, M.	2018	Rimsulfuron-toxicity to Honey bees (<i>Apis mellifera</i> L.) larvae after repeated exposure under <i>in vitro</i> laboratory conditions 20170301 Innovative Environmental Services (IES) Ltd GLP: Yes Published: No	N	DuPont	Y
KCP, 5.1.2/04	xxxxxxxxxxxxxxxxxxxx	2019	DPX-V4B07 24 WG (rimsulfuron 25 SG + thifensulfuron 50 SG + isoxadifen 50 WG) A blend of paste extruded granules plus isodecylalcohol ethoxylated (DPX-KG691) surfactant: Acute toxicity to the rainbow trout, <i>Oncorhynchus mykiss</i> , determined under static-renewal test conditions DuPont-49948, Revision No. 1 xxxxxxxxxxxxxxxxxxxxxxxxxxxx GLP: Yes Published: No	Y	DuPont	Y
KCP, 5.1.2/05	Goudie, O.J.	2019	DPX-V4B07 24 WG (Rimsulfuron 25 SG + Thifensulfuron 50 SG + Isoxadifen 50 WG) A blend of paste extruded granules plus isodecylalcohol ethoxylated (DPX-KG691) surfactant: 48-Hour static renewal, acute toxicity test with the cladoceran, <i>Daphnia magna</i> DuPont-49949, Revision No. 1 Eurofins EAG Agrosience, LLC GLP: Yes Published: No	N	DuPont	Y
KCP, 5.1.2/06	Goudie, O.J.	2019	DPX-V4B07 24 WG (Rimsulfuron 25 SG + thifensulfuron 50 SG + isoxadifen 50 WG) A blend of paste extruded granules plus crop oil (Codacide): 7-Day growth inhibition test with the freshwater aquatic plant, duckweed, <i>Lemna gibba</i> DuPont-49978 Eurofins EAG Agrosience, LLC GLP: Yes Published: No	N	DuPont	Y
KCP, 5.1.2/07	Hoover, E.	2019	DPX-V4B07 24 WG (Rimsulfuron 25 SG + Thifensulfuron 50 SG + Isoxadifen 50 WG) a blend of paste extruded granules plus isodecylalcohol ethoxylated (DPX-KG691) surfactant: Growth inhibition test with the unicellular green alga, <i>Pseudokirchneriella subcapitata</i> DuPont-49943 Eurofins EAG Agrosience, LLC GLP: Yes Published: No	N	DuPont	Y

Data point	Author(s)	Year	Title Company Report No. Source GLP or GEP Status Published or not	Vertebrate study Y/N	Owner	Relied upon Y/N
KCP, 5.1.2/08	Spence, C.	2020	Magnitude of residues in/on maize following foliar application of DPX-TNS43, a blend of paste extruded granules (62.12% Mesotrione 50WG + 24.24% Rimsulfuron 25SG + 9.09% Thifensulfuron methyl 50SG Active) – EU, initiated 2017 DuPont-49732 Charles River Laboratories Edinburgh Ltd GLP: Yes Published: No	N	DuPont	Y
KCP, 5.1.2/09	Verge, E.	2018	Rimsulfuron 25SG/Thifensulfuron methyl 50SG/Isoxadifen ethyl 50WG (DPX-V4B07) a blend of paste extruded granules (14.82% + 9.26% active) + codacide oil: Acute oral and contact toxicity to the bumble bee, <i>Bombus terrestris</i> L. under laboratory conditions DuPont-48951 Eurofins Agrosience Services EcoChem GmbH / Eurofins Agrosience Services Ecotox GmbH GLP: Yes Published: No	N	DuPont	Y
KCP, 5.1.2/10	Verge, E.	2019	Rimsulfuron 25SG/thifensulfuron methyl 50SG/isoxadifen ethyl 50WG (DPX-V4B07) a blend of paste extruded granules (14.82% + 9.26% active) + surfactant DPX-KG691: Acute oral and contact toxicity to the bumble bee, <i>Bombus terrestris</i> L. under laboratory conditions DuPont-48899, Revision No. 1 Eurofins Agrosience Services EcoChem GmbH / Eurofins Agrosience Services Ecotox GmbH GLP: Yes Published: No	N	DuPont	Y

List of data submitted by the applicant and relied on – vertebrate studies

Data point	Author(s)	Year	Title Company Report No. Source GLP or GEP Status Published or not	Vertebrate study Y/N	Owner	Relied upon Y/N
KCP, 5.1.2/04	xxxxxxxxxxxxxxxxxxxx	2019	DPX-V4B07 24 WG (Rimsulfuron 25 SG + Thifensulfuron 50 SG + Isoxadifen 50 WG) A blend of paste extruded granules plus isodecylalcohol ethoxylated (DPX-KG691) surfactant: Acute toxicity to the rainbow trout, <i>Oncorhynchus mykiss</i> , determined under static-xxxxxxxxxxxxxxxxxxxx GLP: Yes Published: No	Y	DuPont	Y

List of data relied on but not submitted– all documents

The following studies are relied upon and have not been evaluated at the EU level. DuPont has a letter of access for the studies but does not own the studies and therefore they are not submitted.

Data point	Author(s)	Year	Title Company Report No. Source GLP or GEP status Published or not	Vertebrate study Y/N	Owner	Relied upon Y/N
CP, 5.1.2	Dacus, S.C., Neal, J. L., Cole, M.	2001	An analytical method for the determination of residues of Isoxadifen-ethyl (AE F122006) and its major metabolites AE F129431 in corn and rice and AE C637375 in rice by gas chromatography using ion trap mass selective detection, M-238876-02-1 (B003344) GLP: No Published: No	N	Bayer	Not evaluated
CP, 5.1.2	Dacus, S.C., Neal, J. L.	2000	An analytical method for the determination of residues of AE F122006 and its major metabolites AE F129431 and AE F162241 in field corn by gas and liquid chromatography using ion trap mass selective detection: AE F122006 M-238556-01-1 (B002825) GLP: No Published: No	N	Bayer	Not evaluated
CP, 5.1.2	Kaune, A.	2002	Validation of the analytical method AM01/08 for the determination of AE F122006 and its metabolites in maize using LC/MS/MS M-206994-01-1 (C018951) GLP: Yes Published: No	N	Bayer	Not evaluated

Data point	Author(s)	Year	Title Company Report No. Source GLP or GEP status Published or not	Vertebrate study Y/N	Owner	Relied upon Y/N
CP, 5.1.2	Freitag, Th.,	2016	AM01/08 - Analytical method AM01/08 for the determination of AE F122006 and its metabolites in maize using LC/MS/MS M-206993-02-1 (C018950) GLP: Yes Published: No	N	Bayer	Not evaluated
CP, 5.2	Bacher, R.	2006	Isoxadifen-ethyl: Analytical method for the determination of isoxadifen-ethyl in air (validation) M-217537-01-1 (C029624) PTRL Europe GmbH GLP: Yes Published: No	N	Bayer	Not evaluated
CP, 5.2	Cole, M. G.; Neal, J. L.; Dacus, S. C.	2001	An Analytical Method for the Determination of Residues of Residues of Isoxadifenethyl (AE F122006) and its Major Metabolite AE F129431 in Soil by Gas Chromatography Using Nitrogen-Phosphorous or Ion Trap Mass Selective Detection, Revision 1 M-185178-02-1 (B003389) AgrEvo USA Company GLP: No Published: No	N	Bayer	Not evaluated
CP, 5.2	Meseguer, C.	2017	Independent laboratory validation of modification M029 of the analytical method 01300 (based on QuEChERS) for the determination of residues of isoxadifen-ethyl and its metabolites in different matrices of plant origin M-590984-01-1 (S16-04195) Eurofins Agroscience Services, Chem SAS GLP: Yes Published: No	N	Bayer	Not evaluated
CP, 5.2	Winter, O., Amann, S.	2016	Modification M029 of the analytical method 01300 (based on QuEChERS) for the determination of residues of isoxadifen-ethyl and its metabolites in different matrices of plant origin M-573745-01-1 (S16-03605) Eurofins Agroscience Services Chem GmbH GLP: Yes Published: No	N	Bayer	Not evaluated

List of data relied on but not submitted– vertebrate studies

No vertebrate studies relied upon but not submitted.

List of rimsulfuron data submitted or referred to by the applicant and relied on, but already evaluated at EU peer review – all documents

Data point	Author(s)	Year	Title Company Report No. Source GLP or GEP status Published or not	Vertebrate study Y/N	Owner	Relied upon Y/N
CP, 5.1.2	Siripriya, G.	2014	DPX-E9636 (Rimsulfuron): Laboratory study of n-octanol/water partition coefficient DuPont-36445 Advinus Therapeutics Limited GLP: Yes Published: No	N	DuPont	Y
CP, 5.1.2	Bacher, R.	2001	Development and validation of analytical methods for the determination of seven sulfonylurea herbicides in air (Amended) DuPont-4560 PTRL Europe GmbH GLP: Yes Published: No	N	DuPont	Y
CP, 5.2	Cabusas, M.E.Y., Rodgers, C.	2012	Analytical method for the determination of rimsulfuron (DPX-E9636), nicosulfuron (DPX-V9360), and IN-V9367 in crop matrices by HPLC/ESI-MS/MS DuPont-32277 DuPont Stine-Haskell Research Center GLP: No Published: No	N	DuPont	Y
CP, 5.2	Rogers, P.	2012	Independent laboratory validation of DuPont-32277 "Analytical method for the determination of rimsulfuron (DPX-E9636), nicosulfuron (DPX-V9360), and IN-V9367 in crop matrices by HPLC/ESI-MS/MS" DuPont-32278 Alliance Pharma, INC. GLP: Yes Published: No	N	DuPont	Y
CP, 5.2	Cabusas, M.E.Y.	2012	Analytical method for the determination of rimsulfuron (DPX-E9636) in watery, acidic, and dry crop matrices by HPLC/ESI-MS/MS DuPont-15033, Revision No. 2 DuPont Stine-Haskell Research Center GLP: No Published: No	N	DuPont	Y

Data point	Author(s)	Year	Title Company Report No. Source GLP or GEP status Published or not	Vertebrate study Y/N	Owner	Relied upon Y/N
CP, 5.2	Connolly, P.	2005	Independent laboratory validation of the analytical method; DuPont-15033, Analytical method for the determination of rimsulfuron in watery and dry crop matrices by HPLC/ ESI-MS/MS DuPont-15029, Revision No. 1 Exygen Research GLP: Yes Published: No	N	DuPont	Y
CP, 5.2	Cabusas, M.E.Y.	2012	Analytical method for the determination of rimsulfuron in oily crop matrices by HPLC/ESI-MS/MS DuPont-15027, Revision No. 2 DuPont Stine-Haskell Research Center GLP: No Published: No	N	DuPont	Y
CP, 5.2	Platridge, B.	2005	Independent laboratory validation of the analytical method, DuPont-15027, Analytical method for the determination of rimsulfuron in oily crop matrices by HPLC/ESI MS/MS DuPont-15030 Exygen Research GLP: Yes Published: No	N	DuPont	Y
CP, 5.2	Pentz, A.M., Cabusas, M.E.Y.	2012	Analytical method for the determination of DuPont sulfonylurea herbicides in animal matrices using HPLC/MS/MS DuPont-30449 DuPont Stine-Haskell Research Center GLP: No Published: No	N	DuPont	Y
CP, 5.2	Gant, A.G.	2012	Independent laboratory validation of DuPont-30449 "Analytical method for the determination of DuPont sulfonylurea herbicides in animal matrices using HPLC/MS/MS" DuPont-30450 ABC Laboratories, Inc. (Missouri) GLP: Yes Published: No	N	DuPont	Y
CP, 5.2	Pentz, A.M., Cabusas, M.E.Y.	2014	Analytical method for the determination of DuPont sulfonylurea herbicides in animal matrices using HPLC/MS/MS DuPont-30449, Supplement No. 1 DuPont Stine-Haskell Research Center GLP: No Published: No	N	DuPont	Y

Data point	Author(s)	Year	Title Company Report No. Source GLP or GEP status Published or not	Vertebrate study Y/N	Owner	Relied upon Y/N
CP, 5.2	xxxxxxxxxxxxxx	1991	Metabolism study of DPX-E9636 in laying hens AMR 1808-90 xxxxxxxxxxxxxx GLP: No Published: No	Y	DuPont	Y
CP, 5.2	Pentz, A.M., Cabusas, M.E.Y.	2014	Analytical method for the determination of rimsulfuron (DPX-E9636) and its metabolites in soil and water using HPLC/ESI-MS/MS DuPont-38604 DuPont Stine-Haskell Research Center GLP: No Published: No	N	DuPont	Y
CP, 5.2	Fiorito, B.	2014	Independent laboratory validation of DuPont-38604 "Analytical method for the determination of rimsulfuron (DPX-E9636) and its metabolites in soil and water using HPLC/MS/MS" DuPont-38605 Alliance Pharma GLP: Yes Published: No	N	DuPont	Y
CP, 5.2	Taoudi, M.	2015	Method validation – Determination of residues of rimsulfuron and its metabolites IN-70941, IN-70942, IN-J290, IN-E9260, IN-T5831 and IN-JF999 in water FH/14/012 Battelle UK Ltd GLP: Yes Published: No	N	Helm AG, Sapac Agro SA, DuPont*	Y
CP, 5.2	Benotti, M.J.	2015	Independent laboratory validation (ILV) of an analytical method for the determination of rimsulfuron, IN-70941, IN-70942, IN-J290, IN-T5831, IN-JF999 and IN-E9260 in drinking water Report No. 100060226B Battelle, USA GLP: Yes Published: No	N	Helm AG, Sapac Agro SA, DuPont*	Y
CP, 5.2	Pentz, A.M., Cabusas, M.E.Y.	2017	Analytical method for the determination of rimsulfuron (DPX-E9636) in plasma and urine by HPLC/ESI-MS/MS DuPont-48528 DuPont Stine-Haskell Research Center GLP: No Published: No	N	DuPont	Y

*DuPont has Letter of Co-Ownership

List of rimsulfuron data submitted or referred to by the applicant and relied on, but already evaluated at EU peer review – vertebrate studies

Data point	Author(s)	Year	Title Company Report No. Source GLP or GEP status Published or not	Vertebrate study Y/N	Owner	Relied upon Y/N
CP, 5.2	xxxxxxxxxxxxxxxxxxxxx	1991	Metabolism study of DPX-E9636 in laying hens AMR 1808-90 xxxxxxxxxxxxxxxxxxxxx GLP: No Published: No	Y	DuPont	Y

List of thifensulfuron methyl data submitted or referred to by the applicant and relied on, but already evaluated at EU peer review – all documents

Data point	Author(s)	Year	Title Company Report No. Source GLP or GEP status Published or not	Vertebrate study Y/N	Owner	Relied upon Y/N
CP, 5.2	Devine, T.J., Nanita, S.C.	2007	Multiresidue analytical method for the determination of sulfonylurea herbicides in oily, watery, acidic and dry crops using SPE purification and LC/MS/MS detection DuPont-13412, Supplement No. 1 DuPont Stine-Haskell Research Center GLP: No Published: No	N	DuPont	Y
CP, 5.2	Pentz, A.M., Bramble, F.Q.	2005	Analytical method for the determination of nicosulfuron, thifensulfuron-methyl, ethametsulfuron methyl, rimsulfuron, tribenuron methyl, and chlorimuron ethyl in oily crop matrices using SPE purification and LC/MS/MS detection DuPont-13412, Revision No. 1 DuPont Stine-Haskell Research Center GLP: No Published: No	N	DuPont	Y
CP, 5.2	Pentz, A.M., Bramble, F.Q., Devine, T.J., Nanita, S.C., Henze, R.M., Stry, J.J.	2014	Summary of multiresidue analytical method for the determination of sulfonylurea herbicides in oily, watery, acidic and dry crops using SPE purification and LC/MS/MS detection DuPont-13412, Supplement No. 4, Revision No. 1 E.I. du Pont de Nemours and Company GLP: Yes Published: No	N	DuPont	Y

Data point	Author(s)	Year	Title Company Report No. Source GLP or GEP status Published or not	Vertebrate study Y/N	Owner	Relied upon Y/N
CP, 5.2	Platridge, B.	2006	Independent laboratory method validation of a multi-residue method for the analysis of sulfonylurea herbicides in crops DuPont-17207, Revision No. 1 Exygen Research GLP: Yes Published: No	N	DuPont	Y
CP, 5.2	Hill, S.J. Stry, J.J	2001	Analytical method for the determination of 13 DuPont sulfonylurea herbicides in soil using LC/MS/MS DuPont-5082, Revision No. 1 DuPont Stine-Haskell Research Center GLP: No Published: No	N	DuPont	Y
CP, 5.2	Amoo, J.S., Jones, W.	2001	Analytical enforcement method for the determination of Thifensulfuron-methyl, metsulfuron methyl, chlorsulfuron, tribenuron methyl, and flupyrsulfuron methyl in cereals (wheat grain, forage and straw) DuPont Stine-Haskell Research Center DuPont-5367 GLP: No Published: No	N	FMC	Y
CP, 5.2	Brookey, F.M., Westberg, G.L.	2007	Analytical method for the determination of Thifensulfuron-methyl, metsulfuron methyl, chlorsulfuron, tribenuron methyl, and flupyrsulfuron methyl in lettuce and tribenuron methyl and bensulfuron methyl in citrus (oranges) Morse Laboratories, Inc. DuPont-5367, Supplement No. 1 GLP: No Published: No	N	FMC	Y
CP, 5.2	Pentz, A.M. Beamble, F.Q.	2002	Independent Laboratory Validation of DuPont-5367 'Analytical enforcement method for the determination of Thifensulfuron-methyl, metsulfuron methyl, chlorsulfuron, tribenuron methyl, and flupyrsulfuron methyl in cereals (wheat grain, forage and straw)' in wheat grain, barley grain, corn grain and tomato. E.I. du Pont de Nemours and Company DuPont-8054 GLP: Yes Published: No	N	FMC	Y

Data point	Author(s)	Year	Title Company Report No. Source GLP or GEP status Published or not	Vertebrate study Y/N	Owner	Relied upon Y/N
CP, 5.2	Henze, R.M. Stry, J.J.	2013	Analytical method for the determination of thifensulfuron methyl in water using LC/MS/MS DuPont-35704 DuPont Stine Haskell Research Center GLP: No Published: No	N	FMC*	
CP, 5.2	Mason, B.J.	2013	Independent laboratory validation of DuPont-35704, "Analytical method for the determination of thifensulfuron methyl in water using LC/MS/MS" DuPont-36531 Morse Laboratories, Inc. GLP: Yes Published: No	N	FMC*	
CP, 5.2	Bacher, R.	2001	Development and validation of analytical methods for the determination of seven sulfonylurea herbicides in air DuPont-4560 PTRL Europe GLP: Yes Published: No	N	DuPont	Y

*FMC Letter of Access available

List of thifensulfuron methyl data submitted or referred to by the applicant and relied on, but already evaluated at EU peer review – vertebrate studies

No vertebrate studies previously submitted and relied upon.

List of thifensulfuron methyl data submitted or referred to by the applicant and relied on, but not evaluated at EU peer review – all documents

Data point	Author(s)	Year	Title Company Report No. Source GLP or GEP status Published or not	Vertebrate study Y/N	Owner	Relied upon Y/N
CP, 5.2	Charles, E., Doran, A. M., Klems, J. P.	2017	Independent laboratory validation of analytical method DuPont-13412 for the determination of thifensulfuron methyl, ethametsulfuron methyl, rimsulfuron, tribenuron methyl and chlorimuron ethyl in olives and soybean seed using SPE purification and LC/MS/MS detection DuPont-13398, Supplement No. 1 Inveresk GLP: Yes Published: No	N	DuPont	Y

Data point	Author(s)	Year	Title Company Report No. Source GLP or GEP status Published or not	Vertebrate study Y/N	Owner	Relied upon Y/N
CP, 5.2	Pentz, A.M., Cabusas, M.E.Y.	2012	Analytical method for the determination of DuPont sulfonylurea herbicides in animal matrices using HPLC/MS/MS DuPont-30449 DuPont Stine-Haskell Research Center GLP: No Published: No	N	DuPont	Y
CP, 5.2	Gant, A.G.	2012	Independent laboratory validation of DuPont-30449 "Analytical method for the determination of DuPont sulfonylurea herbicides in animal matrices using HPLC/MS/MS" DuPont-30450 ABC Laboratories, Inc. (Missouri) GLP: Yes Published: No	N	DuPont	Y
CP, 5.2	Pentz, A.M., Cabusas, M.E.Y.	2014	Analytical method for the determination of DuPont sulfonylurea herbicides in animal matrices using HPLC/MS/MS DuPont-30449, Supplement No. 1 DuPont Stine-Haskell Research Center GLP: No Published: No	N	DuPont	Y
CP, 5.2	Henze, R. M., Stry J. J.	2016	Analytical method for the determination of chlorsulfuron, metsulfuron methyl, thifensulfuron methyl and tribenuron methyl in plasma and urine by LC/MS/MS DuPont-47394 Stine-Haskell Research Center GLP: No Published: No	N	FMC*	Y
KCP 5.2/02	Henze, R.M., Stry, J.J.,	2013	Analytical method for the determination of thifensulfuron methyl in water using LC/MS/MS DuPont Stine-Haskell Research Center DuPont-35704 GLP: No Published: No	N	DuPont	Y
KCP 5.2/07	Mason, B.J.	2013	Independent laboratory validation of DuPont-35704, "Analytical method for the determination of thifensulfuron methyl in water using LC/MS/MS" Morse Laboratories, Inc. DuPont-36531 GLP: Yes Published: No	N	DuPont	Y

*FMC Letter of Access available

List of data submitted by the applicant and not 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	Owner
-	-	-	-	-	-

List of data relied on not submitted by the applicant but necessary for evaluation

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	Owner
-	-	-	-	-	-

Appendix 2 Detailed evaluation of submitted analytical methods

Unless specifically indicated, all reports in this section are submitted to address mandatory data requirements for the approval of the plant protection product.

A 2.1 Analytical methods for rimsulfuron

A 2.1.1 Methods used for the generation of pre-authorization data (KCP 5.1)

A 2.1.1.1 Study 1, DuPont-49732

Comments of zRMS:	<p>Specimens were analyzed for residues of rimsulfuron using a method based on DuPont Method No. 13412 Revision 1/Supplement 1. The analysis of thifensulfuron methyl and the metabolites IN-L9225 and IN- A4098 was performed using a method based on DuPont Method No. DuPont-28527.</p> <p>Concurrent recoveries for all analytes from untreated samples of all matrices fortified at the LOQ to as high as 0.10 mg/kg ranged from 63-114%. Mean values (\pm standard deviation) per analyte/matrix combination ranged from $85 \pm 14\%$ to $97 \pm 4\%$ for 8 to 18 fortifications per analyte/matrix combination.</p> <p>The determined Limit of Quantification (LOQ) was 0.010 mg/kg for all analytes. The Limit of Detection (LOD) was 0.003 mg/kg for all analytes.</p> <p>No rimsulfuron, thifensulfuron methyl or metabolite residues were detected in unfortified control specimens. Data from the analyses of unfortified controls and fortified controls validated method performance.</p> <p>7-point calibration curves were constructed for rimsulfuron, thifensulfuron methyl and metabolites using peak area counts in integrator units (ac) from injection of known standards versus standard concentrations in ng/mL.</p> <p>All calibration curves generated for each analytical set showed good linearity, i.e., the correlation coefficient R was > 0.99. Standard concentrations for rimsulfuron ranged from 0.15 to 10 ng/mL. Standard concentrations for thifensulfuron methyl and metabolites ranged from 0.10 to 10 ng/mL.</p> <p>The validity criteria for the analytical method according to SANCO/3029/99 rev. 4 have been met.</p> <p>The study is acceptable.</p>
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Reference:	KCP 5.1.2/08
Report:	Spence, C., (2020); Magnitude of residues in/on maize following foliar application of DPX-TNS43, a blend of paste extruded granules (62.12% Mesotrione 50WG + 24.24% Rimsulfuron 25SG + 9.09% Thifensulfuron methyl 50SG Active) – EU, initiated 2017
DuPont Report No.:	DuPont-49732
Testing Facility Report No.:	682133
Guidelines	OECD 509 (2009), SANCO/3029/99 rev. 4 (2000)
Deviations:	None
GLP:	Yes
Acceptability:	Yes

MATERIALS AND METHODS

Method Principle

Residues of rimsulfuron (DPX-E9636) were extracted from maize whole plant, stover, and grain by homogenisation in 75/25 (v/v) acetonitrile/20 mM dibasic potassium phosphate (pH 7) buffer solution. Following centrifugation, extract aliquots were partitioned with hexane and aliquots of the acetonitrile/aqueous layer evaporated to near aqueous for purification using solid-phase extraction (SPE). Rimsulfuron was eluted with an ammonium hydroxide in methanol solution, evaporated to near dryness with a keeper solution of ammonium acetate, and final volumes adjusted using acetonitrile and ammonium acetate. All samples were analysed by liquid chromatography coupled with positive-ion

electrospray tandem mass spectrometry (LC-MS/MS). The limit of detection (LOD) and limit of quantitation (LOQ) were 0.003 mg/kg and 0.01 mg/kg, respectively, in all matrices.

Extraction Efficiency

The extraction solvent used in this method (3/1 (v/v) acetonitrile/water (buffered)) differs in composition by no more than 20 vol% compared to the solvent used in the maize metabolism study AMR 1222-88 (2/1 (v/v) acetonitrile/water (buffered)). Extraction efficiency has therefore been successfully demonstrated.

RESULTS AND DISCUSSION

Mean recovery values at each fortification concentration were within the acceptance range (mean recovery 70-110%; RSD ≤20%). The results obtained are summarised in the following tables.

Table A 1: Recovery results from concurrent recoveries of rimsulfuron (*m/z* 432.3/182.0) using the analytical method

Analyte	Matrix	Fortification level (mg/kg)	Mean Recoveries (%)	RSD (%)	n	Comments
Rimsulfuron	Maize Whole Plant	0.01	89	8	6	*n <5: This is considered to have no impact on the quality of the overall study as results are within acceptable range. Additionally, maize grain and maize stover (dry commodities) have acceptable combined recoveries (n=8) at both fortification levels.
		0.10	94	9	6	
	Maize Stover	0.01	93	5	4*	
		0.10	91	10	4*	
	Maize Grain	0.01	92	6	4*	
		0.10	85	15	4*	

Table A 2: Characteristics for the analytical method used for rimsulfuron residues in maize matrices

	Rimsulfuron
Specificity	<i>m/z</i> 432.3/182.0 blank value <30% LOQ
Calibration (type, number of data points)	linear regression analysis with 1/x weighting $r \geq 0.99$ 7 data points
Calibration range	Concentration range of 0.15-10 ng/mL, equivalent to 0.003-0.2 mg/kg
Limit of determination/quantification	LOQ=0.01 mg/kg

CONCLUSION

This method was successfully validated for the determination of rimsulfuron in maize matrices in accordance with SANCO/3029/99 rev.4 (European Commission, 2000).

A 2.1.1.2 Study 2, DuPont-49948, Revision No. 1

Comments of zRMS:	<p>Concentrations of rimsulfuron and thifensulfuron methyl were determined in in freshwater.</p> <p>Limit of Quantification for rimsulfuron: LOQ=0.0743 mg a.s./L,</p> <p>Limit of Quantification for thifensulfuron methyl: LOQ=0.0464 mg a.s./L.</p> <p>No rimsulfuron and thifensulfuron methyl were detected in the control samples.</p> <p>The mean recoveries for each level for two active substances were in the range 70-110%.</p> <p>The corresponding RSD values were below 20%.</p> <p>The validity criteria for the analytical method according to SANCO/3029/99 rev. 4 have been met.</p> <p>The study is acceptable.</p>
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Reference:	KCP 5.1.2/04
Report:	Dinehart, S., (2019); DPX-V4B07 24 WG (Rimsulfuron 25 SG + Thifensulfuron 50 SG + Isoxadifen 50 WG) A blend of paste extruded granules plus isodecylalcohol ethoxylated (DPX-KG691) surfactant: Acute toxicity to the rainbow trout, <i>Oncorhynchus mykiss</i> , determined under static-renewal test conditions
DuPont Report No.:	DuPont-49948, Revision No. 1
Testing Facility Report No.:	86361
Guidelines	OECD 203 (1992)
Deviations:	None
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

MATERIALS AND METHODS

Method Principle

Residues of rimsulfuron and thifensulfuron methyl, the active substances in GF-3969, are determined from samples of a laboratory freshwater by diluting with acetonitrile (ACN) and further diluting, if necessary, with 50:50 HPLC water:ACN (rimsulfuron analysis) or 0.1:50:50 formic acid:ACN:HPLC water (thifensulfuron methyl analysis). The final sample is analysed for rimsulfuron and thifensulfuron methyl by liquid chromatography coupled with positive-ion electrospray tandem mass spectrometry (LC-MS/MS).

RESULTS AND DISCUSSION

Mean recovery values at each fortification concentration were within the acceptance range (mean recovery 70-110%; RSD ≤20%). The results obtained are summarised in the following tables.

Table A 3: Recovery results from method validation of rimsulfuron (*m/z* 432.00/182.00) using the analytical method

Matrix	Analyte	Fortification level (mg a.s./L)	Mean Recovery (%)	RSD (%)	n	Comments
Freshwater	rimsulfuron	0.0743	86	10	5	5 QC samples from definitive test analyses, ranging from 76 to 96%
Freshwater	rimsulfuron	1.86	97	9	5	5 QC samples from definitive test analyses, ranging from 87 to 105%

Table A 4: Recovery results from method validation of thifensulfuron methyl (m/z 388.00/167.00) using the analytical method

Matrix	Analyte	Fortification level (mg a.s./L)	Mean Recovery (%)	RSD (%)	n	Comments
Freshwater	thifensulfuron methyl	0.0464	101	2	5	5 QC samples from definitive test analyses, ranging from 100 to 104%
Freshwater	thifensulfuron methyl	1.16	103	5	5	5 QC samples from definitive test analyses, ranging from 96 to 110%

Table A 5: Characteristics for the analytical method used for validation of rimsulfuron and thifensulfuron methyl residues in freshwater

	rimsulfuron	thifensulfuron methyl
Specificity	<i>m/z</i> 432.00/182.00 <i>m/z</i> 432.00/325.00 blank value <LOD	<i>m/z</i> 388.00/167.00 <i>m/z</i> 388.00/205.00 <i>m/z</i> 388.00/141.00 blank value <LOD
Calibration (type, number of data points)	linear regression analysis with 1/x weighting $r \geq 0.998$ 7 data points	linear regression analysis with 1/x weighting $r \geq 0.999$ 7 data points
Calibration range	Concentration range of 0.0250-2.50 ng/mL Sample equivalent range of 0.00200 – 0.200 mg a.s./L	Concentration range of 0.0250-2.50 ng/mL Sample equivalent range of 0.00200 – 0.200 mg a.s./L
Limit of quantification	LOQ=0.0743 mg a.s./L	LOQ=0.0464 mg a.s./L

CONCLUSION

This method was successfully validated for the determination of rimsulfuron and thifensulfuron methyl, the active substances in GF-3969, in freshwater.

A 2.1.1.3 Study 3, DuPont-49949, Revision No. 1

Comments of zRMS:	<p>Concentrations of rimsulfuron and thifensulfuron methyl were determined in freshwater.</p> <p>Limit of Quantification for rimsulfuron: LOQ=0.743 mg a.s./L,</p> <p>Limit of Quantification for thifensulfuron methyl: LOQ=0.464 mg a.s./L.</p> <p>No rimsulfuron and thifensulfuron methyl were detected in the control samples.</p> <p>The mean recoveries for each level for two active substances were in the range 70-110%.</p> <p>The corresponding RSD values were below 20%.</p> <p>The validity criteria for the analytical method according to SANCO/3029/99 rev. 4 have been met.</p> <p>The study is acceptable.</p>
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Reference:	KCP 5.1.2/05
Report:	Goudie, O.J., (2019); DPX-V4B07 24 WG (Rimsulfuron 25 SG + Thifensulfuron 50 SG + Isoxadifen 50 WG) A blend of paste extruded granules plus isodecylalcohol ethoxylated (DPX-KG691) surfactant: 48-Hour static renewal, acute toxicity test with the cladoceran, <i>Daphnia magna</i>
DuPont Report No.:	DuPont-49949, Revision No. 1
Testing Facility Report No.:	86363
Guidelines	OECD 202 (2004)
Deviations:	None
GLP:	Yes
Acceptability:	Yes

MATERIALS AND METHODS

Method Principle

Residues of rimsulfuron and thifensulfuron methyl, the active substances in GF-3969 are determined from samples of a laboratory freshwater by diluting with acetonitrile (ACN) and further diluting, if necessary, with 50:50 HPLC water:ACN (rimsulfuron analysis) or 0.1:50:50 formic acid:ACN:HPLC water (thifensulfuron methyl analysis). The final sample is analysed for rimsulfuron and thifensulfuron methyl by liquid chromatography coupled with positive-ion electrospray tandem mass spectrometry (LC-MS/MS).

RESULTS AND DISCUSSION

Mean recovery values at each fortification concentration were within the acceptance range (mean recovery 70-110%; RSD ≤20%). The results obtained are summarised in the following tables.

Table A 6: Recovery results from method validation of rimsulfuron (*m/z* 432.00/182.00) using the analytical method

Matrix	Analyte	Fortification level (mg a.s./L)	Mean Recovery (%)	RSD (%)	n	Comments
Freshwater	rimsulfuron	0.743	92	3	5	5 QC samples from definitive test analyses, ranging from 89 to 95%
Freshwater	rimsulfuron	18.3	93	2	5	5 QC samples from definitive test analyses, ranging from 90 to 95%

Table A 7: Recovery results from method validation of thifensulfuron methyl (m/z 388.00/167.00) using the analytical method

Matrix	Analyte	Fortification level (mg a.s./L)	Mean Recovery (%)	RSD (%)	n	Comments
Freshwater	thifensulfuron methyl	0.464	109	4	5	5 QC samples from definitive test analyses, ranging from 102 to 112%
Freshwater	thifensulfuron methyl	11.4	109	3	5	5 QC samples from definitive test analyses, ranging from 105 to 114%

Table A 8: Characteristics for the analytical method used for validation of rimsulfuron and thifensulfuron methyl residues in freshwater

	rimsulfuron	thifensulfuron methyl
Specificity	<i>m/z</i> 432.00/182.00 <i>m/z</i> 432.00/325.00 blank value <LOD	<i>m/z</i> 388.00/167.00 <i>m/z</i> 388.00/205.00 <i>m/z</i> 388.00/141.00 blank value <LOD
Calibration (type, number of data points)	linear regression analysis with 1/x weighting $r \geq 0.998$ 7 data points	linear regression analysis with 1/x weighting $r \geq 0.999$ 7 data points
Calibration range	Concentration range of 0.0250-2.50 ng/mL Sample equivalent range of 0.0200 – 2.00 mg a.s./L	Concentration range of 0.0250-2.50 ng/mL Sample equivalent range of 0.0200 – 2.00 mg a.s./L
Limit of determination/quantification	LOD=0.223 mg a.s./L LOQ=0.743 mg a.s./L	LOD=0.139 mg a.s./L LOQ=0.464 mg a.s./L

CONCLUSION

This method was successfully validated for the determination of GF-3969 as rimsulfuron and thifensulfuron methyl in freshwater.

A 2.1.1.4 Study 4, DuPont-49944

Comments of zRMS:	<p>Concentrations of rimsulfuron and thifensulfuron methyl were determined in 20X freshwater algal nutrient medium (20X FWAM).</p> <p>Limit of Quantification for rimsulfuron: LOQ=0.0000148 mg a.s./L.</p> <p>Limit of Quantification for thifensulfuron methyl: LOQ=0.00000925 mg a.s./L.</p> <p>No rimsulfuron and thifensulfuron methyl were detected in the control samples.</p> <p>The mean recoveries for each level for two active substances were in the range 70-110%.</p> <p>The corresponding RSD values were below 20%.</p> <p>The validity criteria for the analytical method according to SANCO/3029/99 rev. 4 have been met.</p> <p>The study is acceptable.</p>
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Reference:	KCP 5.1.2/02
Report:	Bergfield, A., (2019); DPX-V4B07 24 WG (Rimsulfuron 25 SG + Thifensulfuron 50 SG + Isoxadifen 50 WG) A blend of paste extruded granules plus isodecylalcohol ethoxylated (DPX-KG691) surfactant: 7-Day growth inhibition test with the freshwater aquatic plant, duckweed, <i>Lemna gibba</i>
DuPont Report No.:	DuPont-49944
Testing Facility Report No.:	86356
Guidelines	OECD 221 (2006)
Deviations:	None
GLP:	Yes
Acceptability:	Yes

MATERIALS AND METHODS

Method Principle

Residues of GF-3969, based on thifensulfuron methyl and rimsulfuron total active substances, are determined from samples of 20X freshwater algal nutrient medium (20X FWAM) by loading samples into solid phase extraction (SPE) cartridges preconditioned with methanol and HPLC water. After loading the sample, the sample flask is rinsed with HPLC water and this rinse added to the SPE cartridge, using vacuum to remove any residual water. The SPE cartridge is eluted with a known volume of methanol which is collected into a culture tube, using vacuum to remove any residual methanol from the SPE cartridge. For the low quality control (QC) samples, the eluates are concentrated to a known volume with nitrogen. Two separate aliquots are then diluted, one with 10 mM ammonium acetate and the other with 0.2% formic acid in water, for analysis of rimsulfuron and thifensulfuron methyl, respectively. For the high QC samples, the eluate is not concentrated, but separate portions are diluted, one with 30:70 methanol:10 mM ammonium acetate, the other with 0.1:50:50 formic acid:methanol:HPLC water for analysis of rimsulfuron and thifensulfuron methyl, respectively. The final sample is analysed for thifensulfuron methyl and rimsulfuron active substances by liquid chromatography coupled with positive-ion electrospray tandem mass spectrometry (LC-MS/MS).

RESULTS AND DISCUSSION

Mean recovery values at each fortification concentration were within the acceptance range (mean recovery 70-110%; RSD ≤20%). The results obtained are summarised in the following tables.

Table A 9: Recovery results from method validation of rimsulfuron (*m/z* 432.00/182.00) using the analytical method

Matrix	Analyte	Fortification level (mg a.s./L)	Mean Recovery (%)	RSD (%)	n	Comments
20X FWAM	rimsulfuron	0.0000148	90	7	11	5 method validation sample analyses + 6 QC samples from definitive test analyses, ranging from 80 to 100%
20X FWAM	rimsulfuron	0.00222	104	6	11	5 method validation sample analyses + 6 QC samples from definitive test analyses, ranging from 97 to 111%

Table A 10: Recovery results from method validation of thifensulfuron methyl (*m/z* 388.00/167.00) using the analytical method

Matrix	Analyte	Fortification level (mg a.s./L)	Mean Recovery (%)	RSD (%)	n	Comments
20X FWAM	thifensulfuron methyl	0.00000925	98	19	11	5 method validation sample analyses + 6 QC samples from definitive test analyses, ranging from 85 to 151%. NOTE: one fortification had 151% recovery (concurrent study QC for day 1 spent sample analysis). Excluding this sample from statistics, mean recoveries at this fortification level was 93% with an RSD of 7%, and individual recoveries ranged from 85-103%
20X FWAM	thifensulfuron methyl	0.00139	99	2	11	5 method validation sample analyses + 6 QC samples from definitive test analyses, ranging from 97 to 103%

Table A 11: Characteristics for the analytical method used for validation of thifensulfuron methyl and rimsulfuron residues in 20X FWAM

	rimsulfuron	thifensulfuron methyl
Specificity	<i>m/z</i> 432.00/182.00 <i>m/z</i> 432.00/325.00 blank value <LOD	<i>m/z</i> 388.00/167.00 <i>m/z</i> 388.00/205.00 <i>m/z</i> 388.00/141.00 blank value <LOD
Calibration (type, number of data points)	linear regression analysis with 1/x weighting $r \geq 0.999$ 7 data points	Linear regression analysis with 1/x weighting $r \geq 0.999$ 7 data points
Calibration range	Concentration range of 0.0250-2.50 ng/mL Sample equivalent range of 0.00000100 – 0.000100 mg a.s./L	Concentration range of 0.0250-2.50 ng/mL Sample equivalent range of 0.00000100 – 0.000100 mg a.s./L
Limit of determination/quantification	LOD = 0.00000444 mg a.s./L LOQ = 0.0000148 mg a.s./L	LOD = 0.00000277 mg a.s./L LOQ = 0.00000925 mg a.s./L

CONCLUSION

This method was successfully validated for the determination of GF-3969, based on thifensulfuron methyl and rimsulfuron total active substances in 20X FWAM.

A 2.1.1.5 Study 5, DuPont-49978

Comments of zRMS:	<p>Concentrations of rimsulfuron and thifensulfuron methyl were determined in 20X freshwater algal nutrient medium (20X FWAM).</p> <p>Limit of Quantification for rimsulfuron: LOQ=0.0000148 mg a.s./L.</p> <p>Limit of Quantification for thifensulfuron methyl: LOQ=0.00000925 mg a.s./L.</p> <p>No rimsulfuron and thifensulfuron methyl were detected in the control samples.</p> <p>The mean recoveries for each level for two active substances were in the range 70-110%.</p> <p>The corresponding RSD values were below 20%.</p> <p>The validity criteria for the analytical method according to SANCO/3029/99 rev. 4 have been met.</p> <p>The study is acceptable.</p>
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Reference:	KCP 5.1.2/06
Report:	Goudie, O.J., (2019); DPX-V4B07 24 WG (Rimsulfuron 25 SG + Thifensulfuron 50 SG + Isoxadifen 50 WG) A blend of paste extruded granules plus crop oil (Codacide): 7-Day growth inhibition test with the freshwater aquatic plant, duckweed, <i>Lemna gibba</i>
DuPont Report No.:	DuPont-49978
Testing Facility Report No.:	86359
Guidelines	OECD 221 (2006)
Deviations:	None
GLP:	Yes
Acceptability:	Yes

MATERIALS AND METHODS

Method Principle

Residues of GF-3969, based on thifensulfuron methyl (DPX-M6316) and rimsulfuron (DPX-E9636) total active substances, are determined from samples of 20X freshwater algal nutrient medium (20X FWAM), by loading samples into solid phase extraction (SPE) cartridges preconditioned with methanol and HPLC water. After loading the sample, the sample flask is rinsed with HPLC water and this rinse added to the SPE cartridge, using vacuum to remove any residual water. The SPE cartridge is eluted with a known volume of methanol which is collected into a culture tube, using vacuum to remove any residual methanol from the SPE cartridge. For the low quality control (QC) samples, the eluates are concentrated to a known volume with nitrogen. Two separate aliquots are then diluted, one with 10 mM ammonium acetate in water and the other with 0.2% formic acid in water, for analysis of rimsulfuron and thifensulfuron methyl, respectively. For the high QC samples, the eluate is not concentrated, but separate portions are diluted, one with 30:70 HPLC water:methanol:10 mM ammonium acetate, the other with 0.1:50:50 formic acid:methanol:HPLC water for analysis of rimsulfuron and thifensulfuron methyl, respectively. The final sample is analysed for thifensulfuron methyl and rimsulfuron active substances by liquid chromatography coupled with positive-ion electrospray tandem mass spectrometry (LC-MS/MS).

RESULTS AND DISCUSSION

Mean recovery values at each fortification concentration were within the acceptance range (mean recovery 70-110%; RSD ≤20%). The results obtained are summarised in the following tables.

Table A 12: Recovery results from method validation of rimsulfuron (*m/z* 432.00/182.00) using the analytical method

Matrix	Analyte	Fortification level (mg a.s./L)	Mean Recovery (%)	RSD (%)	n	Comments
20X FWAM	rimsulfuron	0.0000148	88	11	11	5 method validation sample analyses + 6 QC samples from definitive test analyses, ranging from 74 to 105%

Matrix	Analyte	Fortification level (mg a.s./L)	Mean Recovery (%)	RSD (%)	n	Comments
20X FWAM	rimsulfuron	0.00222	107	3	11	5 method validation sample analyses + 6 QC samples from definitive test analyses, ranging from 102 to 112%

Table A 13: Recovery results from method validation of thifensulfuron methyl (*m/z* 388.00/167.00) using the analytical method

Matrix	Analyte	Fortification level (mg a.s./L)	Mean Recovery (%)	RSD (%)	n	Comments
20X FWAM	thifensulfuron methyl	0.00000925	95	11	11	5 method validation sample analyses + 6 QC samples from definitive test analyses, ranging from 72 to 113%
20X FWAM	thifensulfuron methyl	0.00139	104	5	11	5 method validation sample analyses + 6 QC samples from definitive test analyses, ranging from 92 to 111%

Table A 14: Characteristics for the analytical method used for validation of rimsulfuron and thifensulfuron methyl residues in 20X FWAM

	rimsulfuron (DPX-E9636)	thifensulfuron methyl (DPX-M6316)
Specificity	<i>m/z</i> 432.00/182.00 <i>m/z</i> 432.00/325.00 blank value <LOD	<i>m/z</i> 388.00/167.00 <i>m/z</i> 388.00/205.00 blank value <LOD
Calibration (type, number of data points)	linear regression analysis with 1/x weighting $r \geq 0.999$ 7 data points	linear regression analysis with 1/x weighting $r \geq 0.999$ 7 data points
Calibration range	Concentration range of 0.0250-2.50 ng/mL Sample equivalent range of 0.00000100 – 0.000100 mg a.s./L	Concentration range of 0.0250-2.50 ng/mL Sample equivalent range of 0.00000100 – 0.000100 mg a.s./L
Limit of determination/quantification	LOD=0.00000444 mg a.s./L LOQ=0.0000148 mg a.s./L	LOD=0.00000277 mg a.s./L LOQ=0.00000925 mg a.s./L

CONCLUSION

This method was successfully validated for the determination of thifensulfuron methyl and rimsulfuron in 20X FWAM.

A 2.1.1.6 Study 6, DuPont-49943

Comments of zRMS:	<p>Concentrations of rimsulfuron and thifensulfuron methyl were determined in freshwater algal medium (FWAM; equivalent to AAP medium).</p> <p>Limit of Quantification for rimsulfuron: LOQ=0.00297 mg a.s./L,</p> <p>Limit of Quantification for thifensulfuron methyl: LOQ=0.00186 mg a.s./L.</p> <p>No rimsulfuron and thifensulfuron methyl were detected in the control samples.</p> <p>The mean recoveries for each level for two active substances were in the range 70-110%.</p> <p>The corresponding RSD values were below 20%.</p> <p>The validity criteria for the analytical method according to SANCO/3029/99 rev. 4 have been met.</p> <p>The study is acceptable.</p>
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Reference:	KCP 5.1.2/07
Report:	Hoover, E., (2019); DPX-V4B07 24 WG (Rimsulfuron 25 SG + Thifensulfuron 50 SG + Isoxadifen 50 WG) a blend of paste extruded granules plus isodecylalcohol ethoxylated (DPX-KG691) surfactant: Growth inhibition test with the unicellular green alga, <i>Pseudokirchneriella subcapitata</i>
DuPont Report No.:	DuPont-49943
Testing Facility Report No.:	86355
Guidelines	OECD 201
Deviations:	None
GLP:	Yes
Acceptability:	Yes

MATERIALS AND METHODS

Method Principle

Residues of GF-3969, based on the combined rimsulfuron (DPX-E9636) and thifensulfuron methyl (DPX-M6316) total active substances, are determined from samples of a freshwater algal medium (FWAM; equivalent to AAP medium) by centrifuging the samples for 10 minutes at approximately 4000 rpm, then taking an aliquot of the supernatant and diluting it with acetonitrile (ACN), and further diluting, if necessary with 50:50 HPLC water:ACN (rimsulfuron analysis) or 0.1:50:50 formic acid:ACN:HPLC water (thifensulfuron methyl analysis). The final sample is analysed for rimsulfuron and thifensulfuron methyl total active substances, by liquid chromatography coupled with positive-ion electrospray tandem mass spectrometry (LC-MS/MS).

RESULTS AND DISCUSSION

Mean recovery values at each fortification concentration were within the acceptance range (mean recovery 70-110%; RSD ≤20%). The results obtained are summarised in the following tables.

Table A 15: Recovery results from method validation of rimsulfuron (*m/z* 432.00/182.00) using the analytical method

Matrix	Analyte	Fortification level (mg a.s./L)	Mean Recovery (%)	RSD (%)	n	Comments
FWAM	rimsulfuron	0.00297	85	3	6	6 QC samples from definitive test analyses, ranging from 82 to 89%
FWAM	rimsulfuron	1.86	88	2	5	5 QC samples from definitive test analyses, ranging from 85 to 91%

Table A 16: Recovery results from method validation of thifensulfuron methyl (*m/z* 388.00/167.00) using the analytical method

Matrix	Analyte	Fortification level (mg a.s./L)	Mean Recovery (%)	RSD (%)	n	Comments
FWAM	thifensulfuron methyl	0.00186	105	3	6	6 QC samples from definitive test analyses, ranging from 101 to 109%
FWAM	thifensulfuron methyl	1.16	100	4	5	5 QC samples from definitive test analyses, ranging from 95 to 103%

Table A 17: Characteristics for the analytical method used for validation of rimsulfuron and thifensulfuron methyl residues in FWAM

	rimsulfuron	thifensulfuron methyl
Specificity	<i>m/z</i> 432.00/182.00 blank value <LOD	<i>m/z</i> 388.00/167.00 blank value <LOD
Calibration (type, number of data points)	linear regression analysis with 1/x weighting $r \geq 0.997$ 7 data points	Linear regression analysis with 1/x weighting $r \geq 0.999$ 7 data points
Calibration range	Concentration range of 0.0250-2.50 ng/mL Sample equivalent range of 0.000125 – 0.0125 mg a.s./L	Concentration range of 0.0250-2.50 ng/mL Sample equivalent range of 0.000125 – 0.0125 mg a.s./L
Limit of determination/quantification	LOD=0.000891 mg a.s./L LOQ=0.00297 mg a.s./L	LOD=0.000558 mg a.s./L LOQ=0.00186 mg a.s./L

CONCLUSION

This method was successfully validated for the determination of GF-3969 based on rimsulfuron and thifensulfuron methyl in FWAM.

A 2.1.1.7 Study 7, DuPont-48899, Revision No. 1

Comments of zRMS:	<p>An analytical method for the determination of thifensulfuron-methyl and rimsulfuron in 50% (w/v) aqueous sucrose solution and deionised water + 0.1% Triton X was validated with regard to recovery, linearity of detector response, repeatability, specificity, limit of quantification and limit of detection. The analytical method fulfils the requirements of guideline SANCO/3029/99 rev. 4.</p> <p>The limit of quantification (LOQ) of the analytical method was 100 mg test item/L (equivalent to 9.26 mg thifensulfuron-methyl/L and 14.8 mg rimsulfuron/L) in both matrices.</p> <p>No rimsulfuron and thifensulfuron methyl were detected in the control samples.</p> <p>The mean recoveries for each level for two active substances were in the range 70-110%.</p> <p>The corresponding RSD values were below 20%.</p> <p>The study is acceptable.</p>
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Reference:	KCP 5.1.2/10
Report:	Verge, E., (2019); Rimsulfuron 25SG/thifensulfuron methyl 50SG/isoxadifen ethyl 50WG (DPX-V4B07) a blend of paste extruded granules (14.82% + 9.26% active) + surfactant DPX-KG691: Acute oral and contact toxicity to the bumble bee, <i>Bombus terrestris</i> L. under laboratory conditions
DuPont Report No.:	DuPont-48899, Revision No. 1
Testing Facility Report No.:	S18-00130
Guidelines	OECD 247 (2017), OECD 246 (2017)
Deviations:	None
GLP:	Yes
Acceptability:	Yes

MATERIALS AND METHODS

Method Principle

Concentrations of GF-3969 plus surfactant, based on concentrations of the active ingredients rimsulfuron and thifensulfuron methyl, were determined in 50% (w/v) aqueous sucrose solution (oral application solution) and in deionised water + 0.1% Triton X (contact application solution) by dilution of the samples with acetonitrile/water (1:1, v/v) prior to analysis by liquid chromatography coupled with tandem mass spectrometry (LC MS/MS). The limit of detection (LOD) and limit of quantitation (LOQ) were 30 mg test item/L (equivalent to 4.44 mg rimsulfuron/L and 2.78 mg thifensulfuron methyl/L) and 100 mg test item/L (equivalent to 14.8 mg rimsulfuron/L and 9.26 mg thifensulfuron methyl/L), respectively, in all matrices.

RESULTS AND DISCUSSION

Mean recovery values at each fortification concentration were within the acceptance range (mean recovery 70-110%; RSD \leq 20%). The results obtained are summarised in the following tables.

Table A 18: Recovery results from method validation of rimsulfuron (*m/z* 388/167) using the analytical method

Matrix	Analyte	Fortification level (mg a.s./L)	Mean Recovery (%)	RSD (%)	n	Comments
50% (w/v) aqueous sucrose solution	rimsulfuron	14.8	90	3	5	Test item fortification level: 100 mg/L
50% (w/v) aqueous sucrose solution	rimsulfuron	2520	105	9	5	Test item fortification level: 17000 mg/L
Deionised water + 0.1% Triton X	rimsulfuron	14.8	96	2	5	Test item fortification level: 100 mg/L
Deionised water + 0.1% Triton X	rimsulfuron	22200	110	7	4*	Test item fortification level: 150000 mg/L

* Outlier of 127% removed via Grubbs' test

Table A 19: Recovery results from method validation of thifensulfuron methyl (*m/z* 432/182) using the analytical method

Matrix	Analyte	Fortification level (mg a.s./L)	Mean Recovery (%)	RSD (%)	n	Comments
50% (w/v) aqueous sucrose solution	Thifensulfuron methyl	9.26	80	2	5	Test item fortification level: 100 mg/L
50% (w/v) aqueous sucrose solution	Thifensulfuron methyl	1570	110	15	5	Test item fortification level: 17000 mg/L
Deionised water + 0.1% Triton X	Thifensulfuron methyl	9.26	82	3	5	Test item fortification level: 100 mg/L
Deionised water + 0.1% Triton X	Thifensulfuron methyl	13900	109	2	5	Test item fortification level: 150000 mg/L

Table A 20: Characteristics for the analytical method used for validation of rimsulfuron and thifensulfuron methyl concentrations in oral and contact application solutions

	Rimsulfuron	Thifensulfuron methyl
Specificity	<i>m/z</i> 388/167 blank value <LOD	<i>m/z</i> 432/182 blank value <LOD
Calibration (type, number of data points)	linear regression analysis with 1/x weighting $r^2 \geq 0.995$ 11 data points	linear regression analysis with 1/x weighting $r^2 \geq 0.995$ 11 data points
Calibration range	Concentration range of 1-250 ng/mL	Concentration range of 1-250 ng/mL
Limit of quantification	LOQ = 14.8 mg a.s./L, diluted into calibration range	LOQ = 9.26 mg a.s./L, diluted into calibration range

CONCLUSION

This method was successfully validated for the determination of GF-3969 plus surfactant, based on rimsulfuron and thifensulfuron methyl concentrations, in samples of 50% (w/v) aqueous sucrose

solution (oral application solution) and in samples of deionised water + 0.1% Triton X (contact application solution) in accordance with SANCO/3029/99 rev.4 (European Commission, 2000).

A 2.1.1.8 Study 8, DuPont-48951

Comments of zRMS:	<p>An analytical method for the determination of thifensulfuron-methyl and rimsulfuron in 50% (w/v) aqueous sucrose solution and deionised water was validated with regard to recovery, linearity of detector response, repeatability, specificity, limit of quantification and limit of detection. The analytical method fulfils the requirements of guideline SANCO/3029/99 rev. 4, 11/07/2000.</p> <p>The limit of quantification (LOQ) of the analytical method was 100 mg test item/L (equivalent to 9.26 mg thifensulfuron-methyl/L and 14.8 mg rimsulfuron/L) in both matrices.</p> <p>No rimsulfuron and thifensulfuron methyl were detected in the control samples.</p> <p>The mean recoveries for each level for two active substances were in the range 70-110%.</p> <p>The corresponding RSD values were below 20%.</p> <p>The study is acceptable.</p>
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Reference:	KCP 5.1.2/09
Report:	Verge, E., (2018); Rimsulfuron 25SG/Thifensulfuron methyl 50SG/Isoxadifen ethyl 50WG (DPX-V4B07) a blend of paste extruded granules (14.82% + 9.26% active) + Codacide oil: Acute oral and contact toxicity to the bumble bee, <i>Bombus terrestris</i> L. under laboratory conditions
DuPont Report No.:	DuPont-48951
Testing Facility Report No.:	S18-00132
Guidelines	OECD 247 (2017), OECD 246 (2017)
Deviations:	None
GLP:	Yes
Acceptability:	Yes

MATERIALS AND METHODS

Method Principle

Concentrations of GF-3969 mixed with Codacide oil, based on concentrations of the active ingredients rimsulfuron and thifensulfuron methyl, were determined in 50% (w/v) aqueous sucrose solution (oral application solution) and in deionised water (contact application solution) by dilution of the samples with acetonitrile/water (1:1, v/v) prior to analysis by liquid chromatography coupled with tandem mass spectrometry (LC MS/MS). The limit of detection (LOD) and limit of quantitation (LOQ) were 30 mg test item/L (equivalent to 4.44 mg rimsulfuron/L and 2.78 mg thifensulfuron methyl/L) and 100 mg test item/L (equivalent to 14.8 mg rimsulfuron/L and 9.26 mg thifensulfuron methyl/L), respectively, in all matrices.

RESULTS AND DISCUSSION

Mean recovery values at each fortification concentration were within the acceptance range (mean recovery 70-110%; RSD ≤20%). The results obtained are summarised in the following tables.

Table A 21: Recovery results from method validation of rimsulfuron (*m/z* 388/167) using the analytical method

Matrix	Analyte	Fortification level (mg a.s./L)	Mean Recovery (%)	RSD (%)	n	Comments
50% (w/v) aqueous sucrose solution	rimsulfuron	14.8	86	4	5	Test item fortification level: 100 mg/L
50% (w/v) aqueous sucrose	rimsulfuron	2520	103	6	5	Test item fortification level: 17000 mg/L

Matrix	Analyte	Fortification level (mg a.s./L)	Mean Recovery (%)	RSD (%)	n	Comments
solution						
Deionised water	rimsulfuron	14.8	77	4	5	Test item fortification level: 100 mg/L
Deionised water	rimsulfuron	19300	99	3	5	Test item fortification level: 130000 mg/L

Table A 22: Recovery results from method validation of thifensulfuron methyl (*m/z* 432/182) using the analytical method

Matrix	Analyte	Fortification level (mg a.s./L)	Mean Recovery (%)	RSD (%)	n	Comments
50% (w/v) aqueous sucrose solution	Thifensulfuron methyl	9.26	97	3	5	Test item fortification level: 100 mg/L
50% (w/v) aqueous sucrose solution	Thifensulfuron methyl	1570	81	11	5	Test item fortification level: 17000 mg/L
Deionised water	Thifensulfuron methyl	9.26	96	2	5	Test item fortification level: 100 mg/L
Deionised water	Thifensulfuron methyl	12000	89	9	5	Test item fortification level: 130000 mg/L

Table A 23: Characteristics for the analytical method used for validation of rimsulfuron and thifensulfuron methyl concentrations in oral and contact application solutions

	Rimsulfuron	Thifensulfuron methyl
Specificity	<i>m/z</i> 388/167 blank value <LOD	<i>m/z</i> 432/182 blank value <LOD
Calibration (type, number of data points)	linear regression analysis with 1/x weighting $r^2 \geq 0.996$ 11 data points	linear regression analysis with 1/x weighting $r^2 \geq 0.996$ 11 data points
Calibration range	Concentration range of 1-250 ng/mL	Concentration range of 1-250 ng/mL
Limit of quantification	LOQ = 14.8 mg a.s./L, diluted into calibration range	LOQ = 9.26 mg a.s./L, diluted into calibration range

CONCLUSION

This method was successfully validated for the determination of GF-3969 mixed with codacide oil, based on rimsulfuron and thifensulfuron methyl concentrations, in samples of 50% (w/v) aqueous sucrose solution (oral application solution) and in samples of deionised water (contact application solution) in accordance with SANCO/3029/99 rev.4 (European Commission, 2000).

A 2.1.1.9 Study 9, 20170301

Comments of zRMS:	<p>The method for the analysis of feeding solution samples was validated in accordance with the working document of SANCO/3029/99 rev.4.</p> <p>The LOQ is at 0.003 g rimsulfuron/L.</p> <p>No rimsulfuron were detected in the control samples.</p> <p>The mean recoveries for each level were in the range 70-110%. The corresponding RSD values were below 20%.</p> <p>The study is acceptable.</p>
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Reference:	KCP 5.1.2/03
Report:	Cornement, M., (2018); Rimsulfuron-toxicity to Honey bees (<i>Apis mellifera</i> L.) larvae after repeated exposure under <i>in vitro</i> laboratory conditions
DuPont Report No.:	20170301
Testing Facility Report No.:	20170301
Guidelines	OECD 239 (2016)
Deviations:	None
GLP:	Yes
Acceptability:	Yes

MATERIALS AND METHODS

Method Principle

Concentrations of rimsulfuron diluted in acetone were determined in larval honey bee diet by vigorously shaking and then diluting into the calibration range with a mixture of acetonitrile/water (50/50, v/v). Following sonication, samples were analysed using high performance liquid chromatography coupled with tandem mass spectrometry (HPLC-MS/MS). The limit of detection (LOD) and limit of quantitation (LOQ) were 0.00065 g a.s./L and 0.0030 g a.s./L respectively.

RESULTS AND DISCUSSION

Mean recovery values at each fortification concentration were within the acceptance range (mean recovery 70-110%; RSD ≤20%). The results obtained are summarised in the following tables.

Table A 24: Recovery results from method validation of rimsulfuron (*m/z* 432/325) using the analytical method

Matrix	Analyte	Fortification level (g a.s./L)	Mean Recovery (%)	RSD (%)	n	Comments
Larval honey bee diet	rimsulfuron	0.0030	95	4.5	5	
Larval honey bee diet	rimsulfuron	0.42	89	0.9	5	

Table A 25: Characteristics for the analytical method used for validation of rimsulfuron in larval honey bee diet

	Rimsulfuron
Specificity	<i>m/z</i> 432/325 blank value <LOD
Calibration (type, number of data points)	Exponential regression analysis $r^2 > 0.999$ 9 data points
Calibration range	Concentration range of 0.00589-0.246 mg/L
Limit of quantification	LOQ = 0.0030 g a.s./L, diluted into calibration range

CONCLUSION

This method was successfully validated for the determination of rimsulfuron in larval honey bee diet in accordance with SANCO/3029/99 rev.4 (European Commission, 2000).

A 2.1.1.10 Study 10, 49942

Comments of zRMS:	<p>The method used in Study 49942 to determine the concentrations of rimsulfuron and thifensulfuron methyl in stock solutions of DPX-V4B07 24 WG, which was also the highest concentration spray mixture, was developed and validated at EAG laboratories as a part of this study.</p> <p>Remark: The lack of fortifications at multiple concentration levels. No LOQ value has been provided.</p> <p>Applicant explanation: Since many risk assessment methods from older studies do not meet current guidelines, SANTE/2020/182830, rev. 1 defined minimum validation requirements for existing risk assessment methods as:</p> <ul style="list-style-type: none"> • Demonstration of linearity <ul style="list-style-type: none"> ○ Acceptable calibration plots with equations are included for both actives in the final report (pgs. 77 - 78). • Demonstration of selectivity and specificity <ul style="list-style-type: none"> ○ Acceptable chromatograms of a matrix blank, a negative control, and a surfactant control are included in the final report (pgs. 81-83). ○ Acceptable chromatograms at or near the method LOQ (Rimsulfuron LOQ = 101 mg a.s./L; Thifensulfuron methyl LOQ = 63.1 mg a.s./L) are included in final report (pgs. 84-85). • Demonstration of acceptable recovery <ul style="list-style-type: none"> ○ SANTE/2020/182830, rev. 1 defines LOQ as the lowest validated level. For this method, only one level was tested a sufficient number of times (n=3) to determine recovery and precision statistics. As such, the LOQs for the method as applied in this study are identical to concentration of actives in the highest spray mixture concentration: Rimsulfuron LOQ = 101 mg a.s./L; Thifensulfuron methyl LOQ = 63.1 mg a.s./L . Acceptable mean and RSD values are given on pgs. 76-77 of the final report. ○ Two matrix fortification samples done at slightly higher than LOQ concentrations (Rimsulfuron matrix fortification: 110 mg a.s./L; Thifensulfuron methyl fortification: 68.7 mg a.s./L) had acceptable recoveries, further demonstrating the suitability of the method for determining spray mixture concentrations. ○ Solutions were diluted into the calibration range (0.500 – 5.00 mg a.s./L) with 50:50 (v/v) acetonitrile:water (report pgs. 73-74). <p>Overall, the method used in this study is considered fit for purpose. The lack of fortifications at multiple concentration levels is considered to have no impact on the overall quality or conclusion of the study.</p> <p>zRMS conclusion: The explanation is acceptable.</p>
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Reference:	KCP 5.1.2/01
Report:	Arnie, J.R., Aufderheidie, J, Lockard, L., Zhang, L., (2020); Isoxadifen ethyl 50WG/Rimsulfuron 25SG/Thifensulfuron methyl 50SG (DPX-V4B07), A blend of paste extruded granules plus isodecylalcohol ethoxylated (DPX-KG691) Surfactant: A greenhouse study to investigate the effects on vegetative vigor of ten terrestrial plants following foliar exposure
DuPont Report No.:	49942
Testing Facility Report No.:	112P-292
Guidelines	Guidelines OECD 227 and OCSP 850.4150
Deviations:	None
GLP:	Yes
Acceptability:	Yes

MATERIALS AND METHODS

Method Principle

Residues of rimsulfuron and thifensulfuron methyl were determined in spray mixtures of GF-3969. Samples were diluted with 50:50 (v/v) acetonitrile:HPLC-grade water. The concentrations of rimsulfuron and thifensulfuron methyl in the samples were determined by high performance liquid chromatography (HPLC) with ultraviolet absorbance detection using external calibration standards.

RESULTS AND DISCUSSION

The results obtained for rimsulfuron and thifensulfuron methyl are summarised in the following tables. Mean recovery values were within the acceptance range (mean recovery 70-100%; RDS $\leq 20\%$). Although overall recoveries were $n < 5$, this is considered to have no impact on the quality of the overall study.

Table A 26: Analytical verification of spray mixtures for rimsulfuron

Sample Collection Date Active Substance	Sample	Spray Mixture Concentration (ppm a.s.)			
	Number (112P-292-)	Nominal	Measured ^a	% of Nominal ^b	Mean % of Nominal
May 17, 2018 Rimsulfuron	MAB-1	0.0	NQ	-	
	MAS-1	110	106	96.4	--
	1 (negative control)	0.0	NQ	-	
	2 (surfactant control)	0.0	NQ	-	
	3	101	96.2	95.2	95.7 \pm 0.404
	4	101	96.8	95.9	CV = 0.422%
	5	101	96.9	95.9	
May 30, 2018 Rimsulfuron	MAB-2	0.0	NQ	-	
	MAS-2	110	111	101	--
	6 (negative control)	0.0	NQ	-	
	7 (surfactant control)	0.0	NQ	-	
	8	101	98.9	97.9	97.2 \pm 0.666
	9	101	97.6	96.6	CV = 0.685%
	10	101	98.0	97.0	

a "NQ" = Not quantifiable or less than the analytical method LOQ.

b Results were generated by Excel 2010 in full precision mode (May 17, 2018 samples) or Empower 2 software (May 30, 2018 samples). Manual calculations may vary.

Table A 27: Analytical verification of spray mixtures for thifensulfuron methyl

Sample Collection Date Active Substance	Sample	Spray Mixture Concentration (ppm a.s.)			
	Number (112P-292-)	Nominal	Measured ^a	% of Nominal ^b	Mean % of Nominal
May 17, 2018 Thifensulfuron methyl	MAB-1	0.0	NQ	-	
	MAS-1	68.7	65.8	95.8	--
	1 (negative control)	0.0	NQ	-	--
	2 (surfactant control)	0.0	NQ	-	
	3	63.1	59.4	94.2	
	4	63.1	59.7	94.6	94.6 ± 0.351
May 30, 2018 Thifensulfuron methyl	5	63.1	59.9	94.9	CV = 0.371%
	MAB-2	0.0	NQ	-	
	MAS-2	68.7	68.0	99.0	--
	6 (negative control)	0.0	NQ	-	
	7 (surfactant control)	0.0	NQ	-	
	8	63.1	60.0	95.0	95.8 ± 0.681
	9	63.1	60.6	96.0	CV = 0.711%
	10	63.1	60.8	96.3	

a "NQ" = Not quantifiable or less than the analytical method LOQ.

b Results were generated by Excel 2010 in full precision mode (May 17, 2018 samples) or Empower 2 software (May 30, 2018 samples). Manual calculations may vary.

Table A 28: Characteristics for the analytical method used for determination of rimsulfuron and thifensulfuron methyl residues in GF-3969 spray mixtures.

	Rimsulfuron	Thifensulfuron methyl
Specificity	blank value <LOQ retention time matching and UV detection at 235 nm	blank value <LOQ retention time matching and UV detection at 235 nm
Calibration (type, number of data points)	linear regression analysis $r \geq 0.999$ 5 data points	linear regression analysis $r \geq 1.000$ 5 data points
Calibration range	Concentration range of 0.500-5.00 mg a.s./L	Concentration range of 0.500-5.00 mg a.s./L
Limit of determination/quantification	Not applicable LOQ = 101 mg a.s./L, diluted into calibration range	Not applicable LOQ = 63.1 mg a.s./L, diluted into calibration range

CONCLUSION

This method was successfully verified for the determination of rimsulfuron and thifensulfuron methyl in spray mixtures of GF-3969.

A 2.1.2 Methods for post-authorization control and monitoring purposes (KCP 5.2)

A 2.1.2.1 Description of analytical methods for the determination of residues in plant matrices (KCP 5.2)

No new or additional studies have been submitted.

A 2.1.2.2 Description of analytical methods for the determination of residues in animal matrices (KCP 5.2)

No new or additional studies have been submitted.

A 2.1.2.3 Description of methods for the analysis of soil (KCP 5.2)

No new or additional studies have been submitted.

A 2.1.2.4 Description of methods for the analysis of water (KCP 5.2)

No new or additional studies have been submitted.

A 2.1.2.5 Description of methods for the analysis of air (KCP 5.2)

No new or additional studies have been submitted.

A 2.1.2.6 Description of methods for the analysis of body fluids and tissues (KCP 5.2)

No new or additional studies have been submitted.

A 2.1.2.7 Other studies/information

No new or additional studies have been submitted.

A 2.2 Analytical methods for thifensulfuron methyl

A 2.2.1 Methods used for the generation of pre-authorization data (KCP 5.1)

A 2.2.1.1 Study 1, DuPont-49732

Comments of zRMS:	<p>Specimens were analyzed for residues of rimsulfuron using a method based on DuPont Method No. 13412 Revision 1/Supplement 1. The analysis of thifensulfuron methyl and the metabolites IN-L9225 and IN- A4098 was performed using a method based on DuPont Method No. DuPont-28527.</p> <p>Concurrent recoveries for all analytes from untreated samples of all matrices fortified at the LOQ to as high as 0.10 mg/kg ranged from 63-114%. Mean values (\pm standard deviation) per analyte/matrix combination ranged from $85 \pm 14\%$ to $97 \pm 4\%$ for 8 to 18 fortifications per analyte/matrix combination.</p> <p>The determined Limit of Quantification (LOQ) was 0.010 mg/kg for all analytes. The Limit of Detection (LOD) was 0.003 mg/kg for all analytes.</p> <p>No rimsulfuron, thifensulfuron methyl or metabolite residues were detected in unfortified control specimens. Data from the analyses of unfortified controls and fortified controls validated method performance.</p> <p>7-point calibration curves were constructed for rimsulfuron, thifensulfuron methyl and metabolites using peak area counts in integrator units (ac) from injection of known standards versus standard concentrations in ng/mL.</p> <p>All calibration curves generated for each analytical set showed good linearity, i.e., the correlation coefficient R was > 0.99. Standard concentrations for rimsulfuron ranged from 0.15 to 10 ng/mL. Standard concentrations for thifensulfuron methyl and metabolites ranged from 0.10 to 10 ng/mL.</p> <p>The validity criteria for the analytical method according to SANCO/3029/99 rev. 4 have been met.</p> <p>The study is acceptable.</p>
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Reference:	KCP 5.1.2/08
Report:	Spence, C., (2020); Magnitude of residues in/on maize following foliar application of DPX-TNS43, a blend of paste extruded granules (62.12% Mesotrione 50WG + 24.24% Rimsulfuron 25SG + 9.09% Thifensulfuron methyl 50SG Active) – EU, initiated 2017
DuPont Report No.:	DuPont-49732
Testing Facility Report No.:	682133
Guidelines	OECD 509 (2009), SANCO/3029/99 rev. 4 (2000)
Deviations:	None
GLP:	Yes
Acceptability:	Yes

MATERIALS AND METHODS

Method Principle

Residues of rimsulfuron (DPX E9636) were extracted from maize whole plant, stover, and grain by homogenisation in 75/25 (v/v) acetonitrile/20 mM dibasic potassium phosphate (pH 7) buffer solution. Following centrifugation, extract aliquots were partitioned with hexane and aliquots of the acetonitrile/aqueous layer evaporated to near aqueous for purification using solid phase extraction (SPE). Rimsulfuron was eluted with an ammonium hydroxide in methanol solution, evaporated to near dryness with a keeper solution of ammonium acetate, and final volumes adjusted using acetonitrile and ammonium acetate. All samples were analysed by liquid chromatography coupled with positive ion electrospray tandem mass spectrometry (LC MS/MS). The limit of detection (LOD) and limit of quantitation (LOQ) were 0.003 mg/kg and 0.01 mg/kg, respectively, in all matrices.

Extraction Efficiency

The extraction solvent used in this method (3/1 (v/v) acetonitrile/water (buffered)) differs in composition by no more than 20 vol% compared to the solvent used in the maize metabolism study AMR 1222 88 (2/1 (v/v) acetonitrile/water (buffered)). Extraction efficiency has therefore been successfully demonstrated.

Method ID	DuPont-28527, “Analytical Method for the Determination of Thifensulfuron Methyl and Metabolites in Crops Using LC/MS/MS”
Analyte(s)	Thifensulfuron Methyl (DPX-M6316), IN-L9225, IN-A4098
Extraction Solvent/Technique	The procedure for the analysis of thifensulfuron methyl (DPXM6316), IN-L9225 and IN-A4098 in maize samples involved extraction with a solution of acetone and water. For all maize samples (whole plant, silage, stover and grain) a 5-mL aliquot of the extract was evaporated to approximately 1-mL and diluted to 10-mL with water.
Cleanup Strategies	The crops extracts were purified using Supelco Envi Chrom-P solid phase extraction cartridges. Aliquots of purified extracts were evaporated under a stream of nitrogen until the volume was less than 1-mL. The extracts were diluted with acetonitrile and water and an aliquot of the extracts was transferred to an auto-sampler vial for LC/MS/MS analysis.
Chromatography	HPLC System: Shimadzu Prominence, Data Acquisition Software: Analyst 1.6.2 for LC/MS/MS Mass Spectrometer : AB Sciex Instruments API5000 Column: Omnisphere C18, 5µm, 4.6 mm × 150 mm
Detection	For detection of the analyte, electrospray ionization (ESI) was used in the positive polarity mode. Two parent-to-daughter ion transitions of thifensulfuron methyl (quantifier 388→167 and confirmatory 388→141), IN-L9225 (quantifier 374→167 and confirmatory 374→141), and IN-A4098 (quantifier 141→57 and confirmatory 141→85) were monitored during LC/MS/MS analysis.
LOQ	0.010 mg/kg for all analytes

RESULTS AND DISCUSSION

Mean recovery values at each fortification concentration were within the acceptance range (mean recovery 70-110%; RSD ≤20%). The results obtained are summarised in the following tables.

Table A 29: Recovery results from concurrent recoveries of rimsulfuron (*m/z* 432.3/182.0) using the analytical method

Analyte	Matrix	Fortification level (mg/kg)	Mean Recoveries (%)	RSD (%)	n	Comments
Rimsulfuron	Maize Whole Plant	0.01	89	8	6	*n<5: This is considered to have no impact on the quality of the overall study as results are within acceptable range. Additionally, maize grain and maize stover (dry commodities) have acceptable combined recoveries (n=8) at both fortification levels.
		0.10	94	9	6	
	Maize Stover	0.01	93	5	4*	
		0.10	91	10	4*	
	Maize Grain	0.01	92	6	4*	
		0.10	85	15	4*	

Table A 30: Recovery results from concurrent recoveries of thifensulfuron methyl, IN-L9225 and IN-A4098 using the analytical method

Analyte	Matrix	Fortification level (mg/kg)	Mean Recoveries (%)	RSD (%)	n	Comments
thifensulfuron methyl	Maize Whole Plant	0.01	85	14	9	
		0.10	89	10	6	
	Maize Stover	0.01	82	15	7	
		0.10	88	12	7	
	Maize Grain	0.01	0	10	7	
		0.10	92	9	7	
IN-L9225	Maize Whole Plant	0.01	83	9	9	
		0.10	91	12	9	
	Maize Stover	0.01	82	6	7	
		0.10	87	8	7	
	Maize Grain	0.01	89	5	7	
		0.10	98	8	7	
IN-A4098	Maize Whole Plant	0.01	89	9	9	
		0.10	89	11	9	
	Maize Stover	0.01	82	5	7	
		0.10	91	6	7	
	Maize Grain	0.01	97	4	7	
		0.10	97	5	7	

Table A 31: Characteristics for the analytical method used for rimsulfuron residues in maize matrices

	Rimsulfuron
Specificity	m/z 432.3/182.0 blank value <30% LOQ
Calibration (type, number of data points)	linear regression analysis with 1/x weighting $r \geq 0.99$ 7 data points
Calibration range	Concentration range of 0.15–10 ng/mL, equivalent to 0.003–0.2 mg/kg
Limit of determination/quantification	LOQ=0.01 mg/kg

Table A 32: Characteristics for the analytical method used for thifensulfuron methyl, IN-L9225 and IN-A4098 residues in maize matrices

	thifensulfuron methyl	IN-L9225	IN-A4098
Specificity	388 → 167 and 388 → 141 blank value <30% LOQ	374 → 167 and 374 → 141 blank value <30% LOQ	141 → 57 and 141 → 85 blank value <30% LOQ
Calibration (type, number of data points)	linear regression analysis with 1/x weighting $r \geq 0.99$ 7 data points	linear regression analysis with 1/x weighting $r \geq 0.99$ 7 data points	linear regression analysis with 1/x weighting $r \geq 0.99$ 7 data points
Calibration range	0.10 to 10 ng/mL, equivalent to 0.002–0.2 mg/kg	0.10 to 10 ng/mL, equivalent to 0.002–0.2 mg/kg	0.10 to 10 ng/mL, equivalent to 0.002–0.2 mg/kg
Limit of determination/quantification	LOQ=0.01 mg/kg	LOQ=0.01 mg/kg	LOQ=0.01 mg/kg

CONCLUSION

This method was successfully validated for the determination of ~~rim~~imsulfuron in maize matrices in accordance with SANCO/3029/99 rev.4 (European Commission, 2000).

A 2.2.1.2 Study 2, DuPont-49948, Revision No. 1

Comments of zRMS:	<p>See point A 2.1.1.2.</p> <p>Concentrations of rimsulfuron and thifensulfuron methyl were determined in freshwater.</p> <p>Limit of Quantification for rimsulfuron: LOQ=0.0743 mg a.s./L,</p> <p>Limit of Quantification for thifensulfuron methyl: LOQ=0.0464 mg a.s./L.</p> <p>No rimsulfuron and thifensulfuron methyl were detected in the control samples.</p> <p>The mean recoveries for each level for two active substances were in the range 70-110%.</p> <p>The corresponding RSD values were below 20%.</p> <p>The validity criteria for the analytical method according to SANCO/3029/99 rev. 4 have been met.</p> <p>The study is acceptable.</p>
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Reference:	KCP 5.1.2/04
Report:	Dinehart, S., (2019); DPX-V4B07 24 WG (Rimsulfuron 25 SG + Thifensulfuron 50 SG + Isoxadifen 50 WG) A blend of paste extruded granules plus isodecylalcohol ethoxylated (DPX-KG691) surfactant: Acute toxicity to the rainbow trout, <i>Oncorhynchus mykiss</i> , determined under static-renewal test conditions
DuPont Report No.:	DuPont-49948, Revision No. 1
Testing Facility Report No.:	86361
Guidelines	OECD 203 (1992)
Deviations:	None
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

The study summary under A 1.1.1.1 contains information for rimsulfuron and thifensulfuron methyl.

A 2.2.1.3 Study 3, DuPont-49949, Revision No. 1

Comments of zRMS:	<p>See point A 2.1.1.3.</p> <p>Concentrations of rimsulfuron and thifensulfuron methyl were determined in freshwater.</p> <p>Limit of Quantification for rimsulfuron: LOQ=0.743 mg a.s./L,</p> <p>Limit of Quantification for thifensulfuron methyl: LOQ=0.464 mg a.s./L.</p> <p>No rimsulfuron and thifensulfuron methyl were detected in the control samples.</p> <p>The mean recoveries for each level for two active substances were in the range 70-110%.</p> <p>The corresponding RSD values were below 20%.</p> <p>The validity criteria for the analytical method according to SANCO/3029/99 rev. 4 have been met.</p> <p>The study is acceptable.</p>
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Reference:	KCP 5.1.2/05
Report:	Goudie, O.J., (2019); DPX-V4B07 24 WG (Rimsulfuron 25 SG + Thifensulfuron 50 SG + Isoxadifen 50 WG) A blend of paste extruded granules plus isodecylalcohol ethoxylated (DPX-KG691) surfactant: 48-Hour static renewal, acute toxicity test with the cladoceran, <i>Daphnia magna</i>
DuPont Report No.:	DuPont-49949, Revision No. 1
Testing Facility Report No.:	86363
Guidelines	OECD 202 (2004)
Deviations:	None
GLP:	Yes
Acceptability:	Yes

The study summary under A 1.1.1.1 contains information for rimsulfuron and thifensulfuron methyl.

A 2.2.1.4 Study 4, DuPont-49944

Comments of zRMS:	<p>Concentrations of rimsulfuron and thifensulfuron methyl were determined in 20X freshwater algal nutrient medium (20X FWAM).</p> <p>Limit of Quantification for rimsulfuron: LOQ=0.0000148 mg a.s./L,</p> <p>Limit of Quantification for thifensulfuron methyl: LOQ=0.00000925 mg a.s./L.</p> <p>No rimsulfuron and thifensulfuron methyl were detected in the control samples.</p> <p>The mean recoveries for each level for two active substances were in the range 70-110%.</p> <p>The corresponding RSD values were below 20%.</p> <p>The validity criteria for the analytical method according to SANCO/3029/99 rev. 4 have been met.</p> <p>The study is acceptable</p>
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Reference:	KCP 5.1.2/02
Report:	Bergfield, A., (2019); DPX-V4B07 24 WG (Rimsulfuron 25 SG + Thifensulfuron 50 SG + Isoxadifen 50 WG) A blend of paste extruded granules plus isodecylalcohol ethoxylated (DPX-KG691) surfactant: 7-Day growth inhibition test with the freshwater aquatic plant, duckweed, <i>Lemna gibba</i>
DuPont Report No.:	DuPont-49944
Testing Facility Report No.:	86356
Guidelines	OECD 221 (2006)
Deviations:	None
GLP:	Yes
Acceptability:	Yes

The study summary under A 2.1.1.4 contains information for rimsulfuron and thifensulfuron methyl.

A 2.2.1.5 Study 5, DuPont-49978

Comments of zRMS:	<p>Concentrations of rimsulfuron and thifensulfuron methyl were determined in 20X freshwater algal nutrient medium (20X FWAM).</p> <p>Limit of Quantification for rimsulfuron: LOQ=0.0000148 mg a.s./L,</p> <p>Limit of Quantification for thifensulfuron methyl: LOQ=0.00000925 mg a.s./L.</p> <p>No rimsulfuron and thifensulfuron methyl were detected in the control samples.</p> <p>The mean recoveries for each level for two active substances were in the range 70-110%.</p> <p>The corresponding RSD values were below 20%.</p> <p>The validity criteria for the analytical method according to SANCO/3029/99 rev. 4 have been met.</p> <p>The study is acceptable.</p>
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Reference:	KCP 5.1.2/06
Report:	Goudie, O.J., (2019); DPX-V4B07 24 WG (Rimsulfuron 25 SG + Thifensulfuron 50 SG + Isoxadifen 50 WG) A blend of paste extruded granules plus crop oil (Codacide): 7-Day growth inhibition test with the freshwater aquatic plant, duckweed, <i>Lemna gibba</i>
DuPont Report No.:	DuPont-49978
Testing Facility Report No.:	86359
Guidelines	OECD 221 (2006)
Deviations:	None
GLP:	Yes
Acceptability:	Yes

The study summary under A 2.2.1.5 contains information for rimsulfuron and thifensulfuron methyl.

A 2.2.1.6 Study 6, DuPont-49943

Comments of zRMS:	<p>Concentrations of rimsulfuron and thifensulfuron methyl were determined in freshwater algal medium (FWAM; equivalent to AAP medium).</p> <p>Limit of Quantification for rimsulfuron: LOQ=0.00297 mg a.s./L,</p> <p>Limit of Quantification for thifensulfuron methyl: LOQ=0.00186 mg a.s./L.</p> <p>No rimsulfuron and thifensulfuron methyl were detected in the control samples.</p> <p>The mean recoveries for each level for two active substances were in the range 70-110%.</p> <p>The corresponding RSD values were below 20%.</p> <p>The validity criteria for the analytical method according to SANCO/3029/99 rev. 4 have been met.</p> <p>The study is acceptable.</p>
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Reference:	KCP 5.1.2/07
Report:	Hoover, E., (2019); DPX-V4B07 24 WG (Rimsulfuron 25 SG + Thifensulfuron 50 SG + Isoxadifen 50 WG) a blend of paste extruded granules plus isodecylalcohol ethoxylated (DPX-KG691) surfactant: Growth inhibition test with the unicellular green alga, <i>Pseudokirchneriella subcapitata</i>
DuPont Report No.:	DuPont-49943
Testing Facility Report No.:	86355
Guidelines	OECD 201
Deviations:	None
GLP:	Yes
Acceptability:	Yes

The study summary under A 1.1.1.1 contains information for rimsulfuron and thifensulfuron methyl.

A 2.2.1.7 Study 7, DuPont-48899, Revision No. 1

Comments of zRMS:	<p>An analytical method for the determination of thifensulfuron-methyl and rimsulfuron in 50% (w/v) aqueous sucrose solution and deionised water + 0.1% Triton X was validated with regard to recovery, linearity of detector response, repeatability, specificity, limit of quantification and limit of detection. The analytical method fulfils the requirements of guideline SANCO/3029/99 rev. 4.</p> <p>The limit of quantification (LOQ) of the analytical method was 100 mg test item/L (equivalent to 9.26 mg thifensulfuron-methyl/L and 14.8 mg rimsulfuron/L) in both matrices.</p> <p>No rimsulfuron and thifensulfuron methyl were detected in the control samples.</p> <p>The mean recoveries for each level for two active substances were in the range 70-110%.</p> <p>The corresponding RSD values were below 20%.</p> <p>The study is acceptable.</p>
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Reference:	KCP 5.1.2/10
Report:	Verge, E., (2019); Rimsulfuron 25SG/thifensulfuron methyl 50SG/isoxadifen ethyl 50WG (DPX-V4B07) a blend of paste extruded granules (14.82% + 9.26% active) + surfactant DPX-KG691: Acute oral and contact toxicity to the bumble bee, <i>Bombus terrestris</i> L. under laboratory conditions
DuPont Report No.:	DuPont-48899, Revision No. 1
Testing Facility Report No.:	S18-00130
Guidelines	OECD 247 (2017), OECD 246 (2017)
Deviations:	None
GLP:	Yes
Acceptability:	Yes

The study summary under A 1.1.1.1 contains information for rimsulfuron and thifensulfuron methyl.

A 2.2.1.8 Study 8, DuPont-48951

Comments of zRMS:	<p>An analytical method for the determination of thifensulfuron-methyl and rimsulfuron in 50% (w/v) aqueous sucrose solution and deionised water was validated with regard to recovery, linearity of detector response, repeatability, specificity, limit of quantification and limit of detection. The analytical method fulfils the requirements of guideline SANCO/3029/99 rev. 4, 11/07/2000.</p> <p>The limit of quantification (LOQ) of the analytical method was 100 mg test item/L (equivalent to 9.26 mg thifensulfuron-methyl/L and 14.8 mg rimsulfuron/L) in both matrices.</p> <p>No rimsulfuron and thifensulfuron methyl were detected in the control samples.</p> <p>The mean recoveries for each level for two active substances were in the range 70-110%.</p> <p>The corresponding RSD values were below 20%.</p> <p>The study is acceptable.</p>
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Reference:	KCP 5.1.2/09
Report:	Verge, E., (2018); Rimsulfuron 25SG/Thifensulfuron methyl 50SG/Isxadifen ethyl 50WG (DPX-V4B07) a blend of paste extruded granules (14.82% + 9.26% active) + codacide oil: Acute oral and contact toxicity to the bumble bee, <i>Bombus terrestris</i> L. under laboratory conditions
DuPont Report No.:	DuPont-48951
Testing Facility Report No.:	S18-00132
Guidelines	OECD 247 (2017), OECD 246 (2017)
Deviations:	None
GLP:	Yes
Acceptability:	Yes

The study summary under A 2.2.1.8 contains information for rimsulfuron and thifensulfuron methyl.

A 2.2.2 Methods for post-authorization control and monitoring purposes (KCP 5.2)

A 2.2.2.1 Description of analytical methods for the determination of residues in plant matrices (KCP 5.2)

A 2.2.2.1.1 Study 1, DuPont-13398, Supplement No. 1

Comments of zRMS:	<p>The residue method DuPont-13412 for the determination of Thifensulfuron-methyl residues in soybean seed (oily crop group), wheat (dry crop), lettuce (high water) and oranges (acidic crop group) involves simple extraction, clean-up, and analytical determination by HPLC/MS/MS detection with a limit of quantification of 0.010 mg/kg. The validation is considered to be in line with the requirements of SANCO guideline 825/00 guidance and is therefore considered applicable for enforcement purposes. Acceptable ILV with minor modifications to the method procedure was provided for the method of analysis: DuPont-13398. The Limit of Quantitation (LOQ) was 0.010 mg/kg. The mean recoveries at each fortification level and for each matrix are all between 70-120% with %RSD < 20.</p> <p>Additionally Applicant provided Supplement No.1.</p> <p>The purpose of this supplement report is to calculate the recoveries of rimsulfuron (DPX-E9636) and chlorimuron ethyl (DPX-F6025) in olives and soybeans using the target (quantitative) and confirmatory ion transition data found in DuPont-13398. In the original report, the recoveries were calculated using TIC resulting from the sum of the target and confirmatory ion transitions.</p> <p>The Limit of Quantitation (LOQ) for rimsulfuron and chlorimuron ethyl in olives and soybeans was 0.010 mg/kg. This method for determination of residues of rimsulfuron and chlorimuron ethyl in olives and soybeans meets the guidelines of SANCO 8/25/00 revision 8.1. The LC/MS/MS method is free of interference above the LOD at the retention time of rimsulfuron and chlorimuron ethyl in the samples tested. The method generated acceptable quantitative and confirmatory recoveries over the concentration levels tested. This method can be used to quantify and confirm residues of rimsulfuron and chlorimuron ethyl in olives and soybeans.</p> <p>The study is acceptable.</p>
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Reference:	KCP 5.2/02
Report:	Charles, E., Doran, A. M., Klems, J. P., (2017); Independent laboratory validation of analytical method DuPont-13412 for the determination of thifensulfuron methyl, ethametsulfuron methyl, rimsulfuron, tribenuron methyl and chlorimuron ethyl in olives and soybean seed using SPE purification and LC/MS/MS detection
DuPont Report No.:	DuPont-13398, Supplement No. 1
Testing Facility Report No.:	303871
Guidelines	OPPTS 860.1340, SANCO/825/00 rev. 8.1
Deviations:	None
GLP:	Yes
Acceptability:	Yes

MATERIALS AND METHODS

Method Scope

This method is applicable for the quantitative determination of residues of thifensulfuron methyl, ethametsulfuron methyl, rimsulfuron, tribenuron methyl and chlorimuron ethyl residues in oily crop matrices. The method was independently validated in olives and soybean seeds over the concentration range of 0.010 and 0.050 mg/g with a validated limit of quantitation of 0.010 mg/kg.

Method Principle

A mostly organic (75% acetonitrile) solution containing pH 7 aqueous buffer (25% 20 mM dibasic potassium phosphate) is used to extract the sulfonylurea analytes from oily crop matrices. The ratio of extraction solution to sample is 9:1 (v:w) and samples are extracted twice by mechanical tissue grinding. A 5% aliquot of the extract is partitioned with hexane to remove oils and some matrix. The hexane fraction is discarded and one-half of the remaining extract is evaporated in a stream of nitrogen to near aqueous in preparation for solid-phase extraction (SPE) purification using an ENV (a high purity styrene divinyl benzene polymer) cartridge. The extract solution is filtered through the SPE cartridges where

the analytes are adsorbed onto the ENV sorbent. The sorbent is washed with hexane and the analytes are eluted from the SPE cartridge with 25 mM ammonium hydroxide in methanol solution into a collection tube containing 0.5 mL of aqueous 50 mM ammonium acetate (keeper solution). The methanol is removed from the collected eluate by evaporation at a controlled temperature (30-35°C). The sample extract is diluted to final composition of 10% acetonitrile/90% aqueous 50 mM ammonium acetate for instrumental analysis. The analytes are resolved by HPLC and detected by TurboIon Spray LC/MS/MS.

Linearity

For each analyte, the linearity of detector response was evaluated using solvent standard solutions. Calibration curves were calculated by linear regression analysis with 1/x weighting.

Table A 33: Linearity

Analyte	Number of stands	Range min (ng/mL)	Range max (ng/mL)	Regression	Correlation coefficient (r)
Thifensulfuron Methyl	6	0.20	10.0	linear with 1/x weighting	0.9969
Ethametsulfuron Methyl	6	0.20	10.0	linear with 1/x weighting	0.9992
Rimsulfuron	6	0.20	10.0	linear with 1/x weighting	0.9998
Tribenuron Methyl	6	0.20	10.0	linear with 1/x weighting	0.9999
Chlorimuron Ethyl	6	0.20	10.0	linear with 1/x weighting	0.9997

Selectivity

The LC-MS/MS method is highly selective for both the quantitation and confirmation of rimsulfuron and its metabolites. Significant peak response (>30% of the LOQ peak area) is not observed in reagent blank and extracts of untreated blank control samples at the expected retention times of the analytes. Unambiguous identification is ensured by monitoring two MS/MS transitions characteristic of each analyte as follows in the table below.

Table A 34: Transitions monitored

Thifensulfuron Methyl (DPX-M6316)	m/z Q1/Q3 388.1/167.2Q (quantitative)
Thifensulfuron Methyl (DPX-M6316)	m/z Q1/Q3 388.1/204.9C (confirmatory)
Ethametsulfuron methyl (DPX-A7881)	m/z Q1/Q3 411.0/168.2Q (quantitative)
Ethametsulfuron methyl (DPX-A7881)	m/z Q1/Q3 411.0/168.2C (confirmatory)
Rimsulfuron (DPX-E9636)	m/z Q1/Q3 432/182Q (quantitative)
Rimsulfuron (DPX-E9636)	m/z Q1/Q3 432/325.4C (confirmatory)
Tribenuron Methyl (DPX-L5300)	m/z Q1/Q3 396.0/155.1Q (quantitative)
Tribenuron Methyl (DPX-L5300)	m/z Q1/Q3 396.0/181.0C (confirmatory)
Chlorimuron Ethyl (DPX-F6025)	m/z Q1/Q3 415/186Q (quantitative)
Chlorimuron Ethyl (DPX-F6025)	m/z Q1/Q3 415/213C (confirmatory)

Confirmation

Confirmation of the presence of thifensulfuron methyl, ethametsulfuron methyl, rimsulfuron, tribenuron methyl and chlorimuron ethyl was by comparison of retention times (liquid chromatography) of recovery samples with the retention times of the calibration standards as well as by monitoring two structurally characteristic MS/MS transitions for each analyte by tandem mass spectrometry. Validation data obtained using the confirmatory MS/MS transitions met the same acceptance criteria as the validation data generated using the quantitative MS/MS transitions, therefore demonstrating that the analyte signal of the quantitative MS/MS transition is correct and not affected by any other compound.

Limits of Detection and Quantitation

The limit of quantitation, defined as the lowest concentration of an analyte tested at which an unambiguous identification of the analyte can be proven and at which an acceptable mean recovery with an acceptable relative standard deviation is obtained, is 0.01 mg/kg for all analytes in all tested matrices.

The limit of detection, defined as 30% of the LOQ, is 0.003 mg/kg for all analytes in all tested matrices.

RESULTS AND DISCUSSION

Summary of Recovery

Results obtained were within guideline requirements (mean recovery 70-110%; RSD \leq 20%). For each analyte, the two ion mass transitions could be used interchangeably for quantification and confirmation. The results obtained are summarised in the following tables.

Table A 35: Summary of quantitative recovery of Thifensulfuron Methyl (m/z 388.1/167.2Q)

Matrix group	Matrix	Fortification level	Recovery (%)		SD	RSD	n
		(mg/kg)	mean	range	(%)	(%)	
Crop	Olives	0.010	86	78-95	-	9	5
		0.050	83	75-94	-	9	5
Crop	Soybean Seeds	0.010	75	71-78	-	4	5
		0.050	79	73-86	-	7	5

Table A 36: Summary of quantitative recovery of Ethametsulfuron Methyl (m/z 411/168.2Q)

Matrix group	Matrix	Fortification level	Recovery (%)		SD	RSD	n
		(mg/kg)	mean	range	(%)	(%)	
Crop	Olives	0.010	85	76-86	-	6	5
		0.050	78	68-83	-	9	5
Crop	Soybean Seeds	0.010	84	79-86	-	3	5
		0.050	77	71-81	-	6	5

Table A 37: Summary of quantitative recovery of Rimsulfuron (m/z 432/182Q)

Matrix group	Matrix	Fortification level	Recovery (%)		SD	RSD	n
		(mg/kg)	mean	range	(%)	(%)	
Crop	Olives	0.010	104	81-112	7.2	6.9	5
		0.050	92	71-119	18	19	5
Crop	Soybean Seeds	0.010	101	83-113	6.6	6.5	5
		0.050	103	81-112	8.2	8.0	5

Table A 38: Summary of confirmatory recovery of Rimsulfuron (m/z 432/325.4C)

Matrix group	Matrix	Fortification level	Recovery (%)		SD	RSD	n
		(mg/kg)	mean	range	(%)	(%)	
Crop	Olives	0.010	98	81-112	12	13	5
		0.050	92	71-119	18	20	5
Crop	Soybean Seeds	0.010	97	83-113	13	13	5
		0.050	100	93-108	6.9	7.0	5

Table A 39: Summary of quantitative recovery of Tribenuron Methyl (m/z 396/181Q)

Matrix group	Matrix	Fortification level	Recovery (%)		SD	RSD	n
		(mg/kg)	mean	range	(%)	(%)	
Crop	Olives	0.010	75	72-80	-	4	5
		0.050	71	69-72	-	2	5
Crop	Soybean Seeds	0.010	85	80-92	-	5	5
		0.050	94	80-114	-	14	5

Table A 40: Summary of quantitative recovery of Chlorimuron Ethyl (m/z 415/213.1Q)

Matrix group	Matrix	Fortification level	Recovery (%)		SD	RSD	n
		(mg/kg)	mean	range	(%)	(%)	
Crop	Olives	0.010	93		2.3	2.4	5
		0.050	87		11	13	5
Crop	Soybean Seeds	0.010	83		7.2	8.6	5
		0.050	81		2.8	3.5	5

Table A 41: Summary of confirmatory recovery of Chlorimuron Ethyl (m/z 415/186.1C)

Matrix group	Matrix	Fortification level	Recovery (%)		SD	RSD	n
		(mg/kg)	mean	range	(%)	(%)	
Crop	Olives	0.010	98	83-113	12	12	5
		0.050	95	81-112	12	13	5
Crop	Soybean Seeds	0.010	84	76-91	6.2	7.4	5
		0.050	82	72-88	6.4	7.8	5

Repeatability

Repeatability was not assessed as a part of this study.

Working Solution Stability

The stability of working solutions was not assessed as a part of this study.

Sample Extract Stability

The stability of sample extracts was not assessed as a part of this study.

Matrix Effects

Matrix effects were not assessed as a part of this study.

Extraction Efficiency

Extraction efficiency was not assessed as a part of this study.

Changes to Method

Minor changes were made to the method procedure on this occasion. The centrifuge specified in the method was Varian 20-mL reservoirs were not used during the solid phase extraction procedure.

The analytical method was run exactly as written except for the following:

1. Step 4 and Step 8 were changed to state that extracts should be centrifuged for 15 min at *ca* 5°C at 4500 rpm to achieve sufficient clarification of the supernatant.
2. The reservoirs were removed from the procedure to ensure that the cartridges did not go to dryness during the SPE procedure. In some instances the use of these reservoirs created air locks, causing some cartridges to run dry or have an irregular flow through the SPE cartridges.
3. An additional step was added at the end of the gradient in order to flush the column of any potential interfering peaks.
4. Step 5 of the analytical method DuPont-13412 (analyte purification procedure) states to evaporate extract solution to near aqueous (~1.0 mL) in a stream of N₂ on N-Evap at 30-35°C. This step was changed to evaporate extract solution to near aqueous (~2.0 mL). This modification was made to improve the recovery of tribenuron methyl within the acceptable range (70-110%).
5. The SPE cartridges were dried under a vacuum for 10 min after application and elution of the samples instead of *ca* 5 min as detailed in Step 9 of the analytical method DuPont-13412 (analyte purification procedure). The cartridges were also dried under a vacuum for 10 min after washing the cartridges with hexane.

CONCLUSION

Method is acceptable based on current guidelines: EPA Residue Chemistry Test Guidelines OPPTS 860.1340, the requirements of SANCO/3029/99 rev.4 and SANCO/825/00 rev.8.1, as well as PMRA Regulatory Directive Dir98-02.

The method passed the independent laboratory validation for thifensulfuron methyl, ethametsulfuron methyl, rimsulfuron, tribenuron methyl and chlorimuron ethyl in olives with minor modifications to the method procedure. The LOQ of 0.010 mg/kg in olives is considered to be valid.

The method passed the independent laboratory validation for thifensulfuron methyl, ethametsulfuron methyl, rimsulfuron and chlorimuron ethyl in soybean seeds with minor modifications to the method procedure. The method passed the independent laboratory validation for tribenuron methyl in soybean seeds with several minor modifications to the method procedure following discussion with an analyst at DuPont that was familiar with the method. The LOQ of 0.010 mg/kg in soybeans is considered to be valid.

A 2.2.2.2 Description of analytical methods for the determination of residues in animal matrices (KCP 5.2)

A 2.2.2.2.1 Study 1, DuPont-30449

Comments of zRMS:	<p>The analytical method has been satisfactorily validated for the determination of residues of thirteen DuPont sulfonylurea (SU) herbicides (incl. thifensulfuron methyl) in animal matrices (egg whole, milk, cream, bovine meat/ beef, bovine liver, kidney and fat) by HPLC/ MS/MS with a LOQ of 0.01 mg/L.</p> <p>The accuracy and precision of the method during sample analysis were considered to be acceptable since mean recoveries at each fortification level were in the range of 70 – 110% with relative standard deviation(s) ≤ 20 % for animal matrices.</p> <p>This method for the determination of residues of sulfonylurea herbicides (incl. thifensulfuron methyl) in animal matrices meets the guidelines of the European Commission SANCO/825/00.</p> <p>The study is acceptable.</p>
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Reference:	CP 5.2/01
Report:	Pentz, A.M., Cabusas, M.E.Y. (2012): Analytical method for the determination of DuPont sulfonylurea herbicides in animal matrices using HPLC/MS/MS
DuPont Report No.:	DuPont-30449
Testing Facility Report No.:	DuPont-30449
Guidelines	OPPTS 860.1340; SANCO/825/00 rev.7, March 17, 2004
Deviations:	None
GLP:	Yes No
Acceptability:	Yes

An analytical method was developed for the detection, quantitative analysis, and confirmation of thirteen DuPont sulfonylurea (SU) herbicides in animal matrices. These sulfonylurea herbicides were azimsulfuron, bensulfuron methyl, chlorimuron ethyl, chlorsulfuron, ethametsulfuron methyl, flupyr-sulfuron methyl, metsulfuron methyl, nicosulfuron, rimsulfuron, sulfometuron methyl, thifensulfuron methyl, tribenuron methyl and triflusulfuron methyl. The method limit of quantification (LOQ) for each SU was 0.010 mg/kg.

DuPont sulfonylureas (SUs) were extracted from milk, cream, and egg samples by vortex mixing and centrifugation in acetonitrile and 90% acetonitrile/10% water. They were extracted from meat (beef), liver, kidney and fat by consecutive homogenization in 90/10 acetonitrile/water. Following centrifugation, sample extracts were diluted 10 fold with 10/90 acetonitrile/water and analyzed by reversed phase HPLC/ESI-MS/MS.

This residue method for the thirteen SUs in animal matrices was validated on milk, cream, egg, meat (beef), liver, kidney and fat. Average recoveries at the LOQ and 10xLOQ for SUs in all animal matrices tested were acceptable, i.e. 70 % - 110% with $\text{rsd} < 20\%$ ($n=5$), except in fat using solvent standards. Six out of the 13 SUs had average recoveries at the LOQ and/or 10xLOQ at $>110\%$ with $\text{rsd} < 20\%$ due to matrix (enhancement) effect. All average recoveries in each fortification level for the SUs in animal matrices analyzed using matrix-matched standards were within 70 % - 110% with $\text{rsd} < 20\%$ ($n=5$). A summary of the fortification recoveries is shown in the tables below.

The method extraction procedure is similar to the ones used in metabolism studies of radiolabeled compounds of nicosulfuron and flupyrsulfuron methyl in lactating goats and/or egg-laying hens. The method extraction procedure should be able to extract efficiently the other SUs from animal matrices due to similarity in chemical functionality and properties.

The confirmatory method is based on detection and the relative ratios of the two MS/MS transition ions monitored during HPLC/MS/MS analysis.

A single analyst can extract, purify, and analyze 12-24 samples for about 1.3-hour working days. The HPLC/MS/MS analysis is about 12 min/sample and is run unattended overnight. Data processing, including residue calculations using an Excel sheet, is done on the following day.

Compound	Level (mg/kg)	Percent Recovery (%RSD) (Solvent Standards)		
		Egg	Whole Milk	Cream
Azimsulfuron (DPX-A8947)	0.01	107 (12.7) *	88 (3.2)	89 (5.5)
	0.10	108 (9.2)	91 (5.9)	89 (5.2)
	Overall	108 (10.1)	89 (4.8)	90 (5.9)
Bensulfuron methyl (DPX-F5384)	0.01	96 (5.9)	98 (3.1)	96 (2.3)
	0.10	91 (7.1)	100 (5.6)	92 (4.1)
	Overall	92 (7.0)	99 (4.4)	94 (3.6)
Chlorimuron ethyl (DPX-F6025)	0.01	95 (13.8)	95 (3.7)	96 (2.3)
	0.10	88 (6.3)	98 (6.1)	95 (4.4)
	Overall	91 (11.2)	96 (5.0)	96 (3.9)
Chlorsulfuron (DPX-W4189)	0.01	91 (8.3)	97 (7.8)	91 (3.2)
	0.10	89 (6.0)	93 (5.1)	86 (3.5)
	Overall	90 (6.9)	95 (6.7)	89 (4.4)
Ethametsulfuron methyl (DPX-A7881)	0.01	93 (6.9)	100 (9.4)	92 (5.5)
	0.10	89 (10.1)	93 (5.1)	87 (6.6)
	Overall	91 (8.5)	97 (8.2)	90 (7.2)
Flupyrasulfuron methyl (DPX-KE459)	0.01	107 (8.1)	100 (6.3)	96 (2.9)
	0.10	105 (4.0)	99 (12.4)	96 (3.4)
	Overall	106 (6.1)	100 (9.3)	96 (3.3)
Metsulfuron methyl (DPX-T6376)	0.01	90 (5.8)	97 (8.5)	93 (7.2)
	0.10	93 (10.7)	96 (5.3)	88 (7.2)
	Overall	91 (8.5)	96 (6.7)	92 (7.6)
Nicosulfuron (DPX-V9360)	0.01	97 (5.3)	97 (4.9)	99 (3.4)
	0.10	84 (4.1)	95 (6.4)	90 (1.3)
	Overall	91 (8.8)	96 (5.6)	94 (5.6)
Rimsulfuron (DPX-E9636)	0.01	94 (11.3)	96 (5.0)	97 (8.2)
	0.10	94 (11.7)	95 (7.6)	99 (4.8)
	Overall	96 (10.6)	95 (6.1)	98 (6.0)
Sulfometuron methyl (DPX-T5648)	0.01	94 (16.4)	100 (10.5)	84 (3.2)
	0.10	90 (15.8)	99 (5.4)	88 (4.0)
	Overall	92 (15.3)	99 (7.9)	88 (5.4)
Tribenuron Methyl (DPX-L5300)	0.01	101 (17.5)	90 (3.0)	91 (5.3)
	0.10	91 (6.1)	88 (6.8)	93 (4.4)
	Overall	96 (13.8)	89 (5.0)	93 (6.1)
Thifensulfuron methyl (DPX-M6316)	0.01	92 (14.2)	94 (5.0)	91 (3.9)
	0.10	97 (12.1)	93 (3.5)	83 (3.6)
	Overall	95 (12.6)	93 (4.1)	87 (5.6)
Triflusaluron methyl (DPX-66037)	0.01	90 (7.2)	89 (8.7)	85 (6.4)
	0.10	98 (6.2)	98 (5.5)	88 (5.0)
	Overall	94 (7.6)	93 (8.5)	88 (6.1)

*The number of samples (n) validated at the LOQ, and 10xLOQ are 5 and 5. For azimsulfuron, n=4 at the LOQ due to one outlier.

Compound	Level (mg/kg)	Percent Recovery (%RSD) (Solvent Standards)		
		Bovine Meat/ Beef	Bovine Kidney	Bovine Fat
Azimsulfuron (DPX-A8947)	0.01	93 (6.7)	91 (5.8)	102 (3.7)
	0.10	91 (8.0)	96 (2.1)	113 (7.3)
	Overall	92 (7.0)	93 (4.8)	108 (8.0)
Bensulfuron methyl (DPX-F5384)	0.01	96 (8.6)	88 (3.0)	116 (3.0)
	0.10	98 (7.2)	90 (3.9)	123 (5.7)
	Overall	97 (7.5)	89 (3.5)	120 (5.6)
Chlorimuron ethyl (DPX-F6025)	0.01	93 (2.9)	97 (8.0)	111 (5.8)
	0.10	97 (5.2)	98 (3.3)	114 (4.3)
	Overall	95 (4.7)	97 (5.8)	112 (4.5)
Chlorsulfuron (DPX-W4189)	0.01	95 (8.2)	94 (4.8)	99 (5.9)
	0.10	91 (7.1)	93 (2.9)	104 (7.2)
	Overall	93 (7.5)	93 (3.8)	101 (6.7)
Ethametsulfuron methyl (DPX-A7881)	0.01	104 (9.2)	90 (5.0)	108 (5.9)
	0.10	90 (10.3)	96 (5.3)	112 (7.4)
	Overall	97 (12.1)	93 (5.9)	110 (6.8)
Flupyrasulfuron methyl (DPX-KE459)	0.01	100 (11.1)	99 (7.9)	100 (7.0)
	0.10	97 (7.8)	105 (2.2)	110 (5.1)
	Overall	99 (9.2)	102 (6.1)	105 (7.4)
Metsulfuron methyl (DPX-T6376)	0.01	97 (5.9)	98 (6.3)	103 (4.0)
	0.10	97 (9.0)	100 (2.8)	110 (7.1)
	Overall	97 (7.2)	99 (4.6)	107 (6.4)
Nicosulfuron (DPX-V9360)	0.01	106 (6.6)	111 (5.4)	121 (6.9)
	0.10	94 (5.0)	99 (3.3)	107 (3.8)
	Overall	100 (8.5)	105 (7.4)	114 (8.6)
Rimsulfuron (DPX-E9636)	0.01	94 (11.3)	87 (7.1)	106 (6.3)
	0.10	94 (11.1)	89 (5.8)	109 (6.6)
	Overall	94 (10.6)	88 (6.3)	108 (6.3)
Sulfometuron methyl (DPX-T5648)	0.01	93 (10.3)	95 (2.5)	105 (8.4)
	0.10	97 (6.5)	96 (3.4)	106 (4.5)
	Overall	95 (8.3)	96 (2.9)	105 (6.3)
Tribenuron Methyl (DPX-L5300)	0.01	97 (6.8)	94 (1.9)	110 (4.6)
	0.10	87 (9.2)	90 (3.1)	106 (2.6)
	Overall	92 (9.7)	92 (3.3)	108 (4.0)
Thifensulfuron methyl (DPX-M6316)	0.01	92 (6.8)	90 (1.9)	94 (7.8)
	0.10	98 (4.2)	94 (4.1)	105 (4.9)
	Overall	95 (6.2)	92 (3.9)	100 (8.2)
Triflurosulfuron methyl (DPX-66037)	0.01	93 (4.5)	89 (4.4)	100 (7.1)
	0.10	98 (2.4)	97 (3.2)	118 (5.1)
	Overall	91 (4.7)	93 (5.6)	88 (6.2)

Compound	Fortification Level (mg/kg)	Percent Recovery (%RSD) Matrix-Matched Standards			
		Bovine Meat/ Beef	Bovine Liver	Bovine Kidney	Bovine Fat
Azimsulfuron (DPX-A8947)	0.01	90 (3.5)	88 (2.4)	91 (1.6)	89 (6.4)
	0.10	95 (2.9)	101 (2.5)	97 (5.8)	97 (6.0)
	Overall	92 (4.1)	94 (7.9)	94 (5.3)	93 (7.4)
Bensulfuron methyl (DPX-F5384)	0.01	95 (6.8)	95 (2.9)	94 (4.9)	91 (5.5)
	0.10	95 (7.1)	102 (4.1)	97 (2.1)	95 (5.1)
	Overall	95 (6.6)	99 (5.0)	95 (3.8)	93 (5.3)
Chlorimuron ethyl (DPX-F6025)	0.01	98 (6.2)	96 (5.1)	93 (2.4)	91 (4.6)
	0.10	98 (5.6)	96 (2.7)	93 (4.9)	94 (7.7)
	Overall	95 (6.5)	96 (3.9)	93 (3.6)	92 (6.2)
Chlorsulfuron (DPX-W4189)	0.01	91 (5.0)	94 (2.6)	92 (6.1)	91 (8.7)
	0.10	97 (4.3)	91 (3.8)	95 (3.7)	95 (8.2)
	Overall	94 (5.5)	92 (3.4)	93 (5.0)	93 (8.3)
Ethametsulfuron methyl (DPX-A7881)	0.01	102 (8.6)	92 (5.9)	90 (4.1)	104 (10.9)
	0.10	90 (14.1)	101 (4.2)	91 (4.3)	95 (11.2)
	Overall	96 (12.5)	97 (7.0)	90 (4.0)	100 (11.5)
Flupyralsulfuron methyl (DPX-KE459)	0.01	94 (5.9)	103 (3.4)	90 (5.9)	87 (6.9)
	0.10	101 (8.2)	105 (4.2)	96 (3.1)	97 (6.9)
	Overall	97 (7.6)	104 (4.3)	93 (5.7)	92 (8.7)
Metsulfuron methyl (DPX-T6376)	0.01	93 (4.0)	91 (10.0)	91 (2.5)	89 (9.3)
	0.10	97 (3.8)	98 (4.2)	94 (4.8)	96 (9.7)
	Overall	95 (4.2)	95 (8.1)	93 (3.9)	93 (9.9)
Nicosulfuron (DPX-V9360)	0.01	110 (6.1)	99 (4.4)	108 (2.5)	105 (8.0)
	0.10	93 (4.8)	96 (4.0)	93 (2.9)	92 (7.4)
	Overall	102 (10.2)	97 (4.4)	101 (8.2)	98 (10.1)
Rimsulfuron ((DPX-E9636)	0.01	87 (6.6)	90 (6.8)	93 (9.1)	86 (9.0)
	0.10	94 (10.3)	100 (3.6)	101 (4.8)	94 (3.7)
	Overall	90 (9.2)	95 (7.2)	97 (8.1)	90 (7.9)
Sulfometuron methyl (DPX-T5648)	0.01	96 (6.7)	92 (4.1)	92 (4.4)	90 (9.5)
	0.10	89 (11.4)	92 (6.0)	95 (2.8)	95 (7.7)
	Overall	92 (9.5)	92 (4.9)	93 (3.8)	92 (8.6)
Tribenuron Methyl (DPX-L5300)	0.01	94 (12.8)	83 (2.2)	90 (5.6)	88 (14.4)
	0.10	92 (4.2)	86 (4.6)	92 (4.4)	89 (13.2)
	Overall	93 (9.2)	84 (4.0)	91 (4.8)	88 (13.0)
Thifensulfuron methyl (DPX-M6316)	0.01	85 (5.0)	87 (3.7)	86 (2.4)	83 (8.3)
	0.10	93 (4.3)	98 (5.1)	95 (2.6)	92 (8.8)
	Overall	89 (6.3)	93 (7.5)	91 (5.8)	88 (10.2)
Triflusalufuron methyl (DPX-66037)	0.01	90 (5.6)	90 (4.2)	92 (5.0)	82 (6.4)
	0.10	103 (6.5)	99 (3.9)	101 (4.5)	96 (7.7)
	Overall	97 (9.1)	95 (6.4)	96 (6.6)	89 (10.4)

A 2.2.2.2.2 Study 2, DuPont-30450

Comments of zRMS:	<p>The DuPont-30449 analytical method for the determination of DuPont sulfonylurea herbicides in animal matrices using HPLC/MS/MS was successfully independently validated for the determination of residues of azimsulfuron (DPX-A8947), bensulfuron methyl (DPX-F5384), Chlorimuron ethyl (DPX-F6025), chlorsulfuron (DPX-W4189), ethametsulfuron methyl (DPX-A7881), flupyrsulfuron methyl (DPX-KE459), metsulfuron methyl (DPX-T6376), nicosulfuron (DPX-V9360), rimsulfuron (DPX-E9636), sulfometuron methyl (DPX-T5648), thifensulfuron methyl (DPX-M6316), tribenuron methyl (DPX-L5300), and triflusulfuron methyl (DPX-66037) in animal matrices (eggs, milk and beef liver) with a LOQ of 0.01 mg/kg using LC-MS/MS. Mean recovery values at each fortification concentration for each matrix were within the acceptance range (mean recovery 70 - 110%; RSD ≤ 20%).</p> <p>The analytical method fulfils the requirements of guideline SANCO/825/00.</p> <p>The study is acceptable.</p>
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Reference:	CP 5.2/02
Report:	Gant, (2012); Independent Laboratory validation of DuPont-30449' Analytical method for the determination of DuPont sulfonylurea herbicides in animal matrices using HPLC/MS/MS'
DuPont Report No.:	DuPont-30450
Testing Facility Report No.:	DuPont-30450
Guidelines	OPPTS 860.1340; EC Directive 96/46/EC; SANCO/825/00 rev. 7 (17/03/2004)
Deviations:	None
GLP:	Yes
Acceptability:	Yes

MATERIALS AND METHODS

Method Scope

This method is applicable for the quantitative determination of residues of azimsulfuron (DPX-A8947), bensulfuron methyl (DPX-F5384), Chlorimuron ethyl (DPX-F6025), chlorsulfuron (DPX-W4189), ethametsulfuron methyl (DPX-A7881), flupyrsulfuron methyl (DPX-KE459), metsulfuron methyl (DPX-T6376), nicosulfuron (DPX-V9360), rimsulfuron (DPX-E9636), sulfometuron methyl (DPX-T5648), thifensulfuron methyl (DPX-M6316), tribenuron methyl (DPX-L5300), and triflusulfuron methyl (DPX-66037) in animal matrices. The method was independently validated in eggs, milk and beef liver over the concentration range of 0.01 – 0.1 mg/kg with a validated limit of quantitation of 0.01 mg/kg.

Method Principle

The residue analytical method described in DuPont-30449 “Analytical Method for the Determination of DuPont Sulfonylurea Herbicides in Animal Matrices Using HPLC/MS/MS” was used for the analyses in this study. Sulfonylureas were extracted twice from 5.0-g egg and milk samples with acetonitrile followed by 90/10 acetonitrile/water by vortex mixing, shaking and centrifugation. They were extracted from 5.0-g liver by consecutive homogenization in 90/10 acetonitrile/water. Aliquots of sample extracts were filtered through a 0.2-µm PTFE filter and diluted 10-fold with 10/90 acetonitrile/water. The diluted sample extracts were analyzed by reversed phase HPLC/MS/MS.

Linearity

For each analyte, the linearity of detector response was evaluated using solvent standard solutions. Calibration curves were calculated by linear regression analysis with 1/x weighting.

Selectivity

The LC-MS/MS method is highly selective for both the quantitation and confirmation of sulfonylurea herbicides. Significant peak response (>30% of the LOQ peak area) is not observed in reagent blank

and extracts of untreated blank control samples at the expected retention times of the analytes. Unambiguous identification is ensured by monitoring two MS/MS transitions characteristic of each analyte as follows in the table below.

Table A 42: Transitions monitored

DPX-V9360 (Nicosulfuron)	m/z Q1/Q3 411.0/182.0Q (quantitative)
DPX-V9360 (Nicosulfuron)	m/z Q1/Q3 411.0/213.0C (confirmatory)
DPX-T5648 (Sulfometuron Methyl)	m/z Q1/Q3 365.0/150.0Q (quantitative)
DPX-T5648 (Sulfometuron Methyl)	m/z Q1/Q3 365.0/199.0C (confirmatory)
DPX-M6316 (Thifensulfuron Methyl)	m/z Q1/Q3 388.0/167.0Q (quantitative)
DPX-M6316 (Thifensulfuron Methyl)	m/z Q1/Q3 388.0/205.0C (confirmatory)
DPX-T6376 (Metsulfuron Methyl)	m/z Q1/Q3 382.0/77.1Q (quantitative)
DPX-T6376 (Metsulfuron Methyl)	m/z Q1/Q3 382.0/167.0C (confirmatory)
DPX-A7881 (Ethametsulfuron Methyl)	m/z Q1/Q3 411.0/168.0Q (quantitative)
DPX-A7881 (Ethametsulfuron Methyl)	m/z Q1/Q3 411.0/196.0C (confirmatory)
DPX-E9636 (Rimsulfuron)	m/z Q1/Q3 432.0/182.0Q (quantitative)
DPX-E9636 (Rimsulfuron)	m/z Q1/Q3 432.0/325.0C (confirmatory)
DPX-W4189 (Chlorsulfuron)	m/z Q1/Q3 358.0/141.0Q (quantitative)
DPX-W4189 (Chlorsulfuron)	m/z Q1/Q3 358.0/167.0C (confirmatory)
DPX-A8947 (Azimsulfuron)	m/z Q1/Q3 425.0/182.0Q (quantitative)
DPX-A8947 (Azimsulfuron)	m/z Q1/Q3 425.0/244.0C (confirmatory)
DPX-F5384 (Bensulfuron Methyl)	m/z Q1/Q3 411.0/149.0Q (quantitative)
DPX-F5384 (Bensulfuron Methyl)	m/z Q1/Q3 411.0/182.0C (confirmatory)
DPX-L5300 (Tribenuron Methyl)	m/z Q1/Q3 396.0/56.0Q (quantitative)
DPX-L5300 (Tribenuron Methyl)	m/z Q1/Q3 396.0/155.0C (confirmatory)
DPX-KE459 (Flupyr-sulfuron Methyl)	m/z Q1/Q3 466.0/83.0Q (quantitative)
DPX-KE459 (Flupyr-sulfuron Methyl)	m/z Q1/Q3 466.0/182.0C (confirmatory)
DPX-F6025 (Chlorimuron Ethyl)	m/z Q1/Q3 415.0/186.0Q (quantitative)
DPX-F6025 (Chlorimuron Ethyl)	m/z Q1/Q3 415.0/213.0C (confirmatory)
DPX-66037 (Trifl-sulfuron Methyl)	m/z Q1/Q3 493.0/96.1Q (quantitative)
DPX-66037 (Trifl-sulfuron Methyl)	m/z Q1/Q3 493.0/264.1C (confirmatory)

Confirmation

Confirmation of the presence of sulfonylurea herbicides was by comparison of retention times (liquid chromatography) of recovery samples with the retention times of the calibration standards as well as by monitoring two structurally characteristic MS/MS transitions for each analyte by tandem mass spectrometry. Validation data obtained using the confirmatory MS/MS transitions met the same acceptance criteria as the validation data generated using the quantitative MS/MS transitions, therefore demonstrating that the analyte signal of the quantitative MS/MS transition is correct and not affected by any other compound.

Limits of Detection and Quantitation

The limit of quantitation, defined as the lowest concentration of an analyte tested at which an unambiguous identification of the analyte can be proven and at which an acceptable mean recovery with an acceptable relative standard deviation is obtained, is 0.010 mg/kg for all analytes in all tested matrices.

The limit of detection, defined as 30% of the LOQ, is 0.003 mg/kg for all analytes in all tested matrices.

RESULTS AND DISCUSSION

Summary of Recovery

Results obtained were within guideline requirements (mean recovery 70-110%; RSD \leq 20%). For each analyte, the two ion mass transitions could be used interchangeably for quantification and confirmation. The results obtained are summarised in the following tables.

Table A 43: Summary of quantitative recovery of DPX-A8947 (m/z 425/182Q)

Matrix group	Matrix	Fortification level	Recovery (%)		SD	RSD	n
		(mg/kg)	mean	range	(%)	(%)	
Animal	Eggs	0.01	93	89-96	2.8	3.0	5
		0.1	93	87-100	4.7	5.0	5
Animal	Milk	0.01	94	88-104	5.9	6.3	5
		0.1	99	86-115	10.6	10.6	5
Animal	Beef Liver	0.01	92	88-95	2.7	3.0	5
		0.1	93	91-95	1.8	1.9	5

Table A 44: Summary of quantitative recovery of DPX-F5384 (m/z 411/149Q)

Matrix group	Matrix	Fortification level	Recovery (%)		SD	RSD	n
		(mg/kg)	mean	range	(%)	(%)	
Animal	Eggs	0.01	90	86-94	3.1	3.4	5
		0.1	86	81-90	3.6	4.2	5
Animal	Milk	0.01	94	79-100	8.5	9.1	5
		0.1	97	90-103	6.6	6.8	5
Animal	Beef Liver	0.01	94	89-97	3.3	3.5	5
		0.1	94	90-97	3.1	3.3	5

Table A 45: Summary of quantitative recovery of DPX-F6025 (m/z 415/186Q)

Matrix group	Matrix	Fortification level	Recovery (%)		SD	RSD	n
		(mg/kg)	mean	range	(%)	(%)	
Animal	Eggs	0.01	90	76-102	11.2	12.5	5
		0.1	86	81-90	4.0	4.7	5
Animal	Milk	0.01	94	87-97	4.0	4.2	5
		0.1	96	94-97	1.3	1.4	5
Animal	Beef Liver	0.01	95	82-105	9.5	10.0	5
		0.1	93	89-98	3.5	3.8	5

Table A 46: Summary of quantitative recovery of DPX-W4189 (m/z 358/167Q)

Matrix group	Matrix	Fortification level	Recovery (%)		SD	RSD	n
		(mg/kg)	mean	range	(%)	(%)	
Animal	Eggs	0.01	89	83-99	6.2	7.0	5
		0.1	87	81-90	3.9	4.4	5
Animal	Milk	0.01	83	81-90	6.7	8.0	5
		0.1	89	86-93	3.1	3.5	5
Animal	Beef Liver	0.01	87	83-96	5.0	5.9	5
		0.1	92	89-94	2.3	2.5	5

Table A 47: Summary of quantitative recovery of DPX-A7881 (m/z 411/168Q)

Matrix group	Matrix	Fortification level	Recovery (%)		SD	RSD	n
		(mg/kg)	mean	range	(%)	(%)	
Animal	Eggs	0.01	88	86-8	1.1	1.2	5
		0.1	88	79-94	5.6	6.3	5
Animal	Milk	0.01	87	78-90	5.3	6.1	5
		0.1	87	81-93	5.2	5.9	5

Matrix group	Matrix	Fortification level	Recovery (%)		SD	RSD	n
		(mg/kg)	mean	range	(%)	(%)	
Animal	Beef Liver	0.01	92	87-96	3.5	3.9	5
		0.1	94	92-95	1.2	1.3	5

Table A 48: Summary of quantitative recovery of DPX-KE459 (m/z 466/83Q)

Matrix group	Matrix	Fortification level	Recovery (%)		SD	RSD	n
		(mg/kg)	mean	range	(%)	(%)	
Animal	Eggs	0.01	110	107-113	2.5	2.3	5
		0.1	108	101-115	5.5	5.1	5
Animal	Milk	0.01	102	92-117	10.2	10.0	5
		0.1	107	94-122	11.8	11.0	5
Animal	Beef Liver	0.01	95	91-98	2.8	2.9	5
		0.1	100	99-101	1.0	1.0	5

Table A 49: Summary of quantitative recovery of DPX-T6376 (m/z 382/77Q)

Matrix group	Matrix	Fortification level	Recovery (%)		SD	RSD	n
		(mg/kg)	mean	range	(%)	(%)	
Animal	Eggs	0.01	89	81-98	6.5	7.3	5
		0.1	88	83-91	3.3	3.7	5
Animal	Milk	0.01	91	87-97	4.5	4.9	5
		0.1	91	85-104	7.5	8.2	5
Animal	Beef Liver	0.01	94	83-104	7.9	8.3	5
		0.1	95	91-97	2.4	2.5	5

Table A 50: Summary of quantitative recovery of DPX-V9360 (m/z 411/106Q)

Matrix group	Matrix	Fortification level	Recovery (%)		SD	RSD	n
		(mg/kg)	mean	range	(%)	(%)	
Animal	Eggs	0.01	77	70-84	5.2	6.8	5
		0.1	76	73-79	2.6	3.4	5
Animal	Milk	0.01	90	83-102	8.6	9.5	5
		0.1	90	86-97	4.0	4.5	5
Animal	Beef Liver	0.01	86	80-91	3.9	4.5	5
		0.1	85	83-88	1.8	2.1	5

Table A 51: Summary of quantitative recovery of DPX-E9636 (m/z 432/182Q)

Matrix group	Matrix	Fortification level	Recovery (%)		SD	RSD	n
		(mg/kg)	mean	range	(%)	(%)	
Animal	Eggs	0.01	93	91-97	2.5	2.7	5
		0.1	92	85-96	3.9	4.2	5
Animal	Milk	0.01	92	85-102	6.8	7.3	5
		0.1	96	91-98	3.1	3.2	5
Animal	Beef Liver	0.01	93	92-95	1.1	1.2	5
		0.1	95	93-97	1.8	1.9	5

Table A 52: Summary of quantitative recovery of DPX-T5648 (m/z 365/150Q)

Matrix group	Matrix	Fortification level	Recovery (%)		SD	RSD	n
		(mg/kg)	mean	range	(%)	(%)	
Animal	Eggs	0.01	88	82-94	5.2	5.9	5
		0.1	89	83-93	3.9	4.4	5
Animal	Milk	0.01	88	81-98	8.5	9.7	5
		0.1	93	90-96	3.0	3.3	5
Animal	Beef Liver	0.01	95	88-103	5.6	5.9	5
		0.1	92	91-95	1.8	2.0	5

Table A 53: Summary of quantitative recovery of DPX-M6316 (m/z 388/167Q)

Matrix group	Matrix	Fortification level	Recovery (%)		SD	RSD	n
		(mg/kg)	mean	range	(%)	(%)	
Animal	Eggs	0.01	88	83-94	4.5	5.1	5
		0.1	89	84-91	2.8	3.2	5
Animal	Milk	0.01	94	90-97	3.0	3.2	5
		0.1	98	94-102	3.3	3.4	5
Animal	Beef Liver	0.01	94	82-98	6.9	7.3	5
		0.1	92	89-94	2.4	2.6	5

Table A 54: Summary of quantitative recovery of DPX-L5300 (m/z 396/155Q)

Matrix group	Matrix	Fortification level	Recovery (%)		SD	RSD	n
		(mg/kg)	mean	range	(%)	(%)	
Animal	Eggs	0.01	94	90-96	3.0	3.2	5
		0.1	94	87-97	4.0	4.3	5
Animal	Milk	0.01	84	75-93	7.4	8.8	5
		0.1	88	86-89	1.3	1.4	5
Animal	Beef Liver	0.01	83	81-84	1.3	1.6	5
		0.1	84	81-85	1.4	1.7	5

Table A 55: Summary of quantitative recovery of DPX-66037 (m/z 493/96.1Q)

Matrix group	Matrix	Fortification level	Recovery (%)		SD	RSD	n
		(mg/kg)	mean	range	(%)	(%)	
Animal	Eggs	0.01	92	83-97	5.6	6.1	5
		0.1	91	83-97	5.3	5.8	5
Animal	Milk	0.01	90	83-96	6.0	6.7	5
		0.1	90	89-95	2.5	2.8	5
Animal	Beef Liver	0.01	97	91-106	5.6	5.8	5
		0.1	101	99-102	1.4	1.4	5

Repeatability

Repeatability was not assessed as a part of this study.

Working Solution Stability

The stability of working solutions was not assessed as a part of this study.

Sample Extract Stability

The stability of sample extracts was not assessed as a part of this study.

Matrix Effects

Matrix effects were evaluated by comparing the response of the analyte fortified in a control extract after processing (for each matrix type) to the response of the analyte fortified in neat solvent. For all matrices, the results demonstrate that matrix effects exceed $\pm 20\%$. Matrix matched standards were used for quantification for all analytes in all matrices for this study.

Extraction Efficiency

Extraction efficiency was not assessed as a part of this study.

Changes to Method

The analytical method was run exactly as written except as follows:

- A Waters Acquity UPLC system controlled by Applied BioSystems/MDS Sciex Analyst Software was used without a diverter valve.
- HPLC/MS/MS. The conditions specified in this section contained a typographical error. The gradient conditions were 70% A at time 0 min to 30% A at 10 min.

- Due to poor response from the DPX-T6376 transition 382.0/77.1, an alternative transition (382.0/199.0) was monitored in lieu of 382.0/77.1 as the confirmatory ion in the analysis of Milk Trial 2. DPX-V9360 (411.2/106.0) and DPX-KE459 (466.0/100.0) transitions were monitored in Milk Trial 2 in addition to the transitions specified in the method. These ion transitions were not evaluated in liver or egg matrix but showed very good response in standards. It is recommended that these transitions be used for analyte confirmation for all matrices. As mentioned previously, reagents and solvents of equivalent or greater purity were used in the extraction and analysis procedures.

CONCLUSION

Method is acceptable based on current guidelines: EPA Residue Chemistry Test Guidelines OPPTS 860.1340, the requirements of SANCO/3029/99 rev.4 and SANCO/825/00 rev.8.1, as well as PMRA Regulatory Directive Dir98-02.

ABC Laboratories, Inc. successfully independently validated the residue analytical method DuPont-30449, without modifications. Additional ion transitions were ascertained and monitored for residue confirmation in milk matrix.

The method was demonstrated to be applicable for the determination of azimsulfuron (DPX-A8947), bensulfuron methyl (DPX-F5384), Chlorimuron ethyl (DPX-F6025), chlorsulfuron (DPX-W4189), ethametsulfuron methyl (DPX-A7881), flupyrsulfuron methyl (DPX-KE459), metsulfuron methyl (DPX-T6376), nicosulfuron (DPX-V9360), rimsulfuron (DPX-E9636), sulfometuron methyl (DPX-T5648), Thifensulfuron methyl (DPX-M6316), tribenuron methyl (DPX-L5300), and triflusulfuron methyl (DPX-66037), in eggs, milk, and beef liver. An LOQ of 0.01 mg/kg was demonstrated for each matrix evaluated for validated analytes.

A 2.2.2.2.3 Study 3, DuPont-30449, Supplement No. 1

Comments of zRMS:	<p>The validation of the method was evaluated in point A 2.2.2.2.1.</p> <p>This Supplement No.1 has been provided to confirm data for the analysis of the 13 sulfonylurea herbicides in animal matrices. Confirmation data collected simultaneously with the data presented in DuPont-30449 were reprocessed to generate the confirmation data in this supplement. Additionally, new sets of analytical data were generated in order to fulfill the required confirmation data. A complete summary of the method used to generate this data is presented in DuPont-30449.</p> <p>The limit of quantitation (LOQ) for each analyte was 0.010 mg/kg . The limit of detection (LOD) was estimated to be 0.003 mg/kg based on the least responsive analyte. The LOQ and LOD data generated from the primary/quantitation ion transitions and confirmation ion transitions were consistent.</p> <p>Acceptable recoveries for the 13 SUs in fortified animal matrices were generated using the primary/quantitation ion transitions and confirmatory ion transitions, i.e., overall recoveries were within 70% - 120% with relative standard deviations of $\leq 20\%$. These results demonstrated that sulfonylurea residues were stable during sample preparation and LC/MS/MS analysis.</p> <p>The study is acceptable.</p>
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Reference:	CP 5.2/03
Report:	Pentz, A.M., Cabusas, M.E.Y., (2014); Analytical method for the determination of DuPont sulfonylurea herbicides in animal matrices using HPLC/MS/MS
DuPont Report No.:	DuPont-30449, Supplement No. 1
Testing Facility Report No.:	DuPont-30449, Supplement No. 1
Guidelines	OPPTS 860.1340; SANCO/825/00 rev. 8.1, November 16, 2010
Deviations:	None
GLP:	Yes No
Acceptability:	Yes

MATERIALS AND METHODS

Method Scope

This method is applicable for the quantitative determination of residues azimsulfuron, bensulfuron methyl, chlorimuron ethyl, chlorsulfuron, ethametsulfuron methyl, flupyrsulfuron methyl, metsulfuron methyl, nicosulfuron, rimsulfuron, sulfometuron methyl, thifensulfuron methyl, tribenuron methyl and triflursulfuron methyl in milk, cream, fat, kidney, liver, meat/beef and egg. The method was validated over the concentration range of 0.01-0.10 mg/kg with a validated limit of quantitation of 0.010 mg/kg.

Method Principle

Residues of sulfonylurea herbicides are extracted twice from 5.0-g of milk, cream and egg samples with acetonitrile followed by 90/10 acetonitrile/water by vortex mixing, shaking and centrifugation. The analytes are extracted from 5.0-g of meat (beef), liver, kidney and fat samples by consecutive homogenization in 90/10 acetonitrile/water. Aliquots of sample extracts are filtered through a 0.2- μ m PTFE filter and diluted 10-fold with 10/90 acetonitrile/water. The diluted sample extracts are analyzed by reversed-phase HPLC using a Zorbax Eclipse® Plus C8; 3.0 \times 50 mm, 1.8 μ m particle size diameter column and a mobile phase of 0.01% formic acid in 0.1 mM ammonium formate (aq) and methanol. Detection of the analytes was by electrospray mass spectrometry/mass spectrometry (ESI-MS/MS). Two parent-to-daughter ion transitions per analyte were monitored during analysis.

The confirmatory method for the HPLC/MS/MS method was based on detection and the relative ratios of the two MS/MS parent-to-daughter ion transitions monitored during the validation.

Linearity

For each analyte, the linearity of detector response was evaluated using solvent standard solutions. Calibration curves were calculated by linear regression analysis with 1/x weighting. Calibration curves resulting from the injection of eight standards over the concentration range of 0.050-5.0 ng/mL demonstrated linearity with correlation coefficients (r) of at least 0.999.

Selectivity

The method is selective for the determination of sulfonylurea herbicides by virtue of the chromatographic separation and MS/MS detection. Significant peak response (>30% of the LOQ peak area) is not observed in reagent blank and extracts of untreated blank control samples at the expected retention times of the analytes. Unambiguous identification is ensured by the monitoring two MS/MS transitions characteristic of each analyte as follows in the table below.

Table A 56: Transitions monitored

DPX-V9360 (Nicosulfuron)	m/z Q1/Q3 411.0/182.0Q (quantitative)
DPX-V9360 (Nicosulfuron)	m/z Q1/Q3 411.0/213.0C (confirmatory)
DPX-T5648 (Sulfometuron Methyl)	m/z Q1/Q3 365.0/150.0Q (quantitative)
DPX-T5648 (Sulfometuron Methyl)	m/z Q1/Q3 365.0/199.0C (confirmatory)
DPX-M6316 (Thifensulfuron Methyl)	m/z Q1/Q3 388.0/167.0Q (quantitative)
DPX-M6316 (Thifensulfuron Methyl)	m/z Q1/Q3 388.0/205.0C (confirmatory)
DPX-T6376 (Metsulfuron Methyl)	m/z Q1/Q3 382.0/77.1Q (quantitative)
DPX-T6376 (Metsulfuron Methyl)	m/z Q1/Q3 382.0/167.0C (confirmatory)
DPX-A7881 (Ethametsulfuron Methyl)	m/z Q1/Q3 411.0/168.0Q (quantitative)
DPX-A7881 (Ethametsulfuron Methyl)	m/z Q1/Q3 411.0/196.0C (confirmatory)
DPX-E9636 (Rimsulfuron)	m/z Q1/Q3 432.0/182.0Q (quantitative)
DPX-E9636 (Rimsulfuron)	m/z Q1/Q3 432.0/325.0C (confirmatory)
DPX-W4189 (Chlorsulfuron)	m/z Q1/Q3 358.0/141.0Q (quantitative)
DPX-W4189 (Chlorsulfuron)	m/z Q1/Q3 358.0/167.0C (confirmatory)
DPX-A8947 (Azimsulfuron)	m/z Q1/Q3 425.0/182.0Q (quantitative)
DPX-A8947 (Azimsulfuron)	m/z Q1/Q3 425.0/244.0C (confirmatory)
DPX-F5384 (Bensulfuron Methyl)	m/z Q1/Q3 411.0/149.0Q (quantitative)
DPX-F5384 (Bensulfuron Methyl)	m/z Q1/Q3 411.0/182.0C (confirmatory)
DPX-L5300 (Tribenuron Methyl)	m/z Q1/Q3 396.0/56.0Q (quantitative)
DPX-L5300 (Tribenuron Methyl)	m/z Q1/Q3 396.0/155.0C (confirmatory)
DPX-KE459 (Flupyralsulfuron Methyl)	m/z Q1/Q3 466.0/83.0Q (quantitative)
DPX-KE459 (Flupyralsulfuron Methyl)	m/z Q1/Q3 466.0/182.0C (confirmatory)
DPX-F6025 (Chlorimuron Ethyl)	m/z Q1/Q3 415.0/186.0Q (quantitative)
DPX-F6025 (Chlorimuron Ethyl)	m/z Q1/Q3 415.0/213.0C (confirmatory)
DPX-66037 (Triflusalufuron Methyl)	m/z Q1/Q3 493.0/96.1Q (quantitative)
DPX-66037 (Triflusalufuron Methyl)	m/z Q1/Q3 493.0/264.1C (confirmatory)

Confirmation

Confirmation of the presence of sulfonylurea herbicides was by comparison of retention times (liquid chromatography) of recovery samples with the retention times of the calibration standards as well as by monitoring two structurally characteristic MS/MS transitions for each analyte by tandem mass spectrometry. Validation data obtained using the confirmatory MS/MS transitions met the same acceptance criteria as the validation data generated using the quantitative MS/MS transitions, therefore demonstrating that the analyte signal of the quantitative MS/MS transition is correct and not affected by any other compound.

Limits of Detection and Quantitation

The limit of quantitation, defined as the lowest concentration of an analyte tested at which an unambiguous identification of the analyte can be proven and at which an acceptable mean recovery with an acceptable relative standard deviation is obtained, is 0.01 mg/kg for all analytes in all tested matrices. The limit of detection, defined as 30% of the LOQ, is 0.003 mg/kg for all analytes in all tested matrices.

RESULTS AND DISCUSSION

Summary of Recovery

Results obtained were within guideline requirements (mean recovery 70-110%; RSD \leq 20%). For each analyte, the two ion mass transitions could be used interchangeably for quantification and confirmation. The results obtained are summarised in the following tables.

Table A 57: Summary of quantitative recovery of DPX-A8947 (m/z 425/182Q)

Matrix group	Matrix	Fortification level	Recovery (%)		SD	RSD	n
		(mg/kg)	mean	range	(%)	(%)	
Animal	Milk	0.01	92	86-97	5	5	5
		0.1	93	88-97	4	4	5
	Cream	0.01	89	82-95	5	6	5
		0.1	89	84-95	5	5	5
	Fat	0.01	98	89-106	6	6	5
		0.1	110	100-120	9	8	5
	Kidney	0.01	91	86-99	6	6	5
		0.1	96	93-97	2	2	5
	Liver	0.01	88	87-92	2	2	5
		0.1	101	97-104	2	2	5
	Meat/Beef	0.01	92	82-100	7	8	5
		0.1	93	83-103	9	9	5
	Egg	0.01	111	96-128	12	11	5
		0.1	102	82-114	12	12	5

Table A 58: Summary of confirmatory recovery of DPX-A8947 (m/z 425/244C)

Matrix group	Matrix	Fortification level	Recovery (%)		SD	RSD	n
		(mg/kg)	mean	range	(%)	(%)	
Animal	Milk	0.01	90	71-116	17	18	5
		0.1	94	87-98	4	5	5
	Cream	0.01	94	88-107	7	8	5
		0.1	99	83-112	12	12	5
	Fat	0.01	98	94-101	3	4	5
		0.1	117	103-128	12	10	5
	Kidney	0.01	93	79-106	12	13	5
		0.1	97	89-102	6	6	5
	Liver	0.01	85	79-92	5	6	5
		0.1	104	96-111	6	5	5
	Meat/Beef	0.01	87	73-99	12	13	5
		0.1	92	79-109	13	14	5
	Egg	0.01	107	87-129	16	15	5
		0.1	94	69-109	17	18	5

Table A 59: Summary of quantitative recovery of DPX-F5384 (m/z 411/149Q)

Matrix group	Matrix	Fortification level	Recovery (%)		SD	RSD	n
		(mg/kg)	mean	range	(%)	(%)	
Animal	Milk	0.01	99	92-104	4	4	5
		0.1	102	98-113	6	6	5
	Cream	0.01	97	90-102	6	6	5
		0.1	92	87-97	4	4	5
	Fat	0.01	101	90-119	15	15	5
		0.1	115	99-128	14	12	5
	Kidney	0.01	87	80-91	4	5	5
		0.1	87	83-90	3	4	5
	Liver	0.01	95	88-100	6	6	5
		0.1	101	96-105	4	4	5
	Meat/Beef	0.01	94	77-106	11	12	5
		0.1	96	81-101	8	9	5
	Egg	0.01	92	77-103	10	11	5
		0.1	90	82-95	5	6	5

Table A 60: Summary of confirmatory recovery of DPX-F5384 (m/z 411/182C)

Matrix group	Matrix	Fortification level	Recovery (%)		SD	RSD	n
		(mg/kg)	mean	range	(%)	(%)	
Animal	Milk	0.01	99	94-104	4	4	5
		0.1	96	86-114	11	11	5
	Cream	0.01	92	75-111	16	17	5
		0.1	91	82-100	7	7	5
	Fat	0.01	104	93-116	11	10	5
		0.1	113	100-125	11	9	5
	Kidney	0.01	94	79-102	9	10	5
		0.1	98	92-107	6	6	5
	Liver	0.01	95	87-104	7	8	5
		0.1	105	96-112	6	6	5
	Meat/Beef	0.01	101	92-113	8	8	5
		0.1	98	89-102	5	5	5
	Egg	0.01	86	68-101	12	14	5
		0.1	88	83-91	4	4	5

Table A 61: Summary of quantitative recovery of DPX-F6025 (m/z 415/186Q)

Matrix group	Matrix	Fortification level	Recovery (%)		SD	RSD	n
		(mg/kg)	mean	range	(%)	(%)	
Animal	Milk	0.01	95	89-98	3	4	5
		0.1	98	92-107	6	6	5
	Cream	0.01	96	92-98	3	3	5
		0.1	95	91-102	4	4	5
	Fat	0.01	111	106-116	5	4	5
		0.1	115	107-121	5	4	5
	Kidney	0.01	97	89-107	8	8	5
		0.1	98	94-104	4	4	5
	Liver	0.01	94	88-101	5	6	5
		0.1	96	92-98	3	3	5
	Meat/Beef	0.01	93	89-99	4	4	5
		0.1	97	89-101	5	5	5
	Egg	0.01	89	86-93	3	3	5
		0.1	86	83-88	2	2	5

Table A 62: Summary of confirmatory recovery of DPX-F6025 (m/z 415/213C)

Matrix group	Matrix	Fortification level	Recovery (%)		SD	RSD	n
		(mg/kg)	mean	range	(%)	(%)	
Animal	Milk	0.01	111	95-124	14	13	5
		0.1	85	81-91	4	4	5
	Cream	0.01	83	61-97	16	19	5
		0.1	87	79-92	6	6	5
	Fat	0.01	97	83-104	8	9	5
		0.1	104	98-116	8	8	5
	Kidney	0.01	96	78-111	13	14	5
		0.1	94	85-105	7	7	5
	Liver	0.01	86	70-112	18	20	5
		0.1	113	100-119	8	7	5
	Meat/Beef	0.01	86	75-104	12	14	5
		0.1	93	91-96	2	2	5
	Egg	0.01	81	62-104	16	20	5
		0.1	83	78-88	4	5	5

Table A 63: Summary of quantitative recovery of DPX-W4189 (m/z 358/141Q)

Matrix group	Matrix	Fortification level	Recovery (%)		SD	RSD	n
		(mg/kg)	mean	range	(%)	(%)	
Animal	Milk	0.01	98	91-109	8	8	5
		0.1	93	86-98	5	6	5
	Cream	0.01	93	87-96	4	4	5
		0.1	86	82-91	4	5	5
	Fat	0.01	100	88-110	9	9	5
		0.1	103	91-115	11	11	5
	Kidney	0.01	94	84-98	5	6	5
		0.1	92	89-98	4	4	5
	Liver	0.01	94	87-103	6	6	5
		0.1	91	86-97	4	5	5
	Meat/Beef	0.01	98	81-114	12	12	5
		0.1	92	80-99	8	8	5
	Egg	0.01	89	83-92	4	5	5
		0.1	86	83-89	3	3	5

Table A 64: Summary of confirmatory recovery of DPX-W4189 (m/z 358/167C)

Matrix group	Matrix	Fortification level	Recovery (%)		SD	RSD	n
		(mg/kg)	mean	range	(%)	(%)	
Animal	Milk	0.01	96	85-103	8	8	5
		0.1	93	85-103	7	7	5
	Cream	0.01	89	84-96	4	5	5
		0.1	85	79-89	5	5	5
	Fat	0.01	96	84-103	8	8	5
		0.1	106	100-110	5	4	5
	Kidney	0.01	95	85-100	6	6	5
		0.1	95	91-100	3	4	5
	Liver	0.01	94	83-111	11	12	5
		0.1	92	85-100	5	6	5
	Meat/Beef	0.01	90	81-96	6	6	5
		0.1	90	82-96	5	6	5
	Egg	0.01	86	82-92	5	6	5
		0.1	86	80-91	5	5	5

Table A 65: Summary of quantitative recovery of DPX-A7881 (m/z 411/168Q)

Matrix group	Matrix	Fortification level	Recovery (%)		SD	RSD	n
		(mg/kg)	mean	range	(%)	(%)	
Animal	Milk	0.01	102	86-111	10	10	5
		0.1	94	86-102	6	6	5
	Cream	0.01	92	82-98	7	8	5
		0.1	86	80-97	7	8	5
	Fat	0.01	99	90-111	10	10	5
		0.1	110	99-118	10	9	5
	Kidney	0.01	88	80-91	5	6	5
		0.1	94	87-100	5	6	5
	Liver	0.01	93	88-104	6	7	5
		0.1	101	97-108	5	5	5
	Meat/Beef	0.01	95	93-97	2	2	5
		0.1	98	93-103	4	4	5
	Egg	0.01	92	83-101	6	7	5
		0.1	85	80-94	5	6	5

Table A 66: Summary of confirmatory recovery of DPX-A7881 (m/z 411/196C)

Matrix group	Matrix	Fortification level	Recovery (%)		SD (%)	RSD (%)	n
		(mg/kg)	mean	range			
Animal	Milk	0.01	91	77-110	12	14	5
		0.1	91	84-101	8	9	5
	Cream	0.01	98	91-110	9	9	5
		0.1	96	86-109	9	9	5
	Fat	0.01	104	88-128	17	17	5
		0.1	116	97-131	14	12	5
	Kidney	0.01	107	102-113	4	4	5
		0.1	107	96-119	9	8	5
	Liver	0.01	87	81-92	4	5	5
		0.1	106	98-115	6	6	5
	Meat/Beef	0.01	94	89-101	5	5	5
		0.1	98	93-06	5	5	5
	Egg	0.01	92	85-99	6	7	5
		0.1	85	74-96	10	11	5

Table A 67: Summary of quantitative recovery of DPX-KE459 (m/z 466/182Q)

Matrix group	Matrix	Fortification level	Recovery (%)		SD (%)	RSD (%)	n
		(mg/kg)	mean	range			
Animal	Milk	0.01	101	96-107	5	5	5
		0.1	99	80-113	13	13	5
	Cream	0.01	96	93-97	2	2	5
		0.1	96	92-99	3	3	5
	Fat	0.01	103	100-110	4	4	5
		0.1	111	105-116	5	4	5
	Kidney	0.01	100	90-110	8	8	5
		0.1	106	101-107	2	2	5
	Liver	0.01	102	97-108	4	4	5
		0.1	107	99-111	5	5	5
	Meat/Beef	0.01	99	84-107	9	9	5
		0.1	98	88-108	9	9	5
	Egg	0.01	104	99-114	6	6	5
		0.1	102	97-108	4	4	5

Table A 68: Summary of confirmatory recovery of DPX-KE459 (m/z 466/83C)

Matrix group	Matrix	Fortification level	Recovery (%)		SD (%)	RSD (%)	n
		(mg/kg)	mean	range			
Animal	Milk	0.01	94	85-99	5	6	5
		0.1	95	84-102	7	8	5
	Cream	0.01	90	78-101	9	10	5
		0.1	94	92-99	3	3	5
	Fat	0.01	97	91-105	6	6	5
		0.1	104	101-110	4	3	5
	Kidney	0.01	96	82-109	11	12	5
		0.1	98	91-104	6	6	5
	Liver	0.01	110	98-115	7	6	5
		0.1	96	90-101	4	4	5
	Meat/Beef	0.01	95	86-101	6	6	5
		0.1	98	91-102	5	5	5
	Egg	0.01	101	82-115	13	13	5
		0.1	97	90-103	5	6	5

Table A 69: Summary of quantitative recovery of DPX-T6376 (m/z 382/167Q)

Matrix group	Matrix	Fortification level	Recovery (%)		SD	RSD	n
		(mg/kg)	mean	range	(%)	(%)	
Animal	Milk	0.01	97	88-108	8	8	5
		0.1	97	89-103	6	6	5
	Cream	0.01	92	87-103	7	7	5
		0.1	88	80-95	6	7	5
	Fat	0.01	103	97-107	4	4	5
		0.1	111	102-119	8	7	5
	Kidney	0.01	99	89-105	6	6	5
		0.1	100	97-105	3	3	5
	Liver	0.01	102	99-107	3	3	5
		0.1	107	102-110	3	3	5
	Meat/Beef	0.01	97	91-106	6	6	5
		0.1	97	85-107	9	10	5
	Egg	0.01	94	88-105	7	7	5
		0.1	102	89-114	11	11	5

Table A 70: Summary of confirmatory recovery of DPX-T6376 (m/z 382/199C)

Matrix group	Matrix	Fortification level	Recovery (%)		SD	RSD	n
		(mg/kg)	mean	range	(%)	(%)	
Animal	Milk	0.01	93	78-114	14	15	5
		0.1	90	71-97	11	12	5
	Cream	0.01	101	94-112	9	9	5
		0.1	83	75-97	9	10	5
	Fat	0.01	115	93-140	17	15	5
		0.1	103	90-110	8	8	5
	Kidney	0.01	80	65-102	15	19	5
		0.1	85	81-93	5	6	5
	Liver	0.01	104	99-113	6	6	5
		0.1	105	99-110	4	4	5
	Meat/Beef	0.01	74	55-92	14	18	5
		0.1	86	77-93	7	9	5
	Egg	0.01	82	71-102	14	17	5
		0.1	80	68-86	8	10	5

Table A 71: Summary of quantitative recovery of DPX-V9360 (m/z 411/182Q)

Matrix group	Matrix	Fortification level	Recovery (%)		SD	RSD	n
		(mg/kg)	mean	range	(%)	(%)	
Animal	Milk	0.01	94	85-100	6	6	5
		0.1	92	86-101	6	6	5
	Cream	0.01	100	95-105	4	4	5
		0.1	91	87-97	4	4	5
	Fat	0.01	104	89-123	16	15	5
		0.1	104	99-108	3	3	5
	Kidney	0.01	107	94-115	9	8	5
		0.1	100	87-107	7	8	5
	Liver	0.01	96	91-102	5	5	5
		0.1	99	92-103	5	5	5
	Meat/Beef	0.01	91	85-95	4	4	5
		0.1	95	87-100	6	6	5
	Egg	0.01	93	85-97	5	5	5
		0.1	85	80-91	5	5	5

Table A 72: Summary of confirmatory recovery of DPX-V9360 (m/z 411/213C)

Matrix group	Matrix	Fortification level	Recovery (%)		SD	RSD	n
		(mg/kg)	mean	range	(%)	(%)	
Animal	Milk	0.01	101	77-120	17	17	5
		0.1	99	81-129	18	18	5
	Cream	0.01	95	89-104	5	6	5
		0.1	88	81-96	6	7	5
	Fat	0.01	108	91-140	21	20	5
		0.1	108	94-121	11	10	5
	Kidney	0.01	116	102-127	10	9	5
		0.1	97	86-108	8	8	5
	Liver	0.01	105	96-117	9	8	5
		0.1	90	85-94	4	4	5
	Meat/Beef	0.01	99	92-109	7	7	5
		0.1	98	89-107	6	6	5
	Egg	0.01	105	85-122	14	13	5
		0.1	82	77-85	4	5	5

Table A 73: Summary of quantitative recovery of DPX-E9636 (m/z 432/182Q)

Matrix group	Matrix	Fortification level	Recovery (%)		SD	RSD	n
		(mg/kg)	mean	range	(%)	(%)	
Animal	Milk	0.01	95	90-105	7	7	5
		0.1	94	82-102	8	8	5
	Cream	0.01	97	88-109	8	9	5
		0.1	99	92-103	5	5	5
	Fat	0.01	107	98-117	7	7	5
		0.1	109	99-116	7	7	5
	Kidney	0.01	86	79-96	6	7	5
		0.1	89	84-95	5	6	5
	Liver	0.01	91	83-97	6	7	5
		0.1	100	95-104	4	4	5
	Meat/Beef	0.01	96	81-111	10	11	5
		0.1	95	81-104	10	11	5
	Egg	0.01	95	89-111	9	10	5
		0.1	102	82-118	14	13	5

Table A 74: Summary of confirmatory recovery of DPX-E9636 (m/z 432/325C)

Matrix group	Matrix	Fortification level	Recovery (%)		SD	RSD	n
		(mg/kg)	mean	range	(%)	(%)	
Animal	Milk	0.01	99	81-106	10	11	5
		0.1	109	94-127	14	13	5
	Cream	0.01	100	71-119	20	20	5
		0.1	96	84-103	8	8	5
	Fat	0.01	90	72-107	14	16	5
		0.1	109	101-120	8	7	5
	Kidney	0.01	98	90-108	9	9	5
		0.1	99	89-113	9	10	5
	Liver	0.01	80	74-91	6	8	5
		0.1	96	85-107	8	9	5
	Meat/Beef	0.01	85	73-103	12	14	5
		0.1	93	87-106	7	8	5
	Egg	0.01	88	70-108	15	17	5
		0.1	96	86-114	12	13	5

Table A 75: Summary of quantitative recovery of DPX-T5648 (m/z 365/150Q)

Matrix group	Matrix	Fortification level	Recovery (%)		SD	RSD	n
		(mg/kg)	mean	range	(%)	(%)	
Animal	Milk	0.01	101	88-112	9	9	5
		0.1	99	90-104	6	6	5
	Cream	0.01	85	79-90	4	5	5
		0.1	88	84-93	4	5	5
	Fat	0.01	104	94-111	6	6	5
		0.1	107	102-115	5	5	5
	Kidney	0.01	96	94-100	3	3	5
		0.1	96	90-100	4	4	5
	Liver	0.01	91	88-98	4	5	5
		0.1	92	83-99	6	7	5
	Meat/Beef	0.01	93	83-108	10	11	5
		0.1	97	88-105	7	7	5
	Egg	0.01	86	82-90	3	3	5
		0.1	86	73-106	12	14	5

Table A 76: Summary of confirmatory recovery of DPX-T5648 (m/z 365/199C)

Matrix group	Matrix	Fortification level	Recovery (%)		SD	RSD	n
		(mg/kg)	mean	range	(%)	(%)	
Animal	Milk	0.01	99	87-114	10	10	5
		0.1	108	86-119	13	12	5
	Cream	0.01	81	65-108	16	20	5
		0.1	89	79-98	7	8	5
	Fat	0.01	96	77-116	16	17	5
		0.1	99	91-105	6	6	5
	Kidney	0.01	93	85-105	8	9	5
		0.1	93	81-101	8	8	5
	Liver	0.01	104	89-120	13	12	5
		0.1	93	86-102	6	6	5
	Meat/Beef	0.01	101	92-108	7	7	5
		0.1	104	91-127	14	14	5
	Egg	0.01	89	72-113	18	20	5
		0.1	93	71-106	14	15	5

Table A 77: Summary of quantitative recovery of DPX-L5300 (m/z 396/155Q)

Matrix group	Matrix	Fortification level	Recovery (%)		SD	RSD	n
		(mg/kg)	mean	range	(%)	(%)	
Animal	Milk	0.01	94	86-99	5	6	5
		0.1	94	89-100	4	4	5
	Cream	0.01	92	86-100	5	6	5
		0.1	82	78-87	4	4	5
	Fat	0.01	104	94-110	7	6	5
		0.1	101	94-107	5	5	5
	Kidney	0.01	94	90-97	3	3	5
		0.1	90	85-94	4	4	5
	Liver	0.01	82	79-86	3	3	5
		0.1	87	80-92	4	5	5
	Meat/Beef	0.01	100	91-109	7	7	5
		0.1	87	77-101	9	11	5
	Egg	0.01	85	77-93	6	8	5
		0.1	92	87-100	5	5	5

Table A 78: Summary of confirmatory recovery of DPX-L5300 (m/z 396/56C)

Matrix group	Matrix	Fortification level	Recovery (%)		SD	RSD	n
		(mg/kg)	mean	range	(%)	(%)	
Animal	Milk	0.01	94	89-103	6	6	5
		0.1	87	79-90	5	5	5
	Cream	0.01	88	76-97	9	10	5
		0.1	85	82-89	4	4	5
	Fat	0.01	106	97-118	9	8	5
		0.1	100	90-106	7	7	5
	Kidney	0.01	91	83-97	6	6	5
		0.1	89	87-95	3	3	5
	Liver	0.01	88	66-102	14	16	5
		0.1	82	75-87	5	6	5
	Meat/Beef	0.01	87	74-106	12	13	5
		0.1	90	83-103	8	8	5
	Egg	0.01	90	86-97	5	6	5
		0.1	88	83-91	3	4	5

Table A 79: Summary of quantitative recovery of DPX-M6316 (m/z 388/167Q)

Matrix group	Matrix	Fortification level	Recovery (%)		SD	RSD	n
		(mg/kg)	mean	range	(%)	(%)	
Animal	Milk	0.01	89	83-92	3	4	5
		0.1	88	78-98	8	9	5
	Cream	0.01	89	82-96	6	6	5
		0.1	92	83-96	5	6	5
	Fat	0.01	90	83-97	5	6	5
		0.1	104	96-108	5	4	5
	Kidney	0.01	90	86-93	3	3	5
		0.1	93	85-98	5	5	5
	Liver	0.01	88	84-92	3	4	5
		0.1	99	92-105	6	6	5
	Meat/Beef	0.01	92	81-99	7	7	5
		0.1	100	90-104	5	5	5
	Egg	0.01	101	69-121	19	19	5
		0.1	90	83-96	5	5	5

Table A 80: Summary of confirmatory recovery of DPX-M6316 (m/z 388/205C)

Matrix group	Matrix	Fortification level	Recovery (%)		SD	RSD	n
		(mg/kg)	mean	range	(%)	(%)	
Animal	Milk	0.01	95	87-101	5	5	5
		0.1	94	85-110	9	10	5
	Cream	0.01	97	86-110	10	10	5
		0.1	99	95-107	4	5	5
	Fat	0.01	95	87-102	6	6	5
		0.1	107	98-115	7	6	5
	Kidney	0.01	93	84-101	7	8	5
		0.1	100	98-105	3	3	5
	Liver	0.01	87	76-103	10	11	5
		0.1	97	92-100	4	4	5
	Meat/Beef	0.01	87	83-91	4	4	5
		0.1	88	75-96	9	10	5
	Egg	0.01	98	86-109	10	10	5
		0.1	99	85-120	14	14	5

Table A 81: Summary of quantitative recovery of DPX-66037 (m/z 493/264Q)

Matrix group	Matrix	Fortification level	Recovery (%)		SD	RSD	n
		(mg/kg)	mean	range	(%)	(%)	
Animal	Milk	0.01	89	79-101	9	10	5
		0.1	96	92-103	4	5	5
	Cream	0.01	90	87-96	3	4	5
		0.1	90	88-94	2	3	5
	Fat	0.01	100	87-109	9	9	5
		0.1	119	112-128	6	5	5
	Kidney	0.01	92	86-97	5	5	5
		0.1	97	91-101	4	4	5
	Liver	0.01	90	84-94	4	4	5
		0.1	99	96-107	5	5	5
	Meat/Beef	0.01	96	88-101	5	6	5
		0.1	100	95-105	4	4	5
	Egg	0.01	86	83-90	3	3	5
		0.1	93	90-98	3	4	5

Table A 82: Summary of confirmatory recovery of DPX-66037 (m/z 493/96C)

Matrix group	Matrix	Fortification level	Recovery (%)		SD	RSD	n
		(mg/kg)	mean	range	(%)	(%)	
Animal	Milk	0.01	92	74-107	14	15	5
		0.1	108	90-126	14	13	5
	Cream	0.01	79	54-104	19	24	5
		0.1	92	77-115	15	16	5
	Fat	0.01	100	93-107	6	6	5
		0.1	114	100-124	9	8	5
	Kidney	0.01	81	69-88	7	9	5
		0.1	96	85-102	7	7	5
	Liver	0.01	92	83-101	7	7	5
		0.1	100	98-102	2	2	5
	Meat/Beef	0.01	81	70-87	6	8	5
		0.1	90	82-102	8	9	5
	Egg	0.01	94	77-117	16	17	5
		0.1	96	74-114	15	16	5

Repeatability

Repeatability was not assessed as part of this study.

Working Solution Stability

Stock and fortification solutions of all 13 sulfonylurea herbicides in acetonitrile were stable for six months when stored capped in a freezer maintained at a temperature of $\leq -10^{\circ}\text{C}$.

Sample Extract Stability

Final sample extracts containing all 13 sulfonylurea herbicides are stable for 72 hours when refrigerated at 4°C , or at least 120 hours when kept frozen $\leq -10^{\circ}\text{C}$.

Matrix Effects

Matrix effects were evaluated by comparing the response of the analyte fortified in a control extract after processing (for each matrix type) to the response of the analyte fortified in neat solvent. For fat, the results demonstrate that matrix effects exceed $\pm 20\%$ therefore, matrix matched standards were used for quantification for all analytes in fat matrix for this study.

Extraction Efficiency

The method extraction procedure is similar to the ones used in metabolism studies of radiolabeled compounds of nicosulfuron and flupyr-sulfuron methyl in lactating goats and/or egg-laying hens. The method extraction procedure is also similar to the method used in cattle feeding study of nicosulfuron that included a storage stability of the compound in various animal matrices. The method extraction

procedure should also be able to extract efficiently the other SUs from the animal matrices due to similarity in chemical functionality and properties.

CONCLUSION

The analytical method described herein is suitable for the determination of the 13 SUs in animal matrices at a limit of quantification of approximately 0.010 mg/kg. Acceptable (average) fortification recoveries and relative standard deviations were obtained at the LOQ and 10xLOQ, i.e. 70-110% with $\text{rsd} \leq 20\%$. HPLC/MS/MS analysis of 13 SUs in fat using solvent standards yielded unacceptable recoveries ($>110\%$) for six of the SUs due to matrix (enhancement) effect. However, acceptable recoveries were obtained using matrix-matched standards. The confirmatory method for the HPLC/MS/MS method is based on detection and the relative ratios of the two MS/MS transition ions monitored.

Method is acceptable based on current guidelines: EPA Residue Chemistry Test Guidelines OPPTS 860.1340 and SANCO/825/00 rev.8.1, as well as PMRA Regulatory Directive Dir98-02.

A 2.2.2.3 Description of Methods for the Analysis of Soil (KCP 5.2)

No new or additional studies have been submitted

A 2.2.2.4 Description of Methods for the Analysis of Water (KCP 5.2)

No new or additional studies have been submitted

A 2.2.2.4.1 Study 1, DuPont-35704

Comments of zRMS:	The analytical method has been developed and successfully validated for the detection, quantitative analysis and confirmation of residues of thifensulfuron methyl in drinking, ground and surface water. The determined limit of quantitation (LOQ) was 0.1 µg/kg. The accuracy and precision of the method during sample analysis were considered to be acceptable since mean recoveries at each fortification level were in the range of 70 – 110% with relative standard deviation(s) $\leq 20\%$ for water matrices. This method for the determination of residues of thifensulfuron methyl in water meets the requirements of the SANCO/825/00 rev. 8.1. The study is acceptable.
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Reference:	KCP 5.2/02
Report:	Henze, R.M., Stry, J.J., (2013); Analytical Method for the determination of thifensulfuron methyl in water using LC/MS/MS
Report No.:	DuPont-35704
Testing Facility Report No.:	DuPont-35704
Guidelines	OCSPP 850.6100, SANCO/825/00 rev. 8.1 (2010)
Deviations:	None
GLP:	No
Acceptability:	Yes

Materials and methods

Thifensulfuron methyl was analysed in water using a dilute and inject approach. A 10 g water sample was measured into a 15-mL centrifuge tube. A 1.0-mL aliquot of the sample was transferred into a 14-mL centrifuge tube and diluted to 2.0 mL with 0.20-mL of methanol and 0.80-mL of aqueous acetic acid solution. Thifensulfuron methyl was separated from possible interferences by reversed phase liquid chromatography (LC) and detected using turbospray mass spectrometry/mass spectrometry (MS/MS).

Results and discussions

This method is submitted as a monitoring/enforcement method therefore an independent laboratory validation was conducted. Two ion transitions were monitored during method validation. Recovery data

was calculated using two transitions as described in the SANCO 825/00 Revision 8.1 guidance document.

The fortification data reported in the method proposed for monitoring thifensulfuron methyl residues in water are summarised below. The average recovery specified in the decision-making criteria is 70 to 120%, with a standard deviation of $\leq 20\%$.

Table A 83: Recovery results from method validation of thifensulfuron methyl using the analytical method

Matrix	Analyte	Fortification level ($\mu\text{g/kg}$) (n = 5)	Mean recovery (%)	RSD (%)
Drinking Water	Thifensulfuron methyl	0.10	101	2
		1.0	94	1
Well Water		0.10	104	3
		1.0	98	1
Surface Water		0.10	105	1
		1.0	101	3

Table A 84: Characteristics for the analytical method used for validation of thifensulfuron methyl residues in drinking, ground and surface water

	Thifensulfuron methyl
Specificity	Mass spectrum is provided and the blank value $<30\%$ LOQ
Calibration (type, number of data points)	Individual calibration data is presented, calibration line equation presented: $y=271321x+142.5$ $R^2=0.9999$. A total of 6 data points for the quantitative ion transition (388 \rightarrow 167).
Calibration range	Good linearity was observed in the range of 0.025 to 2.5 ng/mL for thifensulfuron methyl. This range corresponds to an analyte concentration range of 0.05 to 5.0 $\mu\text{g/L}$ in a water sample.
Assessment of matrix effects is presented	Yes – In data sheets presented in the original report appendix
Limit of determination/quantification	0.10 $\mu\text{g/kg}$

Conclusion

This method is suitable for use by regulatory agencies to detect thifensulfuron methyl in water. The instrumentation required to perform both the analysis and confirmatory methods is available in most well equipped analytical laboratories. No toxic or hazardous reagents are required to prepare the samples, and all of the sample preparation equipment is commercially available. The method does not require the use of untreated commodity to correct for recoveries.

Confirmatory method

During the validation of DuPont-35704 two individual ion transitions were monitored. Recovery data calculated using the quantitative transition is presented below. Recovery data using the confirmatory transition are presented below. This approach is consistent with the SANCO 825 Revision 8.1 Guidance document.

Materials and methods

Same as described in the original method report.

Results and discussions

Table A 3: Recovery results from confirmatory method validation of thifensulfuron methyl using the confirmatory analytical method

Matrix	Analyte	Fortification level (µg/kg) (n = 5)	Mean recovery (%)	RSD (%)
Drinking Water	Thifensulfuron methyl	0.10 1.0	101 92	10 3
Well Water		0.10 1.0	107 94	3 2
Surface Water		0.10 1.0	108 98	5 0

Table A 4: Characteristics for the confirmatory method used for validation of thifensulfuron methyl residues in drinking, ground and surface water

	Thifensulfuron methyl
Specificity	Mass spectrum is provided and the blank value <30% LOQ)
Calibration (type, number of data points)	Individual calibration data is presented, calibration line equation presented: $y=52860-150.3 R^2=0.9997$. A total of 6 data points for the confirmatory ion transition (388→205).
Calibration range	Good linearity was observed in the range of 0.025 to 2.5 ng/mL for thifensulfuron methyl. This range corresponds to an analyte concentration range of 0.05 to 5.0 µg/L in a water sample.
Assessment of matrix effects is presented	Yes – In data sheets presented in original report appendix
Limit of determination/quantification	0.00010 mg/kg

Conclusion

This method is suitable for use by regulatory agencies to detect thifensulfuron methyl in water. The instrumentation required to perform both the analysis and confirmatory methods is available in most well equipped analytical laboratories. No toxic or hazardous reagents are required to prepare the samples, and all of the sample preparation equipment is commercially available. The method does not require the use of untreated commodity to correct for recoveries.

Extraction efficiency

Extraction efficiency is not required for water methods.

A 2.2.2.4.2 Study 1, DuPont-36531 - Independent laboratory validation

Comments of zRMS:	The DuPont-35704 analytical method was successfully independently validated for the determination of thifensulfuron methyl in drinking, ground, and surface water using LC/MS/MS with a LOQ of 0.1 µg/kg using LC-MS/MS. Mean recovery values at each fortification concentration for each matrix were within the acceptance range (mean recovery 70 - 110%; RSD ≤ 20%). The analytical method fulfils the requirements of guideline SANCO/825/00 rev.8.1. The study is acceptable.
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Reference:	KCP 5.2/07
Report:	Mason, B.J., (2013); Independent laboratory validation of DuPont-35704, "Analytical method for the determination of thifensulfuron methyl in water using LC/MS/MS"
Report No.:	DuPont-36531
Testing Facility Report No.:	80141
Guidelines	SANCO/825/00 rev.8.1 (2010), OCSPP 850.6100, ENV/JM/MONO(2007)17
Deviations:	None
GLP:	Yes
Acceptability:	Yes

Materials and methods

The analytical method was run exactly as written with an addition of a liquid-liquid partition procedure, using equivalent equipment and materials where permitted. No communication, other than the approval of equivalent apparatus, reagents, and techniques; correction of typographical errors; extraction and chromatography issues; clarification of some technical aspects of the method; and recovery updates between the Sponsor Monitor/Method Developer and Study Director was required.

Results and discussions

The results generated during the independent laboratory validation study are summarised below.

Table A 5: Recovery results from independent laboratory validation of thifensulfuron methyl using the analytical method

Matrix	Analyte	Fortification level (µg/kg) (n = 5)	Mean recovery (%)	RSD (%)
Drinking Water	Thifensulfuron methyl	0.10	87	8.8
		1.0	99	2.3
Well Water		0.10	78	7.7
		1.0	103	1.3
Surface Water		0.10	101	9.5
		1.0	124	2.0

Table A 6: Characteristics for the analytical method used for independent laboratory validation of thifensulfuron methyl residues in drinking, ground and surface water

	Thifensulfuron methyl
Specificity	A mass spectrum is not provided in report, the blank values are <30% LOQ.
Calibration (type, number of data points)	Individual calibration data is presented. A calibration line equation presented using six of data points, 388 → 167 (Quantitative) $y=271321x+142.53$ ($R^2=0.9999$).
Calibration range	Accepted calibration range in concentration units (ng/mL) Corresponding calibration range in mass ratio units for the sample (e.g. in mg/kg or µg/L) are not presented. The calibration range corresponds to 0.05 to 5.0 µg/L of thifensulfuron methyl in a water sample.
Assessment of matrix effects is presented	Yes
Limit of determination/quantification	limit of quantification 0.10 µg/kg representing the lowest validated level with sufficient recovery and precision

Conclusion

The independent laboratory validation is acceptable for the analysis of thifensulfuron methyl in drinking, ground and surface water.

A 2.2.2.5 Description of Methods for the Analysis of Body Fluids and Tissues (KCP 5.2)

~~No new or additional studies have been submitted~~

A 2.2.2.5.1 Study 1, DuPont-47394

Comments of zRMS:	The analytical method has been developed and successfully validated for the detection, quantitative analysis and confirmation of residues of thifensulfuron methyl (DPX-M6316) in plasma and urine. The determined limit of quantitation (LOQ) was 1.0 µg/kg (ppb) for plasma and 3.0 µg/kg for urine. The study is acceptable.
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Reference:	CP 5.2
Report:	R. M. Henze and J. J. Stry, 2016; Analytical method for the determination of chlorsulfuron, metsulfuron methyl, thifensulfuron methyl and tribenuron methyl in plasma and urine by LC/MS/MS;
DuPont Report No.:	47394
Testing Facility Report No.:	47394
Guidelines	U.S. EPA Residue Chemistry Test Guidelines, August 1996 OPPTS 860.1340 Residue Analytical Method European Commission, Directorate General Health and Consumer Protection. "Guidance Document on Residue Analytical Methods", SANCO/825/00 rev. 8.1, November 16, 2010
Deviations:	None
GLP:	No
Acceptability:	Yes

MATERIALS AND METHODS

Method Principle

An analytical method was developed and validated for the determination of residues of chlorsulfuron (DPX-W4189), metsulfuron methyl (DPX-T6376), thifensulfuron methyl (DPX-M6316) and tribenuron methyl (DPX-L5300) in plasma and urine. The determined limit of quantitation (LOQ) for all analytes was 1.0 µg/kg (ppb) for plasma and 3.0 µg/kg for urine.

Chlorsulfuron, metsulfuron methyl, thifensulfuron methyl and tribenuron methyl were extracted from each plasma sample by homogenization in 70:30 acetonitrile: 20 mM aqueous sodium phosphate (pH=7.5) using a vortex mixer. The acetonitrile was removed from the extract by evaporation on a nitrogen evaporator. The extract was filtered through a carbon solid phase extraction (SPE) cartridge. The SPE cartridge was washed with 1:1 acetone: 20 mM aqueous sodium phosphate (pH=7.5). The filtered extract and the wash solution were collected in the same tube. The purified extract was evaporated using a nitrogen evaporator until only the aqueous phase remained. The extract was diluted with methanol and 20 mM aqueous sodium phosphate (pH=7.5) prior to analysis.

Urine samples (0.5 grams) were filtered through carbon solid phase extraction (SPE) cartridges. Each SPE cartridge was washed with 1:1 acetone: 20 mM aqueous sodium phosphate (pH=7.5). The filtered extract and the wash solution were collected in the same tube. The purified extract was evaporated using a nitrogen evaporator until only the aqueous phase remained. The extract was diluted with methanol and 20 mM aqueous sodium phosphate (pH=7.5). An aliquot from the purified extracts was diluted with 90% 20 mM aqueous sodium phosphate (pH=7.5)/ 10% methanol prior to analysis.

All analyses were performed by reversed-phase LC with electrospray ionization mass spectrometry/mass spectrometry (MS/MS) detection.

RESULTS AND DISCUSSION

Acceptable recoveries for all analytes were obtained from fortified matrices, i.e. average recoveries per fortification level were within 70% to 120% with RSDs ≤ 20% (N=5). The results are summarized in the table below.

Table A 81: Recovery results from concurrent recoveries of thifensulfuron methyl, IN-L9225 and IN-A4098 using the analytical method (for 388/167 and for 388/205)

Thifensulfuron methyl (DPX-M6316) - 388/167				Thifensulfuron methyl (DPX-M6316) - 388/205		
	LEVEL (µg/kg)	Average Percent Recovery	RSD	LEVEL (µg/kg)	Average Percent Recovery	RSD
Plasma	1.0	104%	8.5%	1.0	106%	5.4%
	10	105%	3.3%	10	101%	5.3%
Urine	3.0	101%	5.4%	3.0	100%	11.9%
	30	100%	4.8%	30	97%	3.0%

Table A 85: Characteristics for the analytical method used for determination of thifensulfuron methyl residues in plasma and urine.

	Thifensulfuron methyl
Specificity	blank value <LOQ
Calibration (type, number of data points)	linear regression analysis with 1/x weighting $r \geq 0.99$ 7 data points
Calibration range	Concentration range of 0.01-2.5 ng a.s./L
Limit of determination/quantification	1.0 µg/kg (ppb) for plasma 3.0 µg/kg for urine.

CONCLUSION

This method for the determination of residues of thifensulfuron methyl in plasma and urine samples meets the guidelines of the European Commission, Directorate General Health, and Consumer Protection. "Guidance Document on Residue Analytical Methods", SANCO/825/00 rev. 8.1 as well as the U.S. EPA Residue Chemistry Test Guidelines, August 1996 OPPTS 860.1340 Residue Analytical Method.

A 2.2.2.6 Other Studies/Information

No new or additional studies have been submitted

A 2.3 Analytical methods for isoxadifen-ethyl

No new studies are submitted. See Appendix 3 for summaries of studies relied upon but not submitted.

Appendix 3 Analytical methods for isoxadifen-ethyl

The following studies have not been previously reviewed at the EU level and are relied upon but not submitted. The study summaries have been provided by Bayer CropScience.

A 3.1 Analytical methods for isoxadifen-ethyl

A 3.1.1 Methods used for the generation of pre-authorization data (KCP 5.1)

A 3.1.1.1 Description of analytical methods for the determination of residues in plant matrices (KCP 5.1)

A 3.1.1.1.1 Analytical method RAM CA/01/00

A 3.1.1.1.1.1 Method validation

Reference:	KCP 5.1.2.5
Title:	An analytical method for the determination of residues of AE F122006 and its major metabolites AE F129431 and AE F162241 in field corn by gas and liquid chromatography using ion trap mass selective detection: AE F122006
Report:	Dacus, S. C.; Neal, J. L.; 2000; B002825; M-238556-01-1
Guideline(s):	--
Deviations:	--
GLP/GEP:	no

Materials and methods

The method RAM CA/01/00 was developed to determine the residues of isoxadifen-ethyl (AE F122006) and its major metabolites (isoxadifen (AE F129431) and AE F162241) in maize matrices with a limit of quantification of 0.02 mg/kg for maize grain and 0.05 mg/kg for maize feed commodities with high water content and maize dry feed commodities.

Residues of isoxadifen-ethyl (AE F122006) and its metabolites isoxadifen (AE F129431) and AE F162241 are extracted from crops with blending in a mixture of acetonitrile: 0.1 N HCl (80:20, v:v). The acidic extracts are partitioned with hexane to remove any oils that may be present. Following the addition of a saturated sodium chloride solution, the extracts are partitioned again with dichloromethane. After the phases have separated, the aqueous phase is discarded and the organic extracts rotary evaporated to dryness and re-dissolved in ethyl ether.

The extract is cleaned-up and fractionated using an amino propyl solid phase extraction (SPE) cartridge. The parent compound isoxadifen-ethyl is isolated from the metabolites isoxadifen (AE F129431) and AE F162241 by eluting the amino SPE cartridge first with ethyl ether. The two acidic metabolites isoxadifen and AE F162241 are then eluted from the SPE cartridge with a mixture of methanol: buffer (pH = 7) (1:1, v:v).

The eluate (Fraction A) containing isoxadifen-ethyl is rotary evaporated to dryness and reconstituted in toluene to await analysis by GC/MS/MS ion trap.

The eluate (Fraction B-C) containing the metabolites isoxadifen and AE F162241 is acidified and partitioned with dichloromethane. This extract is evaporated to dryness, and reconstituted in a known volume of methanol. An aliquot (Fraction B) intended for determination of isoxadifen (AE F129431) is methylated with trimethylsilyldiazomethane, concentrated and reconstituted in toluene. The extracts containing the methylated form of isoxadifen (AE F123756) are analysed by GC/MS/MS.

A second aliquot (Fraction C) intended for determination of AE F162241 is diluted with 0.2% acetic

acid, filtered and analysed by negative ion ESI HPLC/MS/MS ion trap.

The quantification is carried out by external standardization using standards in solvent.

Stock solutions of AE F122006 and AE F123756 were prepared in acetone. Stock solutions of AE F129431 and AE F162241 were prepared in methanol: 0.2% acetic acid (1:1, v:v). Fortification stock solutions were prepared from the stock solutions of AE F122006, AE F129431 and AE F162241 by further dilution with methanol: 0.2% acetic acid (1:1, v:v).

GC calibration solutions were prepared from the stock solutions of AE F122006 and AE F123756 by further dilution with toluene. HPLC calibration solutions were prepared from the stock solution of AE F162241 by further dilution with methanol: 0.2% acetic acid (5:95, v:v).

Only one MRM transition per analyte was monitored. The analytes were quantified as follows:

Analyte	Determination principle	Quantitation ion
AE F122006	GC/MS/MS	<i>m/z</i> 232
AE F123756 (methylated derivative of AE F129431)		
AE F162241	HPLC/MS/MS	<i>m/z</i> 197.5

Results and discussions

Standard curves for the GC-MS/MS system (for determination of AE F122006 and the methylated derivative of AE F129431, i.e. AE F123756) were derived by plotting the $\ln(\text{peak area})$ vs. the $\ln(\text{standard concentration})$ and obtaining the least square regression of these data. Standard curves for the HPLC-MS/MS system (for determination of AE F162241) were derived by plotting the peak area vs. the standard concentration and obtaining the least square regression line of these data.

Correlation coefficients were 0.9985 for AE F122006, 0.9993 for AE F129431 (determined as AE F123756) and 0.9970 for AE F162241.

The accepted calibration range was 0.005 – 0.2 µg/mL each for AE F122006 and AE F129431, equivalent to 0.02 – 0.63 mg/kg in maize feed commodities with high water content and maize dry feed commodities, and equivalent to 0.01 – 0.4 mg/kg (for AE F122006) or 0.01 – 0.5 mg/kg (for AE F129431) in maize grain.

For AE F162241, the accepted calibration range was 0.002 – 0.04 µg/mL, equivalent to 0.03 – 0.63 mg/kg in maize feed commodities with high water content and maize dry feed commodities, and equivalent to 0.01 – 0.25 mg/kg in maize grain.

Method validation data are summarised in the following table.

The limit of quantitation (LOQ) for each single analyte is 0.02 mg/kg for maize grain and 0.05 mg/kg for maize feed commodities with high water content and maize dry feed commodities.

For validation of the method, recovery experiments were performed by fortifying samples of maize forage and hay at levels of the LOQ (0.05 mg/kg) and $10 \times \text{LOQ}$ (0.5 mg/kg) for isoxadifen-ethyl, AE F129431 or AE F162241. In maize grain, the fortification levels for each individual analyte were 0.02 mg/kg (LOQ) and 0.2 mg/kg.

The recovery rates were corrected for apparent residue in the control samples. Standard deviations (SD) were given in the reports for all sample materials at fortification levels ranging from 0.02 to 0.50 mg/kg. Relative standard deviation (RSD) were recalculated from these reported standard deviations ($\text{RSD} = \text{SD}/\text{mean} \times 100$).

The mean recoveries were in the range of 70-120% for isoxadifen-ethyl and its metabolites. The mean recoveries for isoxadifen-ethyl at each fortification level were between 82 and 100%. The relative

standard deviations (RSDs) ranged between 11 and 17% for all matrices for isoxadifen-ethyl.

The mean recoveries for isoxadifen (AE F129431) at each fortification level were between 88 and 118%. The RSDs for AE F129431 were between 9 and 19%.

The mean recoveries for AE F162241 at each fortification level were between 72 and 94%. The RSDs for AE F162241 were between 11 and 19% with one exception of 26% in maize grain.

Table A 86: Recovery results from method validation of isoxadifen-ethyl and its metabolites AE F129431 and AE F162241 using the analytical method RAM CA/01/00

Matrix	Fortification level (mg/kg)	n	Mean recovery (%)*	RSD (%)**	Comments
Isoxadifen-ethyl (AE F122006; m/z 232)					
Maize grain	0.02	5	93	17	-
	0.20	6	82	15	-
Maize forage	0.05	8	100	11	-
	0.50	7	96	13	-
Maize hay	0.05	7	89	12	-
	0.50	10	85	13	-
Isoxadifen (AE F129431; m/z 232)					
Maize grain	0.02	5	101	17	-
	0.20	6	88	17	-
Maize forage	0.05	8	118	9	-
	0.50	7	96	19	-
Maize hay	0.05	8	102	14	-
	0.50	11	106	13	-
AE F162241 (m/z 197.5)					
Maize grain	0.02	5	94	26	-
	0.20	6	72	11	-
Maize forage	0.05	7	94	16	-
	0.50	8	79	15	-
Maize hay	0.05	7	80	13	-
	0.50	10	70	19	-

* Analytical recoveries corrected for apparent residue in control samples.

** Recalculated from the reported SD ($RSD = SD/mean \times 100$)

Table A 87: Characteristics for the analytical method RAM CA/01/00 used for validation of AE F122006, AE F129431 and AE F162241 residues in maize shoot, cob and kernel

	AE F122006	AE F129431 (determined as AE F123756)	AE F162241
Specificity	Blank values not reported	Blank values not reported	Blank values not reported
Calibration (type, number of data points)	Representative calibration data including calibration line equation presented in the study report. $r^2 = 0.9985$ (least square regression) Number of data points: 6	Representative calibration data including calibration line equation presented in the study report. $r^2 = 0.9993$ (least square regression) Number of data points: 6	Representative calibration data including calibration line equation presented in the study report. $r^2 = 0.997$ (least square regression) Number of data points: 5
Calibration range	Accepted calibration range in concentration units: 0.005 – 0.2 µg/mL, equivalent to 0.02 - 0.63 mg/kg in maize feed commodities with high water content and maize dry feed commodities and	Accepted calibration range in concentration units: 0.005 – 0.2 µg/mL, equivalent to 0.02 - 0.63 mg/kg in maize feed commodities with high water content and maize dry feed commodities and equivalent to 0.01 – 0.5 mg/kg in maize grain	Accepted calibration range in concentration units: 0.002 – 0.04 µg/mL, equivalent to 0.03 - 0.63 mg/kg in maize feed commodities with high water content and maize dry feed commodities and

	AE F122006	AE F129431 (determined as AE F123756)	AE F162241
	equivalent to 0.01 – 0.4 mg/kg in maize grain		equivalent to 0.01 – 0.25 mg/kg in maize grain
Assessment of matrix effects is presented	No	No	No
Limit of determination/quantification	Maize kernel: 0.02 mg/kg Maize cob and shoot: 0.05 mg/kg	Maize kernel: 0.02 mg/kg Maize cob and shoot: 0.05 mg/kg	Maize kernel: 0.02 mg/kg Maize cob and shoot: 0.05 mg/kg

Conclusion

The method RAM CA/01/00 is considered suitable to determine residues of isoxadifen-ethyl and its metabolites in maize matrices at the LOQs of 0.02 mg/kg (for maize kernel) and of 0.05 mg/kg (for maize forage and hay).

A 3.1.1.1.2 Analytical method RAM CA/01/01

A 3.1.1.1.2.1 Method validation

Reference:	KCP 5.1.2.5
Title:	An analytical method for the determination of residues of Isoxadifen-ethyl (AE F122006) and its major metabolites AE F129431 in corn and rice and AE C637375 in rice by gas chromatography using ion trap mass selective detection, Revision 1
Report:	Dacus, S.; Neal, J.; Cole, M.; 2001; B003344; M-238876-02-1
Guideline(s):	USEPA (=EPA): OPPTS 860.1340
Deviations:	not specified
GLP/GEP:	no

The method RAM CA/01/00 (please refer to A 3.1.1.1.1) was developed to determine the residues of isoxadifen-ethyl (AE F122006) and its major metabolites (isoxadifen (AE F129431) and AE F162241) in maize matrices with a limit of quantification of 0.02 mg/kg for maize grain and 0.05 mg/kg for maize feed commodities with high water content and maize dry feed commodities.

The method RAM CA/01/01 is suitable in order to determine the residues of isoxadifen-ethyl and its major metabolites in rice and maize. In rice, the analytes determined are isoxadifen-ethyl (AE F122006), isoxadifen (AE F129431), and 3-hydroxy-3,3-diphenylpropanenitrile (AE C637375). In maize, the analytes determined are isoxadifen-ethyl and isoxadifen. This method combines the methods for rice and maize into one method for the sake of clarity and brevity. As such, the method is the same as the previous methods and has been shown to successfully account for weathered residues of isoxadifen-ethyl and its metabolites in rice and maize.

Materials and methods

Residues of isoxadifen-ethyl and its metabolites AE F129431 and AE C637375 are extracted from crops with blending in a mixture of acetonitrile: 0.1 N HCl (80:20, v:v). The acidic extracts are partitioned with hexane to remove any oils that may be present. Following the addition of a saturated sodium chloride solution, the extracts are partitioned again with dichloromethane. After the phases have separated, the aqueous phase is discarded and the organic extracts rotary evaporated to dryness and re-dissolved in ethyl ether.

The extract is cleaned-up and fractionated using an amino propyl solid phase extraction (SPE) cartridge. The parent compound isoxadifen-ethyl and the metabolite AE C637375 are isolated from the metabolite AE F129431 by eluting the amino SPE cartridge first with ethyl ether. The acidic metabolite AE F129431 is then eluted from the SPE cartridge with a mixture of methanol: buffer (pH 7) (1:1, v:v). The

eluate (Fraction A) containing isoxadifen-ethyl and AE C637375 is rotary evaporated to dryness and reconstituted in toluene to await analysis by GC/MS/MS ion trap.

The eluate (Fraction B) containing the metabolite AE F129431 is acidified and partitioned with dichloromethane. This extract is evaporated to dryness and reconstituted in a known volume of methanol.

An aliquot is methylated with trimethylsilyldiazomethane, concentrated and reconstituted in toluene. The extracts containing the methylated form of AE F129431 (AE F123756) are analysed by GC/MS/MS.

The quantification is carried out by external standardization using standards in solvent.

Stock solutions of AE F122006, AE F123756 and AE C637375 were prepared in acetone. Stock solutions of AE F129431 were prepared in methanol: 0.2% acetic acid (1:1, v:v). Fortification stock solutions were prepared from the stock solutions of AE F122006, AE F123756 and AE C637375 by further dilution with methanol: 0.2% acetic acid (1:1, v:v).

GC calibration solutions were prepared from the stock solutions of AE F122006, AE F123756 and AE C637375 by further dilution with toluene.

Two MRM transitions per analyte were monitored as detailed below:

Analyte	Quantitation ion	Confirmation ions
AE F122006	<i>m/z</i> 232	<i>m/z</i> 222, 204, 194
AE F123756 (methylated derivative of AE F129431)	<i>m/z</i> 232	<i>m/z</i> 222, 204, 194
AE C637375	<i>m/z</i> 105	<i>m/z</i> 77, 183

Results and discussions

Standard curves for the GC-MS/MS system (for determination of AE F122006 and the methylated derivative of AE F129431, i.e. AE F123756) were derived by plotting the ln(peak area or peak heights) vs. the ln(standard concentration) and obtaining the least square regression of these data.

Correlation coefficients were 0.9985 for AE F122006, 0.9993 for AE F129431 (determined as AE F123756) and 0.9976 for AE C637375.

The accepted calibration range was 0.005 – 0.2 µg/mL each for AE F122006, AE F129431 (determined as AE F123756) and AE C637375, equivalent to 0.02 – 0.63 mg/kg in rice straw. In rice grain, the accepted calibration was 0.005 – 0.2 µg/mL each for AE F122006, AE F129431 (determined as AE F123756) and AE C637375, equivalent to 0.01 – 0.40 mg/kg for AE F122006 and AE C637375 and to 0.01 – 0.25 mg/kg for AE F129431.

Representative recovery data are presented for isoxadifen-ethyl, AE F129431 and AE C637375 in rice grain and straw in Table A 88 down below.

For isoxadifen-ethyl and AE F129431 in maize grain forage and hay, representative recovery data are summarized from study RAM CA/01/00 (M-238556-01-1). These data are shown in A 3.1.1.1.1, Table A 86.

The limit of quantification (LOQ) for each single analyte is 0.02 mg/kg for maize and rice grain and 0.05 mg/kg for maize feed commodities with high water content and maize dry feed commodities and for rice straw.

Apparent residues in control samples were below 30% of the LOQ.

For validation of the method, recovery experiments were performed by fortifying samples of maize forage and hay at levels of the LOQ (0.05 mg/kg) and $10 \times$ LOQ (0.5 mg/kg) for isoxadifen-ethyl and AE F129431. In maize grain, the fortification levels for each individual analyte were 0.02 mg/kg (LOQ) and 0.2 mg/kg ($10 \times$ LOQ). Rice straw samples were fortified at levels of the LOQ (0.05 mg/kg), $10 \times$ LOQ (0.5 mg/kg) and at 2.0 mg/kg for isoxadifen-ethyl, AE F129431 and AE C637375. In rice grain, the fortification levels for each individual analyte were 0.02 mg/kg (LOQ) and 0.2 mg/kg ($10 \times$ LOQ), and an interim level of 0.1 mg/kg.

The recovery rates were corrected for apparent residue in the control samples. Standard deviations (SD) were given in the reports for all sample materials at fortification levels ranging from 0.02 to 0.50 mg/kg. Relative standard deviation (RSD) were recalculated from these reported standard deviations ($RSD = SD/mean \times 100$).

The mean recoveries for isoxadifen-ethyl at each fortification level were between 93 and 109%. The relative standard deviations (RSDs) ranged between 6 - 17% for all matrices for isoxadifen-ethyl.

The mean recoveries for isoxadifen (AE F129431) at each fortification level were between 68 and 118%. The RSDs for AE F129431 ranged between 2 - 22%.

The mean recoveries for AE C637375 (rice grain and straw only) at each fortification level ranged between 108 and 119%. The RSDs for AE C637375 were between 6 and 18%.

Some recovery means were slightly outside the range of 70 – 110%, i.e. 118% for isoxadifen in field maize forage at 0.05 mg/kg, 119% and 115% for AE C637375 in rice grain at 0.02 mg/kg and 0.20 mg/kg, respectively and 112% for AE C637375 in rice straw at 0.05 mg/kg. Relative standard deviations were below 20% for all analytes and sample materials except for isoxadifen in rice straw at 0.50 mg/kg (22%). Overall, the obtained results remain acceptable.

Table A 88: Recovery results from method validation of isoxadifen-ethyl and its metabolites AE F129431 and AE C637375 using the analytical method RAM CA/01/01, Revision 1

Crop	Fortification Level [mg/kg]	Sample size (n)	Mean [%] per fortification level*	RSD (%) **	Comments
Isoxadifen-ethyl (AE F122006) (quantitation; m/z 232)					
Rice grain	0.02	14	99	14	-
	0.10	5	98	8	-
	0.20	9	101	8	-
Rice straw	0.05	7	103	10	-
	0.50	4	94	6	-
	2.00	3	109	13	-
AE F123756 (methylated derivative of AE F129431) (quantitation; m/z 232)					
Rice grain	0.02	12	104	16	-
	0.10	3	98	2	-
	0.20	7	68	15	-
Rice straw	0.05	7	110	15	-
	0.50	4	105	22	-
	2.00	12	102	3	-
AE C637375 (quantitation; m/z 105)					
Rice grain	0.02	13	119	8	-
	0.10	5	108	6	-
	0.20	9	115	18	-
Rice straw	0.05	7	112	9	-
	0.50	4	109	16	-
	2.00	3	109	13	-

*Analytical recoveries corrected for apparent residue in control samples.

** Recalculated from the reported SD ($RSD = SD/mean \times 100$)

Table A 89: Characteristics for the analytical method RAM CA/01/00 used for validation of AE F122006, AE F129431 and AE F162241 residues in maize shoot, cob and kernel

	AE F122006	AE F129431 (determined as AE F123756)	AE C637375
Specificity	Apparent concentrations in control samples were below 30%LOQ.	Apparent concentrations in control samples were below 30%LOQ.	Apparent concentrations in control samples were below 30%LOQ.
Calibration (type, number of data points)	Representative calibration data including calibration line equation presented in the study report. $r^2 = 0.9985$ (least square regression) Number of data points: 6	Representative calibration data including calibration line equation presented in the study report. $r^2 = 0.9993$ (least square regression) Number of data points: 6	Representative calibration data including calibration line equation presented in the study report. $r^2 = 0.9976$ (least square regression) Number of data points:6
Calibration range	Accepted calibration range in concentration units: 0.005 – 0.2 µg/mL, equivalent to 0.02 - 0.63 mg/kg in maize feed commodities with high water content and maize dry feed commodities and rice straw and equivalent to 0.01 – 0.40 mg/kg in maize and rice grain	Accepted calibration range in concentration units: 0.005 – 0.2 µg/mL, equivalent to 0.02 - 0.63 mg/kg in maize feed commodities with high water content and maize dry feed commodities and rice straw and equivalent to 0.01 – 0.25 mg/kg in maize and rice grain	Accepted calibration range in concentration units: 0.005 – 0.2 µg/mL, equivalent to 0.02 - 0.63 mg/kg in maize feed commodities with high water content and maize dry feed commodities and rice straw and equivalent to 0.01 – 0.40 mg/kg in maize and rice grain
Assessment of matrix effects is presented	No	No	No
Limit of determination/quantification	Maize and rice grain: 0.02 mg/kg maize feed commodities with high water content and maize dry feed commodities and rice straw: 0.05 mg/kg	Maize and rice grain: 0.02 mg/kg maize feed commodities with high water content and maize dry feed commodities and rice straw: 0.05 mg/kg	Maize and rice grain: 0.02 mg/kg maize feed commodities with high water content and maize dry feed commodities and rice straw: 0.05 mg/kg

Conclusion

The method RAM CA/01/01 revision 1 is considered suitable to determine residues of isoxadifen-ethyl and its metabolites in maize and rice matrices with LOQs ranging from 0.02 to 0.05 mg/kg.

A 3.1.1.1.3 Analytical method AM01/08 (maize)

A 3.1.1.1.3.1 Method validation

Reference:	KCP 5.1.2.5
Title:	Validation of the analytical method AM01/08 for the determination of AE F122006 and its metabolites in maize using LC/MS/MS
Report:	Kaune, A.; 2002; C018951; M-206994-01-1
Guideline(s):	--
Deviations:	--
GLP/GEP:	yes

Reference:	KCP 5.1.2.5
Title:	Amendment no. 1 to final report no.: AM01/08 - Analytical method AM01/08 for the determination of AE F122006 and its metabolites in maize using LC/MS/MS
Report:	Freitag, T.; 2016; C018950; M-206993-02-1
Guideline(s):	European Commission, Directorate General Health and Consumer Protection Guidance document on residue analytical methods, SANCO/825/00 rev.6 20/06/00
Deviations:	none
GLP/GEP:	yes

Materials and methods

The method was developed for the determination of residues of isoxadifen-ethyl (AE F122006) and its metabolites isoxadifen (AE F129431) and 5-(4-hydroxyphenyl)-5-phenyl-2-isoxazoline-3-carboxylic acid (AE F162241) in maize. Residues are extracted by macerating the sample in mixture of acetonitrile: hydrochloric acid (8:2, v:v). The extract is centrifuged, filtered and partitioned with hexane. The hexane phase is discarded and the acetonitrile: hydrochloric acid phase is extracted with dichloromethane. The organic phase is evaporated to dryness, taken up with a mixture of acetonitrile: water (1:1, v:v), and analysed by LC/MS/MS.

Stock solutions of AE F122006 and AE F129431 were prepared in acetonitrile. Stock solutions of AE F162241 were prepared in acetonitrile: water (1:1, v:v). Working solutions were prepared from the stock solutions by further dilution with water: acetonitrile (1:1, v:v).

One MRM transition was monitored for each analyte:

AE F122006 m/z 296.2 → 232.0
AE F129431 m/z 268.2 → 204.0
AE F162241 m/z 284.2 → 251.0

For all analytes, the HPLC column was a Hypersil ODS. The mass spectrometer was operated in the positive ion mode under MRM conditions.

Residues were determined with matrix matched standards. To establish the calibration curve, matrix test solutions were injected into the LC/MS/MS system.

Results and discussions

A curve of the type $y = bx + cx^2$ is applicable over the tested range of 50 - 2000 pg, corresponding to 0.005 - 0.2 µg/mL, equivalent to 0.005 - 0.2 mg/kg in kernel; and 100 - 5000 pg, corresponding to 0.01 - 0.5 µg/mL, equivalent to 0.02 - 1.0 mg/kg in cob and shoot, of matrix matched standards. Correlation coefficients determined with solvent standards were >0.99 for AE F122006, AE F129431 and AE F162241.

Method validation data are summarised below.

The limit of quantitation (LOQ) for isoxadifen-ethyl, AE F129431 or AE F162241 was established and validated at 0.05 mg/kg in shoot and cob, and at 0.01 mg/kg in kernel, expressed as isoxadifen-ethyl.

No apparent residues of isoxadifen-ethyl, AE F129431 or AE F162241 were present above 30% of the LOQ in any of the control samples.

For validation of the method, recovery experiments were performed by fortifying control samples of maize shoot and cob at levels of the LOQ (0.05 mg/kg) and $10 \times$ LOQ (0.5 mg/kg) for isoxadifen-ethyl, AE F129431 or AE F162241. In maize kernel, the fortification levels for each individual analyte were 0.01 mg/kg (LOQ) and 0.1 mg/kg.

The mean recoveries for isoxadifen-ethyl at each fortification level were between 92 and 109%. The

relative standard deviations (RSDs) ranged between 3 - 11% for all matrices. The mean recoveries for AE F129431 at each fortification level were between 84 and 94%. The RSDs for AE F129431 were between 2 - 6%. The mean recoveries for AE F162241 at each fortification level were between 86 and 103%. The RSDs for AE F162241 were between 1 - 9%.

The recoveries in the fortified samples of the analytes are within acceptable ranges thus, the stability is considered as sufficiently proven.

Table A 90: Recovery results from method validation of isoxadifen-ethyl and its metabolites AE F129431 and AE F162241 using the analytical method AM01/08

Matrix	Fortification level (mg/kg)	N	Mean recovery (%)	RSD (%)	Comments
Isoxadifen-ethyl (quantitation; m/z 296.2 \rightarrow 232.0)					
Maize shoot	0.05	5	101	7	
	0.5	5	95	3	
Maize cob	0.05	5	109	9	
	0.5	5	92	7	
Maize kernel	0.01	5	100	11	
	0.1	5	109	11	
Isoxadifen (AE F129431) (quantitation; m/z 268.2 \rightarrow 204.0)					
Maize shoot	0.05	5	94	2	
	0.5	5	84	5	
Maize cob	0.05	5	94	4	
	0.5	5	84	3	
Maize kernel	0.01	5	92	6	
	0.1	5	88	4	
AE F162241 (quantitation; m/z 284.2 \rightarrow 251.0)					
Maize shoot	0.05	5	103	5	
	0.5	5	92	1	
Maize cob	0.05	5	94	5	
	0.5	5	89	9	
Maize kernel	0.01	5	86	7	
	0.1	5	91	2	

The calculation was done with a calibration function of peak areas.
Residues of AE F129431 and AE F162241 are calculated as isoxadifen-ethyl

Table A 91: Characteristics for the analytical method AM01/08 used for validation of AE F122006, AE F129431 and AE F162241 residues in maize shoot, cob and kernel

	AE F122006	AE F129431	AE F162241
Specificity	Blank value <30% of LOQ)	Blank value <30% of LOQ)	Blank value <30% of LOQ)
Calibration (type, number of data points)	Representative calibration data including calibration line equation presented Regression equation: $y = bx + cx^2$ Number of data points: 6 $R^2 = 0.9998$	Representative calibration data including calibration line equation presented Regression equation: $y = bx + cx^2$ Number of data points: 6 $R^2 = 0.9981$	Representative calibration data including calibration line equation presented Regression equation: $y = bx + cx^2$ Number of data points: 6 $R^2 = 0.9972$
Calibration range	Accepted calibration range in concentration units: Kernel: 50 - 2000 pg, corresponding to 0.005 – 0.2 µg/mL, equivalent to 0.005 - 0.2 mg/kg Cob and shoot: 100 - 5000 pg, corresponding to 0.01 - 0.5 µg/mL, equivalent to 0.02 - 1.0 mg/kg	Accepted calibration range in concentration units: Kernel: 50 - 2000 pg, corresponding to 0.005 – 0.2 µg/mL, equivalent to 0.005 - 0.2 mg/kg Cob and shoot: 100 - 5000 pg, corresponding to 0.01 - 0.5 µg/mL, equivalent to 0.02 - 1.0 mg/kg	Accepted calibration range in concentration units: Kernel: 50 - 2000 pg, corresponding to 0.005 – 0.2 µg/mL, equivalent to 0.005 - 0.2 mg/kg Cob and shoot: 100 - 5000 pg, corresponding to 0.01 - 0.5 µg/mL, equivalent to 0.02 - 1.0 mg/kg
Assessment of matrix effects is presented	Yes, matrix-matched standards were used	Yes, matrix-matched standards were used	Yes, matrix-matched standards were used
Limit of determination/quantification	Maize kernel: 0.01 mg/kg Maize cob and shoot: 0.05 mg/kg	Maize kernel: 0.01 mg/kg Maize cob and shoot: 0.05 mg/kg	Maize kernel: 0.01 mg/kg Maize cob and shoot: 0.05 mg/kg

Conclusion

The method AM01/08 was sufficiently validated for the determination of residues of isoxadifen-ethyl-derived residues in maize with a limit of quantification of 0.05 mg/kg in shoot and cob, and 0.01 mg/kg in kernel, for each analyte, expressed as isoxadifen-ethyl.

A 3.1.1.2 Description of analytical methods for the determination of residues in animal matrices (KCP 5.1)

No new or additional studies have been submitted.

A 3.1.1.3 Description of analytical methods for the determination of residues in support to environmental fate studies (KCP 5.1)

No new or additional studies have been submitted.

A 3.1.1.4 Description of analytical methods for the determination of residues in support to toxicological studies (KCP 5.1)

No new or additional studies have been submitted.

A 3.1.1.5 Description of analytical methods for the determination of residues in support of operator, worker, resident and bystander exposure studies (KCP 5.1)

No new or additional studies have been submitted.

A 3.1.1.6 Description of analytical methods for the determination of residues in of ecotoxicology studies (KCP 5.1)

No new or additional studies have been submitted.

A 3.1.1.7 Description of analytical methods for the determination of residues in support of physical and chemical properties tests (KCP 5.1)

Analytical methods used for the generation of pre-authorization data are the same as the ones described in part B section 5.

A 3.1.2 Methods for post-authorization control and monitoring purposes (KCP 5.2)

A 3.1.2.1 Description of analytical methods for the determination of residues in plant matrices (KCP 5.2)

A 3.1.2.1.1 Analytical method 01300/M029

A 3.1.2.1.1.1 Method validation

Reference:	KCP 5.2.1
Title:	Modification M029 of the analytical method 01300 (based on QuEChERS) for the determination of residues of isoxadifen-ethyl and its metabolites in different matrices of plant origin
Report:	Winter, O.; Amann, S.; 2016; 01300/M029; M-573745-01-1
Guideline(s):	Regulation (EC) No 1107/2009 of the European Parliament and the Council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC • European Commission Guidance Document for Generating and Reporting Methods of Analysis in Support of Pre-Registration data Requirements for Annex II (part A, Section 4) and Annex III (part A, section 5) of directive 91/414, SANCO/3029/99 rev. 4, 11/07/00 • Guidance document SANCO/825/00 rev. 8.1 of 16-Nov-2010, European Commission • US Environmental Protection Agency, Residue Chemistry Test Guidelines, OCSPP 860.1340, Residue Analytical Method, EPA 712-C-96-174, August 1996 • OECD Guidance document on pesticide residue analytical methods, ENV/JM/MONO (2007), 17 2007-08-1
Deviations:	not specified
GLP/GEP:	yes

Materials and methods

The analytical method 01300/M029 based on QuEChERS was validated for the determination of residues of isoxadifen-ethyl and its metabolites isoxadifen and AE C637375 in cucumber (fruit), orange (whole fruit), dry peas, wheat (grain) and oilseed rape (seed).

The analytes were extracted from the matrix with acetonitrile. Water was added to the samples prior to extraction. After the samples were shaken for about 15 min, magnesium sulphate, sodium chloride and sodium citrate were added to the extracts which were then centrifuged. Afterwards an aliquot was diluted with water for measurement by reversed phase HPLC-MS/MS in positive ion mode. For the analyses of isoxadifen-ethyl and isoxadifen in orange (whole fruit), samples and matrix matched standard solutions were further diluted 1:5 with acetonitrile/water (1/1, v/v).

Two MRM transitions were monitored for each analyte and each matrix tested:

Analyte	Quantitation ion [#]	Confirmation ion	Polarity/ionisation mode
Isoxadifen-ethyl	m/z 296 → 204	m/z 296 → 232	positive
Isoxadifen	m/z 268 → 235	m/z 268 → 207	positive
AE C637375	m/z 241 → 165	m/z 241 → 206	positive

[#] proposed for quantification but both of the ion mass transitions listed can be used for quantification

The HPLC-MS/MS method is highly specific and an additional confirmatory method is not necessary.

Results and discussions

Isoxadifen-ethyl and its metabolites isoxadifen and AE C637375 were stable at 1°C to 10°C in the dark for at least seven days in final extracts of cucumber (fruit), eight days in dry peas, ten days in oilseed rape (seed), eleven days in orange (whole fruit) (isoxadifen-ethyl and isoxadifen), twelve days in wheat (grain) and fourteen days in orange (whole fruit) (AE C637375). Recovery experiments were conducted at the targeted LOQ (0.01 mg/kg) and at 10 × LOQ (0.1 mg/kg). Five replicates per fortification level were analysed. The mean recoveries (for both fortification levels and the two MRM transitions) ranged between 70 and 110 with the RSDs of 1.4 – 5.8% for isoxadifen-ethyl and its metabolites isoxadifen and AE C637375 for all tested matrices. All results are summarised in the tables below.

Table A 92: Recovery results from method validation of isoxadifen-ethyl, isoxadifen and AE C637375 using the analytical method 01300/M029

Matrix	Fortification level (mg/kg)	n	Mean recovery (%)	RSD (%)	Comments
Isoxadifen-ethyl (quantitation m/z 296 → 204)					
Cucumber (fruit)	0.01	5	105	2.9	
	0.10	5	97	2.1	
Orange (whole fruit)	0.01	5	110	1.7	
	0.10	5	110	2.4	
Dry peas	0.01	5	104	1.5	
	0.10	5	101	3.2	
Wheat (grain)	0.01	5	92	5.8	
	0.10	5	93	3.7	
Oilseed rape (seed)	0.01	5	76	3.4	
	0.10	5	79	1.6	
Isoxadifen (quantitation m/z 268 → 235)*					
Cucumber (fruit)	0.01	5	77	1.7	
	0.10	5	89	2.7	
Orange (whole fruit)	0.01	5	84	3.4	
	0.10	5	100	2.3	
Dry peas	0.01	5	70	3.4	
	0.10	5	77	3.0	
Wheat (grain)	0.01	5	79	4.1	
	0.10	5	85	3.1	
Oilseed rape (seed)	0.01	5	70	5.5	
	0.10	5	79	1.7	

Matrix	Fortification level (mg/kg)	n	Mean recovery (%)	RSD (%)	Comments
AE C637375 (quantitation m/z 241 → 165)*					
Cucumber (fruit)	0.01	5	106	1.7	
	0.10	5	99	3.7	
Orange (whole fruit)	0.01	5	88	1.4	
	0.10	5	109	3.0	
Dry peas	0.01	5	101	2.7	
	0.10	5	103	3.2	
Wheat (grain)	0.01	5	94	4.1	
	0.10	5	93	4.1	
Oilseed rape (seed)	0.01	5	99	5.4	
	0.10	5	99	1.9	
Isoxadifen-ethyl (confirmation m/z 296 → 232)					
Cucumber (fruit)	0.01	5	105	3.9	
	0.10	5	98	2.2	
Orange (whole fruit)	0.01	5	109	1.4	
	0.10	5	110	2.3	
Dry peas	0.01	5	103	1.9	
	0.10	5	101	2.8	
Wheat (grain)	0.01	5	90	5.6	
	0.10	5	93	3.9	
Oilseed rape (seed)	0.01	5	78	3.5	
	0.10	5	79	1.7	
Isoxadifen (confirmation m/z 268 → 207)*					
Cucumber (fruit)	0.01	5	78	1.7	
	0.10	5	89	2.6	
Orange (whole fruit)	0.01	5	84	3.1	
	0.10	5	99	2.1	
Dry peas	0.01	5	70	3.9	
	0.10	5	76	3.4	
Wheat (grain)	0.01	5	79	4.4	
	0.10	5	85	4.1	
Oilseed rape (seed)	0.01	5	70	4.5	
	0.10	5	77	2.7	

Matrix	Fortification level (mg/kg)	n	Mean recovery (%)	RSD (%)	Comments
AE C637375 (confirmation m/z 241 → 206) *					
Cucumber (fruit)	0.01	5	101	3.2	
	0.10	5	98	3.3	
Orange (whole fruit)	0.01	5	99	5.3	
	0.10	5	108	3.2	
Dry peas	0.01	5	103	5.0	
	0.10	5	102	3.8	
Wheat (grain)	0.01	5	97	3.5	
	0.10	5	94	4.6	
Oilseed rape (seed)	0.01	5	105	8.1	
	0.10	5	99	1.6	

*Fortification level is expressed as parent equivalent
Fortification as: Isoxadifen or AE C637375

Determination as: Isoxadifen or AE C637375
Calculated as: Isoxadifen-ethyl

Table A 93: Characteristics for the analytical method used for validation of isoxadifen-ethyl and isoxadifen residues in cucumber (fruit), orange (whole fruit), dry peas, wheat (grain), oilseed rape (seed)

	Isoxadifen-ethyl	Isoxadifen	AE C637375
Specificity	Representative mass spectrum is provided blank value <30% of the LOQ	Representative mass spectrum is provided blank value <30% of the LOQ	Representative mass spectrum is provided blank value <30% of the LOQ
Calibration (type, number of data points)	individual calibration data presented: linear regression $y = a + b \cdot x$ with 1/x weighting, coefficient of determination (R ²) >0.996 for all tested matrices, number of data points: 7	individual calibration data presented: linear regression $y = a + b \cdot x$ with 1/x weighting, coefficient of determination (R ²) >0.999 for all tested matrices, number of data points: 7	individual calibration data presented: linear regression $y = a + b \cdot x$ with 1/x weighting, coefficient of determination (R ²) >0.993 for all tested matrices, number of data points: 7
Calibration range*	Corresponding calibration range in mass ratio units for the sample: 0.75 ng/mL to 50 ng/mL (corresponding to 0.003 mg/kg - 0.20 mg/kg) and 0.15 ng/mL to 10 ng/mL (corresponding to 0.003 mg/kg - 0.20 mg/kg) in orange only.	Corresponding calibration range in mass ratio units for the sample: 0.75 ng/mL to 50 ng/mL (corresponding to 0.003 mg/kg - 0.20 mg/kg) and 0.15 ng/mL to 10 ng/mL (corresponding to 0.003 mg/kg - 0.20 mg/kg) in orange only.	Corresponding calibration range in mass ratio units for the sample: 0.75 ng/mL to 50 ng/mL (corresponding to 0.003 mg/kg - 0.20 mg/kg).
Assessment of matrix effects is presented	Yes, matrix-matched standards were used	Yes, matrix-matched standards were used	Yes, matrix-matched standards were used
Limit of determination/quantification	0.01 mg/kg	0.01 mg/kg	0.01 mg/kg

(*) expressed as parent equivalent

Conclusion

The method meets all guideline criteria to determine residues of isoxadifen-ethyl and its metabolites isoxadifen and AE C637375 with a LOQ of 0.01 mg/kg (expressed as parent equivalent) in cucumber (fruit), orange (whole fruit), dry peas, wheat (grain) and oilseed rape (seed).

A 3.1.2.1.2 Independent laboratory validation: analytical method 01300/M029

A 3.1.2.1.2.1 Method validation

Reference:	KCP 5.2.1
Title:	Independent laboratory validation of modification M029 of the analytical method 01300 (based on QuEChERS) for the determination of residues of isoxadifen-ethyl and its metabolites in different matrices of plant origin
Report:	Meseguer, C.; 2017; S16-04195; M-590984-01-1
Guideline(s):	Regulation (EC) No 1107/2009 of the European Parliament and the Council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC European Commission Guidance Document for Generating and Reporting Methods of Analysis in Support of Pre-Registration data Requirements for Annex II (part A, Section 4) and Annex III (part A, section 5) of directive 91/414, SANCO/3029/99 rev. 4, 11/07/00 Guidance document on residue analytical methods, SANCO/825/00/rev. 8.1, European Commission, Directorate General Health and Consumer Protection 16/11/2010 US Environmental Protection Agency, Residue Chemistry Test Guidelines, OCSPP 850.6100. OECD Principles on Good Laboratory Practice (OECD ENV/MC/CHEM (98)17)
Deviations:	none
GLP/GEP:	yes

Materials and methods

The analytical method 01300/M029 based on the multi-residue method QuEChERS was independently validated for the determination of residues of isoxadifen-ethyl and its metabolites isoxadifen and AE C637375 in cucumber (fruit), maize (grain) and oilseed rape (seed).

The analytes were extracted from the matrix with acetonitrile. Water was added to the samples prior to extraction. After the samples were shaken for about 15 min, magnesium sulphate, sodium chloride and sodium citrate were added to the extracts which were then centrifuged. Afterwards an aliquot was diluted with water for measurement by reversed phase HPLC-MS/MS in positive ion mode.

Matrix-matched standards were used for determination.

Results and discussions

The stability was determined in within the original validation study (A 3.1.2.1.1). Recovery experiments were conducted at the targeted LOQ (0.01 mg/kg) and at 10 × LOQ (0.1 mg/kg). Five replicates per fortification level were analysed. The mean recoveries (for both fortification levels and the two MRM transitions) ranged between 70 and 110% with the RSDs of 0.9 – 7.6% for isoxadifen-ethyl and its metabolites isoxadifen and AE C637375 for all tested matrices. All results are summarised in the tables below.

Table A 94: Recovery results from method validation of isoxadifen-ethyl, isoxadifen and AE C637375 using the analytical method 01300/M029

Matrix	Fortification level (mg/kg) (n = x)	n	Mean recovery (%)	RSD (%)	Comments
Isoxadifen-ethyl (quantitation m/z 296 → 204)					
Cucumber (fruit)	0.01	5	89	3.2	
	0.10	5	94	1.2	
Maize (grain)	0.01	5	84	2.3	

Matrix	Fortification level (mg/kg) (n = x)	n	Mean recovery (%)	RSD (%)	Comments
	0.10	5	82	1.5	
Oilseed rape (seed)	0.01	5	74	6.2	
	0.10	5	76	1.4	
Isoxadifen (quantitation m/z 268 → 235)*					
Cucumber (fruit)	0.01	5	91	5.3	
	0.10	5	93	2.2	
Maize (grain)	0.01	5	88	2.3	
	0.10	5	89	2.1	
Oilseed rape (seed)	0.01	5	77	2.4	
	0.10	5	75	1.6	
AE C637375 (quantitation m/z 241 → 206)*					
Cucumber (fruit)	0.01	5	88	6.4	
	0.10	5	92	2.9	
Maize (grain)	0.01	5	96	7.6	
	0.10	5	100	3.2	
Oilseed rape (seed)	0.01	5	87	6.7	
	0.10	5	83	2.6	
Isoxadifen-ethyl (confirmation m/z 296-232)					
Cucumber (fruit)	0.01	5	89	3.2	
	0.10	5	94	0.9	
Maize (grain)	0.01	5	89	1.9	
	0.10	5	85	1.5	
Oilseed rape (seed)	0.01	5	75	5.4	
	0.10	5	78	1.5	
Isoxadifen (confirmation m/z 268 → 207)*					
Cucumber (fruit)	0.01	5	98	5.9	
	0.10	5	92	2.6	
Maize (grain)	0.01	5	89	2.9	
	0.10	5	89	1.0	
Oilseed rape (seed)	0.01	5	76	3.4	
	0.10	5	76	1.2	
AE C637375 (confirmation m/z 241 → 165) *					
Cucumber (fruit)	0.01	5	97	2.6	
	0.10	5	101	1.6	
Maize (grain)	0.01	5	100	3.3	
	0.10	5	102	2.3	
Oilseed rape (seed)	0.01	5	91	3.8	
	0.10	5	97	2.1	

*Fortification level is expressed as parent equivalent
Fortification as: Isoxadifen or AE C637375

Determination as: Isoxadifen or AE C637375
Calculated as: Isoxadifen-ethyl

Table A 95: Characteristics for the analytical method used for validation of isoxadifen-ethyl and isoxadifen residues in cucumber (fruit), orange (whole fruit), dry peas, wheat (grain), oilseed rape (seed)

	Isoxadifen-ethyl	Isoxadifen	AE C637375
Specificity	Representative mass spectrum is provided blank value <30% of the LOQ	Representative mass spectrum is provided blank value <30% of the LOQ	Representative mass spectrum is provided blank value <30% of the LOQ
Calibration (type, number of data points)	individual calibration data presented: 1/x weighted linear regression, coefficient of determination (R ²) >0.996 for all tested matrices, number of data points: 7	individual calibration data presented: 1/x weighted linear regression, coefficient of determination (R ²) >0.993 for all tested matrices, number of data points: 7	individual calibration data presented: 1/x weighted linear regression, coefficient of determination (R ²) >0.994 for all tested matrices, number of data points: 7
Calibration range*	Corresponding calibration range in mass ratio units for the sample: 0.75 ng/mL to 50 ng/mL (corresponding to 0.003 mg/kg - 0.20 mg/kg).	Corresponding calibration range in mass ratio units for the sample: 0.75 ng/mL to 50 ng/mL (corresponding to 0.003 mg/kg - 0.20 mg/kg).	Corresponding calibration range in mass ratio units for the sample: 0.75 ng/mL to 50 ng/mL (corresponding to 0.003 mg/kg - 0.20 mg/kg).
Assessment of matrix effects is presented	Yes, matrix-matched standards were used	Yes, matrix-matched standards were used	Yes, matrix-matched standards were used
Limit of determination/quantification	0.01 mg/kg	0.01 mg/kg	0.01 mg/kg

(*) expressed as parent equivalent

Conclusion

The method was successfully independently validated for the determination of isoxadifen-ethyl and its metabolites isoxadifen and AEC637375 in cucumber (fruit), maize (grain) and oilseed rape (seed) at the tested LOQ of 0.01 mg/kg according to the guidance documents SANCO/825/00 rev. 8.1, SANCO/3029/99 rev. 4 and EPA OCSP 850.6100.

A 3.1.2.1.2.2 Confirmatory method (if required)

No new or additional studies have been submitted.

A 3.1.2.1.2.3 Extraction efficiency

A study on extraction efficiency (R. Bongartz, 2006, M-274486-01-2 (MEF-05/504)) was already peer reviewed (DAR, RMS UK, April 2012), even though residues are not expected to be \geq LOQ in cereal grain and sugar beet roots and therefore extraction efficiency is actually not required according to SANCO/825/00 rev. 8.1.

A 3.1.2.2 Description of analytical methods for the determination of residues in animal matrices (KCP 5.2)

No new or additional studies have been submitted.

A 3.1.2.2.1.1 Independent laboratory validation

No new or additional studies have been submitted.

A 3.1.2.2.1.2 Confirmatory method (if required)

No new or additional studies have been submitted.

A 3.1.2.2.1.3 Extraction efficiency

No new or additional studies have been submitted.

A 3.1.2.2.2 Extraction efficiency

No new or additional studies have been submitted.

A 3.1.2.3 Description of Methods for the Analysis of Body Fluids and Tissues (KCP 5.2)

No new or additional studies have been submitted.

A 3.1.2.4 Description of Methods for the Analysis of Soil (KCP 5.2)

A 3.1.2.4.1 Analytical method CA/02/99

A 3.1.2.4.1.1 Method validation

Report:	KCP 5.2.4 Cole, M. G.; Neal, J. L.; Dacus, S. C.; 2001; M-185178-02-1
Title:	An Analytical Method for the Determination of Residues of Isoxadifen ethyl (AE F122006) and its Major Metabolite AE F129431 in Soil by Gas Chromatography Using Nitrogen-Phosphorous or Ion Trap Mass Selective Detection, Revision 1
Report No.:	B003389
Document No.:	M-185178-02-1
Guideline(s):	not specified
Guideline deviation(s):	not specified
GLP/GEP:	no

Materials and methods

This method is suitable for the determination of the total extractable residues of AE F122006 (CA1) and its major metabolite AE F129431 (CA2) in soils. The soil used for this study was classed as a silt loam.

Soil samples are extracted twice with 70:30 acetonitrile:0.1 M ammonium acetate adjusted to pH =5 with acetic acid followed once by 100% acetonitrile. The acetonitrile is then removed from the extracts by rotary evaporation. The analytes AE F122006 (CA1) and AE F129431 (CA2) are separated from the aqueous phase by selective partitioning with organic solvents. The aqueous phase at pH=5 is partitioned with hexane (Fraction A) to remove the CA1 analyte. After the pH of the aqueous phase is lowered to pH <1 with the addition of 5% H₂SO₄, the aqueous phase is partitioned again with dichloromethane (Fraction B) to remove the CA2 analyte.

Fractions A & B are both concentrated by rotary evaporation. The residue from fraction A containing AE F122006 (CA1) is re-dissolved in 20% ethyl acetate in toluene to await amino solid phase extraction (SPE) cleanup. The residue containing AE F129431 (CA2) is re-dissolved in acetone and methylated by heating at 50°C in the presence of iodomethane and tetrabutylammonium hydroxide for 1 hour to convert CA2 into the methylated analytical target AE F123756 (CA4). After derivatization the methylated extracts are decanted into 0.1 M ammonium acetate adjusted to pH =5. The analytical target CA4 is removed from the aqueous phase by partitioning with dichloromethane.

Fraction B containing AE F123756 (CA4) is concentrated by rotary evaporation, and redissolved in a known volume of 20% ethyl acetate in toluene. Extracts from both fractions A & B are then transferred to an amino propyl SPE for cleanup and eluted with additional volumes of 20% ethyl acetate in toluene. The organic extracts are rotary evaporated to dryness and diluted into toluene to await analysis by gas chromatography using nitrogen phosphorous detection. Confirmatory identification may be conducted by capillary gas chromatography with ion-trap mass selective detection.

Results and discussions

In fortification experiments untreated control samples were analysed using the same analytical method described to verify that any co-extracted substances present in the samples do not interfere with the final determination of the analytes of interest.

A conversion factor must be applied to the final residue value for AE F123756 (CA4) in order to express the determined residue concentration as AE F129431 (CA2). This factor is not needed for calculations involving AE F1 22006 (CA1). This molecular weight conversion factor is the ratio of the molecular weights of AE F1 29431 (CA2) over AE F1 23756 (CA4). This factor calculates as 267.29 + 281.31. The mean recovery of AE F1 22006 (CA1) and AE F1 29431 (CA2) was 90% on soil. The mean recovery values for both analytes were between 70% and 120% with a standard deviation of less than ± 20%.

Table A 96: Recovery Data for AE F122006 (CA1) and AE F129431 (CA2) in Soil

Sample Matrix	Fortification Level (ppm)	Analytical Recovery* (%) AE F122006 (CA1)	Analytical Recovery* (%) AE F129431 (CA2)
Soil	0.002	85 93 84 92	85 102 78 132
	0.100	89 90 94 96	94 80 85 74
	0.200	80 89 98 85	95 84 88 84
Number		12	12
Mean (%)		90	90
Std. Dev.		±5	±15

* Analytical recoveries corrected for apparent residue in control samples.

Table A 97: Characteristics of the analytical method for the determination of residues of AE F130619 in water

	AE F122006 (CA1)	AE F123756 (CA4)
Specificity	The method is selective for the analytes of interest due to the selective detector. The selectivity of the method is confirmed by the GC-column separation in combination with MS detection. Representative mass spectra provided. No signals/peaks interfering with the detection of the analytes were observed in solutions of untreated control specimens.	
Calibration	individual calibration data presented: Linear regression equation: $y = 48963.10 x - 85.1957$ $r = 0.9999$	individual calibration data presented: Linear regression equation: $y = 40207 x - 90.9735$ $r = 0.9999$
Calibration range	The correlation between the injected amount of substance and the detector response was linear for solvent solutions ranging from 0.010 µg/g to 0.20 µg/g.	
Assessment of matrix effects is presented	Control chromatograms of representative soil extracts show no interferences. The MS detection is not affected by the matrix.	
Limit of determination/quantification	0.002 ppm (µg/kg)	

Conclusion

The analytical method complies with all criteria according to SANCO/825/00 rev. 8.1 with the exception of the recovery data. Recoveries should be obtained at the LOQ as well as 10 fold of LOQ. Here the fortification levels are higher by two orders of magnitude. Nevertheless, this can be regarded as acceptable as an additional fortification level is presented. Thus, this method can be regarded as fit for purpose showing good accuracy and precision data of available data.

A 3.1.2.5 Description of Methods for the Analysis of Water (KCP 5.2)

No new or additional studies have been submitted.

A 3.1.2.6 Description of Methods for the Analysis of Air (KCP 5.2)

A 3.1.2.6.1 Independent validation of analytical method EM C01/99-0

Reference:	KCP 5.2.6
Title:	Isoxadifen-ethyl: Analytical method for the determination of isoxadifen-ethyl in air (validation)
Report:	Bacher, R.; 2003; C029624; M-217537-01-1
Guideline(s):	--
Deviations:	--
GLP/GEP:	yes

The objective of this study was to additionally validate the analytical method EM CO 1/99-0 for the determination of isoxadifen-ethyl in air with a target limit of quantification (LOQ) of 0.6 µg/m3. The

analytical method used was originally developed and validated for ambient and warm, humid air (Everitt, S. L.; 1999; M-185277-01-1).

Materials and methods

Air sampling uses adsorption tubes filled with two portions of XAD-2 porous polymer. Particles and aerosols are trapped by filtration or impact onto the adsorbent material. After sampling of air (6 hours at about 1.5 L/min), the front adsorbent portion is extracted three times with approx. 2 mL of acetone. The extracts are combined and the extract evaporated to dryness. The final volume is adjusted to 2.0 mL using toluene.

Determination is performed by gas chromatography with mass spectrometric specific detection (GC/MS). The method achieves a limit of quantification (LOQ) of 0.6 µg/m³ isoxadifen-ethyl in air. The analytical method was validated for the determination of isoxadifen-ethyl in warm, humid air (36°C, 100%).

The final extracts were analysed by gas chromatography on a capillary column with a nonpolar J & W Scientific DB-5 ms stationary phase using mass spectrometric detection (GC/MS). The quantitative determination was carried out by external standardization. The intense fragment ion at 204 m/z was used for quantitative GC/MS determination of isoxadifen-ethyl residues. Additional characteristic ions at e.g. 165 and 294 m/z were appropriate for confirmatory purposes.

Results and discussions

In deviation to the original method the following modifications in the procedure of the original method EM CO 1/99-0 were used:

- Specimen extraction with 3x2 mL of acetone, transfer of final extract into 2.0 mL of toluene.
- Quantification using external calibration and a linear calibration curve
- No marker (benazolin methyl) was used for quantification.

Extraction efficiency was examined by fortifying the analyte (at least duplicates at 0.30 µg and at 3.0 µg) onto adsorbent portion A (100 mg adsorption material) of the sampling cartridge. Thereafter both adsorbent portions (adsorbent portion A and back-up sampling portion B, 50 mg adsorption material) were transferred together with the glass wool plugs into the extraction vials. The analyte was extracted, evaporated to dryness and redissolved in toluene.

Sampling cartridges were fortified with 0.30 µg or 3.0 µg of isoxadifen-ethyl and analysed according to the extraction procedure without previous air sampling. The total average recovery was 91 ± 10% with individual recoveries ranging from 80 to 99%. These results demonstrate that the analytical procedure allows satisfactory extraction of the analyte from the adsorption material.

For recovery and breakthrough after sampling (retention efficiency), adsorption tubes (portion A) were fortified with isoxadifen-ethyl at fortification levels at LOQ (0.30 µg) and at 10xLOQ (3.0 µg). Thereafter the sampling of air was performed for 6 hours with warm, humid air (36°C, 100% relative humidity). Five replicates were performed at each fortification level.

After sampling (6 hours) the trap portions A were analysed for recovery. In selected trials at the higher fortification level the portion B was analysed for breakthrough determination.

Retention efficiency was demonstrated by the following results:

- The mean recovery after air sampling amounted to 102% at LOQ and 83% at 10-fold LOQ, with relative standard deviations of <4%. The overall mean recovery amounted to 93%, with an overall relative standard deviation of 11%.
- No breakthrough was observed in the back portion of the adsorption tubes (<1% of the fortified amount).

The results of the method validation are presented in the table below:

Table A 98: Recovery, breakthrough and extraction efficiency of the method

Specimen Type	Fortified µg	Average C _{air} µg/m ³	Average Recovery (%)	RSD (%)	N
Extraction efficiency	0.30	-	99	—	2
	3.0	-	83	—	2
Warm, humid air 36 °C, 100% rel. humidity	0.30	0.57	102	4	5
	3.0	5.5	83	4	5
		Overall	93	11	10

RSD: relative standard deviation.
N: Number of specimens included in calculation.
Average C_{air}: Average concentration of isoxadifen-ethyl in air.

Table A 99: Characteristics of the analytical method for the determination of residues of isoxadifen-ethyl in air

	Isoxadifen-ethyl
Specificity	Quantification is performed by GC/MS using the signal for the fragment ion at 204 m/z. The molecular ion signal at 294 m/z and the fragment ion signal at 165 m/z are used for confirmatory purposes. Applying three ions >100 m/z to the detection of isoxadifen-ethyl residues a high level of specificity is ensured. Thus no additional confirmatory method is required.
Calibration	The function is linear. Calibration equation: $y = 277 \cdot x$; $R^2 = 0.9961$ With: y = Peak area ion 204 m/z in counts; x : Concentration in ng/mL; R^2 : Regression coefficient
Calibration range	15 to 3000 ng/mL 8 concentrations tested.
Assessment of matrix effects is presented	The chromatograms of the control specimens showed no interfering signal at the retention time of isoxadifen-ethyl.
Limit of determination/quantification	0.6 µg/m ³

According to SANCO 825/00 rev. 8.1 the LOQ should comply with the concentration c calculated from the AOEL_{systemic} (in [mg/kg bw d]). The equation according SANCO 825/00 was used:

$c = \text{AOEL}_{\text{systemic}} \cdot (\text{safety factor} \cdot \text{body weight}) / \text{air intake}$ with a safety factor of 0.1; body weight of 60 [kg]; air intake of 20 [m³/day] is

$c = \text{AOEL}_{\text{systemic}} \cdot 300 [\mu\text{g}/\text{m}^3]$ with a AOEL_{systemic} of 0.2 mg/kg/day; $c = 60 \mu\text{g}/\text{m}^3$

Conclusion

The analytical method EM CO 1/99-0 was successfully validated for the determination of isoxadifen-ethyl in air with a limit of quantification of 0.6 µg/m³. All requirements of SANCO 825/00 rev.8.1 are fulfilled.