

FINAL REGISTRATION REPORT

Part B

Section 5

Analytical Methods

Detailed summary of the risk assessment

Product code: CHR/H/IZOXACYP 250 SC

Product name(s):

Metida Plus 250 SC

Taizza Plus 250 SC

Chemical active substance:

Isoxaflutole, 250 g/L

Central Zone

Zonal Rapporteur Member State: Poland

CORE ASSESSMENT

(authorization)

Applicant: Innvigo Sp. z o. o.

Submission date: April 2022, August 2025, September 2025

Finalisation date: December 2022; October 2025; February 2026

Version history

| When | What |
|---------|---|
| 12/2022 | ZRMs Assessment |
| 08/2025 | Applicant update |
| 09/2025 | Applicant update |
| 10/2025 | Assessment |
| 02/2026 | The final Registration Report after the reporting period. |

Table of Contents

| | | |
|-------------------|--|-----------|
| 5 | Analytical methods..... | 4 |
| 5.1 | Conclusion and summary of assessment..... | 4 |
| 5.2 | Methods used for the generation of pre-authorization data (KCP 5.1)..... | 4 |
| 5.3 | Methods for post-authorization control and monitoring purposes (KCP 5.2) | 4 |
| 5.3.1.1 | Description of methods for the analysis of water (KCP 5.2)..... | 4 |
| 5.3.1.2 | Description of methods for the analysis of air (KCP 5.2)..... | 6 |
| 5.3.1.3 | Description of methods for the analysis of body fluids and tissues (KCP 5.2) | 6 |
| Appendix 1 | Lists of data considered in support of the evaluation..... | 8 |
| Appendix 2 | Detailed evaluation of submitted analytical methods | 14 |
| A 2.1 | Analytical methods for isoxaflutole..... | 14 |
| A 2.1.1 | Methods used for the generation of pre-authorization data (KCP 5.1)..... | 14 |
| A 2.1.2 | Methods for post-authorization control and monitoring purposes (KCP 5.2) | 14 |

5 Analytical methods

New and additional information about determination of isoxaflutole and metabolites in drinking water and body fluids, are highlighted in green

New and additional information added in 09/2025 are highlighted in yellow

Data matching studies for isoxaflutole have been evaluated by RMS and as a result of the assessment all reports were accepted and considered as equivalent to protected studies. Therefore, to support the authorization of CHR/H/IZOXACYP 250 SC (Taizza Plus 250 SC, Metida Plus 250 SC) INNVIGO is allowed to refer to EU approved reports.

5.1 Conclusion and summary of assessment

Sufficiently sensitive and selective analytical methods are available for the active substance(s) and relevant impurities in the plant protection product.

Noticed data gaps:

- none

Method Validation for Determination of Isoxaflutole and Metabolites in Water, Independent Laboratory Validation for Determination of Isoxaflutole and Metabolites in Drinking Water and Method Validation for Determination of Isoxaflutole and its Metabolite RPA 202248 in Body Fluids and Tissues were provided. Methods are accepted. The data gap has been filled.

| Commodity/crop | Supported/ Not supported |
|----------------|-----------------------------|
| Maize | Supported |

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

Please refer to Core Assessment.

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

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

An overview on the acceptable methods and possible data gaps for analysis of isoxaflutole in surface and drinking water is given in the following tables. No new studies are necessary.

An overview on the acceptable methods and possible data gaps for analysis of active substance in surface and drinking water is given in the following tables. For the detailed valuation of new/ additional studies it is referred to Appendix 2.

Table 5.3-1: Validated methods for water (if appropriate)

| Component of residue definition: isoxaflutole | | | | |
|---|-----------------------------|--------------------|---|---|
| Matrix type | Method type | Method LOQ | Principle of method (i.e. GC-MS or HPLC-UV) | Author(s), year / missing |
| Drinking water | Primary | LOQ = 2 µg/L | HPLC-UV | Le Gren, 1995a ref. 4.2.3/01 DAR Isoxaflutole (1997) – Volume 3 Annex B.4 – Analytical methods |
| | ILV | N/A LOQ=0.050 µg/L | LC-MS/MS | Ivanov E., 2025 |
| | Confirmatory | LOQ = 2 µg/L | HPLC-UV | Le Gren, 1995b ref. 4.2.3/02 DAR Isoxaflutole (1997) – Volume 3 Annex B.4 – Analytical methods |
| Surface water | Not preformed, not required | | | |

For any special comments or remarkable points concerning the analytical methods for water please refer to Appendix 2.

Table 5.3-2a Validated methods for water (if appropriate)

| Component of residue definition: Sum of isoxaflutole and RPA 202248, expressed as isoxaflutole | | | | |
|--|-----------------------------|----------------|---|---------------------------|
| Matrix type | Method type | Method LOQ | Principle of method (i.e. GC-MS or HPLC-UV) | Author(s), year / missing |
| Drinking water | Primary | LOQ= 0.05 µg/L | LC-MS/MS | Asekunowo J., 2025 |
| | ILV | LOQ= 0.05 µg/L | LC-MS/MS | Ivanov E., 2025 |
| Surface water | Not preformed, not required | | | |

For any special comments or remarkable points concerning the analytical methods for water please refer to Appendix 2.

Table 5.3-3b Validated methods for water (if appropriate)

| Component of residue definition: RPA 205834, RPA 203328 | | | | |
|---|-------------|---------------|---|---------------------------|
| Matrix type | Method type | Method LOQ | Principle of method (i.e. GC-MS or HPLC-UV) | Author(s), year / missing |
| Drinking water | Primary | LOQ= 0.1 µg/L | LC-MS/MS | Asekunowo J., 2025 |
| | ILV | LOQ= 0.1 µg/L | LC-MS/MS | Ivanov E., 2025 |

| Component of residue definition: RPA 205834, RPA 203328 | | | | |
|---|-----------------------------|------------|--|---------------------------|
| Matrix type | Method type | Method LOQ | Principle of method (i.e. GC-MS or HPLC-UV) | Author(s), year / missing |
| Surface water | Not preformed, not required | | | |

For any special comments or remarkable points concerning the analytical methods for water please refer to Appendix 2.

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

An overview on the acceptable methods and possible data gaps for analysis of isoxaflutole in air is given in the following tables. No new studies are necessary.

Table 5.3-4: Validated methods for air (if appropriate)

| Component of residue definition: isoxaflutole | | | |
|---|---------------------------|--|---|
| Method type | Method LOQ | Principle of method (i.e. GC-MS or HPLC-UV) | Author(s), year / missing |
| Primary | LOQ = 2 µg/m ³ | LC-UV | DAR Isoxaflutole (1997) – Volume 3 Annex B.4 – Analytical methods |
| Confirmatory | Not required | | |

For any special comments or remarkable points concerning the analytical methods for air it is referred to Appendix 2.

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

No methods were supplied for the determination of isoxaflutole or metabolites in these matrices. A case has been made for not providing these as the active substance is not classified as toxic or very toxic which is acceptable.

An overview on the acceptable methods and possible data gaps for analysis of active substance in body fluids and tissues is given in the following table. For the detailed evaluation of new/ additional studies it is referred to Appendix 2.

Table 5.3-5: Methods for body fluids (if appropriate)

| Component of residue definition: Sum of isoxaflutole and RPA 202248, expressed as isoxaflutole | | | |
|--|---------------|--|---------------------------|
| Method type | Method LOQ | Principle of method (i.e. GC-MS or HPLC-UV) | Author(s), year / missing |
| Primary | LOQ=0.01 mg/L | LC-MS/MS | Asekunowo J., 2025-2024 |

| Component of residue definition: Sum of isoxaflutole and RPA 202248, expressed as isoxaflutole | | | |
|--|------------|--|---------------------------|
| Method type | Method LOQ | Principle of method (i.e. GC-MS or HPLC- UV) | Author(s), year / missing |
| Confirmatory | | | |

For any special comments or remarkable points concerning the analytical methods for body fluids please refer to Appendix 2.

Appendix 1 Lists of data considered in support of the evaluation

List of data submitted by the applicant and 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 |
|-----------------|-------------------------------|--------------|---|-------------------------|----------|
| KCP 5.1.1/01 | Patrzalek M. | 2021 | Validation of analytical method for Izoaxflutole 250 SC (CHR/H/IZOXACYP 250 SC) for determination of isoxaflutole Company Report No ICB/10/2021 ICB Pharma, 10 Lema Street, 43-600 Jaworzno Poland GLP Unpublished | N | Chemiroł |
| KCP 5.1.1/02 | Łebek B. | 2022 | Validation of analytical method for Izoaxflutole 250 SC (CHR/H/IZOXACYP 250 SC) for determination of cyprosulfamide Company Report No ICB/6/2022 ICB Pharma, 10 Lema Street, 43-600 Jaworzno Poland GLP Unpublished | N | Chemiroł |
| KCP 5.2/01 | Ivanoc E., Ezeobi- Chris C | 2025 | Independent Laboratory Validation for Determination of Isoxaflutole and Metabolites in Drinking Water S23-104465(EAG-2301V) Eurofins Agrosience ServicesChem GmbH GLP Unpublished | N | Chemiroł |
| KCP 5.2/02 | Asekunowo J., | 2025 2024 | Method Validation for Determination of Isoxaflutole and its Metabolite RPA 202248 in Body Fluids and Tissues S23-104329 Eurofins Agrosience ServicesChem GmbH GLP | N | Chemiroł |

| 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 |
|---------------|---------------|------|--|----------------------------|----------|
| | | | Unpublished | | |
| KCP 5.2/03 | Asekunowo J., | 2025 | Report Amendment No.1 Method Validation for Determination of Isoxaflutole and Metabolites in Water S23-104328 Eurofins Agrosience ServicesChem GmbH GLP Unpublished | N | ChemiroI |

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

| 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 |
|-----------------|---------------------------------|-------|---|----------------------------|-------------------|
| KCA 5.1.2/01 | Manley, J. D. | 1995 | Analytical method for the determination of residues of RPA 201772, RPA 202248 and RPA 203328 in maize forage, grain and fodder Rhone-Poulenc Agriculture Ltd., Ongar, Essex, United Kingdom Bayer CropScience, Report No.: C021001, Edition Number: M-210419-01-1 Date: 1995-10-24 GLP/GEP: yes, unpublished ...also filed: KCA 4.2 /05 | N | Bayer CropScience |
| KCA 5.1.2/02 | Austin, D. J.; Manley, J. D. | 1995 | Herbicides: RPA201772: The evaluation of alternative procedures to diazomethane for the preparation of esters of RPA203328 for the "Analytical method for the determination of residues of RPA201772, RPA202248 and RPA203328 in maize grain an Rhone-Poulenc Agriculture Ltd., Ongar, Essex, United Kingdom Bayer CropScience, Report No.: R000352, Report includes Trial Nos.: P94/110 Edition Number: M-158450-01-1 Date: 1995-04-28 GLP/GEP: yes, unpublished | N | Bayer CropScience |
| KCA 5.1.2/03 | Schuster, L.L., | 1995a | PR Notice 88-5 Enforcement Method Confirmation for RPA 201772 and its Metabolites RPA 202248 and RPA 203328 in Corn Grain | N | Bayer CropScience |

| 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 |
|-------------------|--|-------------|--|-------------------------------------|----------------------|
| | | | Generated by: ABC Laboratories, Pan-Ag Division, Madera, California, USA Submitted by: Rhône-Poulenc Agro, France Report/file N°: 94454, Docmap n° 44663 Date of report: February 23, 1995 | | |
| KCA 5.1.2/04 | Guillet, M., Venet, C. & Simonin, B., | 1995 | Isoxaflutole and Metabolites: Analytical Method for the Determination of Residues in Soil. Generated by: Rhône-Poulenc Secteur Agro, Lyon, France Submitted by: Rhône-Poulenc Agro, France Report/file N°: AR 106-94 (E), Docmap n° 437490, Date of report: February 2, 1995. | N | Bayer CropScience |
| KCA 5.1.2/05 | Schuster, L.L., | 1995 | Method Confirmation for RPA 201772 and its Metabolites RPA 202248, RPA 203328 and RPA 205834 in Soil. Generated by: ABC Laboratories, Pan-Ag Division, Madera, California, USA Submitted by: Rhône-Poulenc Agro, France Report/file N°: EC-95-305 Date of report: November 7, 1995 | N | Bayer CropScience |
| KCA 5.1.2/06 | Plaisance, R.S., 1995 | 1995 | Validation of Method of Analysis for Isoxaflutole and its Metabolites in/on Agricultural Soil. Generated by: Rhône-Poulenc Ag, Co., USA. Submitted by: Rhône-Poulenc Agro, France. Report/file N°: EC-95-299 Date of report: November 6, 1995 | N | Bayer CropScience |
| KCP 5.1.2/07 | Corgier, M. and Turier, G., | 1995 | Analytical Method for the Determination of Isoxaflutole in Air. Generated by: Rhône-Poulenc Agro, France. Submitted by: Rhône-Poulenc Agro, France. Report/file N°: 94-115 Docmap 438832 Date of report: June 15, 1995 | N | Bayer CropScience |
| KCP 5.2/1 | Guillet, M., Venet, C. & Simonin, B., | 1995 | Isoxaflutole and Metabolites: Analytical Method for the Determination of Residues in Soil. Generated by: Rhône-Poulenc Secteur Agro, Lyon, France Submitted by: Rhône-Poulenc Agro, France Report/file N°: AR 106-94 (E), Docmap n° 437490, Date of report: February 2, 1995. | N | Bayer CropScience |

| 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 |
|-------------------|--|-------------|--|-------------------------------------|----------------------|
| KCP 5.2/2 | Schuster, L.L., | 1995 | Method Confirmation for RPA 201772 and its Metabolites RPA 202248, RPA 203328 and RPA 205834 in Soil. Generated by: ABC Laboratories, Pan-Ag Division, Madera, California, USA Submitted by: Rhône-Poulenc Agro, France Report/file N°: EC-95-305 Date of report: November 7, 1995 | N | Bayer CropScience |
| KCP 5.2/3 | Plaisance, R.S., 1995 | 1995 | Validation of Method of Analysis for Isoxaflutole and its Metabolites in/on Agricultural Soil. Generated by: Rhône-Poulenc Ag, Co., USA. Submitted by: Rhône-Poulenc Agro, France. Report/file N°: EC-95-299 Date of report: November 6, 1995 | N | Bayer CropScience |
| KCP 5.2/4 | Corgier, M. and Turier, G., | 1995 | Analytical Method for the Determination of Isoxaflutole in Air. Generated by: Rhône-Poulenc Agro, France. Submitted by: Rhône-Poulenc Agro, France. Report/file N°: 94-115 Docmap 438832 Date of report: June 15, 1995 | N | Bayer CropScience |
| KCP 5.2/5 | Guillet, M. , Diot, R. & Le Gren, I., | 1995b | Isoxaflutole and/or Metabolites: Analytical Method for the Determination of Residues in Animal Products Generated by: Rhône-Poulenc Agro, France Submitted by: Rhône-Poulenc Agro, France Report/file N°: AR 109-95 (E) Date of report: April 12, 1995 | N | Bayer CropScience |
| KCP 5.2/6 | Guillet, M. , & Le Gren, I., | 1995d | Isoxaflutole and/or Metabolites: Analytical Method for the Determination of Residues in Animal Products Complementary Report Generated by: Rhône-Poulenc Agro, France Submitted by: Rhône-Poulenc Agro, France Report/file N°: 9515587 Date of report: May 23, 1995 | N | Bayer CropScience |
| KCP 5.2/7 | Guillet, M. , Diot, R. & Le Gren, I., | 1995b | Isoxaflutole and/or Metabolites: Analytical Method for the Determination of Residues in Animal Products Complementary Report n°:2 | N | Bayer CropScience |

| 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 |
|-------------------|------------------------------|-------------|---|-------------------------------------|----------------------|
| | | | Generated by: Rhône-Poulenc Agro, France Submitted by: Rhône-Poulenc Agro, France Report/file N°: 9516279 Date of report: September 20, 1995 | | |
| KCP 5.2/8 | Hunt, T.W. and Lopes, A., | 1995 | Isoxaflutole - Validation of Method of Analysis for Isoxaflutole and its Metabolites in Animal Tissues. Generated by: Rhône-Poulenc Ag Co., USA Submitted by: Rhône-Poulenc Agro, France Report/file N°: EC-95-313 Date of report: December 28, 1995 | N | Bayer CropScience |
| KCP 5.2/9 | Shaffer, S. | 1995 | Independent Laboratory Validation of the Rhône-Poulenc Methods entitled, "Method of Analysis for the determination of Isoxaflutole and its Metabolites (RPA 203328, RPA 202248 and RPA 205834 in Milk" and "Method of Determination for Isoxaflutole and its Metabolite (RPA 202248) in/on Bovine and Poultry Tissues" Generated by: Horizon Laboratories Inc. Columbia, Missouri, USA Submitted by: Rhône-Poulenc Agro, France Report/file N°: EC-95-233 Date of report: December 22, 1995 | N | Bayer CropScience |

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

A 2.1 Analytical methods for isoxaflutole

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

No new or additional studies have been submitted

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

zRMS: Method is accepted

Reference: KCP 5.2/03

Report Method Validation for Determination of Isoxaflutole and Metabolites in Water

Guideline(s): SANTE/2020/12830, rev.2 for risk assessment and/or monitoring

Deviations: No

GLP: Yes

Acceptability:

STUDY OBJECTIVE

The objective was to validate a method for the determination of Isoxaflutole and its metabolites RPA 202248, RPA 205834 and RPA 203328 in drinking and surface water in accordance with guidance document SANTE/2020/12830, rev.2 for monitoring.

METHOD SUMMARY

Principle of the Analytical Procedure

Homogenisation for drinking and surface water: Shaking by hand

Extraction: No extraction necessary, the water samples were fortified if necessary and vortexed for 20 sec

Quantification: The analytes were analysed by direct injection (LC-MS/MS).

LC-MS/MS determination was conducted with evaluation of two mass transitions for Isoxaflutole (m/z 360→251 and m/z 360→144), RPA 202248 (m/z 358→79 and m/z 358→64), RPA 205834 (m/z 360→280 and m/z 360→183) and RPA 203328 (m/z 267→223 and m/z 267→159). Due to enhanced sensitivity mass transition m/z 360→251 for Isoxaflutole, m/z 358→79, RPA 202248, m/z 360→280 for RPA 205834 and m/z 267→223 for RPA 203328 were proposed to be used for quantification but both mass transitions are applicable interchangeably for quantification and confirmation.

A reagent blank and two (2) control samples per matrix and analyte were extracted and analysed according to the method to investigate the presence of residue and/or background interference at the retention time of the analytes. For both mass transitions, the samples showed no significant interference that would correspond to 30 % of LOQ at the retention time of the analytes in any investigated matrices surface and drinking water, therefore showing that the method is highly specific.

Blank correction was not performed.

SUMMARY

| Item | Activity, Result, Assessment |
|---------------------------------------|--|
| Study Scope | Validation of an analytical method according to guidance document SANTE/2020/12830, rev. 2 for risk assessment and/or monitoring. |
| Analytes | Isoxaflutole, RPA 202248 (expressed as Isoxaflutole), RPA 205834, RPA 203328 |
| Matrix | Drinking water, Surface water |
| Method Reference | The method was developed at the test facility. |
| LOQ | Isoxaflutole and RPA 202248 (expressed as Isoxaflutole) 0.05 µg/L (lowest validated fortification level) RPA 205834 and RPA 203328 0.10 µg/L (lowest validated fortification level) |
| LOD | 30 % of the LOQ (lowest calibration standard) |
| Principle of the Analytical Procedure | Homogenisation for drinking and surface water: Shaking by hand Extraction: No extraction necessary, the water samples were fortified directly if necessary and vortexed Quantification: The analytes were analysed by direct injection (LC-MS/MS). |
| Selectivity and Specificity | Demonstrated by validation of two mass transitions for all analytes. Analyte levels or chromatographic interferences of unknown compounds in a reagent blank and control sample extracts were below of what would correspond to an analyte level of 30 % of the LOQ. |

| | |
|---|---|
| Matrix Effects on Analyte Detection | <p>Insignificant (< 20 %) for drinking water and surface water for Isoxaflutole, RPA 205834 and RPA 203328 Significant (> 20 %) for drinking water and surface water for RPA 202248</p> |
| Calibration | <p>Matrix-matched calibration standards A minimum of five (5) concentration levels Single determination Injection of standard solutions spread over the whole acquisition batch Isoxaflutole and RPA 202248 (expressed as Isoxaflutole) Concentration range: 0.015 ng/mL to 0.60 ng/mL Corresponding mass fraction range: 0.015 µg/L to 0.60 µg/L Coverage: 30 % of the LOQ to at least + 20 % of the highest analyte concentration level detected in a sample extract RPA 205834 and RPA 203328 Concentration range: 0.030 ng/mL to 1.2 ng/mL Corresponding mass fraction range: 0.030 µg/L to 1.2 µg/L Coverage: 30 % of the LOQ to at least + 20 % of the highest analyte concentration level detected in a sample extract The validated range does not exceed two orders of magnitude</p> |
| Quantification | <p>No quantification was performed for recovery determination. Because of the direct measurement of the samples, recovery rates cannot be calculated. Thus, repeatability (precision) data and retention time are presented. Calibration data are only presented to demonstrate the linearity according to SANTE/2020/12830 rev. 2. The injection of standard solutions was spread over the whole analytical sequence. Linear regression with 1/x weighting Regression residuals randomly distributed Correlation coefficients (R) ≥ 0.99</p> |
| Accuracy and Precision | <p>Isoxaflutole and RPA 202248 (expressed as Isoxaflutole) Five (5) fortifications at 0.05 µg/L (LOQ) Five (5) fortifications at 0.5 µg/L (10x LOQ) RPA 205834 and RPA 203328 Five (5) fortifications at 0.10 µg/L (LOQ) Five (5) fortifications at 1.0 µg/L (10x LOQ) Analytes fortified jointly Mean relative standard deviations (RSD ≤ 20 %) for the two evaluated mass transitions comply with the standard acceptance criteria of the guidance document SANTE/2020/12830, rev. 2.</p> |
| Stability of Analytes in Standard Solutions | <p>Within ± 10 % for 69 days when prepared in acetonitrile and stored at typically 1 °C to 10 °C in the dark. Within ± 10 % for 69 days when prepared in acetonitrile/water (20:80, v:v) containing 0.22 ml/L formic acid and stored at typically 1 °C to 10 °C in the dark.</p> |
| Stability of Analytes in Sample Extracts | <p>Recoveries within 70 % - 120 % in drinking water extracts for at least 37 days when stored at typically 1 °C to 10 °C in the dark. Recoveries within 70 % - 120 % in surface water extracts for at least 36 days when stored at typically 1 °C to 10 °C in the dark.</p> |
| Conclusion | <p>The method was found to be valid according to the guidance document</p> |

| | |
|--|---|
| | SANTE/2020/12830, rev. 2 for risk assessment and/or monitoring. |
|--|---|

zRMS: Method is accepted

| | |
|----------------|---|
| Reference: | KCP 5.2/01 |
| Report | Independent Laboratory Validation for Determination of Isoxaflutole and Metabolites in Drinking Water, Ivanoc E., Ezeobi-Chris C, 2025 Study code: S23-104465 |
| Guideline(s): | SANTE/2020/12830, rev.2 for risk assessment and/or monitoring |
| Deviations: | No |
| GLP: | Yes |
| Acceptability: | |

STUDY OBJECTIVE

The objective was to independently validate (ILV) the analytical method for the determination of isoxaflutole and its metabolites RPA 202248 (expressed as isoxaflutole), RPA 205834 and RPA 203328 in/on drinking water in accordance with guidance document SANTE/2020/12830, rev.2 for monitoring.

METHOD SUMMARY

LC-MS/MS determination was conducted with evaluation of two mass transitions for Isoxaflutole (m/z 360→251 and m/z 360→144), RPA 202248 (m/z 358→79 and m/z 358→64), RPA 205834 (m/z 360→280 and m/z 360→183) and RPA 203328 (m/z 267→223 and m/z 267→159). Due to enhanced sensitivity mass transition m/z 360→251 for Isoxaflutole, m/z 358→79, RPA 202248, m/z 360→280 for RPA 205834 and m/z 267→223 for RPA 203328 were proposed to be used for quantification but both mass transitions are applicable interchangeably for quantification and confirmation.

A reagent blank and two control samples were extracted and analysed according to the method to investigate the presence of residue and/or background interference at the retention time of the analytes. For both mass transitions, the samples showed no significant interference that would correspond to 30 % of LOQ at the retention time of the analytes in any investigated matrix drinking water, therefore showing that the method is highly specific.

Blank correction was not performed.

Principle of the Analytical Procedure

Homogenisation for drinking: Shaking by hand Extraction: No extraction necessary, the water samples were fortified if necessary and vortexed for 20 sec Quantification: The analytes were analysed by direct injection (LC-MS/MS).

No addition or modification to the original method other than optimisation of instrumental parameters and no communication with the method developers or others familiar with the method was necessary in order to carry out the analysis.

SUMMARY

| Item | Activity, Result, Assessment |
|---------------------------------------|--|
| Study Scope | Independent Laboratory Validation of an analytical method according to guidance document SANTE/2020/12830, rev. 2 for monitoring |
| Analytes | Isoxaflutole, RPA 202248 (expressed as Isoxaflutole), RPA 205834, RPA 203328 |
| Matrix | Drinking water |
| Method Reference | The method was developed and validated in Study no. S23-104328 |
| LOQ | Isoxaflutole and RPA 202248 (expressed as Isoxaflutole) 0.05 µg/L (lowest validated fortification level) RPA 205834 and RPA 203328 0.10 µg/L (lowest validated fortification level) |
| LOD | 30 % of the LOQ (lowest calibration standard) |
| Principle of the Analytical Procedure | Homogenisation: Flush the water from the tap for 2 minutes. Shaking by hand. Extraction: No extraction necessary, the water samples were fortified directly if necessary and vortexed Quantification: The analytes were analysed by direct injection (LC-MS/MS). |
| Selectivity and Specificity | Demonstrated by validation of two (2) mass transitions for all analytes. Analyte levels or chromatographic interferences of unknown compounds in a reagent blank and control sample extracts were below of what would correspond to an analyte level of 30 % of the LOQ. |
| Matrix Effects on Analyte Detection | Insignificant (< 20 %) for drinking water for RPA 205834, RPA 203328 and RPA 202248. Significant (> 20 %) for drinking water for Isoxaflutole. |
| Calibration | Matrix-matched calibration standards A minimum of five (5) concentration levels Single determination Injection of standard solutions spread over the whole acquisition batch Isoxaflutole and RPA 202248 (expressed as Isoxaflutole) Concentration range: 0.015 ng/mL to 0.60 ng/mL Corresponding mass fraction range: 0.015 µg/L to 0.60 µg/L Coverage: 30 % of the LOQ to at least + 20 % of the highest analyte concentration level detected in a sample extract RPA 205834 and RPA 203328 Concentration range: 0.030 ng/mL to 1.2 ng/mL Corresponding mass fraction range: 0.030 µg/L to 1.2 µg/L Coverage: 30 % of the LOQ to at least + 20 % of the highest analyte concentration level detected in a sample extract The validated range does not exceed two (2) orders of magnitude |
| Quantification | Linear regression with 1/x weighting Regression residuals randomly distributed Coefficients of determination (R^2) ≥ 0.98 |
| Accuracy and Precision | Isoxaflutole and RPA 202248 (expressed as Isoxaflutole) Five (5) fortifications at 0.05 µg/L (LOQ) Five (5) fortifications at 0.5 µg/L (10x LOQ) RPA 205834 and RPA 203328 Five (5) fortifications at 0.10 µg/L (LOQ) Five (5) fortifications at 1.0 µg/L (10x LOQ) Analytes fortified jointly. Mean relative standard deviations ($RSD \leq 20 \%$) for the two (2) evaluated mass transitions comply with the standard acceptance criteria of the guidance document SANTE/2020/12830, rev. 2. |

| | |
|---|---|
| Stability of Analytes in Standard Solutions | The stability of the analyte(s) in standard solutions was not investigated in this study, since data were reported in study report no. S23-104328. |
| Stability of Analytes in Sample Extracts | Stability testing was not part of this study, since final extracts for both analyte/matrix combinations were found to be stable for at least 37 days as reported in study no. S23-104328. |
| Independent Laboratory Validation | Primary validation and independent laboratory validation were carried out at different locations and by different study personnel. No addition or modification to the original method other than optimisation of instrumental parameters was done. No communication with the method developers or others familiar with the method was necessary to carry out the analysis. |
| Conclusion | The method was found to be valid according to the guidance document SANTE/2020/12830, rev. 2 for monitoring. |

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

zRMS: Method is accepted

Reference: KCP 5.2/02

Report Method Validation for Determination of Isoxaflutole and its Metabolite RPA 202248 in Body Fluids and Tissues, Asekunowo J., 2024 Study code: S23-104329

Guideline(s): SANTE/2020/12830, rev.2 for risk assessment and/or monitoring

Deviations: No

GLP: Yes

Acceptability:

STUDY OBJECTIVE:

The objective of the study is to validate the multi-residue method QuEChERS for the determination of Isoxaflutole and its metabolite RPA 202248 in body fluids and in animal tissues in accordance to guidance document SANTE/2020/12830, rev.2 for monitoring

METHOD SUMMARY

In brief, the samples of human urine were extracted with acetonitrile. The samples of bovine (minced muscle) meat were extracted with acetonitrile containing 1 % of formic acid (after addition of water containing 1 % of formic acid). Then the magnesium sulfate, sodium chloride and buffering citrate salt mixture was added and shaken intensively followed by centrifugation for phase separation. Clean-up of

the extract was performed by dispersive dSPE with primary/secondary amine (PSA). In addition, for (for bovine (minced muscle) meat, the sample extracts were frozen to eliminate the fat (prior to SPE clean-up). Finally, the extracts were diluted with water/acetonitrile (20/80, v/v) containing 0.1% formic acid. Quantification was performed by use of LC MS/MS.

LC-MS/MS determination was conducted with evaluation of two mass transitions for Isoxaflutole

(m/z 360→251 and m/z 360→144) and for RPA 202248 (m/z 358→79 and m/z 358→64). Due to enhanced

sensitivity mass transition m/z 360→251 for Isoxaflutole and m/z 358→79 for RPA 202248 were proposed to be used for quantification but both mass transitions are applicable interchangeably for quantification and confirmation.

A reagent blank and two control samples per matrix and analyte were extracted and analysed according to the method to investigate the presence of residue and/or background interference at the retention time of the analytes. For both mass transitions, the samples showed no significant interference that would correspond to 30 % of LOQ at the retention time of the analytes in any investigated matrix bovine (minced muscle) meat and human urine, therefore showing that the method is highly specific.

Blank correction was not performed.

SUMMARY

| Item | Activity, Result, Assessment |
|---------------------------------------|--|
| Study Scope | Validation of an analytical method according to guidance document SANTE/2020/12830, rev. 2 for risk assessment and/or monitoring. |
| Analytes | Isoxaflutole, RPA 202248 |
| Matrices | Bovine (minced muscle) meat, Human Urine |
| Method Reference | The method was developed at the test facility. The validated method is based on the principles of the multi-residue method QuEChERS. |
| LOQ | 0.01 mg/kg for Bovine (minced muscle) meat 0.01 mg/L for Human Urine |
| LOD | 30 % of the LOQ (lowest calibration standard) |
| Principle of the Analytical Procedure | <p>Homogenisation for bovine (minced muscle) meat: Minced meat was used. No further homogenisation.</p> <p>Homogenisation for human urine: Mix well</p> <p>Extraction for Bovine (minced muscle) meat: Acetonitrile containing 1% formic acid and addition of water containing 1% formic acid, shaking by shaker</p> <p>Extraction for Human Urine Acetonitrile, shaking by shaker</p> <p>For all matrices: Supel QE Citrate (EN) Tube Clean-up: PSA SPE clean tube Additional clean-up only for bovine (minced muscle) meat: Freeze-out (performed prior to PSA SPE clean up) For Bovine (minced muscle) meat: Sample concentration in final extract: 0.01 g sample per mL of extract Quantification: LC-MS/MS</p> <p>For Human Urine: Sample concentration in final extract: 0.01 mL sample per mL of extract Quantification: LC-MS/MS</p> |

| Selectivity and Specificity | Demonstrated by validation of two (2) mass transitions. Analyte levels or chromatographic interferences of unknown compounds in a reagent blank and control sample extracts were below of what would correspond to an analyte level of 30 % of the LOQ. | | | | | | | | | |
|---|---|-------------------------------|------------------------------|-------------------------------|--------|----------|------|----------------|----------|------|
| Matrix Effects on Analyte Detection | Significant (> 20 %) for bovine (minced muscle) meat for both analytes Insignificant (< 20 %) for human urine for both analytes | | | | | | | | | |
| Calibration | Matrix-matched calibration standards A minimum of five (5) concentration levels Single determination Injection of standard solutions spread over the whole acquisition batch. Concentration range: 0.030 ng/mL to 1.2 ng/mL Corresponding mass fraction range for bovine (minced muscle) meat: 0.003 mg/kg to 0.12 mg/kg Corresponding mass fraction range for human urine: 0.003 mg/L to 0.12 mg/L Coverage: 30 % of the LOQ to at least + 20 % of the highest analyte concentration level detected in a sample extract The validated range does not exceed two (2) orders of magnitude | | | | | | | | | |
| Quantification | Linear regression with 1/x weighting Regression residuals randomly distributed. Correlation coefficients (R) ≥ 0.99 | | | | | | | | | |
| Accuracy and Precision | <p>For bovine (minced muscle) meat:</p> <p>Five (5) fortifications at 0.01 mg/kg (LOQ) Five (5) fortifications at 0.1 mg/kg (10x LOQ) Analytes fortified jointly. Mean recoveries for the two (2) evaluated mass transitions comply with the standard acceptance criteria of the guidance document SANTE/2020/12830, rev. 2 .</p> <table><tr><th>Concentration Level (mg/kg)</th><th>Range of Mean Recoveries (%)</th><th>Precision, Rel. Std. Dev. (%)</th></tr><tr><td>≤ 0.01</td><td>60 - 120</td><td>≤ 30</td></tr><tr><td>> 0.01 - ≤ 0.1</td><td>70 - 120</td><td>≤ 20</td></tr></table> <p>For human urine:</p> <p>Five (5) fortifications at 0.01 mg/L (LOQ) Five (5) fortifications at 0.1 mg/L (10x LOQ) Analytes fortified jointly. Mean recoveries for the two (2) evaluated mass transitions were in the range of 70 % -120 % with an RSD ≤20% and comply with the standard acceptance criteria of the guidance document SANTE/2020/12830, rev. 2.</p> | Concentration Level (mg/kg) | Range of Mean Recoveries (%) | Precision, Rel. Std. Dev. (%) | ≤ 0.01 | 60 - 120 | ≤ 30 | > 0.01 - ≤ 0.1 | 70 - 120 | ≤ 20 |
| Concentration Level (mg/kg) | Range of Mean Recoveries (%) | Precision, Rel. Std. Dev. (%) | | | | | | | | |
| ≤ 0.01 | 60 - 120 | ≤ 30 | | | | | | | | |
| > 0.01 - ≤ 0.1 | 70 - 120 | ≤ 20 | | | | | | | | |
| Stability of Analyte(s) in Standard Solutions | Within ± 10 % for 69 days when prepared in acetonitrile and stored at typically 1 °C to 10 °C in the dark. Within ± 10 % for 69 days when prepared in acetonitrile/water (20:80 v,v) + 0.22 ml/L formic acid) and stored at typically 1 °C to 10 °C in the dark. | | | | | | | | | |
| Stability of Analyte(s) | Recoveries within 70 % - 120 % in human urine extracts for 29 days when stored at typically 1 °C to 10 °C in the dark. Recoveries within 70 % - 120 % in bovine (minced muscle) meat extracts for 15 days when stored at typically 1 °C to 10 °C in the dark. | | | | | | | | | |
| Conclusion | The method was found to be valid according to the guidance document SANTE/2020/12830, rev. 2 for risk assessment and/or monitoring. | | | | | | | | | |

A 2.1.2.7 A.2.A.9 Other Studies/ Information

No new or additional studies have been submitted