

# **FINAL REGISTRATION REPORT**

## **Part B**

### **Section 5**

#### **Analytical Methods**

Detailed summary of the risk assessment

Product code: K-300 SL-RR

Product name(s): Faworyt 300 SL

Chemical active substance:

Clopyralid, 300 g/L

Central Zone

Zonal Rapporteur Member State: Poland

CORE ASSESSMENT – Art. 43

(Renewal of authorisation)

Applicant: CIECH Sarzyna S.A.

Submission date: 12/2021

Correction: 05/2022

MS Finalisation date: 07/2022; 10/2022

## Version history

When	What
December 2021	dRR version 1 submitted by applicant
<b>May 2022</b>	<b>First correction for product authorization</b>
July 2022	zRMS first assessment
October 2022	Final Registration Report

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## 5 Analytical methods

### 5.1 Conclusion and summary of assessment

CIECH Sarzyna S.A. possess Letter of Access from the Task Force Clopyralid to alternative data package for active substance Clopyralid. Task Force Clopyralid has submitted its Data Matching List to zRMS, Finland. This Data Matching List covers all the protected studies from the main notifier.

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

Sufficiently sensitive and selective analytical methods are available for all analytes included in the residue definitions.  
Minor data gap: extraction efficiency (for plant and animal matrices). Not provided during the EU review.

Commodity/crop	Supported/ Not supported
Winter wheat	Supported
Winter rape	Supported
Sugar beet	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 clopyralid in plant protection product is provided as follows:

Reference:	KCP 5.1.1
Report	Faworyt 300 SL Method validation for determination of the active substance content (clopyralid) in the preparation, Gutowska I., 2019, BA-01/19
Guideline(s):	Yes (SANCO/3030/99 rev. 4)
Deviations:	No
GLP:	Yes
Acceptability:	Yes

#### Materials and methods

The method is based on determination of clopyralid using reversed phase high performance liquid chromatography (RP-HPLC) with UV-DAD detection at wavelength 223 nm and external standard.

Test Item FAWORYT 300 SL  
Active substance Clopyralid 300 g/L

Chromatographic conditions:

Oven temperature 30 °C  
Mobile phase flow  $v = 1.0 \text{ mL/min}$   
Wavelength  $\lambda = 223 \text{ nm}$   
Injection volume 2  $\mu\text{L}$   
Mobile phase composition Acetonitrile + 0.1%  $\text{H}_3\text{PO}_4$  (aq) (20 + 80, V/V)

Under the above conditions retention time of the examined substance is about 7.1 min. Total analysis time is 20 minutes.

Validation - Results and discussions

**Table 5.2-1: Methods suitable for the determination of clopyralid in plant protection product Faworyt 300 SL**

	Clopyralid										
Author(s), year	Gutowska I., 2019										
Principle of method	The method is based on determination of clopyralid using reversed phase high performance liquid chromatography (RP-HPLC) with UV-DAD detection at wavelength 223 nm and external standard.										
Linearity (correlation coefficient, expressed as r)	The linearity of the analytical method was assessed using ten solutions of clopyralid in the concentration range between 0.6134 – 1.2344 mg/mL. y= 5184114.1643x + 139 465.3810  R <sup>2</sup> =0.9995 Acceptance criteria: R <sup>2</sup> ≥ 0.999										
Precision – Repeatability Mean n = 6 (%RSD)	%RSD = 0.64  Acceptance criteria: %RSD ≤ 1.64  Horrat value: Hr = %RSD/%RSDr H <sub>r</sub> = 0.39  acceptance criterion: Hr ≤ 1										
Accuracy	Accuracy of determination of clopyralid content in FAWORYT 300 SL preparation was assessed by recovery at two concentration (five replicates) <table><tr><td></td><td>Avarage Recovery (%) n=5</td><td>Mean Recovery (%)</td></tr><tr><td>Level 1</td><td>100.6</td><td rowspan="2">100.1</td></tr><tr><td>Level 2</td><td>99.6</td></tr></table> The result of 100.1% fulfils the acceptance criterion (98 – 102%) *				Avarage Recovery (%) n=5	Mean Recovery (%)	Level 1	100.6	100.1	Level 2	99.6
	Avarage Recovery (%) n=5	Mean Recovery (%)									
Level 1	100.6	100.1									
Level 2	99.6										
Interference/ Specificity	No interference										
Comment	Provided method is accepted										

\*Result meet the acceptance criteria 97-100% as stated in SANCO/3030/99 rev.5

## Conclusion

Validation criteria are compliant with EU requirements given in SANCO/3030/99 Rev.4.  
The analysis of the validation results was also carried out in terms of the requirements of SANCO3030/99 Rev. 5.  
Compliance with the above-mentioned guideline has been confirmed.

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

Faworyt 300 SL does not contain relevant impurities which are of toxicological, ecotoxicological or environmental concern which could be arisen in the manufacturing process or as a result of degradation during storage of the product.

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

The other formulants and also components of other formulants of Faworyt 300 SL are not of toxicological and/or ecotoxicological or environmental concern and therefore it is not necessary to submit the analytical methods for determination of other formulants or components of other formulants of above product.

### 5.2.1.4 Applicability of existing CIPAC methods (KCP 5.1.1)

There is no CIPAC method available for the determination of clopyralid.

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

An overview on the acceptable methods and possible data gaps for analysis of residues of Clopyralid for the generation of pre-authorization data is given in the following table. Only analytical methods not previously reviewed and accepted by the RMS and EFSA were summarized. The analytical method summaries include the recovery, precision, limit of quantitation, specificity and linearity of the method as outlined in SANCO/3029/99 rev.4 for validation of a data generation method. For the detailed evaluation of the new studies it is referred to Appendix 2.

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

Component of residue definition: Clopyralid				
Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing / EU agreed
Plant residue (wheat grain, straw, germ, flour, bread, total bran)	Primary	0.01 mg/kg	LC-MS/MS	White T., 2021, S19-01810 Analytical phase: Sayed S., Hamoum N., S19-01810-L2; Hernandez Ch., S19-01810-L3
Plant residue (wheat grain, straw, germ)	Primary	0.044 mg/kg	LC-MS/MS	White T., 2021, S20-04397 Analytical Phase: Sayed S. Souchier M.

Component of residue definition: Clopyralid				
Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing / EU agreed
50% sucrose solution (Ecotoxicology)	Primary	2.988467 mg/kg	HPLC	Świstak M., 2019, 0016/0051/FA
Deionized water (Ecotoxicology)	Primary	0.054727 mg/kg	HPLC	Świstak M., 2019, 0016/0055/FA

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

#### 5.3.1 Analysis of the plant protection product (KCP 5.2)

Analytical methods for the determination of the active substance and relevant impurities in the plant protection product shall be submitted, unless the applicant shows that these methods already submitted in accordance with the requirements set out in point 5.2.1 can be applied.

#### 5.3.2 Description of analytical methods for the determination of residues of clopyralid (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 new proposition for legal residue definition has been presented (EFSA Journal, 10.2903/j.efsa.2021.6389).

Existing enforcement residue definition for commodities of plant origin: Clopyralid

Proposed enforcement residue definition for commodities of plant origin (by the EU pesticides peer review): clopyralid common moiety (sum of clopyralid, its salts and conjugates expressed as clopyralid).

Existing enforcement residue definition for commodities of animal origin: Clopyralid

Proposed enforcement residue definition for commodities of animal origin (by the EU pesticides peer review): clopyralid and its salts OR clopyralid common moiety (sum of clopyralid, its salts and glycine conjugates expressed as clopyralid).

**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 (sugar beet)	Clopyralid	1 mg/kg LOQ = 0.01 mg/kg	COMMISSION REGULATION (EU) 2021/1807
Plant, high protein/high starch content (dry commodities) (wheat)		3 mg/kg LOQ = 0.01 mg/kg	COMMISSION REGULATION (EU) 2021/1807



Matrix	Residue definition	MRL / limit	Reference for MRL/level Remarks
Plant, high oil content (oilseed rape)		0.5 mg/kg LOQ = 0.01 mg/kg	COMMISSION REGULATION (EU) 2021/1807
Muscle (bovine)	Clopyralid	0.08 mg/kg LOQ = 0.01 mg/kg	COMMISSION REGULATION (EU) 2021/1807
Milk (bovine)		0,05 mg/kg LOQ = 0.01 mg/kg	COMMISSION REGULATION (EU) 2021/1807
Eggs (poultry)		0,05 mg/kg LOQ = 0.01 mg/kg	COMMISSION REGULATION (EU) 2021/1807
Fat		0.05 mg/kg LOQ = 0.01 mg/kg	COMMISSION REGULATION (EU) 2021/1807
Liver (bovine)		0.15 mg/kg LOQ = 0.01 mg/kg	COMMISSION REGULATION (EU) 2021/1807
Kidney (bovine)		1.5 mg/kg LOQ = 0.01 mg/kg	COMMISSION REGULATION (EU) 2021/1807
Soil (Ecotoxicology)	Clopyralid	0.5 µg/kg	EFSA Journal 2018;16(8):5389, 21 pp.
Drinking water (Human toxicology)	Clopyralid	0.1 µg/L	general limit for drinking water EFSA Journal 2018;16(8):5389, 21 pp.
Surface water (Ecotoxicology)	Clopyralid	LOQ = 0.05 µg/kg	EFSA Journal 2018;16(8):5389, 21 pp.
Air	Clopyralid	LOQ = 4.5 µg/m <sup>3</sup>	AOEL: 0.15 mg/kg bw per day EFSA Journal 2018;16(8):5389, 21 pp.
Tissue (meat or liver) (bovine muscle)	Clopyralid	0.08 mg/kg LOQ = 0.01 mg/kg	COMMISSION REGULATION (EU) 2021/1807
Body fluids		LOQ = 0.05 mg/kg	EFSA Journal 2018;16(8):5389, 21 pp.

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

~~CIECH Sarzyna S.A. possess Letter of Access from the Task Force Clopyralid to alternative data package for active substance Clopyralid. Equivalent analytical methods for the determination of residues in plant matrices were included in Data Matching List. This Data Matching List covers all the protected studies from the main notifier.~~

An overview on the acceptable methods and possible data gaps for analysis of Clopyralid in plant matri-

ces is given in the following tables. For the detailed evaluation of new studies it is referred to Appendix 2.

**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: Clopyralid (and X36538 - clopyralid-glycine conjugate)				
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 (tomato)	Primary	0.01 mg/kg	LC-MS/MS	Vogl E., 2012, Report No. 120610, 2013, Report No. 130729 Knop M., 2020, S19-00446
	ILV	0.01 mg/kg	LC-MS/MS	Austin, R., 2012, Report No. 120614, 2014, Report No. 130728 Richer S. 2020, S19-00438
High acid content (grape)	Primary	0.01 mg/kg	LC-MS/MS	Vogl E., 2012, Report No. 120610, 2013, Report No. 130729 Knop M., 2020, S19-00446
	ILV	0.01 mg/kg	LC-MS/MS	Richer S. 2020, S19-00438
High oil content (olive)	Primary	0.01 mg/kg	LC-MS/MS	Vogl E., 2012, Report No. 120610, 2013, Report No. 130729 Knop M., 2020, S19-00446
	ILV	0.01 mg/kg	LC-MS/MS	Austin, R., 2012, Report No. 120614, 2014, Report No. 130728 Richer S. 2020, S19-00438
High protein/high starch content (rice)	Primary	0.01 mg/kg	LC-MS/MS	Vogl E., 2012, Report No. 120610, 2013, Report No. 130729 Knop M., 2020, S19-00446
	ILV	0.01 mg/kg	LC-MS/MS	Richer S. 2020, S19-00438

For any special comments or remarkable points concerning the analytical methods for the determination of residues in plant matrices, please refer to Appendix 2.

**Table 5.3-3: Statement on extraction efficiency**

	Method for products of plant origin
Required, available from:	-
Not required, because:	In compliance with SANCO/825/00 rev. 8.1 the extraction procedure has to be addressed for all matrix groups for which residues $\geq$ LOQ are expected. For Clopyralid non of residue value exceed LOQ.

**zRMS comment:**

Data gap: extraction efficiency. Residues above LOQ are expected.

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

CIECH Sarzyna S.A. possess Letter of Access from the Task Force Clopyralid to alternative data package for active substance Clopyralid. Equivalent analytical methods for the determination of residues in animal matrices were included in Data Matching List. This Data Matching List covers all the protected studies from the main notifier.

An overview on the acceptable methods and possible data gaps for analysis of Clopyralid in animal matrices is given in the following tables. For the detailed evaluation of the studies it is referred to Appendix 2.

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

Component of residue definition: Clopyralid				
Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing
Milk	Primary	0.01 mg/kg	LC-MS/MS	-, 2012, 120483 -, 2013, Report No. 130729 Abe Ch., 2019a, S19-00447
	ILV	0.01 mg/kg	LC-MS/MS	-, 2012, Report No. 120484 -, 2014, Report No. 130728 Schweizer M., 2019, P 5210 G
Eggs	Primary	0.01 mg/kg	LC-MS/MS	-, 2012, 120483 -, 2013, Report No. 130729 Abe Ch., 2019a, S19-00447
	ILV	0.01 mg/kg	LC-MS/MS	-, 2012, Report No. 120484 -, 2014, Report No. 130728 Schweizer M., 2019, P 5210 G
Muscle	Primary	0.01 mg/kg	LC-MS/MS	-, 2012, 120483 -, 2013, Report No. 130729 Abe Ch., 2019a, S19-00447
	ILV	0.01 mg/kg	LC-MS/MS	-, 2012, Report No. 120484 -, 2014, Report No. 130728 Schweizer M., 2019, P 5210 G
Fat	Primary	0.01 mg/kg	LC-MS/MS	-, 2012, 120483 -, 2013, Report No. 130729 Abe Ch., 2019a, S19-00447
	ILV	0.01 mg/kg	LC-MS/MS	Schweizer M., 2019, P 5210 G
Kidney	Primary	0.01 mg/kg	LC-MS/MS	-, 2012, 120483 -, 2013, Report No. 130729 Abe C., 2019a, S19-00447
	ILV	0.01 mg/kg	LC-MS/MS	-, 2012, Report No. 120484 -, 2014, Report No. 130728 Schweizer M., 2019, P 5210 G
Liver	Primary	0.01 mg/kg	LC-MS/MS	-, 2012, 120483 -, 2013, Report No. 130729 Abe Ch., 2019a, S19-00447
	ILV	0.01 mg/kg	LC-MS/MS	Schweizer M., 2019, P 5210 G

For any special comments or remarkable points concerning the analytical methods for the determination

of residues in animal matrices, please refer to Appendix 2.

**Table 5.3-5: Statement on extraction efficiency**

	Method for products of animal origin
Required, available from:	-
Not required, because:	In compliance with SANCO/825/00 rev. 8.1 the extraction procedure has to be addressed for all matrix groups for which residues $\geq$ LOQ are expected. For tribenuron-methyl clopyralid non of residue value exceed LOQ.

**zRMS comment:**

Data gap: extraction efficiency. Residues above LOQ are expected.

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

~~CIECH Sarzyna S.A. possess Letter of Access from the Task Force Clopyralid to alternative data package for active substance Clopyralid. Equivalent analytical methods for the determination of residues in soil were included in Data Matching List. This Data Matching List covers all the protected studies from the main notifier.~~

An overview on the acceptable methods and possible data gaps for analysis of Clopyralid in soil is given in the following tables. For the detailed evaluation of study it is referred to Appendix 2.

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

Component of residue definition: Clopyralid			
Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing
Primary	0.5 µg/kg	LC-MS/MS	Vincent, T. P., 2013, Report No. 120612 Knop M., 2019a, S19-00448

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

~~CIECH Sarzyna S.A. possess Letter of Access from the Task Force Clopyralid to alternative data package for active substance Clopyralid. Equivalent analytical methods for the determination of residues in water were included in Data Matching List. This Data Matching List covers all the protected studies from the main notifier.~~

An overview on the acceptable methods and possible data gaps for analysis of Clopyralid in soil is given in the following tables. For the detailed evaluation of study it is referred to Appendix 2.

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

Component of residue definition: Clopyralid				
Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing
Drinking water	Primary	0.05 µg/L	LC-MS/MS	Shaffer S., 2012, Report No. 120611 Knop M., 2019b, S19-00449

Component of residue definition: Clopyralid				
Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing
	ILV	0.05 µg/L	LC-MS/MS	Austin, R., Turner, R., 2013, Report No. 120613 Richter S., 2019, P 5211 G
Surface water	Primary	0.05 µg/L	LC-MS/MS	Shaffer S., 2012, Report No. 120611 Knop, M., 2019b, S19-00449

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

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

~~CIECH Sarzyna S.A. possess Letter of Access from the Task Force Clopyralid to alternative data package for active substance Clopyralid. Equivalent analytical methods for the determination of residues in air were included in Data Matching List. This Data Matching List covers all the protected studies from the main notifier.~~

An overview on the acceptable methods and possible data gaps for analysis of Clopyralid in air is given in the following tables. For the detailed evaluation of study please refer to Appendix 2.

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

Component of residue definition: Clopyralid			
Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing
Primary	4.5 µg/m3	LC-MS/MS	Bacher, R., 2012, Report No. 120601 Kirchherr M., 2019, S19-00451

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

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

~~CIECH Sarzyna S.A. possess Letter of Access from the Task Force Clopyralid to alternative data package for active substance Clopyralid. Equivalent analytical methods for the determination of residues in body fluids and tissues were included in Data Matching List. This Data Matching List covers all the protected studies from the main notifier.~~

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

**Table 5.3-9: Methods for body fluids and tissues (if appropriate)**

Component of residue definition: Clopyralid			
Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing
Primary	0.05 mg/kg / mg/L	LC-MS/MS	<del>-, 2014, Report No. 1307297</del> Abe Ch. 2019b, S19-00450

#### 5.3.2.8 Other studies/ information

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## Appendix 1 Lists of data considered in support of the evaluation

Tables considered not relevant can be deleted as appropriate.

MS to blacken authors of vertebrate studies in the version made available to third parties/public.

### 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	Gutowska I.	2019	FAWORYT 300 SL Method validation for determination of the active substance content (clopyralid) in the preparation BA-01/19 INSTITUTE OF INDUSTRIAL ORGANIC CHEMISTRY GLP Unpublished	N	CIECH Sarżyna S.A.
KCP 5.1.2/01 (KCA 6.5.2-6.5.3/01)	White T. Analytical phase: Sayed S., Hamoum N., Hernandez Ch.	2021	Determination of Residues of Clopyralid after One Application of Major 300 SL (CHR/H/CPD 300SL) in Winter Wheat. One site in Northern France and One Site in Southern France During 2019 S19-01810 Eurofins Agroscience Services Ltd. GLP Unpublished	N	Proplan, Plant Protection Company SL  PUH Chemirol Sp. zo.o
KCP 5.1.2/02 (KCA 6.5.2-6.5.3/02)	White T. Analytical Phase: Sayed S. Souchier M.	2021	Determination of Residues of Clopyralid after One Application of Major 300 SL (CHR/H/CPD 300SL) in Spring Wheat. One site in Northern France During 2020 S20-04397 Eurofins Agroscience Services Ltd. GLP Unpublished	N	Proplan, Plant Protection Company SL  PUH Chemirol Sp. zo.o
KCP 5.1.2/04	Świstak M.	2019	Validation of analytical method for the determination of test item Faworyt 300 SL in 50% sucrose solution	N	CIECH

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 5.1.2/03			0016/0051/FA SORBOLAB Research Laboratory LLC GLP Unpublished		Sarzyna S.A.
KCP 5.1.2/02 KCP 5.1.2/04	Świstak M.	2019	Validation of analytical method for the determination of test item Faworyt 300 SL in media for breeding aquatic organisms and deionized water 0016/0055/FA SORBOLAB Research Laboratory LLC GLP Unpublished	N	CIECH Sarzyna S.A.
KCP 5.2/01	Knop M.	2019	Validation of the Multi-Residue Method QuEChERS for the Determination of Clopyralid and X36538 in Different Plant Matrices S19-00446 Eurofins Agroscience Services EcoChem GmbH GLP Unpublished	N	Proplan, Plant Protection Company, SL PUH Chemirol Sp. zo.o.
KCP 5.2/02	Richer S.	2020	Independent Laboratory Validation of an Analytical Method for the Determination of Clopyralid and X36538 in Different Plant Matrices S19-00438 EAG Laboratories GmbH GLP Unpublished	N	Proplan, Plant Protection Company, SL PUH Chemirol Sp. zo.o.
KCP 5.2/03	Abe Ch.	2019	Validation of an Analytical Method for the Determination of Clopyralid in Different Matrices of Animal Origin S19-00447 Eurofins Agroscience Services EcoChem GmbH GLP Unpublished	N	Proplan, Plant Protection Company, SL PUH Chemirol Sp. zo.o.



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 5.2/04	Schweizer M.	2019	Independent Laboratory Validation of an Analytical Method for the Determination of Clopyralid in Different Matrices of Animal Origin P 5210 G EAG Laboratories GmbH GLP Unpublished	N	Proplan, Plant Protection Company, SL  PUH Chemirol Sp. zo.o.
KCP 5.2/05	Knop M.	2019	Validation of an Analytical Method for the Determination of Clopyralid in Soil S19-00448 Eurofins Agrosience Services EcoChem GmbH GLP Unpublished	N	Proplan, Plant Protection Company, SL  PUH Chemirol Sp. zo.o.
KCP 5.2/06	Knop M.	2019	Validation of an Analytical Method for the Determination of Clopyralid in Water S19-00449 Eurofins Agrosience Services EcoChem GmbH GLP Unpublished	N	Proplan, Plant Protection Company, SL  PUH Chemirol Sp. zo.o.
KCP 5.2/07	Richter S.	2019	Independent Laboratory Validation of an Analytical Method for the Determination of Clopyralid in Water P 5211 G EAG Laboratories GmbH GLP Unpublished	N	Proplan, Plant Protection Company, SL  PUH Chemirol Sp. zo.o.
KCP 5.2/08	Kirchherr M.	2019	Clopyralid Validation of an Analytical Method for the Determination in Air S19-00451	N	Proplan, Plant Protection

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
			Eurofins Agroscience Services EcoChem GmbH GLP Unpublished		Company, SL  PUH Chemirol Sp. zo.o.
KCP 5.2/09	Abe Ch.	2019	Development and Validation of an Analytical Method for the Determination of Clopyralid in Body Fluids S19-00450 Eurofins Agroscience Services EcoChem GmbH GLP Unpublished	N	Proplan, Plant Protection Company, SL  PUH Chemirol Sp. zo.o.

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
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The following tables are to be completed by MS

**List of data submitted by the applicant and not relied on**

<b>Data point</b>	<b>Author(s)</b>	<b>Year</b>	<b>Title</b> <b>Company Report No.</b> <b>Source (where different from company)</b> <b>GLP or GEP status</b> <b>Published or not</b>	<b>Vertebrate study Y/N</b>	<b>Owner</b>

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

<b>Data point</b>	<b>Author(s)</b>	<b>Year</b>	<b>Title</b> <b>Company Report No.</b> <b>Source (where different from company)</b> <b>GLP or GEP status</b> <b>Published or not</b>	<b>Vertebrate study Y/N</b>	<b>Owner</b>

## Appendix 2 Detailed evaluation of submitted analytical methods

### A 2.1 Analytical methods for Clopyralid

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

##### A 2.1.1.1 Description of analytical methods for the determination of residues to support residue studies (KCP 5.2)

##### A 2.1.1.1.1 Determination of test item Faworyt 300 SL in winter wheat

##### A 2.1.1.1.1.1 Method validation

Comments of zRMS:	The method is acceptable
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**Reference:** KCP 5.1.2/01; (KCA 6.5.2-6.5.3/01)

**Report** Determination of Residues of Clopyralid after One Application of Major 300 SL (CHR/H/CPD 300SL) in Winter Wheat. 2021, T. White, S19-01810

**Guideline(s):** Yes (SANCO/3029/99 rev.4), SANCO/825/00 rev. 8.1

**Deviations:** No

**GLP:** Yes

**Acceptability:** Yes

##### Materials and methods

The analytical method was initially validated for the determination of clopyralid in wheat grain, wheat straw, wheat germ, white flour, white bread and total bran according to SANCO/3029/99, rev. 4 within analytical phase S19-01810-L2 by fortification of control (untreated) test portions of the respective matrix and subsequent determination of the recoveries. Five fortifications of untreated control samples at the level of LOQ (0.01 mg/kg) and five fortifications at the level of tenfold LOQ (0.1 mg/kg) were performed.

As the objective of the study was to determine the residue levels of total clopyralid (clopyralid and clopyralid conjugate (X36538)), a further analytical phase was included within the study (S19-01810-L3).

##### Analytical phase S19-01810-L2

The analyte was determined by use of LC-MS/MS detection.

##### Chromatographic conditions:

HPLC System	LC30AD, Shimadzu Nexera 2 + Shimadzu SIL 30AC Nexera 2 LC20ADXR, Shimadzu + SIL20ADXR, Shimadzu
Oven	CTO-20AC
Tandem Mass Spectrometer	API 5500 SCIEX
Column HPLC	Agilent ZORBAX Eclipse XDB-C 8 250x4.6mm, 5µm
Column temperature	60°C
Injection volume	10µL
Mobile phase condition	A: ultra-pure water + 0.1% acetic acid

Retention time B: methanol +0.1% acetic acid  
Approx. 4.5min

Mass Spectrometer conditions and MRM transitions:  
MS System API 5500 SCIEX  
Ionisation type Electrospray (ESI, Turbolon Spray)  
Polarity Negative ion mode  
Scan type MS/MS, MRM

## Results and discussions

**Table A 1: Recovery results from method validation of clopyralid using the analytical method**

Matrix	Analyte	Fortification level (mg/kg) (n = 5)	Mean recovery on levels (%)	RSD (%)	Method recovery [%]	Comments
Mass Transition (m/z) 190→ 146 (Proposed for Quantification)						
Wheat grain	clopyralid	0.01	77	3	74	!
		0.1	71	1		!
Wheat germs	clopyralid	0.01	89	3	87	!
		0.1	84	2		!
Wheat straw	clopyralid	0.01	80	2	81	!
		0.1	82	2		!
White flour	clopyralid	0.01	93	2	94	!
		0.1	95	1		!
Total bran	clopyralid	0.01	71	18	74	!
		0.1	77	7		!
White bread	clopyralid	0.01	94	2	95	!
		0.1	96	2		!
Mass Transition (m/z) 192→ 148 (Proposed for Confirmation)						
Wheat grain	clopyralid	0.01	78	4	74	!
		0.1	70	2		!
Wheat germs	clopyralid	0.01	92	3	89	!
		0.1	85	1		!
Wheat straw	clopyralid	0.01	76	3	79	!
		0.1	83	3		!
White flour	clopyralid	0.01	94	2	95	!
		0.1	95	1		!
Total bran	clopyralid	0.01	73	12	76	!
		0.1	80	7		!
White bread	clopyralid	0.01	91	2	94	!
		0.1	97	1		!

**Table A 2: Characteristics for the analytical method used for validation of clopyralid residues in winter wheat**

	<b>Clopyralid</b>
<b>Specificity</b>	For each analyte one mass transition was evaluated. A second mass transition was monitored for confirmation. The blank values at the expected retention times of the analyte of the control sample material that were used for determination of the procedural recoveries did not exceed 30% of the LOQ. Example chromatograms for each matrix and analyte representing at least control samples, the lowest calibration level, sample fortified at the LOQ and treated residue samples showing also reagent blank extracts were presented.
<b>Calibration</b>	The linearity of the detector response was demonstrated by single determination of matrix-matched calibration standards at a minimum of five concentration levels.
<b>Calibration range</b>	- 0.75 ng/mL to 50 ng/mL (wheat grain, wheat germs, total bran, white bread, white flour, white wholemeal flour, wholemeal bread, wheat shorts and wheat middling) - 0.003 mg/kg to 0.50 mg/kg (wheat straw)  Thus covers the range from no more than 30% of the LOQ and at least +20% of the highest analyte concentration detected in any sample extract.  Linear, $R^2 \geq 0.99$
<b>Assessment of matrix effects is presented</b>	Matrix-matched calibration were used
<b>Limit of determination/quantification</b>	LOQ = 0.01 mg/kg

### **Analytical phase S19-01810-L3**

In the analytical phase S19-01810-L3 of the study samples of winter wheat (straw, grain) and its proceed fractions (total bran, shorts, middlings, white bread, wholemeal bread, wheat germs, white flour and wholemeal flour) were analysed for residues of total clopyralid (clopyralid and clopyralid conjugate X36538).

#### **Chromatographic conditions, quantification analysis:**

<b>HPLC System</b>	LC30AD, Nexera X2 + Shimadzu SIL 30AC (Injector) Nexera, Shimadzu (UPLC, $\leq 1000$ bar)
<b>Oven</b>	CTO-20AC
<b>Column HPLC</b>	Agilent ZORBAX Eclipse XDB-C 8 250x4.6mm, 5 $\mu$ m
<b>Column temperature</b>	60°C
<b>Injection volume</b>	10 $\mu$ L
<b>Indicative pressure</b>	80 bar
<b>Mobile phases</b>	Eluent A: Water containing 0.1% (v/v) acetic acid Eluent B: Methanol containing 0.1% (v/v) acetic acid
<b>Retention time</b>	Approx.. 5.84 min

#### **Mass Spectrometer conditions and MRM transitions, quantification analysis:**

<b>MS System</b>	SCIEX TripleQuad 5500 System, SCIEX
<b>Ionisation type</b>	Electrospray (ESI, Turbolon Spray)

Polarity Negative ion mode  
Scan type MS/MS, MRM

Chromatographic conditions, confirmation analysis:

HPLC System LC30AD, Nexera X2 + Shimadzu SIL 30AC (Injector) Nexera, Shimadzu (UPLC, ≤ 1000bar)  
Column HPLC Accucore Phenyl-Hexyl (50 mm x 4.6 mm, 2.6µm, ThermoFisher)  
Column temperature 30°C  
Injection volume 10µL  
Indicative pressure N.R.  
Mobile phases Eluent A: Methanol containing 0.1% (v/v) acetic acid  
Eluent B: Water containing 0.1% (v/v) acetic acid  
Retention time Approx.. 1.8 min

Mass Spectrometer conditions and MRM transitions, confirmation analysis:

MS System SCIEX TripleQuad 5500 System, SCIEX  
Ionisation type Electrospray (ESI, Turbolon Spray)  
Polarity Negative ion mode  
Scan type MS/MS, MRM

Results and discussions

Table A 3: Recovery results from method validation of clopyralid using the analytical method

Matrix*	Analyte	Fortification level (mg/kg) (n = 5)	Mean recovery on levels (%)	RSD (%)	Method recovery [%]	Comments
Mass Transition (m/z) 190→ 146 (Proposed for Quantification)						
White flour	clopyralid	0.044	103	2	103	!
		0.44	104	1		!
Total bran	clopyralid	0.044	98	2	98	!
		0.44	98	2		!
White bread	clopyralid	0.044	95	2	96	!
		0.44	96	2		!
Mass Transition (m/z) 190→146 (Proposed for Confirmation using alternative chromatographic conditions)						
White flour	clopyralid	0.044	105	4	101	!
		0.44	97	2		!
Total bran	clopyralid	0.044	84	1	87	!
		0.44	90	2		!
White bread	clopyralid	0.044	109	3	100	!
		0.44	91	3		!

\*Due to their similarity, the validation results generated for white flour, white bread and total bran are considered to be representative respectively for wholemeal flour, wholemeal bread, shorts and middlings.

**Table A 4: Characteristics for the analytical method used for validation of clopyralid residues in winter wheat**

	<b>Clopyralid</b>
<b>Specificity</b>	One mass transition was evaluated. Example chromatograms for each matrix representing at least control samples, the lowest calibration level, samples fortified at the LOQ, samples fortified at 10xLOQ level and treated residue samples showing also reagent blank extracts were presented.
<b>Calibration</b>	The linearity of the detector response was demonstrated by single determination of matrix-matched calibration standards at a minimum of five concentration levels.
<b>Calibration range</b>	1.5ng/mL to 300 ng/mL which corresponds to fortification level of 0.015 mg/kg to 3.0 mg/kg and thus covers the range from no more than 34% of the LOQ and at least +20% of the highest analyte concentration detected in any sample extract.  Linear, $R^2 \geq 0.990$  The linearity of the detector was extended ranging from 1.0ng/mL to 300 ng/mL, this range corresponds to a fortification level of 0.010 mg/kg (23% of the LOQ) to 3.0 mg/kg.
<b>Assessment of matrix effects is presented</b>	Matrix-matched calibration were used
<b>Limit of determination/quantification</b>	LOQ = 0.044 mg/kg LOD = 0.013 mg/kg

### Conclusion

During the validation of the analytical method the following parameters: selectivity, linearity, accuracy, precision, limit of detection and limit of quantification were determined.  
The analytical method therefore meets the requirements of guideline SANCO/3029/99 rev. 4

## A 2.1.1.1.2 Determination of test item Faworyt 300 SL in spring wheat

### A 2.1.1.1.2.1 Method validation

Comments of zRMS: The method is acceptable

**Reference:** KCP 5.1.2/02; (KCA 6.5.2-6.5.3/02)

**Report** Determination of Residues of Clopyralid after One Application of Major 300 SL (CHR/H/CPD 300SL) in Spring Wheat. T. White, 2021, S20-04397

**Guideline(s):** Yes (SANCO/3029/99 rev.4), SANCO/825/00 rev. 8.1

**Deviations:** No

**GLP:** Yes



Acceptability: Yes

## Materials and methods

The analyte was determined by use of LC-MS/MS detection.

### Chromatographic conditions, quantification analysis:

HPLC System LC30AD, Nexera X2 + Shimadzu SIL 30AC (Injector) Nexera, Shimadzu (UPLC, ≤ 1000bar)  
Oven CTO-20AC  
Column HPLC Agilent ZORBAX Eclipse XDB-C 8 250x4.6mm, 5µm  
Column temperature 60°C  
Injection volume 10µL  
Indicative pressure 80 bar  
Mobile phases Eluent A: Water containing 0.1% (v/v) acetic acid  
Eluent B: Methanol containing 0.1% (v/v) acetic acid  
Retention time Approx.. 5.84 min

### Mass Spectrometer conditions and MRM transitions, quantification analysis:

MS System SCIEX TripleQuad 5500 System, SCIEX  
Ionisation type Electrospray (ESI, Turbolon Spray)  
Polarity Negative ion mode  
Scan type MS/MS, MRM

### Chromatographic conditions, confirmation analysis:

HPLC System LC30AD, Nexera X2 + Shimadzu SIL 30AC (Injector) Nexera, Shimadzu (UPLC, ≤ 1000bar)  
Column HPLC Accucore Phenyl-Hexyl (50 mm x 4.6 mm, 2.6µm, ThermoFisher)  
Column temperature 30°C  
Injection volume 10µL  
Indicative pressure N.R.  
Mobile phases Eluent A: Methanol containing 0.1% (v/v) acetic acid  
Eluent B: Water containing 0.1% (v/v) acetic acid  
Retention time Approx.. 1.8 min

### Mass Spectrometer conditions and MRM transitions, confirmation analysis:

MS System SCIEX TripleQuad 5500 System, SCIEX  
Ionisation type Electrospray (ESI, Turbolon Spray)  
Polarity Negative ion mode  
Scan type MS/MS, MRM

## Results and discussions

**Table A 5: Recovery results from method validation of clopyralid using the analytical method**

Matrix	Analyte	Fortification level (mg/kg) (n = 5)	Mean recovery on levels (%)	RSD (%)	Method recovery [%]	Comments
Mass Transition (m/z) 190→ 146 (Proposed for Quantification)						
Wheat	clopyralid	0.044	89	3	91	!

Matrix	Analyte	Fortification level (mg/kg) (n = 5)	Mean recovery on levels (%)	RSD (%)	Method recovery [%]	Comments
straw		0.44	93	2		!
Wheat grain	clopyralid	0.044	107	8	102	!
		0.44	97	1		!
Wheat germs	clopyralid	0.044	97	18	98	!
		0.44	99	4		!
Mass Transition (m/z) 190→146 (Proposed for Confirmation using alternative chromatographic conditions)						
Wheat straw	clopyralid	0.044	102	6	94	!
		0.44	86	4		!
Wheat grain	clopyralid	0.044	109	5	96	!
		0.44	83	2		!
Wheat germs	clopyralid	0.044	105	13	102	!
		0.44	99	4		!

**Table A 6: Characteristics for the analytical method used for validation of clopyralid residues in spring wheat**

	Clopyralid
Specificity	For each analyte one mass transition was evaluated. A second mass transition was monitored for confirmation. The blank values at the expected retention times of the analyte of the control sample material that were used for determination of the procedural recoveries did not exceed 30% of the LOQ. Example chromatograms for each matrix and analyte representing at least control samples, the lowest calibration level, sample fortified at the LOQ and treated residue samples showing also reagent blank extracts were presented.
Calibration	The linearity of the detector response was demonstrated by single determination of matrix-matched calibration standards at a minimum of five concentration levels.
Calibration range	1.50 ng/mL to 300 ng/mL which corresponds to a fortification level of 0.015 mg/kg to 3.0 mg/kg  The linearity of the detector was extended ranging from 1.0ng/mL to 300 ng/mL, this range corresponds to a fortification level of 0.010 mg/kg (23% of the LOQ) to 3,0 mg/kg.  Thus covers the range from no more than 30% of the LOQ and at least +20% of the highest analyte concentration detected in any sample extract.  Linear, $R^2 \geq 0.99$
Assessment of matrix effects is presented	Matrix-matched calibration were used
Limit of determination/quantification	LOQ = 0.044 mg/kg

	LOD = 0.01 mg/kg
--	------------------

## Conclusion

The method was successfully validated for determination of total clopyralid (clopyralid and clopyralid conjugate X36538) in all matrices with LOQ = 0.044 mg/kg and up to 0.44 mg/kg according to the guidance SANCO/3029/99 rev.4.

## A 2.1.1.2 Description of analytical methods for the determination of residues to support ecotoxicological studies (KCP 5.2)

### A 2.1.1.2.1 Determination of test item Faworyt 300 SL in 50% sucrose solution

#### A 2.1.1.2.1.1 Method validation

Comments of zRMS:	The method is acceptable
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Reference:	KCP 5.1.2/03
Report	Validation of analytical method for the determination of test item Faworyt 300 SL in 50% sucrose solution, Świstak M., 2019, 0016/0051/FA
Guideline(s):	Yes (SANCO/3029/99 rev.4)
Deviations:	No
GLP:	Yes
Acceptability:	Yes

## Materials and methods

Determination of test item was performed by high performance liquid chromatography with diode array detection. Identification of active substance in the test item was based on comparison retention times and UV spectra of standard (clopyralid) solution and the test item. The analysis was performed under following chromatographic conditions:

Column Gemini	C18, 3 µm, 4,6x150 mm
Detection	225 nm
Injection volume	30 µL
Column thermostat temperature	35°C
Mobile phase	A – H <sub>2</sub> O (0.1% H <sub>3</sub> PO <sub>4</sub> ) B – acetonitrile, C – H <sub>2</sub> O B : C 25 : 75 0 – 4 min A : B 75 : 25 4 – 14 min B : C 25 : 75 14 – 19.5 min
Flow of mobile phase	1.1 mL/min

## Results and discussions

**Table A 37:** Recovery results from method validation of Clopyralid using the analytical method

Matrix	Analyte	Fortification level (mg/kg) (n = 5)	Mean recovery on levels (%)	RSD (%)	Method recovery [%]	Comments
sucrose solution	clopyralid	Level I	98.0	0.08	95	-
		Level II	92.4	0.01		-

**Table A 48:** Characteristics for the analytical method used for validation of clopyralid residues in sucrose solution

	Clopyralid
Specificity	In time of the peak from the standard of the active substance no interfering peaks from other substances larger than 3% of the peak area of the active substance in the solution of the test item; under the conditions of analysis, retention time of the active substance in a solution of standard and solution of the test item is comparable (does not differ by more than 2%); registered UV spectrum of active substance standard allow for the identification of active substance in the test item
Calibration (type, number of data points)	Function was linear in full range.  A calibration curve was described by equation: $f(x) = 19411.3 * x + 22343.5$
Calibration range	Solutions with concentrations: 7.69166 mg/L; 14.93255 mg/L; 73.84417 mg/L; 147.02783 mg/L and 221.53494 mg/L were obtained.  Correlation coefficient was equal: $r=0.999$ Criterion of acceptance $r \geq 0.99$ was fulfilled
Precision	Precision of method was designated as repeatability (%RSD) Level 1 = 0.08 Level 2 = 0.01
Assessment of matrix effects is presented	no
Limit of determination/quantification	Limit of detection is: 0.986194 mg/L, and limit of quantification: 2.988467 mg/L

## Conclusion

During the validation of the analytical method the following parameters: selectivity, linearity, accuracy, precision, limit of detection and limit of quantification were determined.

The analytical method therefore meets the requirements of guideline SANCO/3029/99 rev. 4

### A 2.1.1.2.2 Determination of test item Faworyt 300 SL in media for breeding aquatic organisms and deionized water

#### A 2.1.1.2.2.1 Method validation

Comments of zRMS: The method is acceptable

Reference: KCP 5.1.2/04

Report Validation of analytical method for the determination of test item Faworyt 300 SL in in media for breeding aquatic organisms and deionized water, Świstak M., 2019, 0016/0055/FA

Guideline(s): Yes (SANCO/3029/99 rev.4)

Deviations: No

GLP: Yes

Acceptability: Yes

#### Materials and methods

Determination of test item was performed by high performance liquid chromatography with diode array detection. Identification of active substance in the test material was based on comparison retention times of standard (clopyralid) solution and the test item. The analysis was performed under following chromatographic conditions:

Column Gemini C18, 3 µm, 4,6x150 mm  
Detection 225 nm  
Injection volume 100 µL  
Column thermostat temperature 35°C  
Mobile phase ACN:H<sub>2</sub>O (0.1% H<sub>3</sub>PO<sub>4</sub>) 20:80  
Flow of mobile phase 1 mL/min

#### Results and discussions

**Table A 59:** Recovery results from method validation of clopyralid using the analytical method

Matrix	Analyte	Fortification level (mg/kg) (n = 5)	Mean recovery on levels (%)	Method recovery [%]	RSD (%)	Comments
M7	clopyralid	Level I	105.5	97	1.98	-
		Level II	88.9		4.15	-
F2+Si		Level I	93.7	99	0.07	-
		Level II	105.0		6.13	-
Smart&Barco		Level I	98.3	99	0.27	-
		Level II	99.5		13.15	-
Water		Level I	100.9	103	1.92	-
		Level II	104.4		5.85	-

**Table A6-10: Characteristics for the analytical method used for validation of clopyralid residues in different media**

	Clopyralid
Specificity	In time of the peak from the standard of the active substance no interfering peaks from other substances larger than 3% of the peak area of the active substance in the solution of the test item; under the conditions of analysis, retention time of the active substance in a solution of standard and solution of the test item is comparable (does not differ by more than 2%)
Calibration (type, number of data points)	Function was linear in full range.  A calibration curve was described by equation: $f(x)=72393.8 \cdot x + 1392.96$
Calibration range	Solutions with concentrations: 0.10013; 0.25032; 0.50064; 1.00128; 1.66880 mg/L were obtained.  Correlation coefficient was equal: $r=0.999$ Criterion of acceptance $r \geq 0.99$ was fulfilled
Precision	Precision of method was designated as repeatability (% RSD). Results are presented in the Table A3.  In the method used, precision in the analyzed concentration levels were not exceed the value of RSD [%] $\leq 20\%$ .
Assessment of matrix effects is presented	no
Limit of determination/quantification	Limit of detection is: 0.014664 mg/L, and limit of quantification: 0.054727 mg/L

### Conclusion

During the validation of the analytical method the following parameters: selectivity, linearity, accuracy, precision, limit of detection and limit of quantification were determined.

The analytical method therefore meets the requirements of guideline SANCO/3029/99 rev. 4

The validation method 0016/0055/FA was also used for the other studies from the Section B9:

1. Chronic Toxicity Test for Bee Larvae, 0016/0056/E, submitted under the point KCP 10.3.1/3.
2. Reproduction test of *Daphnia magna* according to guideline OECD 211, 0016/0058/E, submitted under the point KCP 10.2.2.
3. Freshwater alga growth inhibition test according to OECD 201, 0016/0057/E, submitted under the point KCP 10.2.1/2.
4. Water-sediment *Myriophyllum spicatum* toxicity test according to OECD 239, 0016/0061/E, submitted under the point KCP 10.2.1/3
5. Seedling emergence and seedling growth test according to OECD 208, 0016/0059/E, submitted under the point KCP 10.6/1
6. Vegetative Vigour Test according to OECD 227, 0016/0060/E, submitted under the point KCP 10.6/2

## 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)

#### A 2.1.2.1.1 Analytical Method Validation for the determination of Clopyralid and X36538 in Different Plant Matrices

Comments of zRMS: The method is acceptable

Reference: KCP 5.2/01

Report Validation of the Multi-Residue Method QuEChERS for the Determination of Clopyralid and X36538 in Different Plant Matrices, M. Knop, 2020 S19-00446

Guideline(s): SANCO/825/00, rev. 8.1

Deviations: No

GLP: Yes

Acceptability: Yes

#### Materials and methods

Samples of tomato, olive, grape and rice were extracted with acetonitrile after addition of water. A salt mixture containing magnesium sulphate, sodium chloride and sodium citrate was added and the extract was shaken. After centrifugation an aliquot of the acetonitrile phase was diluted with water/acetonitrile. Quantification was performed by use of LC-MS/MS detection. For the determination of X36538 the test item was hydrolysed by addition of sodium hydroxide prior to extraction with acetonitrile.

Test Item 1	Clopyralid
Test Item 2	X36538
	(3,6-Dichloropicolinoyl)glycine
Method Reference(s)	Multi-residue method QuEChERS

#### Chromatographic conditions for clopyralid for tomato, grape, olive and rice

Column Oven temperature	40 °C
Mobile phase flow	600 µL/min
Injection volume	10µL
Mobile phase composition	Eluent A: Water containing 1%(v/v) acetic acid Eluent B: Methanol containing 1%(v/v) acetic acid
Retention time(s)	Clopyralid: approx. 2.1 min

#### Chromatographic conditions for Clopyralid after Hydrolysis of X36538 for tomato, grape, olive and rice

Column Oven temperature	40°C
Mobile phase flow	800 µL/min
Injection volume	10 µL
Mobile phase composition	Eluent A: Water containing 1%(v/v) acetic acid

Retention time(s)

Eluent B: Methanol containing 1%(v/v) acetic acid  
Clopyralid: approx. 2.4 min

Mass spectrometric conditions for Clopyralid and Clopyralid after Hydrolysis of X36538 for tomato, grape, olive and rice

MS system

Triple quadrupole mass spectrometer

Ionisation type

Electrospray ionisation

Polarity

Negative ion mode

Scan type

MS/MS, Multiple Reaction Monitoring (MRM)

Capillary voltage (IS)

-4500 V

Mass transition monitored (m/z)

Clopyralid: 192→148  
190→146

## Results and discussions

**Table A 11: Recovery results from method validation of Clopyralid and X36538 using the analytical method**

Matrix	Analyte	Fortification level (mg/kg) (n = 5)	Mean recovery on levels (%)	RSD (%)	Overall Mean Recovery (%)	Overall RSD (%)
Mass Transition <i>m/z</i> 192→148 (Quantification)						
Tomato	Clopyralid	0.01	100	4	96	11
		0.1	92	14		
Olive	Clopyralid	0.01	91	7	92	5
		0.1	92	1		
Grape	Clopyralid	0.01	101	11	98	9
		0.1	94	3		
Rice	Clopyralid	0.01	105	2	99	7
		0.1	92	3		
Mass Transition <i>m/z</i> 190→146 (Confirmation)						
Tomato	Clopyralid	0.01	98	3	95	9
		0.1	92	13		
Olive	Clopyralid	0.01	93	10	93	7
		0.1	93	1		
Grape	Clopyralid	0.01	103	9	99	7
		0.1	95	3		
Rice	Clopyralid	0.01	97	4	93	6
		0.1	89	4		
Mass Transition <i>m/z</i> 192→148 (Quantification)						
Tomato	X36538	0.01	82	4	77	8
		0.1	71	4		
Olive	X36538	0.01	107	7	98	10



Matrix	Analyte	Fortification level (mg/kg) (n = 5)	Mean recovery on levels (%)	RSD (%)	Overall Mean Recovery (%)	Overall RSD (%)
		0.1	90	2		
Grape	X36538	0.01	96	6	86	15
		0.1	75	8		
Rice	X36538	0.01	85	11	88	9
		0.1	92	7		
Mass Transition <i>m/z</i> 190→146 (Confirmation)						
Tomato	X36538	0.01	93	3	88	7
		0.1	83	5		
Olive	X36538	0.01	94	9	92	7
		0.1	90	3		
Grape	X36538	0.01	91	6	85	10
		0.1	78	8		
Rice	X36538	0.01	82	15	84	11
		0.1	86	7		

**Table A 12: Characteristics for the analytical method used for validation of Clopyralid and X36538 residues in tomato, olive, grape and rice**

	Clopyralid and X36538																																	
Specificity	Two (2) mass transitions were evaluated in order to demonstrate that the method achieves a high level of selectivity. No significant interference above 30 % of LOQ was detected in any of the reagent blanks or the control sample extracts of each matrix																																	
Calibration	<p>The linearity of the detector response was demonstrated by single determination of matrix-matched calibration standards at a minimum of six (6) concentration levels ranging from no more than 30 % of the LOQ and at least + 20 % of the highest analyte concentration level detected in a sample extract.</p> <p>The calibration curves obtained for both mass transitions and all matrices were linear since correlation coefficients (R) were <math>\geq 0.995</math>. Linear regression was performed with 1/x-weighting.</p>																																	
Calibration range	<table><tr><th>Matrix</th><th>Calibration range</th><th>Corresponding fortification level</th></tr><tr><td colspan="3">Clopyralid</td></tr><tr><td>Tomato</td><td>0.3 - 25 ng/mL</td><td>0.003 - 0.25 mg/kg</td></tr><tr><td>Olive</td><td>0.15 - 25 ng/mL</td><td>0.003 - 0.50 mg/kg</td></tr><tr><td>Grape</td><td>0.3 - 25 ng/mL</td><td>0.003 - 0.25 mg/kg</td></tr><tr><td>Rice</td><td>0.15 - 25 ng/mL</td><td>0.003 - 0.50 mg/kg</td></tr><tr><td colspan="3">Clopyralid after hydrolysis of X36538</td></tr><tr><td>Tomato</td><td>0.3 - 100 ng/mL</td><td>0.003 – 1.0 mg/kg</td></tr><tr><td>Olive</td><td>0.15 - 25 ng/mL</td><td>0.003 – 2.0 mg/kg</td></tr><tr><td>Grape</td><td>0.3 - 100 ng/mL</td><td>0.003 – 1.0 mg/kg</td></tr><tr><td>Rice</td><td>0.06 - 100 ng/mL</td><td>0.003 – 5.0 mg/kg</td></tr></table>	Matrix	Calibration range	Corresponding fortification level	Clopyralid			Tomato	0.3 - 25 ng/mL	0.003 - 0.25 mg/kg	Olive	0.15 - 25 ng/mL	0.003 - 0.50 mg/kg	Grape	0.3 - 25 ng/mL	0.003 - 0.25 mg/kg	Rice	0.15 - 25 ng/mL	0.003 - 0.50 mg/kg	Clopyralid after hydrolysis of X36538			Tomato	0.3 - 100 ng/mL	0.003 – 1.0 mg/kg	Olive	0.15 - 25 ng/mL	0.003 – 2.0 mg/kg	Grape	0.3 - 100 ng/mL	0.003 – 1.0 mg/kg	Rice	0.06 - 100 ng/mL	0.003 – 5.0 mg/kg
Matrix	Calibration range	Corresponding fortification level																																
Clopyralid																																		
Tomato	0.3 - 25 ng/mL	0.003 - 0.25 mg/kg																																
Olive	0.15 - 25 ng/mL	0.003 - 0.50 mg/kg																																
Grape	0.3 - 25 ng/mL	0.003 - 0.25 mg/kg																																
Rice	0.15 - 25 ng/mL	0.003 - 0.50 mg/kg																																
Clopyralid after hydrolysis of X36538																																		
Tomato	0.3 - 100 ng/mL	0.003 – 1.0 mg/kg																																
Olive	0.15 - 25 ng/mL	0.003 – 2.0 mg/kg																																
Grape	0.3 - 100 ng/mL	0.003 – 1.0 mg/kg																																
Rice	0.06 - 100 ng/mL	0.003 – 5.0 mg/kg																																

Accuracy and Precision	Accuracy was determined by fortification of control samples with known amounts of the test items and subsequent determination of the recoveries when applying the analytical method. Precision was determined by repeatability (relative standard deviation). The analytes were fortified and quantified separately. Results are presented in Table A11.
Assessment of matrix effects is presented	Yes Matrix suppression or enhancement was < 20 % for clopyralid in extracts of tomato and for clopyralid after hydrolysis of X36538 in extracts of olive and thus deemed to be insignificant. However, matrix-matched standards were used for quantification throughout the study. In the remaining matrices, matrix suppression or enhancement was ≥ 20 %, the matrix effect was deemed significant. Again, matrix-matched standards were used for quantification throughout the study.
Limit of determination/quantification	The LOQ is the lowest validated fortification level for each analyte and was thus successfully established at 0.01 mg/kg in tomato, olive, grape and rice for the two (2) mass transitions. The LOD was set at 0.003 mg/kg for all matrices, which is 30 % of the LOQ.
Stability of Clopyralid in Stock and Fortification Solutions	Clopyralid was found to be stable for 17 days when prepared in acetonitrile and stored at 1 °C to 10 °C in the dark.
Stability of Analyte(s) in Sample Extracts	When extracted without hydrolysis, clopyralid was found to be stable in final extracts of all matrices for at least 8 days when stored at 1 °C to 10 °C in the dark. When X36538 is hydrolyzed, clopyralid was found to be stable in final extracts of olive for 8 days when stored at 1 °C to 10 °C in the dark. However, clopyralid was found to be instable in extracts of tomato, grape and rice after storage for at least 8 days at 1 °C to 10 °C in the dark.

## Conclusion

The method was found to be valid according to the guidance document SANCO/825/00, rev 8.1 for the determination of clopyralid in tomato, olive, grape and rice with the tested LOQ of 0.01 mg/kg. All mean recovery values at the fortification levels of 0.01 mg/kg and 0.1 mg/kg for both mass transitions comply with the standard acceptance criteria of SANCO/825/00, rev. 8.1 since mean recoveries were in the range of 70 - 120% with relative standard deviations of ≤ 20% for all analytes and matrices at each level.  
LC-MS-MS method can be regarded as highly specific when two ion transitions have been validated and, thus an additional confirmatory method is not necessary.

### A 2.1.2.1.2 Independent Laboratory Validation for the determination of Clopyralid and X36538 in Different Plant Matrices

Comments of zRMS: The method is acceptable

Reference: KCP 5.2/02

Report: Independent Laboratory Validation of an Analytical Method for the Determination of Clopyralid and X36538 in Different Plant Matrices Steffi Richer, 2020, S19-00438

Guideline(s): SANCO/825/00, rev. 8.1

Deviations: No

GLP: Yes

Acceptability: Yes

## Materials and methods

Samples of plant matrices were extracted with acidified acetonitrile, after addition of water. A salt mixture containing magnesium sulphate and sodium chloride was added and the extract was shaken. After centrifugation, an aliquot of the acetonitrile phase was diluted with acidified water/acetonitrile. For the determination of X36538 the test item was hydrolysed by addition of sodium hydroxide followed by addition of sulphuric acid to quench the reaction, prior to extraction with acidified acetonitrile. Liquid chromatography with tandem mass spectrometry (LC-MS/MS) was used for determination of Clopyralid in Plant Matrices and Clopyralid conjugate X36538 in Plant Matrices.

Test Item 1	Clopyralid
Test Item 2	X36538
	(3,6-Dichloropicolinoyl)glycine
Method Reference(s)	Multi-residue method QuEChERS

## Chromatographic conditions for Clopyralid in Tomato, Olive and Rice

Column Oven temperature	40 °C
Mobile phase flow	500 µL/min
Injection volume	50 µL
Mobile phase composition	Eluent A: Water containing 1% (v/v) acetic acid Eluent B: Methanol containing 1% (v/v) acetic acid
Retention time(s)	Clopyralid: approx. 3.4 min

## Mass spectrometric conditions Clopyralid in Tomato, Olive and Rice

MS system	SCIEX API 5500 QTrap System
Ionisation type	Electrospray (ESI, TurboIon Spray)
Polarity	Negative ion mode
Scan type	MS/MS, Multiple Reaction Monitoring (MRM)
Capillary voltage (IS)	-4500 V
Mass transition monitored (m/z)	Clopyralid: 192→148 190→146

## Results and discussions

**Table A 13: Recovery results from the independent method validation of Clopyralid and X36538 using the analytical method**

Matrix	Analyte	Fortification level (mg/kg) (n = 5)	Mean recovery on levels (%)	RSD (%)	Overall Mean Recovery (%)	Overall RSD (%)
Mass Transition m/z 192→148						
Tomato	Clopyralid	0.01	94	15	101	12
		0.1	108	1.9		
Olive	Clopyralid	0.01	105	3.7	101	8.3
		0.1	98	11		
Rice	Clopyralid	0.01	90	14	91	12
		0.1	93	11		

Matrix	Analyte	Fortification level (mg/kg) (n = 5)	Mean recovery on levels (%)	RSD (%)	Overall Mean Recovery (%)	Overall RSD (%)
Mass Transition <i>m/z</i> 190→146						
Tomato	Clopyralid	0.01	94	15	101	12
		0.1	109	1.9		
Olive	Clopyralid	0.01	102	3.6	101	7.6
		0.1	100	11		
Rice	Clopyralid	0.01	84	14	89	13
		0.1	94	11		
Mass Transition <i>m/z</i> 192→148 (Quantification)						
Tomato	X36538	0.01	94	2.2	96	4.5
		0.1	98	5.4		
Olive	X36538	0.01	73	4.1	73	3.7
		0.1	73	3.8		
Rice	X36538	0.01	Not evaluable			
		0.1				
Mass Transition <i>m/z</i> 190→146 (Confirmation)						
Tomato	X36538	0.01	94	2.1	95	4.0
		0.1	96	5.0		
Olive	X36538	0.01	Not evaluable			
		0.1				
Rice	X36538	0.01	103	7.1	89	18
		0.1	76	12		

**Table A 14: Characteristics for the independent method validation of Clopyralid and X36538 residues in tomato, olive and rice.**

	Clopyralid and X36538
Specificity	Quantification was performed by use of LC-MS/MS detection. Two (2) mass transitions were evaluated in order to demonstrate that the method achieves a high level of selectivity. No significant interference above 20 % of LOQ was detected in any of the reagent blanks or the control sample extracts (except for the analysis of X36538 in olive and rice), so that a high level of selectivity was demonstrated.
Calibration	<p>The linearity of the detector response was demonstrated by single determination of matrix-matched calibration standards at least seven (7) concentration levels ranging from 0.060, 0.15 or 0.30 ng/mL to 25 or 100 ng/mL.</p> <p>These ranges correspond to 0.003 mg/kg to 1.0, 2.0 or 10 mg/kg and thus covers the range from no more than 30 % of the LOQ and at least + 20 % of the highest analyte concentration level detected in a sample extract.</p> <p>The calibration curves obtained for both mass transitions and all matrices were linear since correlation coefficients (R) were <math>\geq 0.99</math>, except for the analysis of X36538 in rice and olive where only 1 mass transitions could be evaluated due to high inferences. Linear regression was performed with 1/x-weighting.</p>

Calibration range	<table border="1"> <thead> <tr> <th>Matrix</th><th>Calibration range (mg/kg)</th></tr> </thead> <tbody> <tr> <td>Tomato</td><td>0.003 - 1.0</td></tr> <tr> <td>Olive</td><td>0.003 - 2.0</td></tr> <tr> <td>Rice</td><td>0.003 - 2.0</td></tr> <tr> <td colspan="2">after hydrolysis of X36538</td></tr> <tr> <td>Tomato</td><td>0.003 - 1.0</td></tr> <tr> <td>Olive</td><td>0.003- 0.50 (m/z 192→148) 0.002- 0.30 (m/z 190→146)</td></tr> <tr> <td>Rice</td><td>0.003 - 5.0</td></tr> </tbody> </table>	Matrix	Calibration range (mg/kg)	Tomato	0.003 - 1.0	Olive	0.003 - 2.0	Rice	0.003 - 2.0	after hydrolysis of X36538		Tomato	0.003 - 1.0	Olive	0.003- 0.50 (m/z 192→148) 0.002- 0.30 (m/z 190→146)	Rice	0.003 - 5.0
Matrix	Calibration range (mg/kg)																
Tomato	0.003 - 1.0																
Olive	0.003 - 2.0																
Rice	0.003 - 2.0																
after hydrolysis of X36538																	
Tomato	0.003 - 1.0																
Olive	0.003- 0.50 (m/z 192→148) 0.002- 0.30 (m/z 190→146)																
Rice	0.003 - 5.0																
Accuracy and Precision	Accuracy was determined by fortification of control samples with known amounts of the test / reference item and subsequent determination of the recoveries when applying the analytical method. Precision was determined by repeatability (relative standard deviation). Results are presented in Table A 13.																
Assessment of matrix effects is presented	Yes Matrix effects on the detection of Clopyralid in extracts of plant origin were found to be mostly significant (> 20 %). Thus, matrix-matched standards were used for quantification.																
Limit of determination/quantification	The LOQ is the lowest validated fortification level and was successfully established at 0.01 mg/kg in Tomato, Olive and Rice. The LOD was set at 0.003 mg/kg for all matrices, which is 30 % of the LOQ.																
Stability of Clopyralid in Stock and Fortification Solutions	Stability of Clopyralid in stock and fortification solutions was shown in the original method validation study. Clopyralid was found to be stable for at least 17 days when prepared in acetonitrile/water (8/2, v/v) and stored at 1 °C to 10 °C in the dark. Stability of X36538 in stock solution was shown in the present study. X36538 was found to be stable for at least 22 days when prepared in acetonitrile containing 0.1% formic acid and stored at 1 °C to 10 °C in the dark.																
Stability of Analyte(s) in Sample Extracts	Stability of the analyte in sample solutions was shown in the original method validation study. Clopyralid was found to be stable in final extracts of Tomato, Olive and Rice for at least 8 days when stored at 1 °C to 10 °C in the dark. For the extraction of Clopyralid after hydrolysis of X36538, extracts of olive were considered to be stable for at least 8 days when stored at 1 °C to 10 °C in the dark.																

### Conclusion

The method was successfully independently validated for the determination of Clopyralid and X36538 in plant matrices from the tested LOQ of 0.01 mg/kg up to 0.10 mg/kg according to the guidance document SANCO/825/00, rev. 8.1.

All mean recovery values at fortification levels of 0.01 mg/kg and 0.10 mg/kg for two (2) mass transitions (for the analysis of X36538 in olive and rice only one (1) transition) are within 70 – 120 % with relative standard deviations ≤ 20 % and thereby comply with the standard acceptance criteria of the guidance document SANCO/825/00, rev. 8.1.

LC-MS-MS method can be regarded as highly specific when two ion transitions have been validated and, thus an additional confirmatory method is not necessary.

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

### A 2.1.2.2.1 Analytical Method Validation for the determination of Clopyralid in animal matrices

Comments of zRMS:	The method is acceptable
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Reference:	KCP 5.2/03
Report	Validation of an Analytical Method for the Determination of Clopyralid in Different Matrices of Animal Origin, Abe Ch., 2019, S19-00447
Guideline(s):	SANCO/825/00, rev. 8.1
Deviations:	No
GLP:	Yes
Acceptability:	Yes

### Materials and methods

Samples of animal tissues (bovine milk, poultry's egg, bovine fat, bovine meat, bovine liver and bovine kidney) were extracted with acidified acetonitrile after addition of water. A salt mixture containing magnesium sulphate and sodium chloride was added and the extract was shaken. After centrifugation an aliquot of the acetonitrile phase was freeze out over night at about -18°C, then diluted with acetonitrile/water containing 0.1% formic acid (1/9 v/v).

Test Item	Clopyralid
Detection	Liquid chromatography with tandem mass spectrometry (LC-MS/MS)
Method Reference(s)	Multi-residue method QuEChERS

### Chromatographic conditions for Clopyralid in animal matrices

Column Oven temperature	40 °C
Mobile phase flow	600 µL/min
Injection volume	30µL
Mobile phase composition	Eluent A: Water containing 1% (v/v) Acetic acid Eluent B: Methanol containing 1% (v/v) Acetic acid
Retention time(s)	Clopyralid: approx. 2.1 min

### Mass spectrometric conditions for Clopyralid in animal matrices

MS system	SCIEX TripleQuad 5500 System, SCIEX
Ionisation type	Electrospray ionisation (ESI, TurboIonSpray)
Polarity	Negative ion mode
Scan type	MS/MS, Multiple Reaction Monitoring (MRM)
Capillary voltage (IS)	-4500 V
Mass transition monitored (m/z)	Clopyralid: 190→146 192→148

### Results and discussions

**Table A 15: Recovery results from the method validation of Clopyralid using the analytical method**

Matrix	Analyte	Fortification level (mg/kg) (n = 5)	Mean recovery on levels (%)	RSD (%)	Overall Mean Recovery (%)	Overall RSD (%)
Mass Transition <i>m/z</i> 190→146 (Quantification)						
Bovine meat	Clopyralid	0.01	88.9	2.5	85.7	4.3
		0.1	82.6	1.0		
Poultry's egg	Clopyralid	0.01	99.8	3.7	92.3	9.0
		0.1	85.8	6.4		
Bovine whole milk	Clopyralid	0.01	91.2	0.9	91.7	1.6
		0.1	92.2	2.1		
Bovine fat	Clopyralid	0.01	98.2	5.1	97.3	3.6
		0.1	96.3	1.0		
Bovine kidney	Clopyralid	0.01	106	1.7	95.9	10.8
		0.1	86.3	3.0		
Bovine liver	Clopyralid	0.01	83.7	2.4	84.6	2.5
		0.1	85.6	2.3		
Mass Transition <i>m/z</i> 192→148 (Confirmation)						
Bovine meat	Clopyralid	0.01	87.5	6.1	85.2	5.1
		0.1	82.8	1.0		
Poultry's egg	Clopyralid	0.01	91.9	3.7	89.0	5.9
		0.1	86.1	6.3		
Bovine whole milk	Clopyralid	0.01	85.3	4.1	89.8	6.1
		0.1	94.4	1.2		
Bovine fat	Clopyralid	0.01	99.6	5.4	97.9	4.1
		0.1	96.2	1.1		
Bovine kidney	Clopyralid	0.01	89.0	12.6	86.5	9.6
		0.1	83.9	4.3		
Bovine liver	Clopyralid	0.01	105	5.9	95.2	11.7
		0.1	85.9	6.0		

**Table A 16: Characteristics for the analytical method used for validation of Clopyralid residues in animal matrices.**

	Clopyralid																				
Specificity	<p>LC-MS/MS determination was conducted by monitoring two (2) mass transitions (<math>m/z</math> 190→146 and <math>m/z</math> 192→148). Mass transition 190→146 <math>m/z</math> is proposed to be used for quantification but both mass transitions are applicable interchangeably for quantification and confirmation.</p> <p>A reagent blank and two (2) control samples per matrix were extracted and analysed according to the method to investigate the presence of residue and/or background interference at the retention time of the analyte. For both mass transitions, the reagent blank showed no significant interference above 30 % of LOQ at the retention time of the Clopyralid, therefore showing that the method is highly specific.</p>																				
Calibration	<p>The linearity of the detector response was demonstrated by single determination of matrix-matched calibration standards at seven (7) concentration levels ranging from 0.10 ng/mL to 15 ng/mL. This range corresponds to a fortification level of 0.002 mg/kg to 0.3 mg/kg and thus covers the range from no more than 30 % of the limit of quantification (LOQ) and at least + 20 % of the highest analyte concentration detected in a (diluted) sample extract.</p> <p>The calibration curves obtained for both ion mass transitions and all matrices were linear since coefficients of determination correlation coefficients (R) were <math>\geq 0.999</math>. Linear regression was performed with 1/x-weighting.</p>																				
Calibration range	<table><tr><th>Matrix</th><th>Calibration range (mg/kg)</th></tr><tr><td colspan="2">Quantification; <math>m/z</math> 190→146</td></tr><tr><td>Bovine meat</td><td rowspan="6">0.002 - 0.3</td></tr><tr><td>Poultry's Egg</td></tr><tr><td>Bovine whole milk</td></tr><tr><td>Bovine fat</td></tr><tr><td>Bovine kidney</td></tr><tr><td>Bovine liver</td></tr><tr><td colspan="2">Qualification; <math>m/z</math> 192→148</td></tr><tr><td>Bovine meat</td><td rowspan="6">0.002 - 0.3</td></tr><tr><td>Poultry's Egg</td></tr><tr><td>Bovine whole milk</td></tr><tr><td>Bovine fat</td></tr><tr><td>Bovine kidney</td></tr><tr><td>Bovine liver</td></tr></table>	Matrix	Calibration range (mg/kg)	Quantification; $m/z$ 190→146		Bovine meat	0.002 - 0.3	Poultry's Egg	Bovine whole milk	Bovine fat	Bovine kidney	Bovine liver	Qualification; $m/z$ 192→148		Bovine meat	0.002 - 0.3	Poultry's Egg	Bovine whole milk	Bovine fat	Bovine kidney	Bovine liver
Matrix	Calibration range (mg/kg)																				
Quantification; $m/z$ 190→146																					
Bovine meat	0.002 - 0.3																				
Poultry's Egg																					
Bovine whole milk																					
Bovine fat																					
Bovine kidney																					
Bovine liver																					
Qualification; $m/z$ 192→148																					
Bovine meat	0.002 - 0.3																				
Poultry's Egg																					
Bovine whole milk																					
Bovine fat																					
Bovine kidney																					
Bovine liver																					
Accuracy and Precision	<p>Accuracy was determined by fortification of control samples with known amounts of Clopyralid and subsequent determination of the recoveries when applying the analytical method. Precision was determined by repeatability (relative standard deviation).</p> <p>Five (5) recovery determinations were performed at 0.01 mg/kg and at 0.1 mg/kg, respectively. Analysis was performed by single extraction and single injection. Results are presented in Table A 15.</p>																				
Assessment of matrix effects is presented	<p>Yes</p> <p>Matrix effects were <math>\geq \pm 20</math> % and deemed to be significant for bovine liver and bovine kidney. Therefore, matrix-matched standards were used for quantification throughout the study.</p> <p>Matrix suppression or enhancement was <math>&lt; 20</math> % for bovine meat, poultry's egg, bovine whole milk and bovine fat and thus deemed to be insignificant. However, matrix-matched standards were used for quantification throughout the study.</p>																				
Limit of determination/quantification	<p>The LOQ of the method is defined as the lowest analyte concentration at which the methodology had been successfully validated. Thus, an LOQ of 0.01 mg/kg was confirmed for Clopyralid in animal tissues.</p> <p>The LOD was set at 20 % of the LOQ which is 0.002 mg/kg.</p>																				



Stability of Clopyralid in Stock and Fortification Solutions	<p>The stock solution: The mean peak area of the stored diluted stock solution was within <math>\pm 20</math> % of the mean peak area of the freshly prepared diluted stock solutions indicating that stock solutions are stable when stored at 1 °C to 10 °C in the dark for 55 days.</p> <p>The fortification solutions: The mean peak area of the stored diluted stock solution was within <math>\pm 20</math> % of the mean peak area of the freshly prepared diluted stock solutions indicating that stock solutions are stable when stored at 1 °C to 10 °C in the dark for 21 days.</p>
Stability of Analyte(s) in Sample Extracts	<p>The final extracts of samples fortified at the 10xLOQ level together with two (2) control sample extract were stored at typically 1 °C to 10 °C in the dark for at least 7 days. After this period, the final extracts were re-analysed against freshly prepared calibration standards.</p> <p>The mean recovery value(s) of the re-analysed extracts were in the range of 70 - 120 % and within <math>\pm 20</math> % of the original result. Therefore, extracts are considered to be stable when stored at 1 °C to 10 °C for at least 7 days in the dark.</p>

### Conclusion

The method was successfully validated for the determination of Clopyralid in animal tissues from the tested LOQ of 0.01 mg/kg up to 0.1 mg/kg according to the guidance document SANCO/825/00, rev. 8.1. All mean recovery values at the fortification levels of 0.01 mg/kg and 0.1 mg/kg for both mass comply with the standard acceptance criteria of SANCO/825/00, rev. 8. since mean recoveries were in the range of 70 - 120 % with relative standard deviations of  $\leq 20$  at each level.

LC-MS-MS method can be regarded as highly specific when two ion transitions have been validated and, thus an additional confirmatory method is not necessary.

### A 2.1.2.2.2 Independent Laboratory Validation for the determination of Clopyralid in animal matrices

Comments of zRMS:	The method is acceptable
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Reference:	KCP 5.2/04
Report	Independent Laboratory Validation of an Analytical Method for the Determination of Clopyralid in Different Matrices of Animal Origin, Schweizer M., 2019, P 5210 G
Guideline(s):	SANCO/825/00, rev. 8.1
Deviations:	No
GLP:	Yes
Acceptability:	Yes

### Materials and methods

Samples of animal matrices (poultry's eggs, bovine fat, bovine meat) were extracted with acidified acetonitrile, after addition of water, hydrolysis with sodium hydroxide and neutralisation with sulphuric acid. A salt mixture containing magnesium sulphate, sodium chloride was added and the extract was shaken. After centrifugation, an aliquot of the acetonitrile phase was cleaned by freezing out at  $\leq 18^{\circ}\text{C}$ .

Test Item	Clopyralid
Detection	Liquid chromatography with tandem mass spectrometry (LC-MS/MS)
Method Reference(s)	Multi-residue method QuEChERS

#### Chromatographic conditions for Clopyralid in animal matrices

Column Oven temperature	40 °C
Mobile phase flow	600 µL/min
Injection volume	50 µL
Mobile phase composition	Eluent A: Water containing 1 % (v/v) Acetic acid Eluent B: Methanol containing 1 % (v/v) Acetic acid
Retention time(s)	Clopyralid: approx. 2.2 min

#### Mass spectrometric conditions for Clopyralid in animal matrices

MS system	SCIEX API 5500 QTrap System
Ionisation type	Electrospray (ESI, TurboIon Spray)
Polarity	Negative ion mode
Scan type	MS/MS, Multiple Reaction Monitoring (MRM)
Capillary voltage (IS)	-4500 V
Mass transition monitored (m/z)	Clopyralid: 192→148 190→146

### Results and discussions

**Table A 17: Recovery results from the independent method validation of Clopyralid using the analytical method**

Matrix	Analyte	Fortification level (mg/kg) (n = 5)	Mean recovery on levels (%)	RSD (%)	Overall Mean Recovery (%)	Overall RSD (%)
Mass Transition m/z 192→148 Fragment m/z 148 (Proposed for Quantification)						
Bovine meat	Clopyralid	0.01	72	2.2	72	1.8
		0.1	71	1.3		
Poultry's egg	Clopyralid	0.01	74	2.7	77	6.4
		0.1	81	5.8		
Bovine fat	Clopyralid	0.01	106	3.6	103	4.1
		0.1	100	0.77		
Mass Transition m/z 190→146 Fragment m/z 146 (Proposed for Confirmation)						
Bovine meat	Clopyralid	0.01	77	4.5	75	5.1
		0.1	72	1.9		
Poultry's egg	Clopyralid	0.01	76	4.5	79	7.0
		0.1	83	6.4		
Bovine fat	Clopyralid	0.01	93	5.4	95	4.3
		0.1	96	1.9		

**Table A 18: Characteristics for the independent method validation of Clopyralid residues in animal matrices.**

	Clopyralid														
Specificity	Quantification was performed by use of LC-MS/MS detection. Two (2) mass transitions were evaluated in order to demonstrate that the method achieves a high level of selectivity. No significant interference above 20 % of LOQ was detected in any of the reagent blanks or the control sample extracts of Bovine Meat, Bovine Fat and Poultry's Egg, so that a high level of selectivity was demonstrated.														
Calibration	The linearity of the detector response was demonstrated by single determination of matrix-matched calibration standards at seven (7) concentration levels ranging from 0.10 ng/mL to 15 ng/mL. This range corresponds to 0.002 mg/kg to 0.3 mg/kg and thus covers the range from no more than 20 % of the LOQ and at least + 20 % of the highest analyte concentration level detected in a sample extract. The calibration curves obtained for both mass transitions and all matrices were linear since correlation coefficients (R) were ≥ 0.99. Linear regression was performed with 1/x-weighting.														
Calibration range	<table><tr><td>Matrix</td><td>Calibration range (mg/kg)</td></tr><tr><td colspan="2">Quantification; m/z 192→148</td></tr><tr><td>Bovine meat</td><td rowspan="3">0.002 - 0.3</td></tr><tr><td>Poultry's Egg</td></tr><tr><td>Bovine fat</td></tr><tr><td colspan="2">Qualification; m/z 190→146</td></tr><tr><td>Bovine meat</td><td rowspan="3">0.002 - 0.3</td></tr><tr><td>Poultry's Egg</td></tr><tr><td>Bovine fat</td></tr></table>	Matrix	Calibration range (mg/kg)	Quantification; m/z 192→148		Bovine meat	0.002 - 0.3	Poultry's Egg	Bovine fat	Qualification; m/z 190→146		Bovine meat	0.002 - 0.3	Poultry's Egg	Bovine fat
Matrix	Calibration range (mg/kg)														
Quantification; m/z 192→148															
Bovine meat	0.002 - 0.3														
Poultry's Egg															
Bovine fat															
Qualification; m/z 190→146															
Bovine meat	0.002 - 0.3														
Poultry's Egg															
Bovine fat															
Accuracy and Precision	Accuracy was determined by fortification of control samples with known amounts of the test / reference item and subsequent determination of the recoveries when applying the analytical method. Precision was determined by repeatability (relative standard deviation) Results are presented in Table A 17.														
Assessment of matrix effects is presented	Yes Matrix effects on the detection of clopyralid in extracts of animal origin were found to be insignificant (< 20 %). However, matrix-matched standards were used for quantification.														
Limit of determination/quantification	The LOQ is the lowest validated fortification level and was successfully established at 0.01 mg/kg in Bovine Meat, Bovine Fat and Poultry's Egg for the two (2) mass transitions. The LOD was set at 0.002 mg/kg for all matrices, which is 20 % of the LOQ.														
Stability of Clopyralid in Stock and Fortification Solutions	Stability of the analyte in stock and fortification solutions was shown in the original method validation study. Clopyralid was found to be stable for at least 21 when prepared in acetonitrile/water (8/2, v/v) and stored at 1 °C to 10 °C in the dark.														
Stability of Analyte(s) in Sample Extracts	Stability of the analyte in sample solutions was shown in the original method validation study. Clopyralid was found to be stable in final extracts of Bovine Meat and Poultry's Egg for at least 7 days and at least 12 days for Bovine Fat extracts when stored at 1 °C to 10 °C in the dark.														

## Conclusion

The method was successfully independently validated for the determination of clopyralid in different Matrices of Animal Origin from the tested LOQ of 0.01 mg/kg up to 0.10 mg/kg according to the guidance document SANCO/825/00, rev. 8.1.

All mean recovery values at the fortification levels of 0.01 mg/kg and 0.1 mg/kg for both mass transitions comply with the standard acceptance criteria of SANCO/825/00, rev. 8.1 since mean recoveries were in the range of 70 - 120 % with relative standard deviations of  $\leq 20$  % for clopyralid at each level. LC-MS-MS method can be regarded as highly specific when two ion transitions have been validated and, thus an additional confirmatory method is not necessary.

### A 2.1.2.3 Description of Methods for the Analysis of Soil (KCP 5.2)

#### A 2.1.2.3.1 Analytical Method Validation for the determination of Clopyralid in Soil

Comments of zRMS:	The method is acceptable
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Reference:	KCP 5.2/05
Report	Validation of an Analytical Method for the Determination of Clopyralid in Soil, 2019, Knop M., S19-00448
Guideline(s):	SANCO/825/00, rev. 8.1
Deviations:	No
GLP:	Yes
Acceptability:	Yes

#### Materials and methods

Clopyralid was applied to 10.0 g soil samples in 50 mL tubes. The samples were extracted twice with 10 mL acetonitrile/1 % formic acid (1/9, v/v) followed by 30 minutes shaking at 150 rpm and centrifugation for 5 minutes at 4000 rpm. Sample extracts were decanted and collected in a 50 mL tube and the volume was adjusted to 25 mL with the extraction solvent. Quantification of the undiluted extracts was performed by LCMS/MS detection.

Test Item	Clopyralid
Detection	Liquid chromatography with tandem mass spectrometry (LC-MS/MS)
Method Reference(s)	Multi-residue method QuEChERS

#### Chromatographic conditions for Clopyralid in Soil

Column Oven temperature	40 °C
Mobile phase flow	600 µL/min
Injection volume	30 µL
Mobile phase composition	Eluent A: Water containing 1 % (v/v) Acetic acid Eluent B: Methanol containing 1 % (v/v) Acetic acid
Retention time(s)	Clopyralid: approx. 2.2 min

#### Mass spectrometric conditions for Clopyralid in Soil

MS system	API 5500™ LC-MS/MS System (Sciex)
Ionisation type	Electrospray (ESI, TurboIon Spray)
Polarity	Negative ion mode
Scan type	MS/MS, Multiple Reaction Monitoring (MRM)
Capillary voltage (IS)	-4500 V

Mass transition monitored (m/z)

Clopyralid: 192→148  
190→146

## Results and discussions

**Table A 19: Recovery results from method validation of Clopyralid using the analytical method**

Matrix	Analyte	Fortification level (µg/kg) (n = 5)	Mean recovery on levels (%)	RSD (%)	Overall Mean Recovery (%)	Overall RSD (%)
Mass Transition <i>m/z</i> 192→148 (Quantification)						
Soil	Clopyralid	0.5	87	7	86	5
		5.0	85	2		
Mass Transition <i>m/z</i> 190→146 (Confirmation)						
Soil	Clopyralid	0.5	79	2	83	5
		5.0	87	2		

**Table A 20: Characteristics for the analytical method used for validation of Clopyralid residues in Soil**

	Clopyralid										
Specificity	Quantification was performed by use of LC-MS/MS detection. Two (2) mass transitions ((m/z 192→148 and m/z 190→146) were evaluated in order to demonstrate that the method achieves a high level of selectivity. No significant interference above 30 % of LOQ was detected in any of the reagent blanks or the control sample extracts, so that a high level of selectivity was demonstrated.										
Calibration	The linearity of the detector response was demonstrated by single determination of matrix-matched calibration standards at nine (9) concentration levels ranging from 0.06 ng/mL to 20 ng/mL. This range corresponds to 0.15 µg/kg to 50 µg/kg and thus covers the range from no more than 30 % of the LOQ and at least + 20 % of the highest analyte concentration level detected in a sample extract. The calibration curves obtained for both mass transitions and all matrices were linear since correlation coefficients (R) were ≥ 0.99. Linear regression was performed with 1/x-weighting.										
Calibration range	<table border="1"> <tr> <th>Matrix</th><th>Calibration range (µg/kg)</th></tr> <tr> <td colspan="2">Quantification; m/z 192→148</td></tr> <tr> <td>Soil</td><td>0.15 - 50</td></tr> <tr> <td colspan="2">Qualification; m/z 190→146</td></tr> <tr> <td>Soil</td><td>0.15 - 50</td></tr> </table>	Matrix	Calibration range (µg/kg)	Quantification; m/z 192→148		Soil	0.15 - 50	Qualification; m/z 190→146		Soil	0.15 - 50
Matrix	Calibration range (µg/kg)										
Quantification; m/z 192→148											
Soil	0.15 - 50										
Qualification; m/z 190→146											
Soil	0.15 - 50										
Accuracy and Precision	Accuracy was determined by fortification of control samples with known amounts of the test item and subsequent determination of the recoveries when applying the analytical method. Precision was determined by repeatability (relative standard deviation). Results are presented in Table A 19.										
Assessment of matrix effects is presented	Yes Matrix effects on the detection of Clopyralid in extracts of soil LUFA F 2.1 were found to be significant (≥ 20 %). Therefore, matrix-matched standards were used for quantification.										

Limit of determination/quantification	The LOQ is the lowest validated fortification level and was thus successfully established at 0.5 µg/kg in soil for the two (2) mass transitions. The LOD was set at 0.15 µg/kg, which is 30 % of the LOQ.
Stability of Clopyralid in Stock and Fortification Solutions	Clopyralid was found to be stable in stock solutions for 11 days when prepared in acetonitrile/ water (8/2, v/v) and stored at typically 1 °C to 10 °C in the dark. Stability of fortification solutions was not tested since the solutions were prepared in the same solvent as the stock solution.
Stability of Analyte(s) in Sample Extracts	Clopyralid was found to be stable in final extracts of for 14 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 SANCO/825/00, rev 8.1 for the determination of Clopyralid in soil with the tested LOQ of 0.5 µg/kg.  
All mean recovery values at the fortification levels of 0.5 µg/kg and 5.0 µg/kg for both mass transitions comply with the standard acceptance criteria of SANCO/825/00, rev. 8.1 since mean recoveries were in the range of 70 - 120 % with relative standard deviations of ≤ 20 % for Clopyralid in soil at each level.  
LC-MS-MS method can be regarded as highly specific when two ion transitions have been validated and, thus an additional confirmatory method is not necessary.

### A 2.1.2.4 Description of Methods for the Analysis of Water (KCP 5.2)

#### A 2.1.2.4.1 Analytical Method Validation for the determination of Clopyralid in Water

Comments of zRMS:	The method is acceptable
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Reference:	KCP 5.2/06
Report	Validation of an Analytical Method for the Determination of Clopyralid in Water, Knop M., 2019, S19-00449
Guideline(s):	SANCO/825/00, rev. 8.1
Deviations:	No
GLP:	Yes
Acceptability:	Yes

## Materials and methods

Samples of drinking and surface water were analysed by direct injection using LC-MS/MS detection, with no need of performing an extraction step.

Test Item	Clopyralid
Detection	Liquid chromatography with tandem mass spectrometry (LC-MS/MS)
Chromatographic conditions for Clopyralid in Water	
Column Oven temperature	40 °C
Mobile phase flow	600 µL/min
Injection volume	30 µL
Mobile phase composition	Eluent A: Water containing 1% (v/v) Acetic acid Eluent B: Methanol containing 1% (v/v) Acetic

Retention time(s)	acid Clopyralid: approx. 2.2 min
Mass spectrometric conditions for Clopyralid in Water	
MS system	API 5500™ LC-MS/MS System (Sciex)
Ionisation type	Electrospray (ESI, TurboIon Spray)
Polarity	Negative ion mode
Scan type	MS/MS, Multiple Reaction Monitoring (MRM)
Capillary voltage (IS)	-4500 V
Mass transition monitored (m/z)	Clopyralid: 192→148 190→146

## Results and discussions

**Table A 21: Recovery results from method validation of Clopyralid using the analytical method**

Matrix	Analyte	Fortification level (µg/L) (n = 5)	Mean recovery on levels (%)	RSD (%)	Overall Mean Recovery (%)	Overall RSD (%)
Mass Transition <i>m/z</i> 192→148 (Quantification)						
Drinking Water	Clopyralid	0.05	79	6	87	11
		0.5	96	2		
Surface Water	Clopyralid	0.05	85	5	92	8
		0.5	98	2		
Mass Transition <i>m/z</i> 190→146 (Confirmation)						
Drinking Water	Clopyralid	0.05	96	9	94	6
		0.5	93	3		
Surface Water	Clopyralid	0.05	96	8	95	6
		0.5	94	2		

**Table A 22: Characteristics for the analytical method used for validation of Clopyralid residues in Water**

	Clopyralid
Specificity	Quantification was performed by use of LC-MS/MS detection. Two (2) mass transitions were evaluated in order to demonstrate that the method achieves a high level of selectivity. No significant interference above 30 % of LOQ was detected in any of the reagent blanks or the control sample extracts of each matrix, so that a high level of selectivity was demonstrated.
Calibration	The linearity of the detector response was demonstrated by single determination of matrix-matched calibration standards at eight (8) concentration levels ranging from 0.015 ng/mL to 10 ng/mL. This range corresponds to 0.015 µg/L to 10 µg/L and thus covers the range from no more than 30 % of the LOQ and at least + 20 % of the highest analyte concentration level detected in a sample extract. The calibration curves obtained for both mass transitions and all matrices were linear since correlation coefficients (R) were ≥ 0.99. Linear regression was performed with 1/x-weighting.

Calibration range	<table border="1"> <tr> <th>Matrix</th><th>Calibration range (µg/L)</th></tr> <tr> <td colspan="2">Quantification; <math>m/z</math> 192→148</td></tr> <tr> <td>Water (drinking and surface)</td><td>0.015 – 10</td></tr> <tr> <td colspan="2">Qualification; <math>m/z</math> 190→146</td></tr> <tr> <td>Water (drinking and surface)</td><td>0.015 – 10</td></tr> </table>	Matrix	Calibration range (µg/L)	Quantification; $m/z$ 192→148		Water (drinking and surface)	0.015 – 10	Qualification; $m/z$ 190→146		Water (drinking and surface)	0.015 – 10
Matrix	Calibration range (µg/L)										
Quantification; $m/z$ 192→148											
Water (drinking and surface)	0.015 – 10										
Qualification; $m/z$ 190→146											
Water (drinking and surface)	0.015 – 10										
Accuracy and Precision	Accuracy was determined by fortification of control samples with known amounts of the test item and subsequent determination of the recoveries when applying the analytical method. Precision was determined by repeatability (relative standard deviation). Results are presented in Table A 21.										
Assessment of matrix effects is presented	Yes Matrix effects on the detection of Clopyralid in drinking and surface water were found to be insignificant (< 20 %). However, matrix-matched standards were used for quantification.										
Limit of determination/quantification	The LOQ is the lowest validated fortification level and was thus successfully established at 0.05 µg/L in drinking and surface water for the two (2) mass transitions. The LOD was set at 0.015 µg/L, which is 30 % of the LOQ.										
Stability of Clopyralid in Stock and Fortification Solutions	Clopyralid was found to be stable for 11 days when prepared in acetonitrile/water (8/2, v/v) and stored at typically 1 °C to 10 °C in the dark. Stability of fortification solutions was not tested since the solutions were prepared in the same solvent as the stock solution.										
Stability of Analyte(s) in Sample Extracts	Clopyralid was found to be stable in final extracts of for 13 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 SANCO/825/00, rev 8.1 for the determination of Clopyralid in water with the tested LOQ of 0.05 µg/L.

All mean recovery values at the fortification levels of 0.05 µg/L and 0.5 µg/L, for both mass transitions comply with the standard acceptance criteria of SANCO/825/00, rev. 8.1 since mean recoveries were in the range of 70 - 120 % with relative standard deviations of ≤ 20 % for Clopyralid in all matrices at each level.

LC-MS-MS method can be regarded as highly specific when two ion transitions have been validated and, thus an additional confirmatory method is not necessary.

### A 2.1.2.4.2 Independent method Validation for the determination of Clopyralid in Water

Comments of zRMS:	The method is acceptable
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Reference: KCP 5.2/07

Report: Independent Laboratory Validation of an Analytical Method for the Determination of Clopyralid in Water, Richter S., 2019, P 5211 G

Guideline(s): SANCO/825/00, rev. 8.1

Deviations: No

GLP: Yes

Acceptability: Yes



## Materials and methods

Samples of drinking water were analysed by direct injection using LC-MS/MS detection, with no need of performing an extraction step.

Test Item	Clopyralid
Detection	Liquid chromatography with tandem mass spectrometry (LC-MS/MS)
Chromatographic conditions for Clopyralid in drinking water	
HPLC system	1290 Infinity Binary LC System, Agilent Technologies
Column Oven temperature	40 °C
Mobile phase flow	600 µL/min
Injection volume	50 µL
Mobile phase composition	Eluent A: Water containing 1 % (v/v) Acetic acid Eluent B: Methanol containing 1 % (v/v) Acetic acid
Retention time(s)	Clopyralid: approx. 2.3 min
Mass spectrometric conditions for Clopyralid in drinking water	
MS system	SCIEX API 5500 QTrap System
Ionisation type	Electrospray (ESI, TurboIon Spray)
Polarity	Negative ion mode
Scan type	MS/MS, Multiple Reaction Monitoring (MRM)
Capillary voltage (IS)	-4500 V
Mass transition monitored (m/z)	Clopyralid: 192→148 190→146

## Results and discussions

**Table A 23: Recovery results from method validation of Clopyralid using the analytical method**

Matrix	Analyte	Fortification level (µg/L) <i>(n = 5)</i>	Mean recovery on levels (%)	RSD (%)	Overall Mean Recovery (%)	Overall RSD (%)
Mass Transition <i>m/z</i> 192→148 (Quantification)						
Drinking Water	Clopyralid	0.05	79	6	87	11
		0.5	96	2		
Surface Water	Clopyralid	0.05	85	5	92	8
		0.5	98	2		
Mass Transition <i>m/z</i> 190→146 (Confirmation)						
Drinking Water	Clopyralid	0.05	96	9	94	6
		0.5	93	3		
Surface Water	Clopyralid	0.05	96	8	95	6
		0.5	94	2		

**Table A 24: Characteristics for the analytical method used for validation of Clopyralid residues in drinking water**

	<b>Clopyralid</b>										
<b>Specificity</b>	Quantification was performed by use of LC-MS/MS detection. Two (2) mass transitions were evaluated in order to demonstrate that the method achieves a high level of selectivity. No significant interference above 30 % of LOQ was detected in any of the reagent blanks or the control sample extracts of each matrix, so that a high level of selectivity was demonstrated.										
<b>Calibration</b>	The linearity of the detector response was demonstrated by single determination of matrix-matched calibration standards at eight (8) concentration levels ranging from 0.015 ng/mL to 10 ng/mL. This range corresponds to 0.015 µg/L to 10 µg/L and thus covers the range from no more than 30 % of the LOQ and at least + 20 % of the highest analyte concentration level detected in a sample extract. The calibration curves obtained for both mass transitions and all matrices were linear since correlation coefficients (R) were ≥ 0.99. Linear regression was performed with 1/x-weighting.										
<b>Calibration range</b>	<table border="1"> <thead> <tr> <th>Matrix</th><th>Calibration range (µg/L)</th></tr> </thead> <tbody> <tr> <td colspan="2">Quantification; <math>m/z</math> 192→148</td></tr> <tr> <td>Water (drinking and surface)</td><td>0.015 – 10</td></tr> <tr> <td colspan="2">Qualification; <math>m/z</math> 190→146</td></tr> <tr> <td>Water (drinking and surface)</td><td>0.015 – 10</td></tr> </tbody> </table>	Matrix	Calibration range (µg/L)	Quantification; $m/z$ 192→148		Water (drinking and surface)	0.015 – 10	Qualification; $m/z$ 190→146		Water (drinking and surface)	0.015 – 10
Matrix	Calibration range (µg/L)										
Quantification; $m/z$ 192→148											
Water (drinking and surface)	0.015 – 10										
Qualification; $m/z$ 190→146											
Water (drinking and surface)	0.015 – 10										
<b>Accuracy and Precision</b>	Accuracy was determined by fortification of control samples with known amounts of the test item and subsequent determination of the recoveries when applying the analytical method. Precision was determined by repeatability (relative standard deviation). Results are presented in Table A 23.										
<b>Assessment of matrix effects is presented</b>	Yes Matrix effects on the detection of Clopyralid in drinking and surface water were found to be insignificant (< 20 %). However, matrix-matched standards were used for quantification.										
<b>Limit of determination/quantification</b>	The LOQ is the lowest validated fortification level and was thus successfully established at 0.05 µg/L in drinking and surface water for the two (2) mass transitions. The LOD was set at 0.015 µg/L, which is 30% of the LOQ.										
<b>Stability of Clopyralid in Stock and Fortification Solutions</b>	Clopyralid was found to be stable for 11 days when prepared in acetonitrile/water (8/2, v/v) and stored at typically 1 °C to 10 °C in the dark. Stability of fortification solutions was not tested since the solutions were prepared in the same solvent as the stock solution.										
<b>Stability of Analyte(s) in Sample Extracts</b>	Clopyralid was found to be stable in final extracts of for 13 days when stored at typically 1°C to 10°C in the dark.										

## Conclusion

The method was successfully independently validated for the determination of clopyralid in drinking water from the tested LOQ of 0.05µg/L up to 0.50µg/L according to the guidance document SANCO/825/00, rev. 8.1.

All mean recovery values at fortification levels of 0.05 µg/L and 0.50 µg/L for two (2) mass transitions are within 70 – 120% with relative standard deviations ≤ 20% and thereby comply with the standard acceptance criteria of the guidance document SANCO/825/00, rev. 8.1.

LC-MS-MS method can be regarded as highly specific when two ion transitions have been validated and, thus an additional confirmatory method is not necessary.

## A 2.1.2.5 Description of Methods for the Analysis of Air (KCP 5.2)

### A 2.1.2.5.1 Analytical Method Validation for the determination of Clopyralid in Air

Comments of zRMS: The method is acceptable

Reference: KCP 5.2/08

Report: Clopyralid Validation of an Analytical Method for the Determination in Air, Kirchherr M., 2019, S19-00451

Guideline(s): SANCO/825/00, rev. 8.1

Deviations: No

GLP: Yes

Acceptability: Yes

#### Materials and methods

Clopyralid was spiked onto the front filter of an adsorbent tube consisting of two units (front and backup bed) filled with adsorbent material. Air was passed through the filter at a constant flow rate of 1 L/min with a sampling period of 8 hours. Clopyralid was extracted from the adsorbent material with 5 mL methanol on a flatbed shaker for 60 min at 150 rpm.

Test Item	Clopyralid
Detection	Liquid chromatography with tandem mass spectrometry (LC-MS/MS)

Chromatographic conditions	
HPLC system	Shimadzu HPLC pump
Column Oven temperature	40 °C
Mobile phase flow	600 µL/min
Injection volume	20 µL
Mobile phase composition	Eluent A: Water containing 1 % Acetic acid Eluent B: Methanol containing 1 % Acetic acid
Retention time(s)	Clopyralid: approx. 2.2 min

Mass spectrometric conditions	
MS system	API 5500™ LC-MS/MS System (Sciex)
Ionisation type	Electrospray (ESI, Turbo Ion Spray)
Polarity	Negative ion mode
Scan type	MS/MS, Multiple Reaction Monitoring (MRM)
Capillary voltage (IS)	-4500 V
Mass transition monitored (m/z)	Clopyralid: 192→148 190→146

#### Results and discussions

Table A 25: Recovery results from the method validation of Clopyralid using the analytical

## method

Matrix	Analyte	Fortification level (µg/m³) <i>(n = 5)</i>	Mean recovery on levels (%)	RSD (%)	Overall Mean Recovery (%)	Overall RSD (%)
Mass Transition <i>m/z</i> 192→148 (Quantification)						
Air	Clopyralid	4.5	94	0	96	3
		45	98	4		
Mass Transition <i>m/z</i> 190→146 (Confirmation)						
Air	Clopyralid	4.5	95	1	97	4
		45	99	4		

**Table A 26: Characteristics for the analytical method used for validation of Clopyralid residues in Air**

	Clopyralid				
Specificity	Two selected ion mass transitions were evaluated in order to demonstrate that the method achieves a high level of selectivity. No significant interference above 30 % of LOQ was detected in the control samples, so that a high level of selectivity was demonstrated.				
Calibration	The linearity of the detector response was demonstrated by single determination of calibration standards at 7 concentration levels ranging from 0.4 ng/mL to 20 ng/mL. This range corresponds to 0.833 µg/m³ to 41.7 µg/m³ and thus covers the range from no more than 30 % of the LOQ and at least + 20 % of the highest Clopyralid concentration level detected in a diluted sample. The LOQ and 10x LOQ fortification samples were diluted (factor 200 and factor 2000) with methanol/0.1 % formic acid (10/90, v/v) prior LC-MS/MS analysis. The calibration curves obtained for both ion mass transitions were linear with coefficients of correlation (r) greater than 0.999. Linear regression was performed with 1/x weighting.				
Calibration range	<table border="1"> <tr> <th>Matrix</th><th>Calibration range (ng/L)</th></tr> <tr> <td>Air</td><td>0.4 – 20</td></tr> </table>	Matrix	Calibration range (ng/L)	Air	0.4 – 20
Matrix	Calibration range (ng/L)				
Air	0.4 – 20				
Accuracy and Precision	Accuracy was determined by fortification of control samples with known amounts of Clopyralid and subsequent determination of the recoveries when applying the extraction procedure. Precision was determined by repeatability (relative standard deviation). Results are presented in Table A 25.				
Assessment of matrix effects is presented	Yes Matrix effects on the detection of analyte in extracts of matrix air were found to be insignificant (< 20 %). Therefore, solvent standards were used for quantification.				
Limit of determination/quantification	The limit of quantification (LOQ) is the lowest validated fortification level for Clopyralid and was thus successfully established at 4.5 µg/m³ for both ion mass transitions. The limit of detection (LOD) was set at 20 % of the limit of quantification, which was 0.9 µg/m³ Clopyralid.				
Stability of Clopyralid in Stock and Fortification	Clopyralid was found to be stable in tenax tubes (at room temperature, in a refrigerator or in a freezer) and extracts (in a refrigerator or in a freezer) for at				

Solutions	least 7 days in the dark without any significant loss of test item.
Stability of Analyte(s) in Sample Extracts	Extracts were stored for 7 days in a refrigerator or a freezer after sampling. The mean recoveries ranged from 92 % to 98 % at the quantifier ion transition and from 94 % to 98 % at the qualifier ion transition. Samples in the form of solvent extracts may therefore be stored under these conditions up to 7 days without significant loss.
Breakthrough	Under the sampling conditions described, no breakthrough of Clopyralid into the backup bed of the tubes was observed and therefore the point of breakthrough could only be estimated. The point of breakthrough must therefore be greater than 400 µg Clopyralid or higher than a concentration of 833 µg/m <sup>3</sup> Clopyralid at a total volume of 480 L air within 8 hours.

### Conclusion

The method was successfully validated for Clopyralid at 4.5 µg/m<sup>3</sup> (LOQ) and 45 µg/m<sup>3</sup> (10x LOQ) fortification level according to the guidance document SANCO/825/00 rev 8.1. All mean recovery values at the fortification levels of 4.5 µg/m<sup>3</sup> (LOQ) and 45 µg/m<sup>3</sup> (10x LOQ) for both ion mass transitions comply with the standard acceptance criteria of SANCO/825/00 rev 8.1, since mean recoveries were in the range of 70 - 110 % with a relative standard deviation of ≤ 20 %. LC-MS-MS method can be regarded as highly specific when two ion transitions have been validated and, thus an additional confirmatory method is not necessary.

## A 2.1.2.6 Description of Methods for the Analysis of Body Fluids and Tissues (KCP 5.2)

### A 2.1.2.6.1 Analytical Method Validation for the determination of Clopyralid in Body Fluids

Comments of zRMS: The method is acceptable

Reference:	KCP 5.2/09
Report	Development and Validation of an Analytical Method for the Determination of Clopyralid in Body Fluids, Abe Ch., 2019, S19-00450
Guideline(s):	SANCO/825/00, rev. 8.1
Deviations:	No
GLP:	Yes
Acceptability:	Yes

### Materials and methods

Samples of body fluids were extracted with acetonitrile. A salt mixture containing magnesium sulphate, sodium chloride and sodium citrate was added and the extract was shaken. After centrifugation the acetonitrile phase was diluted with acetonitrile / water + 0.1% formic acid (v/v) for analysis. The final determination was performed by HPLC-MS/MS.

Test Item	Clopyralid
Method Reference(s)	Modified Multi-residue method QuEChERS
Detection	Liquid chromatography with tandem mass spectrometry (LC-MS/MS)
Method Reference	Multi-residue method QuEChERS
Chromatographic conditions for Clopyralid in body fluids	
HPLC system	Shimadzu HPLC System

Column Oven temperature	40 °C
Mobile phase flow	600 µL/min
Injection volume	30µL
Mobile phase composition	Eluent A: Water containing 1 % (v/v) Acetic acid Eluent B: Acetonitrile containing 1 % (v/v) Acetic acid
Retention time(s)	Clopyralid: approx. 2.2 min

#### Mass spectrometric conditions for Clopyralid in body fluids

MS system	SCIEX TripleQuad 5500 System, SCIEX
Ionisation type	Electrospray ionisation (ESI, TurboIonSpray)
Polarity	Negative ion mode
Scan type	MS/MS, Multiple Reaction Monitoring (MRM)
Capillary voltage (IS)	-4500 V
Mass transition monitored (m/z)	Clopyralid: 190→146 192→148

## Results and discussions

**Table A 21: Recovery results from the analytical method validation of Clopyralid using the analytical method**

Matrix	Analyte	Fortification level (mg/L) (n = 5)	Mean recovery on levels (%)	RSD (%)
Mass Transition 190→146 m/z (Quantification)				
Urine	Clopyralid	0.05	79.1	4.3
Mass Transition 192→148 m/z (Confirmation)				
Urine	Clopyralid	0.05	78.8	3.9

**Table A 27: Characteristics for the analytical method used for validation of Clopyralid residues in Body Fluids**

	Clopyralid
Specificity	Quantification was performed by use of LC-MS/MS detection. Two (2) mass transitions were evaluated in order to demonstrate that the method achieves a high level of selectivity. No significant interference above 30 % of LOQ was detected in any of the reagent blanks or the control sample extracts of body fluids, so that a high level of selectivity was demonstrated.
Calibration	The linearity of the detector response was demonstrated by single determination of solvent calibration standards at six (6) concentration levels ranging from 0.5 ng/mL to 15 ng/mL. This range corresponds to 0.005mg/L to 0.15 mg/L and thus covers the range from no more than 30 % of the LOQ and at least + 20 % of the highest analyte concentration level detected in a diluted sample extract. The calibration curves obtained for both mass transitions and were linear since coefficients of determination (R <sup>2</sup> ) were ≥ 0.9999. Linear regression was performed

	with 1/x-weighting.										
Calibration range	<table border="1"> <tr> <td>Matrix</td><td>Calibration range (mg/L)</td></tr> <tr> <td colspan="2">Quantification; <math>m/z</math> 190→146</td></tr> <tr> <td>Urine</td><td>0.005 -0.15</td></tr> <tr> <td colspan="2">Qualification; <math>m/z</math> 192→148</td></tr> <tr> <td>Urine</td><td>0.005 -0.15</td></tr> </table>	Matrix	Calibration range (mg/L)	Quantification; $m/z$ 190→146		Urine	0.005 -0.15	Qualification; $m/z$ 192→148		Urine	0.005 -0.15
Matrix	Calibration range (mg/L)										
Quantification; $m/z$ 190→146											
Urine	0.005 -0.15										
Qualification; $m/z$ 192→148											
Urine	0.005 -0.15										
Accuracy and Precision	The accuracy was determined by fortification of control samples with known amounts of the test / reference item and subsequent determination of the recoveries when applying the analytical method. The precision was determined by repeatability (relative standard deviation). Results are presented in Table A 26.										
Assessment of matrix effects is presented	Yes Matrix effects on the detection of clopyralid in extracts of body fluids were found to be insignificant (< 20 %). Therefore, solvent standards were used for quantification.										
Limit of determination/quantification	The LOQ is the lowest validated fortification level for clopyralid and was thus successfully established at 0.05 mg/L in body fluids for the two (2) mass transitions. The LOD was set at 0.01 mg/L for the matrix, which was 20 % of the LOQ.										
Stability of Clopyralid in Stock and Fortification Solutions	The analyte Clopyralid was found to be stable for at least 12 days when prepared in acetonitrile / water (80/20 v/v) and stored at typically 1 °C to 10 °C in the dark.										
Stability of Analyte(s) in Sample Extracts	The analyte Clopyralid was found to be stable in final extracts of body fluids for 8 days when stored at typically 1 °C to 10 °C in the dark.										

## Conclusion

The method was successfully validated for the determination of Clopyralid in body fluids from the tested LOQ of 0.05 mg/L according to the guidance document SANCO/825/00, rev. 8.1 and OECD ENV/JM/MONO(2007)17.

The mean recovery values at the fortification levels of 0.05 mg/L for both mass transitions comply with the standard acceptance criteria of SANCO/825/00, rev. 8.1 since mean recoveries were in the range of 70 - 120 % with relative standard deviations of ≤ 20 %.

LC-MS-MS method can be regarded as highly specific when two ion transitions have been validated and, thus an additional confirmatory method is not necessary.

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

No new or additional studies have been submitted