FINAL REGISTRATION REPORT

Part B

Section 5

Analytical Methods

Detailed summary of the risk assessment

Product code: PP-113H

Product name(s): BARILOCHE

Chemical active substance:

Clopyralid 100 g/L (10% w/v) SL

Central Zone

Zonal Rapporteur Member State: Poland

CORE ASSESSMENT

Applicant: PROPLAN Plant Protection Company, S.L.U.

Submission date: December 2021

Correction on: 06/2022

MS Finalization date: 07/2022, April 2023

Version history

When	What
February 2019	Initial version
December 2021	Version 2, Update for the renewal.
June 2022	Correction of update for the renewal
July 2022	Assessment by the expert
April 2023	The final version of RR after commenting period.

The product PP-113H (Clopyralid 10% w/v SL), is registered in several European countries under the brands Bariloche and Bariloche 100.

The product BARILOCHE is currently registered in Italy (16096), Spain (ES-00493), UK (Re. No. 17577), Poland (Reg. No. R-26/2018wu), Germany (Reg. No. 008865-00), Czech Republic (Reg. No. 5583-0) and Romania (Reg. No. 466PC) in Sugar beet.
Bariloche 100 is registered in France (Reg. No. 2150085) and Spain (Reg. No. 25.909) for sugar beet and oilseed rape.

This new dossier has been carried out to support the renewal of the approval of the active substance Clopyralid.

All the changes that have been made in this section, with respect to the original dossier, have been highlighted in yellow. It must be taken into account that the format of the dossier has changed.

Table of Contents

5 Analytical methods

Introduction

This document reviews the analytical methods for the product PP-113H containing Clopyralid which was included into Annex I of Directive 91/414 (Directive 2006/64/EC of 18 July).

Where appropriate this document refers to the conclusions of the EU review of the Clopyralid.
This will be where:

- the active substance data is relied upon in the risk assessment of the formulation; or when
- the EU review concluded that additional data/information should be considered at national re-registration.

Note: this Part B document only reviews data (Annex II or Annex III) and additional information that has not previously been considered within the EU review process, as part of the Annex I inclusion decision. New annex II data must only be included if they are considered essential for the evaluation and in this case a full study summary must be provided. In the case where the formulation has been previously evaluated, at European level, detailed summaries have not been provided.

PP-113H was not the representative formulation. The product has not been previously evaluated in other Countries according to Uniform Principles.

The Final Renewal report for the active substance clopyralid (SANTE/10206/2021 Rev 1,20 May 2021) and the Peer review of the pesticide risk assessment of the active substance clopyralid (EFSA Journal 2018;16(8):5389) are considered to provide the relevant review information or a reference to where such information can be found.

The Annex I Inclusion Directive for Clopyralid provides specific provisions under Part B which need to be considered by the applicant in the preparation of their submission and by the MS prior to granting an authorisation.

For the implementation of the uniform principles of Annex VI, the conclusions of the review report on the Clopyralid, and in particular Appendices I and II thereof, as finalised in the Standing Committee on the Food Chain and Animal Health on 4/04/2006 shall be taken into account. In this overall assessment:

Member States should/must/may pay particular attention to the:

- the protection of non-target plants and ground water under vulnerable conditions. Conditions of authorization should include risk mitigation measures and monitoring programs should be initiated to verify potential groundwater contamination in vulnerable zones, where appropriate.

Information on the detailed composition of PP-113H can be found in the confidential dossier of this submission (Registration Report - Part C).

5.1 Conclusion and summary of assessment

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

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

- method for body fluids with the required LOQ of 0.01 mg/L set in SANTE/2020/12830 rev.1. (minor data gaps are to be completed after registration)

Commodity/crop	Supported/ Not 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:

Comments of zRMS:	The a.s. clopyralid was renewed in 2021. The conclusions are summarised in Final renewal report for the active substance clopyralid (SANTE/10206/2021 Rev 1, 20 May 2021). The active ingredient source was approved at EU level in August 2018 (RMS Spain) and the equivalence report is available on CIRCA. As the equivalence is considered still valid the analytical method developed for the determination of a.s. in plant protection product Bariloche submitted during the first registration of the product and is still acceptable. The analytical method meets the specificity, linearity, precision/repeatability and accuracy criteria specified in SANCO/3030/99 rev. 4 (11/07/00). It fulfils also the requirements of SANCO/3030/99 rev. 5.

Method:

An analytical method has been developed for the determination of the active substance, Clopyralid, in PP-113H.

The following analytical method for the determination of the active substance in the plant protection product performed on PP-113H has not previously been reviewed and is provided in support of this assessment.

Report:	**KCP 5.1.1-01; Pardo, M., 2011.**
Title:	PP-113H (Clopyralid 10 % w/v SL): Validation of the analytical method for the determination of the active ingredient content
Document No:	CH-397/2011
Guidelines:	EEC: SANCO/3030/99 rev 4.
GLP	Yes

Conclusion: the method is acceptable.

INTRODUCTION

The Test Facility conducted a study to adjust and validate the analytical method for determining the Clopyralid content in a PP-113H (Clopyralid 10 % w/v SL) formulation sample supplied by the Sponsor.
The study was performed in compliance with Study Plan CH - 397/2011 and the following guidelines:
EEC guideline SANCO/3030/99 rev. 4 dated 11/07/00:
Working document "Technical Material and Preparations: Guidance for generating and reporting methods of analysis in support of pre and post registration data requirements for
Annex II (part A, Section 4) and Annex III (part A, Section 5) of Directive 91/414".
and the following data requirements:
- Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009, concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC.

- Commission Regulation (EU) No 545/2011 of 10 June 2011, implementing Regulation (EC) No 1107/2009 of the European Parliament and of the Council as regards the data requirements for plant protection products.

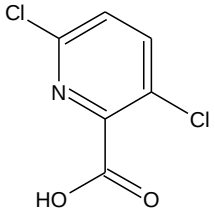
The experimental phase of this study started on October 05, 2011 and was completed on October 07, 2011.

Deviation from the Study Plan
As consequence of a typing error, the C.A.S. number of Clopyralid is "1702-17-6" instead of "57754-85-5", as predicted in the Study Plan.
The Study Director declares that this deviation does not affect the outcome of the study.

MATERIALS

Test Item
A sample of PP-113H (Clopyralid 10 % w/v SL) contained in a HDPE bottle, was received by the Test Facility on August 03, 2011. Information on the test item is as follows:

Test item identification	:	PP-113H (Clopyralid 10 % w/v SL)
Nominal active ingredient	:	Clopyralid 10 % w/v
Active ingredient content	:	Clopyralid 9.9 % w/v (from Certificate of Analysis)
Batch number	:	20110713
Internal number	:	2906075-001
Manufacturing date	:	July 2011
Expiry date	:	July 2014
Common name	:	Clopyralid (BSI, ANSI, draft E-ISO, (m) draft F-ISO)
Chemical name (IUPAC)	:	3,6-dichloro-2-pyridinecarboxylic acid

Chemical structure : 

Chemical formula : $C_6H_3Cl_2NO_2$

Chemical Class	:	Herbicide
C.A.S. number	:	1702-17-6
Molecular weight	:	192.0

Storage condition and Stability: The test item was stored at room temperature in a dark, cool, dry and well-ventilated area in accordance with the Material Safety Data Sheet (MSDS) supplied by the Sponsor.

Placebo

The Placebo of the PP-113H (Clopyralid 10 % w/v SL) formulation, contained in a HDPE bottle, was received by the Test Facility on May 10, 2011.

Batch number	:	Blank Formulation (from Placebo's label supplied by Sponsor)
Manufacturing date	:	January 2011
Expiry date	:	January 2014(*)
Internal code	:	2905180-005

(*) In accordance with the Internal Standard Operating Procedure G04_2, the expiry date was set as three years from the test item manufacturing date.
The Placebo was stored at room temperature in the dark, in accordance with Internal Standard Operating Procedure G04_2.

Reference material

Common name	:	Clopyralid, analytical standard (supplied by Sigma-Aldrich)
C.A.S. number	:	1702-17-6
Molecular weight	:	192.00
Purity	:	98.7 %
Batch number	:	SZBA166X
Preparation date	:	July 15, 2010
Expiry date	:	July 15, 2015
Internal code	:	STU 452.1

To avoid degradation, the reference material was stored at -20°C in the freezer, Internal code No. 153, even if the relevant Certificate of Analysis recommends storage at room temperature.

EQUIPMENT

- HPLC Agilent mod. 1200 equipped with UV detector, autosampler and Agilent software for data processing, Internal code No. 366
- Analytical balance, Mettler ML204, Internal code No. 417
- Refrigerator Fiocchetti, mod. Labor Lux 1000, Internal code No. 418
- Freezer Whirlpool, mod. Easytronic, Internal code No. 153
- Ultrasonic Bath, VWR International Ultrasonic Cleaner, Internal code No. 479
- Laboratory Water Purification Systems, Sartorius Arium® 611, Internal code No. 355
- Volumetric glassware: pipettes, flasks, measuring cylinders
- Usual laboratory glassware.

REAGENTS

- Water, HPLC grade obtained from the Laboratory Water Purification System
- Acetonitrile, HPLC grade (VWR International)
- Phosphoric acid 85% (H_3PO_4), reagent grade (Merck)
- Ethyl 4-Hydroxybenzoate (Ethyl paraben), analytical grade used as internal standard (Sigma-Aldrich)

EXPERIMENTAL

Preliminary non GLP tests on the test item were performed to find the best chromatographic conditions to avoid any interference.

Specificity

The SANCO/3030/99 rev. 4 guideline requires any interference from other substances present in the preparation should not contribute more than 3 % to the total peak area measured for each active substance.
The specificity test was conducted injecting, in the adjusted chromatographic conditions, a solvent wash, Clopyralid reference material, Ethyl paraben internal standard, Placebo solution and test item solution and comparing the chromatograms in order to check possible cross contaminations.
These solutions' concentrations were in the range of this method, but their exact values were not reported, since they were not used in calculations.

Linearity and System Precision

Linear regression analysis was performed using the least squares method.
The correlation coefficient was calculated using regression analysis.

Preparation of the stock reference material solution

Using the analytical balance, a 1018.6 µg/mL stock standard solution was prepared, taking into account its 98.7 % purity, by weighing 25.8 mg of Clopyralid reference material into a 25.00 mL volumetric flask and then dissolving to volume with acetonitrile.

Preparation of the stock internal standard solutions

Using the analytical balance, a 1016.0 µg/mL stock internal standard solution was prepared by weighing 25.4 mg of Ethyl paraben internal standard into a 25.00 mL volumetric flask, and dissolving to volume with acetonitrile.

Preparation of the working standard solutions

Using volumetric flasks and volumetric pipettes, five working standard solutions for linear calibration were prepared as follows:
WSS 1. 030 mL of the stock reference material solution and 0.50 mL of the stock internal standard solution were transferred into a 20.00 mL volumetric flask, making to volume with acetonitrile (working standard solution at 15.28 µg/mL and 25.40 µg/mL, respectively).
WSS 2. 0.40 mL of the stock reference material solution and 0.50 mL of the stock internal standard solution were transferred into a 20.00 mL volumetric flask, making to volume with acetonitrile (working standard solution at 20.37 µg/mL and 25.40 µg/mL, respectively).
WSS 3. 0.50 mL of the stock reference material solution and 0.50 mL of the stock internal standard solution were transferred into a 20.00 mL volumetric flask, making to volume with acetonitrile (working standard solution at 25.46 µg/mL and 25.40 µg/mL, respectively).
WSS 4. 0.60 mL of the stock reference material solution and 0.50 mL of the stock internal standard solution were transferred into a 20.00 mL volumetric flask, making to volume with acetonitrile (working standard solution at 30.56 µg/mL and 25.40 µg/mL, respectively).
WSS 5. 0.70 mL of the stock reference material solution and 0.50 mL of the stock internal standard solution were transferred into a 20.00 mL volumetric flask, making to volume with acetonitrile (working standard solution at 35.65 µg/mL and 25.40 µg/mL, respectively).
The linearity test was performed with solutions from 15.28 to 35.65 µg/mL, corresponding to ± 40 % of the nominal concentration in the sample solutions.

From the lowest to the highest concentration, four series of injections were performed and a solvent wash was injected after each highest standard concentration solution, in order to verify if memory peaks were detected.
Means and standard deviations for each level were calculated using the data from the four replicate injections.

Repeatability (Precision)
Preparation of the standard solutions
Standard 1. Using the analytical balance, 25.3 mg of Clopyralid reference material and 24.8 mg of Ethyl paraben internal standard were weighed into a 50 mL conical flask and dissolved with 25 mL of acetonitrile. The resulting solution was diluted 1:40 with acetonitrile.
Standard 2. Using the analytical balance, 25.3 mg of Clopyralid reference material and 25.2 mg of Ethyl paraben internal standard were weighed into a 50 mL conical flask and dissolved with 25 mL of acetonitrile. The resulting solution was diluted 1:40 with acetonitrile.
Six solutions of the test item (labelled from A to F) were prepared and injected as described in Internal Analytical Method No. 397/2011.

These injections were alternated with those of the Standard 1 and 2 according to the sequence of analysis reported in Table 6.
Precision (repeatability) of the analytical method was assessed with the obtained data.

Recovery (Accuracy)
The test was performed by spiking six aliquots of the Placebo (2905180-005) with the Clopyralid reference material at three levels in duplicate, corresponding to additions of 75 %, 100 % and 125 % of the nominal concentration of active ingredient.

Fortified sample preparation and analysis
Using the analytical balance, six Placebo aliquots, about 900 mg each, and six internal standard aliquots, about 100 mg each, were weighed into six 100 mL conical flasks.
To obtain Placebo fortification at three levels, corresponding to nominal additions of 75 %, 100 % and 125 % of the active ingredient, nominal aliquots of 75 mg, 100 mg and 125 mg of the reference material were added respectively, as summarized in Table 1.

TABLE 1 Fortified sample preparation for recovery test

Fortification level	Placebo (mg)	W_{pl} (mg)*(1)*	Ethyl paraben (mg)	Clopyralid ref. mat. W_{std} (mg)	Clopyralid W_{added} (mg)*(2)*
Low level A	928.8	0.0	101.4	76.6	75.6
Low level B	927.2	0.0	101.3	76.1	75.1
Medium level A	910.4	0.0	101.9	100.9	99.6
Medium level B	908.1	0.0	101.5	100.4	99.1
High level A	870.2	0.0	100.3	129.3	127.6
High level B	869.1	0.0	100.6	128.9	127.2

(1) Calculated taking into consideration the absence of Clopyralid content in Placebo.
(2) Calculated taking into consideration the added weights of the Clopyralid reference material having a 98.7 % purity.

The fortified samples were treated and analysed as described for the test item in Internal Analytical Method No. 397/2011.

The percentage recovery was calculated as follows:

$$\text{Recovery (\%)} = \frac{W_{found}}{W_{added} + W_{pl}} \times 100$$

where:

W_{found}	=	Active ingredient found in the fortified test item (mg)
W_{pl}	=	Active ingredient already present in the Placebo (mg)
W_{added}	=	Active ingredient added using the reference material (mg)

Accuracy (recovery) of the analytical method was assessed with the obtained data.

RESULTS

Note. Data in the tables are rounded values taken from Excel spreadsheets which will be archived together with the raw data. The use of Excel spreadsheets for calculations produces more accurate endpoints. These endpoints may slightly differ from the values derived by replacing the rounded values in the formulae given in the methods section.
The .pdf version of chromatograms is not obtained from the scanning of hard copies, but from a direct conversion to .pdf file. Therefore, a slight gap between printing and conversion times may be registered.

Specificity
A comparison of the chromatograms of the solvent wash, Clopyralid reference material, Ethyl paraben internal standard, Placebo solution and test item solution, shows that, following the operating conditions recommended in the analytical method, the active ingredient and internal standard peaks are well separated and there is not evidence of interferences with the test item peaks.
Therefore, by using the conditions stated in the method, interferences can be avoided and the active ingredient can be reliably determined in PP-113H (Clopyralid 10 % w/v SL) formulation samples.

Linearity and System Precision
To check linearity and system precision, five working standard solutions were prepared as previously described in the experimental section and each solution was injected four times.
The peak areas obtained were used to determine the mean value, the standard deviation (S.D.) and the relative standard deviation (RSD%) of the analytical method at each concentration.
The results of the linearity regression test are summarized in Tables 2 and 3 and the relevant.
These results showed that the analytical method is linear over the range tested for the active ingredient (correlation coefficient > 0.99).

TABLE 2 Linearity test on Clopyralid reference material

Clopyralid	**WSS 1 15.28 µg/mL** (Peak area)	**WSS 2 20.37 µg/mL** (Peak area)	**WSS 3 25.46 µg/mL** (Peak area)	**WSS 4 30.56 µg/mL** (Peak area)	**WSS 5 35.65 µg/mL** (Peak area)
1st injection	4740959	6470796	7941720	9785292	11400915
2nd injection	4749826	6494698	8026391	9771576	11467456
3rd injection	4738851	6492955	7970101	9765181	11383292
4th injection	4761615	6440071	7971504	9746852	11392927
Mean	**4747813**	**6474630**	**7977429**	**9767225**	**11411148**
S.D.	10358	25479	35408	15964	38224
RSD%	*0.22%*	*0.39%*	*0.44%*	*0.16%*	*0.33%*
***Linear calibration: y* = m*x* + q**					
Parameter **m** (slope)	326321	Parameter **q** (intercept)	-233983	Parameter **R^2** (correlation)	0.99948

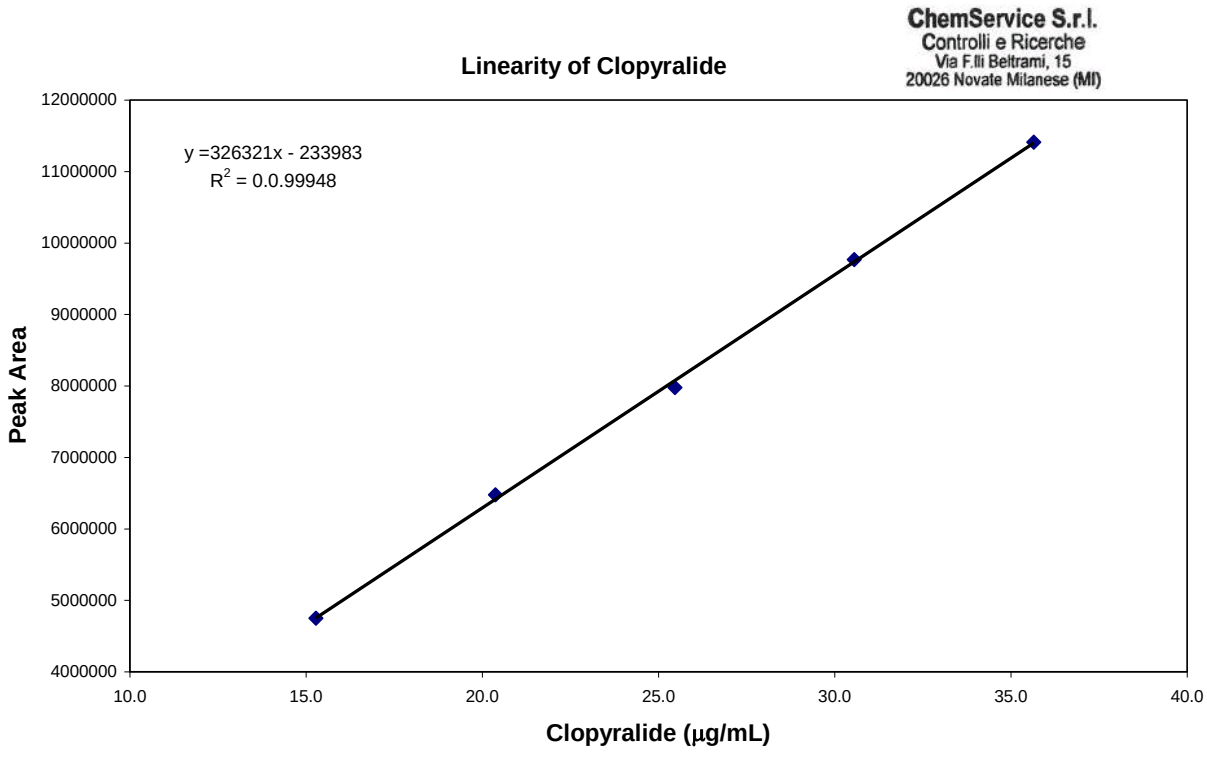


Figure 6 Linearity test graphic: Clopyralid reference material

TABLE 3 Linearity test. Ratio of Clopyralid reference material with internal standard (I.S.)

Clopyralid /S.I.	**WSS 1 0.60** (area ratio)	**WSS 2 0.80** (area ratio)	**WSS 3 1.00** (area ratio)	**WSS 4 1.20** (area ratio)	**WSS 5 1.40** (area ratio)
1st injection	0.5059	0.6993	0.8731	1.0319	1.2163
2nd injection	0.5076	0.7004	0.8804	1.0295	1.2235
3rd injection	0.5058	0.7012	0.8759	1.0281	1.2131
4th injection	0.5082	0.6962	0.8747	1.0278	1.2142
Mean	**0.5069**	**0.6993**	**0.8760**	**1.0293**	**1.2168**
S.D.	0.0012	0.0022	0.0031	0.0018	0.0047
RSD%	*0.24%*	*0.31%*	*0.36%*	*0.18%*	*0.38%*
***Linear calibration: y* = m*x* + q**					
Parameter **m** (slope)	0.8727	Parameter **q** (intercept)	-0.0093	Parameter **R^2** (correlation)	0.99873

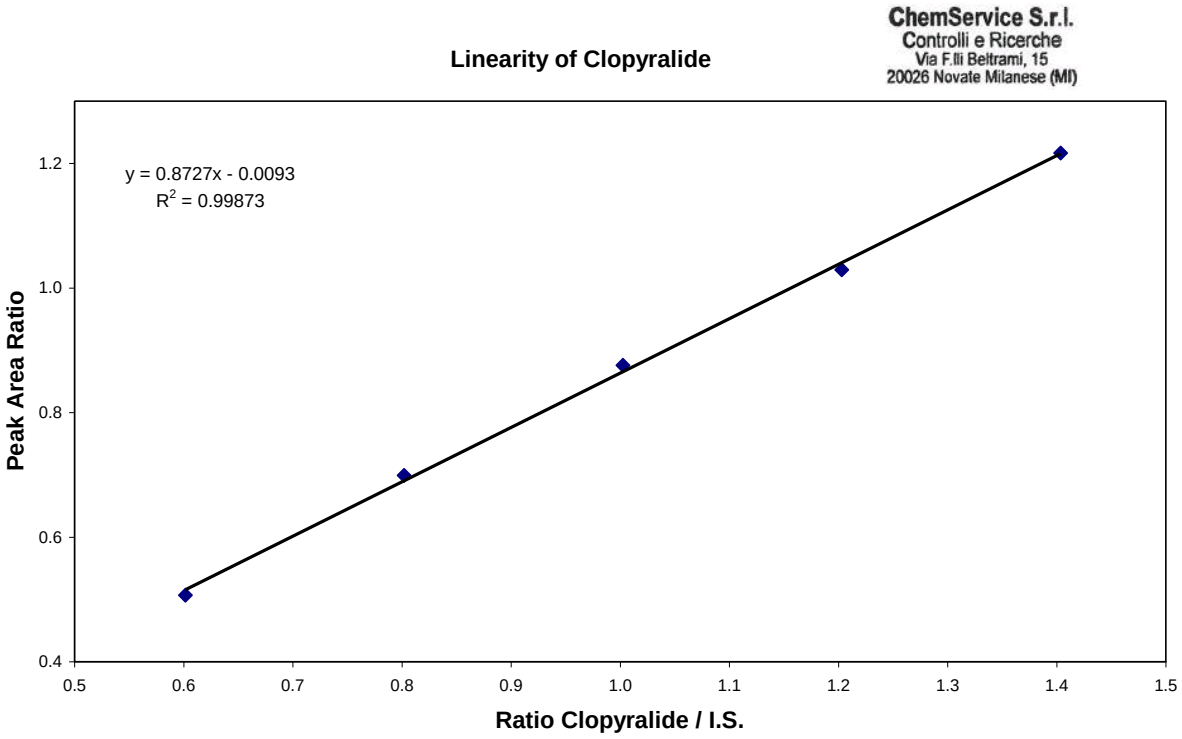


Figure 7 Linearity test graphic: ratio Clopyralid / I.S.

Repeatability (Precision)

The test was performed by six determinations of the test item (labelled A to F).
Data and results are reported in Tables 4 and 5. Table 6 shows the analysis sequence.

The relative standard deviation was 0.41% for Clopyralid and the Horwitz RSDr was 1.91 at a Clopyralid concentration of 9.5 % w/w. Since the relative standard deviation was less than the Horwitz RSDr, the repeatability test for this active ingredient was acceptable.

The value of 0.1 % w/w (0.1 % w/v, 1 g/L) for the precision of the analytical method for Clopyralid, calculated as twice the standard deviation, can be considered acceptable for this test item with a declared nominal purity of 10 % w/v.

Data and results were used to determine the following precision.

Clopyralid : 9.5 ± 0.1 % w/w 9.9 ± 0.1 % w/v 99 ± 1 g/L

In order to obtain the precision expressed in % w/v and g/L, a 1.0516 g/mL density value, calculated in GLP Study CH – 392/2011, was used.

TABLE 4 Repeatability test: F factor calculation for Clopyralid

Standard Code	**W_{std} (mg)**	**W_{is} (std) (mg)**	**A_{std} / A_{is} (std) mean of 2 injections**	**P purity (%)**	**FACTOR F**
Standard 1	25.3	24.8	0.8964	98.7	0.8902
Standard 2	25.3	25.2	0.8757	98.7	0.8837
Standard 1	25.3	24.8	0.8919	98.7	0.8858
Standard 2	25.3	25.2	0.8781	98.7	0.8861
				Mean value :	**0.8865**
				Relative Standard Deviation (RSD%) :	*0.31%*

TABLE 5 Repeatability test: test item analytical resultsfor Clopyralid

Internal Number	**W_S (mg)**	**W_{is} (mg)**	**A_S / A_{is} mean of 2 inj.**	**F**	**Clopyralid (% w/w)**	**Clopyralid (g/L)** (1)
2906075-001 A	1025.1	99.4	0.8683	0.8865	9.497	99.9
2906075-001 B	1044.6	119.2	0.7361	0.8865	9.476	99.6
2906075-001 C	1038.8	99.0	0.8840	0.8865	9.503	99.9
2906075-001 D	1017.1	100.2	0.8503	0.8865	9.449	99.4
2906075-001 E	1049.3	101.4	0.8658	0.8865	9.438	99.3
2906075-001 F	1052.0	100.4	0.8734	0.8865	9.403	98.9
				Mean value :	**9.5**	**99**
				Standard deviation (S.D.) :	0.038	0.4
				Relative Standard Deviation (RSD%) :	*0.41%*	*0.41%*
				Precision (2 x S.D.) :	**0.1**	**1**
				Horwitz RSDr [0.67 x 2 $^{(1-0.5 \log(Mean/100))}$]:	1.91	1.91

Legenda can be found in the calculation paragraph.
(1) Calculated taking into consideration the 1.0516 g/mL density value obtained in GLP Study No. 392/2011.

TABLE 6 Repeatability test: analysis sequence

Vial identification	**Number of inj.**	**Vial identification**	**Number of inj.**
Solvent Wash	1	2906075-001 D	2
Standard 1	2	2906075-001 E	2
Standard 2	2	2906075-001 F	2
Solvent Wash	1	Solvent Wash	1
2906075-001 A	2	Standard 1	2
2906075-001 B	2	Standard 2	2
2906075-001 C	2	Solvent Wash	1

Recovery (Accuracy)

The test was performed by spiking the Placebo (2905180-005) with the Clopyralid reference material at three levels in duplicate, corresponding to additions of 75 %, 100 % and 125 % of the nominal concentration of active ingredient.

Data of the injections of both the standards and the fortified samples for the recovery test are reported in Tables from 7 to 9; Table 10 shows the analysis sequence.

For the accuracy, the SANCO/3030/99 rev. 4 guideline requires mean recovery values in the range 98 to 102 % for active ingredient content equal or higher than 10 % w/w.

From obtained data, these criteria were fulfilled and therefore accuracy of the analytical method can be considered acceptable.

TABLE 7 Recovery test: F factor calculation for Clopyralid

Standard Code	W_{std} (mg)	W_{is} (std) (mg)	A_{std} / A_{is} (std) mean of 2 injections	P purity (%)	FACTOR F
Standard 1	25.3	24.8	0.8937	98.7	0.8858
Standard 2	25.3	25.2	0.8735	98.7	0.8815
Standard 1	25.3	24.8	0.8934	98.7	0.8875
Standard 2	25.3	25.2	0.8731	98.7	0.8810
				Mean value :	**0.8840**
				Relative Standard Deviation (RSD%) :	*0.36%*

TABLE 8 Recovery test: fortified Placebo analysis for Clopyralid

Spike Code	W_{pl} (mg)	W_{is} (mg)	A_s / A_{is} mean of 2 injections	F	W_{found} (mg)
Spike Low A	928.8	101.4	0.6556	0.8840	75.2
Spike Low B	927.2	101.3	0.6540	0.8840	74.9
Spike Medium A	910.4	101.9	0.8613	0.8840	99.3
Spike Medium B	908.1	101.5	0.8622	0.8840	99.0
Spike High A	870.2	100.3	1.1164	0.8840	126.7
Spike High B	869.1	100.6	1.1138	0.8840	126.8

TABLE 9 Recovery test: recovery results on fortified Placebo

Spike Code	W_{found} (mg)	W_{added} (mg) *(1)*	W_{pl} (mg) *(1)*	Recovery (%)
Spike Low A	75.2	75.6	0.0	99.47
Spike Low B	74.9	75.1	0.0	99.78
			Mean recovery (%) :	**99.6**
Spike Medium A	99.3	99.6	0.0	99.69
Spike Medium B	99.0	99.1	0.0	99.90
			Mean recovery (%) :	**99.8**
Spike High A	126.7	127.6	0.0	99.26
Spike High B	126.8	127.2	0.0	99.63
			Mean recovery (%) :	**99.4**
			Total Mean recovery (%) :	**99.6**

(1) see in experimental section, recovery paragraph, the calculation of these values.

TABLE 10 Recovery test: analysis sequence

Vial identification	Number of inj.	Vial identification	Number of inj.
Solvent Wash	1	Spike Medium B	2
Standard 1	2	Spike High A	2
Standard 2	2	Spike High B	2
Solvent Wash	1	Solvent Wash	1
Spike Low A	2	Standard 1	2
Spike Low B	2	Standard 2	2
Spike Medium A	2	Solvent Wash	1

CONCLUSIONS

The analytical method was shown to be specific for Clopyralid active ingredient in PP-113H (Clopyralid 10 % w/v SL) formulation samples.

The range tested for Clopyralid, from 15.28 to 35.65 µg/mL (± 40 % of the solution concentration used for the quantification analysis), was found to be linear (correlation coefficient > 0.99).

The relative standard deviation was 0.41% for Clopyralid and the Horwitz RSDr was 1.91 at a Clopyralid concentration of 9.5 % w/w. Since the relative standard deviation was less than the Horwitz RSDr, the repeatability test for this active ingredient was acceptable. Horrat value is 0.21 which is acceptable.

The value of 0.1 % w/w (0.1 % w/v, 1 g/L) for the precision of the analytical method for Clopyralid, calculated as twice the standard deviation, can be considered acceptable for this test item with a declared nominal purity of 10 % w/v.

Data and results were used to determine the following precision.

Clopyralid : 9.5 ± 0.1 % w/w 9.9 ± 0.1 % w/v 99 ± 1 g/L

In order to obtain the precision expressed in % w/v and g/L, a 1.0516 g/mL density value, calculated in GLP Study CH – 392/2011, was used.

For the accuracy, the SANCO/3030/99 rev. 4 guideline requires mean recovery values in the range 98 to 102 % for active ingredient content equal or higher than 10 % w/w. The SANCO/3030/99 rev. 5 guidance requires main recovery values in the range from 97 to 103%.
Since all recovery values fulfilled these criteria, the accuracy of the analytical method can be considered acceptable.

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

No method is required because PP-113H does not contain impurities of toxicological, ecotoxicological or environmental concern which appear at a significant level.

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

No method is required because PP-113H does not contain impurities of toxicological, ecotoxicological or environmental concern which appear at a significant level.

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.

Table 5.2-1: Validated methods for the generation of pre-authorization data

Component of residue definition: CLOPYRALID				
Matrix type	**Method type**	**Method LOQ**	**Principle of method**	**Author(s), year / missing / EU agreed**
Plant origin (Aqueous, Dry, Oily and Acidic	Primary	LOQ 0.01 mg/kg	LC-MS/MS	Vogl, E. 2012 DAS 120610 DRAR Clopyralid 2017

Component of residue definition: CLOPYRALID				
Matrix type	**Method type**	**Method LOQ**	**Principle of method**	**Author(s), year / missing / EU agreed**
Crops)				
Plant origin (Grass, Cereal Grain and Straw)	Primary	LOQ 0.20 mg/kg in grass and straw; 0.05 mg/kg in grain	GC-MS	Clements, B,; Harrington, R. (1997) ERC 97.10 DRAR Clopyralid 2017
Plant origin (Barley, Forage Grain Straw)	Primary	LOQ 0.01 mg/kg	GC/MSD Pre-registration and Post-Registration	Hastings 2002a GRM 01.16 (O97) DAR Clopyralid 2005
Plant origin (Wheat, Grain Straw; Sugar beet)	Confirmatory	LOQ 0.01 mg/kg	GC/MSD ILV of GRM 01.16 (O97)	Rawle 2002 GHE-P-9567 (O95) DAR Clopyralid 2005
Plant origin (Barley, Forage Grain Straw)	Primary	LOQ 0.10 mg/kg LOQ 0.05 mg/kg LOQ 0.10 mg/kg	GC-ECD Pre-registration	Kutschinski 1979 ACR 79.5 O38 DAR Clopyralid 2005
Plant origin (Barley, Grain Straw)	Primary	LOQ 0.1 mg/kg	GC-ECD Pre-registration	Jones 1975a ERC 75.1 (O23) DAR Clopyralid 2005
Plant origin (Grass, Forage Hay Seed)	Primary	LOQ 0.01 mg/kg	GC-ECD Pre-registration and Post-registration	Hastings 2002a GRM 01.16 (O97) DAR Clopyralid 2005
Plant origin (Grass)	Primary	LOQ 0.05 mg/kg	GC-ECD Pre-registration and Post-registration	Wood 1994 ERC 94.8 (O47) DAR Clopyralid 2005
Plant origin (Oilseed Rape, Grain Straw)	Primary	LOQ 0.01 mg/kg	GC/MSD Post-registration	Hastings 2002a GRM 01.16 (O97) DAR Clopyralid 2005
Plant origin (Oilseed Rape, Cake, Oil Seed, Straw)	Primary	LOQ 0.1 mg/kg	GC-ECD Pre-registration	Jones 1975b ERC 75.3 (O29) DAR Clopyralid 2005
Plant origin (Sorghum, Forage, Grain, Straw)	Primary	LOQ 0.01 mg/kg	GC/MSD Pre-registration and Post-registration	Hastings 2002a GRM 01.16 (O97) DAR Clopyralid 2005
Plant origin (Sugarbeets, Roots, Tops)	Primary	LOQ 0.01 mg/kg	GC/MSD Pre-registration and Post-registration	Hastings 2002a GRM 01.16 (O97) DAR Clopyralid 2005
Plant origin (Sugarbeets, Molasses, Pulp,	Primary	LOQ 0.05 mg/kg	GC/MSD Post-registration	Kubitschek & Kyle 1994 GRM 94.04 (O102)

Component of residue definition: CLOPYRALID				
Matrix type	**Method type**	**Method LOQ**	**Principle of method**	**Author(s), year / missing / EU agreed**
Sugar)				DAR Clopyralid 2005
Plant origin (Sugarbeets, Refined Sugar)	Primary	LOQ 0.005 mg/kg	GC-ECD Pre-registration	Anon. 1989 ERC 88.2 (O34) DAR Clopyralid 2005
Plant origin (Sugarbeets, Molasses)	Primary	LOQ 0.05 mg/kg	GC-ECD Pre-registration	Anon. 1988 ERC 88.1 (O31) DAR Clopyralid 2005
Plant origin (Sugarbeets, Roots, Tops)	Primary	LOQ 0.10 mg/kg	GC-ECD Pre-registration	Jones 1977 ERC 77.4 (O27) DAR Clopyralid 2005
Plant origin (Wheat, Forage Grain, Straw)	Primary	LOQ 0.01 mg/kg	GC/MSD Pre-registration and Post-registration	Hastings 2002a GRM 01.16 (O97) DAR Clopyralid 2005
Plant origin (Wheat, Grain, Straw)	Primary	LOQ 0.05 mg/kg LOQ 0.10 mg/kg	GC-ECD Pre-registration	Freeman & Smith 1983 ERC 83.23 (O25)
Plant origin (Wheat, Forage, Grain, Straw)	Primary	LOQ 0.10 mg/kg LOQ 0.05 mg/kg LOQ 0.10 mg/kg	GC-ECD Pre-registration	Kutschinski 1979 ACR 79.5 (O38) DAR Clopyralid 2005
Plant origin (Wheat, Grain, Straw)	Primary	LOQ 0.1 mg/kg	GC-ECD Pre-registration	Jones 1975a ERC 75.1 (O23) DAR Clopyralid 2005
Animal products (Beef Tissues, Fat, Kidney, Liver, Muscle)	Primary	LOQ 0.05 mg/kg	GC-ECD Pre-registration	Kuper 1974b ACR 74.9 (O40) DAR Clopyralid 2005
Animal product (Milk and Cream)	Primary	LOQ 0.01 mg/kg	GC-ECD Pre-registration	Kutschinski 1974 ACR 74.3 (012) DAR Clopyralid 2005
Animal product (Chicken Tissues, Eggs, Fat, Liver, Muscle)	Primary	LOQ 0.05 mg/kg	GC-ECD Pre-registration	Kuper 1975 ACR 75.2 (O13) DAR Clopyralid 2005
Animal product (Chicken Tissues, Eggs, Fat, Kidney, Liver, Muscle)	Primary	LOQ 0.05 mg/kg	GC-ECD Pre-registration	Kuper 1974a ACR 74.2 (O11) DAR Clopyralid 2005

Component of residue definition: CLOPYRALID				
Matrix type	**Method type**	**Method LOQ**	**Principle of method**	**Author(s), year / missing / EU agreed**
Animal product (Animal Tissues and Products, Fat, Kidney, Liver, Muscle, Eggs, Milk)	Primary	LOQ 0.01 mg/kg	GC/MSD Post-registration	Hastings 2002b GRM 02.14 (O96) DAR Clopyralid 2005
Soil	Primary	LOQ 0.5 µg/kg	GC/MSD Post-registration	Hastings and Schauerman, M. 2001 GRM 00.18 (O92) DAR Clopyralid 2005
Soil	Primary	LOQ 0.5 µg/kg	GC/MSD Pre-registration	Butler, R.E., Hastings, M. and Lyons, S. ERC 98.18 (O90) DAR Clopyralid 2005
Water (Drinking, ground, surface)	Primary	LOQ 0.05 µg/l	GC/MSD Pre-registration and Postregistration	Hastings, M.J. and Schauerman, M. 2001b GRM 00.17 (091) DAR Clopyralid 2005
Air	Primary	15 µg/m^3	GC/MSD Post-registration	Devine & Rawle 2002 GRM 02.06 (O103) DAR Clopyralid 2005
Air	Primary	17 µg/m^3	GC/MSD Pre-registration and Postregistration	Long 1994 ERC 94.18 (O44) DAR Clopyralid 2005

Notes
Pre registration: Methods of analysis to support pre-registration data requirements.
Post-registration: Methods of analysis for post-registration monitoring and control.
Confirmation: confirmatory technique/method.
Residue: parent = clopyralid.

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 Renewal Assessment Report (incl. its addenda) the current legal residue definition is identical.

In the Draft Renewal Assessment Report of Clopyralid (2017), the definitions of residues were the following:

- Definition of residue in plants: Clopyralid common moiety (sum of clopyralid, its salts and conjugates expressed as clopyralid) pending the outstanding clarification on the nature of "polar clopyralid"
- Definition of residue in animal product: Clopyralid and its salts.
- Soil: Clopyralid.
- Ground water: Clopyralid.
- Surface water: Clopyralid.
- Air: Clopyralid.
- Animal tissues: Clopyralid.

The current legal residue definitions are summarized in the following table 5.3-1.

Table 5.2-2: Current residue definitions for monitoring and risk assessment *(Reg. (EU) 2018/1514)*

Matrix	Residue definition	MRL/limit	Reference for MRL/level Remarks
Plant, high water content	Clopyralid	LOQ 0.5 mg/kg	Reg. (EU) 2021/1807
Plant, high acid content		LOQ 0.5 mg/kg	
Plant, high protein/high starch content (dry commodities)		Cereal grain: LOQ 2.0 mg/kg Rapeseed: LOQ 0.5 mg/kg. Sugar beet: LOQ 1.0 mg/kg.	
Plant, high oil content		LOQ 0.5 mg/kg	
Plant, difficult matrices (hops, spices, tea)		LOQ 0.5 mg/kg	
Muscle		Muscle (swine, bovine, sheep, goat) LOQ 0.08 mg/kg Muscle (equine, poultry) LOQ 0.05 mg/kg Milk & eggs LOQ 0.05 mg/kg Fat & liver (swine, equine, poultry) LOQ 0.05 mg/kg Fat (bovine) LOQ 0.15 mg/kg Fat & liver (sheep, goat) LOQ 0.2 mg/kg Liver (bovine) LOQ mg/kg Kidney (poultry & equine) LOQ 0.05 mg/kg	
Milk			
Eggs			
Fat			

Matrix	Residue definition	MRL/limit	Reference for MRL/level Remarks
Liver, kidney	Clopyralid	Kidney (swine) LOQ 0.6 mg/kg Kidney (bovine) LOQ 1.5 mg/kg Kidney (sheep & goat) LOQ 2 mg/kg	Reg. (EU) 2021/1807
Soil (Ecotoxicology)		LOQ 0.05 µg/kg	EFSA Journal 2018;16(7):5389
Drinking water (Human toxicology)		LOQ 0.05 µg/L	
Surface water (Ecotoxicology)			
Air		LOQ 4.5 µg/m^3	
Tissue (meat or liver)		LOQ 0.01 mg/kg	
Body fluids		LOQ 0.05 mg/L	

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

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

Table 5.2-3: 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				
Matrix type	**Method type**	**Method LOQ**	**Principle of method**	**Author(s), year / missing / EU agreed**
Dry crops: ~~wheat grain~~ (rice)	Primary	LOQ 0.01 mg/kg	LC-MS/MS	~~Vogl, E. 2012~~ ~~DAS 120610~~ ~~DRAR Clopyralid 2017~~ Knop M., 2020, S19-00446
	ILV	LOQ 0.01 mg/kg	LC-MS/MS	Richer S. 2020, S19-00438
Wet crops: ~~Wheat forage~~ (tomato)	Primary	LOQ 0.01 mg/kg	LC-MS/MS	~~Vogl, E. 2012~~ ~~DAS 120610~~ ~~DRAR Clopyralid 2017~~ Knop M., 2020, S19-00446
	ILV	LOQ 0.01 mg/kg	LC-MS/MS	~~Austin, R. 2012~~ ~~DAS 120614~~ ~~DRAR Clopyralid 2017~~ Richer S. 2020, S19-00438
Acidic crops: ~~Orange~~ (grape)	Primary	LOQ 0.01 mg/kg	LC-MS/MS	~~Vogl, E. 2012~~ ~~DAS 120610~~ ~~DRAR Clopyralid 2017~~ Knop M., 2020, S19-00446
	ILV	LOQ 0.01 mg/kg	LC-MS/MS	Richer S. 2020, S19-00438

Component of residue definition: Clopyralid				
Matrix type	**Method type**	**Method LOQ**	**Principle of method**	**Author(s), year / missing / EU agreed**
Oily crops: ~~Oilseed Rape Seeds~~ (olive)	Primary	LOQ 0.01 mg/kg	LC-MS/MS	~~Vogl, E. 2012~~ ~~DAS 120610~~ ~~DRAR Clopyralid 2017~~ Knop M., 2020, S19-00446
	ILV	LOQ 0.01 mg/kg	LC-MS/MS	~~Austin, R. 2012~~ ~~DAS 120614~~ ~~DRAR Clopyralid 2017~~ 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 5.2-4: 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 adressed for all matrix groups for which residues ≥ 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)

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 5.2-5: 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**~~	~~**Author(s), year / missing**~~
~~Beef Fat~~ ~~Beef Kidney~~ ~~Beef Liver~~ ~~Beef Muscle~~	~~Primary~~	~~LOQ 0.05 mg/kg~~	~~GC/ECD~~	~~Kuper 1974b~~ ~~O40~~ ~~DAR Clopyralid 2005~~
~~Cream~~ ~~Milk~~	~~Primary~~	~~LOQ 0.01 mg/kg~~	~~GC/ECD~~	~~Kutschinski 1974~~ ~~O12~~ ~~DAR Clopyralid 2005~~

~~Component of residue definition: Clopyralid~~				
~~Matrix type~~	~~Method type~~	~~Method LOQ~~	~~Principle of method~~	~~Author(s), year / missing~~
~~Chicken eggs~~ ~~Chicken fat~~ ~~Chicken liver~~ ~~Chicken muscle~~	~~Primary~~	~~LOQ 0.05 mg/kg~~	~~GC/ECD~~	~~Kuper 1975~~ ~~DAR Clopyralid 2005~~
~~Chicken muscle~~ ~~Chichen liver~~ ~~Chicken Kidney~~ ~~Chichen fat~~ ~~Whole eggs~~ ~~Eggs whites~~ ~~Eggs yolks~~	~~Primary~~	~~LOQ 0.05 mg/kg~~	~~GC/ECD~~	~~Kuper 1974a~~ ~~O11~~ ~~DAR Clopyralid 2005~~
~~Bovine kidney~~ ~~Bovine liver~~ ~~Bovine fat~~ ~~Bovine milk~~ ~~Chicken fat~~ ~~Chicken muscle~~ ~~Chicken eggs~~ ~~Bovine tissues~~ ~~Chicken tissues~~ ~~Animal tissues*~~	~~Primary~~	~~LOQ 0.01 mg/kg~~	~~GC/MSD~~	~~Hastings 2002b~~ ~~O96~~ ~~DAR Clopyralid 2005~~

~~*Animal tissues included bovine (kidney, liver, fat, milk) and chicken (fat, muscle, eggs).~~

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	Abe Ch., 2019a, S19-00447
	ILV	0.01 mg/kg	LC-MS/MS	Schweizer M., 2019, P 5210 G
Eggs	Primary	0.01 mg/kg	LC-MS/MS	Abe Ch., 2019a, S19-00447
	ILV	0.01 mg/kg	LC-MS/MS	Schweizer M., 2019, P 5210 G
Muscle	Primary	0.01 mg/kg	LC-MS/MS	Abe Ch., 2019a, S19-00447
	ILV	0.01 mg/kg	LC-MS/MS	Schweizer M., 2019, P 5210 G
Fat	Primary	0.01 mg/kg	LC-MS/MS	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	Abe C., 2019a, S19-00447
	ILV	0.01 mg/kg	LC-MS/MS	Schweizer M., 2019, P 5210 G
Liver	Primary	0.01 mg/kg	LC-MS/MS	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 5.2-6: 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 adressed for all matrix groups for which residues ≥ LOQ are expected. For clopyralid non of residue value exceed LOQ.

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

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

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

Table 5.2-7: Validated methods for soil (if appropriate)

Component of residue definition: Clopyralid *(EFSA Journal 2018;16(7):5389)*			
Method type	**Method LOQ**	**Principle of method**	**Author(s), year / missing**
Primary	LOQ 0.5 µg/kg	LC-MS/MS	~~Vincent, T. P. (2013) DAS 120612~~ ~~DRAR Clopyralid 2017~~ Knop M., 2019a, S19-00448
~~ILV~~	~~LOQ 0.5 µg/kg~~	~~LC-MS/MS~~	~~Austin, R., Turner, R. (2014)~~ ~~DAS 140079~~ ~~DRAR Clopyralid 2017~~

~~Residues of clopyralid are extracted from soil samples by adding 25 mL of acetone:1N hydrochloric acid (90:10) then shaking and centrifuging, followed by 10 mL of additional acetone:1N hydrochloric acid (90:10) and further shaking and centrifuging. The acetone is then evaporated using nitrogen and brought to 8 mL final volume with 1N sodium hydroxide before vortexing and sonication. Approximately 8 mL of dichloromethane is added, with sonication, vortexing, and centrifuging to mix well, and the upper 6 mL extract layer is transferred to a clean glass tube and 6 mL of 1N hydrochloric acid is added. The sample is then passed through a pre-conditioned Waters HLB solid phase extraction (SPE) column. The sample bottle is then rinsed with 1N hydrochloric acid which is used to rinse the SPE column. The sample bottle is then rinsed with acetonitrile/1N formic acid (15:85) solution which is then used to rinse the SPE column, followed by drying under full vacuum. The SPE column is eluted with dichloromethane, which is evaporated to dryness using a gentle steam of nitrogen. The sample residue is reconstituted with a methanol/0.1% formic acid (10:90) solution filtered through a 0.2 µm PTFE syringe filter and then analyzed by liquid chromatography (Accucore Phenyl hexyl column, 4.6x50 mm, 2.6 µm; Mobile Phase: A) water containing 0.01% formic acid, B) 60:40 methanol:acetonitrile containing 0.01% formic acid, gradient elution) coupled with negative ion electrospray ionization tandem mass spectrometry (ESI LC-MS-MS). (Vincent, T. P. (2013) DAS 120612)~~

~~The objective of this study was to assess and to independently validate Dow AgroSciences Method 120612 for the determination of clopyralid in soil.~~

~~Residues of clopyralid are extracted from soil samples by adding 25 mL of acetone/1N hydrochloric acid (90:10) solution followed by shaking and centrifugation. The solvent is decanted before an additional 10 mL of acetone/1N hydrochloric acid (90:10) solution is added to the sample followed by further shaking and centrifugation. The two solvent extracts are combined, and the acetone is evaporated using nitrogen before being brought to a final volume of 8 mL using a 1N sodium hydroxide solution. The sample is vortex mixed and sonicated. Approximately 8 mL of dichloromethane is added and the sample is mixed well using vortex mixing and sonication. The sample is centrifuged before a 6 mL aliquot of the upper extract layer is transferred to a new glass tube and 6 mL of 1N hydrochloric acid is added to the upper extract layer. The acidified upper extract layer is then passed through a pre-conditioned Waters HLB SPE cartridge, and the sample tube is rinsed with 1N HCl which is then transferred to and passed through the SPE cartridge. This is followed by rinsing the sample tube with acetonitrile/1N formic acid (15:85) and passing this rinse through the SPE cartridge also. The cartridge is dried under full vacuum for 30 minutes before elution of the analytes with dichloromethane. The sample is evaporated to dryness using nitrogen and reconstituted in 1.0 mL of methanol/0.1% formic acid (10:90) solution before being filtered through a 0.2 µm PTFE syringe filter. The final sample is analysed for clopyralid by liquid chromatography (Accucore Phenyl hexyl column, 4.6x50 mm, 2.6 µm; Mobile Phase: A) water containing 0.01% formic acid, B) 60:40 methanol:acetonitrile containing 0.01% formic acid, gradient elution) coupled with negative ion electrospray tandem mass spectrometry (LC-MS/MS).
(Austin, R., Turner, R. (2014) DAS 140079)~~

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

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

Table 5.2-8: Validated methods for water (if appropriate)

Component of residue definition: Clopyralid *(EFSA Journal 2018;16(7):5389)*				
Matrix type	**Method type**	**Method LOQ**	**Principle of method**	**Author(s), year / missing**
Drinking water ~~Ground water~~ Surface water	Primary	0.050 µg/L	LC-MS/MS	~~Shaffer, S. (2012)~~ ~~DAS 120611~~ ~~DRAR Clopyralid 2017~~ Knop M., 2019b, S19-00449
Drinking water ~~Ground water~~ ~~Surface water~~	ILV	0.050 µg/L	LC-MS/MS	~~Austin, R., Turner, R. (2013)~~ ~~DAS 120613~~ ~~DRAR Clopyralid 2017~~ Richter S., 2019, P 5211 G

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

~~Residues of clopyralid and picloram are extracted from water samples by passing 100 mL of water through a pre-conditioned Waters HLB solid phase extraction (SPE) column after adjusting the pH to below 2 with 1N HCl. The sample bottle is then rinsed with 1N HCl which is used to rinse the SPE column. The sample bottle is then rinsed with acetonitrile/1N formic acid (15:85) solution which is then used to rinse the SPE column, followed by drying under full vacuum. The SPE column is eluted with dichloromethane, which is evaporated to dryness using a gentle steam of nitrogen. The sample residue is reconstituted with a methanol/0.1% formic acid (10:90) solution filtered through a 0.2 µm PTFE syringe filter and then analyzed by liquid chromatography (Accucore Phenyl hexyl column, 4.6x50 mm, 2.6 µm; Mobile Phase: A) water containing 0.01% formic acid, B) 60:40 methanol:acetonitrile containing 0.01% formic acid, gradient elution) coupled with negative ion electrospray ionization tandem mass spectrometry (ESI LC-MS-MS).~~

~~(Shaffer, S. (2012) DAS 120611)~~

~~The objective of this study was to assess and to independently validate the method described in the Dow AgroSciences Method 120611, "Method Validation Study for the Determination of Residues of Clopyralid and picloram in Drinking Water, Ground Water, and Surface Water by LC-MS/MS.~~
~~Residues of clopyralid are extracted from water matrices by acidifying with 1 N hydrochloric acid (5 mL) followed by a solid phase extraction (SPE) clean up. The sample is transferred onto a conditioned 0.2 g Waters HLB column at an approximate rate of 2 mL/min. The sample bottle is rinsed with 1 N hydrochloric acid (1 mL) followed by 15:85 acetonitrile/1 N formic acid (5 mL) and the column washed with the rinse before drying under full vacuum for at least 30 minutes. The column is eluted with 14 mL of dichloromethane (DCM). The extract is evaporated to dryness using nitrogen and reconstituted in methanol/0.1% formic acid in water (10:90). The final extract is filtered through a 0.2 µm PTFE syringe filter and then analyzed by liquid chromatography (Accucore Phenyl hexyl column, 4.6x50 mm, 2.6 µm; Mobile Phase: A) water containing 0.01% formic acid, B) 60:40 methanol:acetonitrile containing 0.01% formic acid, gradient elution) coupled with negative ion electrospray ionization tandem mass spectrometry (ESI LC-MS/MS).~~
~~(Austin, R., Turner, R. (2013) DAS 120613)~~

Water samples from aquatic ecotoxicological studies

Report:	**KCP 5.2-01, Garagna, D. and Tediosi, E., 2011**
Title:	Validation of the analytical method for the determination of Clopyralid residues in water samples from aquatic ecotoxicological studies.
Document No:	CH-606/2011
Guidelines:	EEC: SANCO/3029/99 rev 4. EEC: SANCO/825/00 rev 8.1.
GLP	Yes

CONCLUSIONS

The analytical method was shown to be specific for Clopyralid residues in water samples from ecotoxicological tests.
The Clopyralid tested concentration in injected solutions ranged from 0.10 µg/mL to 4.98 µg/mL, corresponding to the same concentration in water samples and was found to be linear (correlation coefficient > 0.99).
The SANCO/3029/99 rev. 4 guideline requires any interference from the untreated control sample to be lower than 30 % at the L.O.Q. In the sequence of analysis of fortified samples at the two fortification levels (Low at 0.15 µg/mL and High at 1.50 µg/mL), the analysis of the control samples showed no significant interference.
The limit of quantification (L.O.Q.) of this method is defined as the lowest fortification level of 0.15 µg/mL (ppm) in water matrix samples, corresponding to an injected solution at the same concentration.
The limit of detection (L.O.D.) of this method is defined as 50% of the lowest calibration level, i.e.0.05 µg/mL, corresponding to the same concentration in water matrix samples.

Residue results calculated as values < 0.05 µg/mL are classified as not detected (n.d.).
Residue results calculated as higher than the limit of detection but less than limit of quantification, are designated as <L.O.Q. (< 0.15 µg/mL).

Spike add	**Mean Found**	**Test No.**	**RSD%**	**Recoveries**
Reconstituted water				
L) 0.15 µg/mL	0.15 µg/mL	6 det.	1.75 %	96.86 % - 101.82 % ; mean 98.61 %
H) 1.50 µg/mL	1.49 µg/mL	6 det.	1.18 %	98.13 % - 101.19 % ;

				mean 99.61 %
Algal growth medium				
L) 0.15 µg/mL	0.15 µg/mL	6 det.	4.56 %	92.96 % - 105.52 % ; mean 100.90 %
H) 1.50 µg/mL	1.47 µg/mL	6 det.	1.58 %	96.47 % - 100.66 % ; mean 98.48 %
***Lemna* growth medium**				
L) 0.15 µg/mL	0.15 µg/mL	6 det.	4.49 %	94.45 % - 107.02 % ; mean 98.58 %
H) 1.50 µg/mL	1.49 µg/mL	6 det.	3.58 %	95.60 % - 104.04% ; mean 99.89 %

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)

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

Table 5.2-9: Validated methods for air (if appropriate)

Component of residue definition: Clopyralid *(EFSA Journal 2018;16(7):5389)*			
Method type	**Method LOQ**	**Principle of method**	**Author(s), year / missing**
Primary	LOQ 4.5 µg/m^3	LC-MS/MS	~~Bacher, R. (2012) DAS 120601 DRAR Clopyralid 2017~~ Kirchherr M.. 2019, S19-00451

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

~~Air sampling used adsorption tubes filled with two portions of XAD adsorption material. Particles and aerosols were trapped by filtration or impact onto the adsorbent material. After sampling of air (6 hours), the front and the back adsorbent portions of the adsorption material were separated and both sections were extracted separately three times, each time with 3 mL of acetonitrile. The three extracts from the front portion were combined, and the volumes wereadjusted to 10 mL with acetonitrile. Extracts obtained from recoveries fortified at 100xLOQ (front portion) were further diluted by a factor of 50 using acetonitrile/water (2/8). Combined extracts of the blank control, LOQ, and 100xLOQ from the back portion of the tubes used to check for breakthrough were diluted by a factor of 5 using acetonitrile/water (2/8). Final determination of clopyralid was performed by LC-MS/MS (YMC Triart C18 column, 150 x 3.0 mm, 3.0 µm particle size, Securityguard: Phenomenex, C18, 4 x 3 mm; mobile phase: A – water with 0.1% formic acid, B – methanol with 0.1% formic acid, gradient elution), using the transition 192 m/z => 146 m/z as the primary transition ion of the analyte for quantification and the transition 192 m/z => 110 m/z as the secondary transition ion for confirmation of the presence of the analyte. (Bacher, R. (2012) DAS 120601)~~

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

An overview on the acceptable methods and possible data gaps for analysis of Clopyralid in body fluids

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

Table 5.2-10: Methods for body fluids and tissues (if appropriate)

Component of residue definition: Clopyralid *(EFSA Journal 2018;16(7):5389)*			
Method type	**Method LOQ**	**Principle of method**	**Author(s), year / missing**
Primary	LOQ 0.05 mg/L	LC-MS/MS	~~(2014)~~ ~~DAS 130727~~ ~~DRAR Clopyralid 2017~~ Abe Ch. 2019b, S19-00450

~~This method is applicable for the quantitative determination of residues of clopyralid in human blood and urine with a limit of quantitation of 0.05 mg/L.~~

~~For the method validation study, untreated control samples of human blood and urine were fortified at 0.05 mg/L with clopyralid. Urine was acidified and cleaned up over Isolut HM-N cartridges and eluted with dichloromethane. An aliquot of the dichloromethane phase was evaporated to dryness, and the residue was reconstituted in acetonitrile / water (2/8, v/v). Blood was extracted with acetone; an aliquot was evaporated to dryness and dissolved in acetonitrile/water (2/8, v/v). The extracts were analyzed by liquid chromatography with positive ion electrospray ionization (ESI) tandem mass spectrometry (LC-MS/MS) (YMC, J`Sphere ODS-H 80, C18 column, 150 x 3.0 mm, 4.0 µm particle size, Pre-column Phenomenex C18, 4 x 3 mm; mobile phase: A – water with 0.1% formic acid, B – methanol with 0.1% formic acid, gradient elution).~~
~~((2014) DAS 130727)~~

zRMS: The LOQ for body fluids in the method by Abe (2019) does not comply with the required limit of 0.01 mg/L set in SANTE/2020/12830 rev.1. (minor data gap to be completed after registration)

5.3.2.8 Other studies/ information

Not required.

5.3.3 Description of analytical methods for the determination of residues of active substance 2 (KCP 5.2)

Not required. The product PP-113H only has one active substance.

Appendix 1 Lists of data considered in support of the evaluation

List of data submitted by the applicant and relied on

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 5.1.1	Pardo, M.	2011	PP-113H (Clopyralid 10 % w/v SL): Validation of the analytical method for the determination of the active ingredient content. ChemService S.r.I. (Italy) Report No: CH-397/2011 GLP, Unpublished	N	Proplan, Plant Protection Company, SL
KCP 5.2	Garagna, D. and Tediosi, E.	2011	Validation of the analytical method for the determination of Clopyralid residues in water samples from aquatic ecotoxicological studies. ChemService S.r.I. (Italy) Report No: CH-606/2011 GLP, Unpublished	N	Proplan, Plant Protection Company, SL
KCP 5.2.~~1~~/01	Matthias Knop	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.~~1~~/02	Steffi Richer	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.~~1~~/03	Chizuko Abe	2019	Validation of an Analytical Method for the Determination of Clopyralid in Different Matrices of Animal Origin S19-00447 Eurofins Agroscience Services EcoChem GmbH	N	Proplan, Plant Protection Company, SL

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
			GLP Unpublished		PUH Chemirol Sp. zo.o.
KCP 5.2.~~1~~/04	Martin Schweizer	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.~~1~~/05	Matthias Knop	2019	Validation of an Analytical Method for the Determination of Clopyralid in Soil S19-00448 Eurofins Agroscience Services EcoChem GmbH GLP Unpublished	N	Proplan, Plant Protection Company, SL PUH Chemirol Sp. zo.o.
KCP 5.2.~~1~~/06	Matthias Knop	2019	Validation of an Analytical Method for the Determination of Clopyralid in Water S19-00449 Eurofins Agroscience Services EcoChem GmbH GLP Unpublished	N	Proplan, Plant Protection Company, SL PUH Chemirol Sp. zo.o.
KCP 5.2.~~1~~/07	Steffi Richter	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.~~1~~/08	Monika Kirchherr	2019	Clopyralid Validation of an Analytical Method for the Determination in Air S19-00451	N	Proplan, Plant Protection Company, SL

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		PUH Chemi-rol Sp. zo.o.
KCP 5.2.~~1~~09	Chizuko Abe	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 Chemi-rol Sp. zo.o.
KCP 5.3	Antón, B.	2020	Determination of Residues of Clopyralid (Common Moiety Method- Sum of Clopyralid, its Salts and Conjugates Expressed as Clopyralid) in Honey, after One Application of PP-113H (Clopyralid 100 g/L SL) in Phacelia tanacetifolia under semi- field conditions, at 4 Sites in Central and Southern Europe in 2020. Analytical phase report. Eurofins. Report No: S20-01463 GLP, Unpublished	N	Proplan, Plant Protection Company, SL

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
KCP 5.1.2	Hastings, M.J.	2002a	Determination of residues of clopyralid on agricultural crops by gas chromatography with negative-ion chemical ionization mass spectrometry. Dow AgroSciences LLC, Indianapolis, Indiana, USA	N	DAS

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
			Report No.: GH-C 5439, Date:19.04.2002, GLP, Non Published DAS No. O97		
KCP 5.1.2	Rawle, N.	2002	Independent laboratory validation of a method for the determination of clopyralid residues in crops. CEM Analytical Services Ltd, North Ascot, UK Report No.: CEMS-1671, Date:12.03.2002, GLP, Non Published DAS No. O95	N	DAS
KCP 5.1.2	Clements, B,; Harrington, R.	1997	Determination of Residues of MCPA. Clopyralid and Fluroxypyr in Grass and Cereal Grain and Straw Dow AgroSciences, LLC, Letcombe, UK Dow AgroSciences Study Number: ERC 97.10 15.10.1997 GLP, Non Published	N	DAS
~~KCP 5.1.2~~	~~Vogl, E.~~	~~2012~~	~~Method Validation Study for the Determination of Residues of Clopyralid and Picloram in Agricultural Commodities by LC-MS/MS~~ ~~ABC Laboratories, Inc., Columbia, Missouri, USA~~ ~~DAS Report No. 120610 21.9.2012~~ ~~GLP/GEP (Y/N): Yes~~ ~~Published (Y/N): No~~	~~N~~	~~DAS~~
~~KCP 5.1.2~~	~~Austin, R.~~	~~2012~~	~~Independent Laboratory Validation of Dow AgroSciences Method 120610, "Method Validation Study for the Determination of Residues of Clopyralid and Picloram in Agricultural Commodities by LC-MS/MS"~~ ~~Battelle UK Ltd, Ongar, Essex, United Kingdom~~ ~~DAS Report No. 120614 12.10.2012~~ ~~GLP/GEP (Y/N): Yes~~ ~~Published (Y/N): No~~	~~N~~	~~DAS~~
KCP 5.1.2	Kutschinski, A.H.	1979	Determination of residues of 3,6-dicloropicolinic acid and 2,4-D in barley and wheat by gas chromatography.	N	DAS

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**
			Dow Chemical USA, Agricultural Products Department Midland, Michigan, USA Report No.: ACR 79.5, Date:18.04.1979, Non GLP, Non Published DAS No. O38		
KCP 5.1.2	Jones, E.M.	1975a	Determination of 3,6-dicloropicolinic acid (DOWCO 290) in wheat and barley grain and straw by gas chromatography. Dow Chemical Company, Agricultural Research and Development, King´s Lynn, Norfolk, UK Report No.: ERC 77.4, Date: .5.1975, Non GLP, Non Published DAS No. O23	N	DAS
KCP 5.1.2	Wood, S.J.	1994	Determination of residues of clopyralid in grass. Dow Elanco Europe, Letcombe Laboratory, Wantage, Oxon, UK, Report No.: RV94.08, Date:30.08.1994, GLP, Non Published DAS No. O47	N	DAS
KCP 5.1.2	Jones, E.M.	1975b	Determination of 3,6-dicloropicolinic acid (DOWCO 290) in rape seed oil, cake and straw by gas chromatography. Dow Chemical Company, Agricultural Research and Development, King´s Lynn, Norfolk, UK Report No.: ERC 75.3, Date: .9.1975, Non GLP, Non Published	N	DAS

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
			DAS No. O29		
KCP 5.1.2	Kubitschek, C.E. and Kyle, J.A.	1994	Determination of clopyralid in sugar beets and sugar beet processed products by capillary gas chromatography/mass spectrometry. North American Environmental Chemistry Laboratory, Dow Elanco, Indianapolis, Indiana, USA Report No.: GRM 94.04, Date:16.06.1994, Non GLP, Non Published DAS No. O102	N	DAS
KCP 5.1.2	Anon.	1989	Analytical method: Determination of clopyralid residues in refined sugar. Dow Elanco Europe, Letcombe Laboratory, Wantage, Oxon, UK, Report No.: ERC 88.2, Date:25.10.1989, Non GLP, Non Published DAS No. O34	N	DAS
KCP 5.1.2	Anon.	1988	Analytical method: Determination of clopyralid residues in sugar beet molasses. Dow Elanco Europe, Letcombe Laboratory, Wantage, Oxon, UK, Report No.: ERC 88.1, Date:25.10.1988, Non GLP, Non Published DAS No. O31	N	DAS
KCP 5.1.2	Jones, E.M.	1977	Determination of 3,6-dicloropicolinic acid (DOWCO 290) in sugarbeet by gas chromatography. Dow Chemical Company, Agricultural Research and Development, King´s Lynn, Norfolk, UK Report No.: ERC 77.4, Date: .4.1977, Non GLP, Non Published	N	DAS

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**
			DAS No. O27		
KCP 5.1.2	Freeman, J.M.H. and Smith, D.W.	1983	Analytical method: Determination of clopyralid residues in wheat grain and straw. Dow Chemical Company, Agricultural Research and Development, King´s Lynn, Norfolk, UK Report No.: ERC 83.23, Date: .11.1983, Non GLP, Non Published DAS No. O25	N	DAS
KCP 5.1.2	Kuper, A.W	1974b	Determination of 3,6-dicloropicolinic acid in bovine tissues by gas chromatography. Dow Chemical USA, Agricultural Products Department Midland, Michigan, USA Report No.: ACR 74.9, Date:27.11.1974, Non GLP, Non Published DAS No. O40	N	DAS
KCP 5.1.2	Kutschinski, A.H.	1974	Determination of DOWCO 290 (3,6-dicloropicolinic acid) in milk and cream by gas chromatography. Dow Chemical USA, Agricultural Products Department Midland, Michigan, USA Report No.: ACR 74.3, Date:03.05.1974, Non GLP, Non Published DAS No. O12	N	DAS
KCP 5.1.2	Kuper, A.W.	1975	Determination of 3,6-dicloropicolinic acid in chicken tissues and eggs by gas chromatography. Dow Chemical USA, Agricultural Products Department Midland, Michigan, USA Report No.: ACR 75.2,	N	DAS

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**
			Date:27.03.1975, Non GLP, Non Published DAS No. O13		
KCP 5.1.2	Kuper, A.W.	1974a	Determination of residues of 3,6-dicloropicolinic acid in chicken tissues and eggs by gas chromatography. Dow Chemical USA, Agricultural Products Department Midland, Michigan, USA Report No.: ACR 74.2, Date:11.04.1974, Non GLP, Non Published DAS No. O11	N	DAS
KCP 5.1.2	Hastings, M.J.	2002b	Determination of residues of clopyralid in animal tissues by gas chromatography with negative-ion chemical ionization mass spectrometry. Dow AgroSciences LLC, Indianapolis, Indiana, USA Report No.: GH-C 5440 (GRM 02.14), Date:19.04.2002, GLP, Non Published DAS No. O96	N	DAS
~~KCP 5.1.2~~	~~Vincent, T. P.~~	~~2013~~	~~Method Validation Study for the Determination of Residues of Clopyralid and Picloram in Soil by LC-MS/MS~~ ~~ABC Laboratories, Inc., Columbia, Missouri, USA~~ ~~DAS Report No. 120612 20.2.2013~~ ~~GLP/GEP (Y/N): Yes~~ ~~Published (Y/N): No~~	~~N~~	~~DAS~~
~~KCP 5.1.2~~	~~Austin, R., Turner, R.~~	~~2014~~	~~Independent Laboratory Validation of a Dow AgroSciences Method for the Determination of Residues of Clopyralid and Picloram in Soil by LC-MS/MS~~ ~~Battelle UK Ltd, Chelmsford, Essex, United Kingdom~~ ~~DAS Report No. 140079 19.5.2014~~ ~~GLP/GEP (Y/N): Yes~~ ~~Published (Y/N): No~~	~~N~~	~~DAS~~

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~~	~~Shaffer, S.~~	~~2012~~	~~Method Validation Study for the Determination of Residues of Clopyralid and Picloram in Drinking Water, Ground Water, and Surface Water by LC-MS/MS~~ ~~ABC Laboratories, Inc., Columbia, Missouri, USA~~ ~~DAS Report No. 120611 4.12.2012~~ ~~GLP/GEP (Y/N): Yes~~ ~~Published (Y/N): No~~	N	DAS
~~KCP 5.1.2~~	~~Austin, R., Turner, R.~~	~~2013~~	~~Independent Laboratory Validation of Dow AgroSciences Method 120611, "Method Validation Study for the Determination of Residues of Clopyralid and Picloram in Drinking Water, Ground Water, and Surface Water by LC-MS/MS"~~ ~~Battelle UK Ltd, Ongar, Essex, United Kingdom~~ ~~DAS Report No. 120613 5.4.2013~~ ~~GLP/GEP (Y/N): Yes~~ ~~Published (Y/N): No~~	N	DAS
~~KCP 5.1.2~~	~~Bacher, R.~~	~~2012~~	~~The Development and Validation of a Method for the Analysis of Clopyralid in Air~~ ~~PTRL Europe GmbH, D-89081 Ulm, Germany DAS Report No. 120601 4.10.2012~~ ~~GLP/GEP (Y/N): Yes~~ ~~Published (Y/N): No~~	N	DAS
~~KCP 5.1.2~~	~~-~~	~~2014~~	~~Development and Validation of an Analytical Method for the Determination of Clopyralid in Body Fluid(s)~~ ~~DAS Report No. 130727 9.7.2014~~ ~~GLP/GEP (Y/N): Yes~~ ~~Published (Y/N): No~~	N	DAS

The following tables are to be completed by MS

List of data submitted by the applicant and not relied on

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

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

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

Appendix 2 Detailed evaluation of submitted analytical methods

A 2.1 Analytical methods for the active substance 1

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

New studies to support Ecotoxicology section are described below, not previously evaluated in a peer reviewed process at EU level:

zRMS: the method is acceptable

Reference:	KCP 5.3 Antón, B. (2020)
Report	Determination of Residues of Clopyralid (Common Moiety Method- Sum of Clopyralid, its Salts and Conjugates Expressed as Clopyralid) in Honey, after One Application of PP-113H (Clopyralid 100 g/L SL) in Phacelia tanacetifolia under semi- field conditions, at 4 Sites in Central and Southern Europe in 2020. Analytical phase report.
Report No.	S20-01463
Guideline(s):	SANCO/3029/99 rev.4 (2000), SANCO/825/00 rev. 8.1, SANTE/11956/2016 rev. 9
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Aim of the Study

To determine residue levels of Clopyralid (common moiety method – sum of Clopyralid, its salts and conjugates expressed as Clopyralid) in honey from Phacelia tanacetifolia after one application of PP-113H (Clopyralid 100 g/L SL), under semi-field conditions. The study will be conducted as four separate field trials in Spain and Germany, in 2020.

1. Analytical method validation for clopyralid quantification

AIM

The aim of the test is to validate the analytical method for the quantification of the Clopyralid content in honey following the guideline SANCO/3029/99 rev. 4 and SANCO/825/00 rev. 8.1.

The Clopyralid content was determined by UPLC-MS/MS.

EXPERIMENTAL/PROCEDURE

Reagents, materials, equipment and apparatus

- Water
- Acetonitrile LCMS
- Methanol LCMS
- Formic acid LCMS

- Acetic acid LCMS
- QUECHERS extraction reagent (without citrate)
- Sodium hydroxide 5 N
- Sulphuric acid 5 N
- Honey
- 5, 50, 100, 500 and 1000 ml volumetric flasks
- 1, 5 and10 ml pipette
- Beakers
- Cylinders
- Glass vials and plastic tubes
- Analytical balance, Mettler Toledo, XA105DU, serial nr. B121145611
- UPLC-MS/MS, Waters Acquity UPLC H-Class
- Ultrasonic bath, Bandelin electronic, Sonorex Super RK100 3L serial nr. 301.00098580.007 and Sonorex Super RK510 9.7L serial nr. 327.00098578.006
- Micropipette Brand Transferpette S digital serial nr. 16A06848 and 20C49914
- Micropipette PL200 Pluripet (Kartell) serial nr. 756850029
- Micropipette Kartell PL5000 serial nr. 656880100
- Centrifuge apparatus, Selecta Macrotonic-BL serial nr. 478482
- Dispenser, Brand Dispensette Organic, Dig, Easy calibration serial nr. 04L 21831
- Vortex, Heidolph REAX top serial nr. 080626378
- Agitax, Cirtalab SR1-CP57 serial nr. 034CP57
- Chronometer, Oregon scientific TR118

Preparation of the reference item solutions

SM solution: In a 50 ml volumetric flask, 9.8 mg of reference item were weighed. It was dissolved with methanol in the ultrasonic bath and the final volume was adjusted with methanol.

SMLOQ solution: In a 50 ml volumetric flask, 125 µl SM were added. Final volume was made with methanol.

SM10LOQ solution: In a 5 ml volumetric flask, 125 µl SM were added. Final volume was made with methanol.

The proper amounts indicated in the following table of the indicated reference item solution and matrix were added, using suitable micropipettes, into a glass vial.

SOLUTION	AMOUNT OF REFERENCE ITEM	MATRIX	THEORETICAL CONCENTRATION (µg/l)
P1	100 µl of P4	900 µl	0.5
P2	100 µl of P5	900 µl	1.0
P3	100 µl of P6	900 µl	2.5
P4	100 µl of P7	900 µl	5.0
P5	100 µl of P8	900 µl	10
P6	50 µl of SMLOQ	950 µl	25
P7	100 µl of SMLOQ	900 µl	50
P8	200 µl of SMLOQ	800 µl	100

Preparation of matrix

In a plastic tube, 5 g of honey and 9 ml of water were added and it was dispersed. 300 µl of sodium hydroxide 5 N were added and it was let to rest for approx. 30 min at room temperature. 300 µl of sulphuric acid 5 N were added. Then 10 ml of acetonitrile were added. It was shaken and QUECHERS extraction reagent were added. It was stirred approx. 20 s. in the vortex and then in the agitax for approx. 5 min. It was centrifuged (5 min, 3000 rpm) at room temperature. The upper liquid was diluted 1:1 with water and filtered.

Agitax conditions for all the study:

Amplitude (mm)	Speed (m/s)	Acceleration (m/s^2)	Jerk (m/s^3)·1000	Delay (s)	Time (s)	Reps (Nr)
90	2.0	99	5	0	300	0

Preparation of acid acetonitrile

In a 500 ml volumetric flask, 5 ml of formic acid were added. Final volume was made up with acetonitrile.

Preparation of eluent a

In a 1 l volumetric flask, 0.1 ml acetic acid and 50 ml of acetonitrile were added. Final volume was made up with water.
It was degasified in an ultrasonic bath.

Preparation of eluent b

In a 1 l volumetric flask, 0.1 ml acetic acid and 50 ml of water were added. Final volume was made up with acetonitrile.
It was degasified in an ultrasonic bath.

Method conditions

LC System	Waters ACQUITY UPLC H-Class
LC Column	C18 Zorbax Eclipse Plus (4.6x100mm), Temp. column: 40 ºC
LC Method	Solvent A : Eluent A Solvent B : Eluent B Flow : 1.4 ml/min Injection Volume : 20 µl Analysis time : 6 min Sample Temp. : 12 º C

Time (min)	A (%)	B (%)
0	80	20
1.0	70	30
3.0	50	50
3.5	50	50
3.6	80	20
6	80	20

Ion Source Conditions ESI Negative Polarity	Source Temperature	:	130 ºC
	Desolvation temperature	:	500 ºC
	Source Voltages		
	Capillary	:	1.0 (kV)
	Source Gas Flow		
	Desolvation	:	800 (L/Hr)
	Cone	:	50 (L/Hr)
MS/MS Conditions in negative mode	Span (Da)	:	0.7
	MS/MS transition for quantification		
	189.71 m/z > 145.88 m/z		
	Cone potential (V)	:	22
	Collision potential (eV)	:	10
	Dwell time (s)	:	0.1
	MS/MS transition for confirmation		
	191.71 m/z > 147.88 m/z		
	Cone potential (V)	:	22
	Collision potential (eV)	:	10
	Dwell time (s)	:	0.1

Active ingredient quantification

For the quantification of the Clopyralid, the regression curve had the following expression:

$$y = b \cdot x + a \rightarrow area\ (y) = b \cdot conc.(x) + a$$

However, to adjust properly the regression curve to the residue determination, in which the determination was done between a big range of standard concentrations, a weighting factor *w=1/x* was applied. Then for the calculation of the weighted straight line the following equations were followed in order to obtain the desired parameters:

$$b = \frac{\sum w_i \cdot \sum w_i x_i y_i - \sum w_i x_i \cdot \sum w_i y_i}{\sum w_i \cdot \sum w_i x_i^2 - (\sum w_i x_i)^2}$$

$$a = \frac{\sum w_i x_i^2 \cdot \sum w_i y_i - \sum w_i x_i \cdot \sum w_i x_i y_i}{\sum w_i \cdot \sum w_i x_i^2 - (\sum w_i x_i)^2}$$

$$r = \frac{\sum w_i \cdot \sum w_i x_i y_i - \sum w_i x_i \cdot \sum w_i y_i}{\sqrt{\sum w_i \cdot \sum w_i x_i^2 - (\sum w_i x_i)^2} \cdot \sqrt{\sum w_i \cdot \sum w_i y_i^2 - (\sum w_i y_i)^2}}$$

Where:

w_i: is the weighting factor chosen 1/x, where *x* means nominal concentration (µg/ml)

x_i, y_i: is the *i* th data pair of the *n* total points.

To calculate the (µg/g) Clopyralid, the following expression was used:

$$(\mu g/g)\ Clopyralid = ((A_M - b) / a) \cdot (V_M / W_M \cdot D)$$

Where:

A_M: area of Clopyralid in the sample
a: slope of the curve
b: interception of the curve
W_M: weight in g of the specimen
V_M: Volume of the specimen solution (ml)
D: Dilution

SPECIFICITY

Principle

Specificity is the ability to measure accurately and specifically the analyte of interest in the presence of other components that may be expected to be present in the sample matrix.

Preparation of the solutions

Matrix was used.

Determination procedure

To determine the specificity of the analytical method, the following solution was analysed:

- Matrix

Results and data processing

Matrix values obtained for the Validation

REPLICATE Nr.	AREA CLOPYRALID	
	Quantification Trace	Confirmation Trace
1	2.6810	0.1980
2	1.7860	0.4540
3	3.0490	0
Average	2.51	0.22
% Matrix Interference:	**3.36**	**0.41**

Conclusion

The interferences do not contribute in ± 30 % of LOQ and no overlap of peaks was observed.

LINEARITY

Principle

Linearity is the ability of the method to elicit test results that are directly proportional to analyte concentration within a given range. Linearity is generally reported as the variance of the slope of the regression line.

Preparation of the solutions

The reference item solutions preparation was explained before.

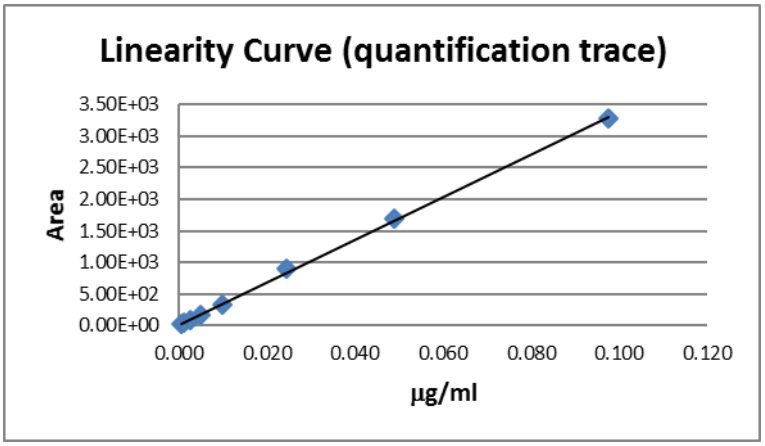
Determination procedure

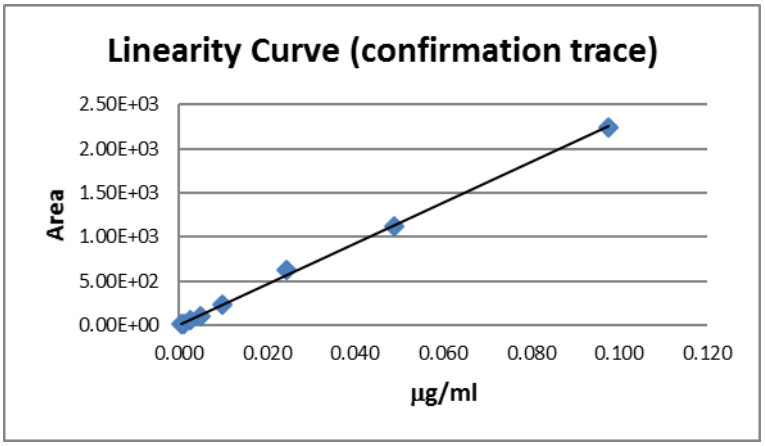
To verify the linearity, 8 reference item solutions were prepared inside the range defined. These concentrations were distributed as symmetrically as possible inside the range.

Each one of the levels of concentration was measured by one injection.

Results and data processing

Solution ID	Concentration (µg/ml)	Quantification trace	Confirmation trace
		Area	Area
P1	0.0005	18.1750	16.2260
P2	0.0010	40.6540	24.4910
P3	0.0024	90.9130	57.2180
P4	0.0049	173.2840	105.6990
P5	0.0098	326.9610	236.2690
P6	0.0244	903.9220	635.6730
P7	0.0488	1685.0130	1125.9290
P8	0.0977	3280.4460	2244.7740
Equation:		$y = 34218.99\ x + 4.23$	$y = 23316.62\ x + 3.38$
Correlation Coefficient: r		0.9994	0.9988





Conclusion

From experimental results obtained we could say that the method presents linearity inside the range of 0.49– 97.7 µg/l of Clopyralid due to a correlation coefficient ≥ 0.99.

ACCURACY

Principle

Accuracy is the measure of exactness of an analytical method, or the closeness of agreement between the value which is accepted either as a conventional, true value or an accepted reference value and the value found. It is measured as the percent of analyte recovered by assay, by spiking samples in a blind study.

Preparation of the solutions

For the quantification of the samples, reference item solutions prepared for the linearity were used.

Accuracy was done by means of two levels of fortification, one at the limit of quantification LOQ and the other at a higher concentration. In both cases, 5 solutions of each level were prepared.
The LOD was calculated from LOQ results where it is the 30 % of the LOQ.

SOLUTION	REFERENCE ITEM SOLUTION	HONEY (g)	FINAL VOLUME (ml)	DILUTION	THEORETICAL CONCENTRATION (µg/l)
MA (LOQ) 0.01 µg/g	100 µl of SMLOQ	5	10	1:1	2.5
MB (10xLOQ) 0.1 µg/g	100 µl of SM10LOQ	5	10	1:1	25

In a plastic tube, approx. 5 g of honey, reference item and 9 ml of water were added, it was dispersed. 300 µl of sodium hydroxide 5 N were added and it was let to rest for approx. 30 min at room temperature. 300 µl of sulphuric acid 5 N were added. Then 10 ml of acid acetonitrile were added. It was shaken and QUECHERS extraction reagent was added. It was stirred approx. 20 s. in the vortex and then in the agitax for approx. 5 min (see agitax conditions in A3/2.3). It was centrifuged (5 min, 3000 rpm) at room temperature.
The upper liquid was diluted 1:1 with water and then it was filtered.

Determination procedure

1 injection was carried out for each one of the samples. The linearity described before was used to quantify the samples.

Results and data processing

Quantification Trace				
LOQ (calculated content = 0.0024 µg/ml, equivalent to 0.01 mg/kg honey)				
SAMPLE	Area	Experimental Clopyralid Concentration		Recovery (%)
		µg/ml extract	mg/kg honey	
LOQ 1	81.7830	0.0023	0.0089	91.18
LOQ 2	78.8620	0.0022	0.0086	88.48
LOQ 3	65.2610	0.0018	0.0071	72.76
LOQ 4	73.0850	0.0020	0.0080	82.09
LOQ 5	73.3190	0.0020	0.0080	82.02
Average		0.0021	0.0081	83.31
Standard Deviation		0.0002	0.0007	7.12

Relative Standard Deviation (%)		9.0	8.55	8.55
10xLOQ (calculated content = 0.0244 µg/ml, equivalent to 0.10 mg/kg honey)				
SAMPLE	**Area**	**Experimental Clopyralid Concentration**		**Recovery (%)**
		µg/ml extract	**mg/kg honey**	
10xLOQ 1	698.5380	0.0203	0.0812	83.12
10xLOQ 2	690.5100	0.0201	0.0785	80.33
10xLOQ 3	660.9260	0.0192	0.0746	76.38
10xLOQ 4	717.8650	0.0209	0.0835	85.47
10xLOQ 5	731.8770	0.0213	0.0836	85.55
Average		0.0203	0.0803	82.17
Standard Deviation		0.0008	0.0038	3.88
Relative Standard Deviation (%)		3.9	4.72	4.72
Confirmation Trace				
LOQ (calculated content = 0.0024 µg/ml, equivalent to 0.01 mg/kg honey)				
SAMPLE	**Area**	**Experimental Clopyralid Concentration**		**Recovery (%)**
		µg/ml extract	**mg/kg honey**	
LOQ 1	49.5740	0.0020	0.0078	79.70
LOQ 2	52.2280	0.0021	0.0083	84.99
LOQ 3	49.1040	0.0020	0.0078	80.00
LOQ 4	62.3630	0.0025	0.0101	103.20
LOQ 5	51.3250	0.0021	0.0082	83.52
Average		0.0021	0.0084	86.28
Standard Deviation		0.0002	0.0009	9.72
Relative Standard Deviation (%)		11.0	11.27	11.27
10xLOQ (calculated content = 0.0244 µg/ml, equivalent to 0.10 mg/kg honey)				
SAMPLE	**Area**	**Experimental Clopyralid Concentration**		**Recovery (%)**
		µg/ml extract	**mg/kg honey**	
10xLOQ 1	484.7050	0.0206	0.0826	84.57
10xLOQ 2	494.9790	0.0211	0.0825	84.45
10xLOQ 3	460.6260	0.0196	0.0762	78.05
10xLOQ 4	485.3360	0.0207	0.0828	84.71
10xLOQ 5	489.1920	0.0208	0.0819	83.82
Average		0.0206	0.0812	83.12
Standard Deviation		0.0006	0.0028	2.86
Relative Standard Deviation (%)		2.7	3.44	3.44

- Accuracy calculation:

Recovery (%): (Obtained Content / Calculated Content) * 100

SAMPLE	RECOVERY (%)	
	Quantification Trace	Confirmation Trace
LOQ	83.3	86.3
10xLOQ	82.2	83.1
Mean recovery (%)	**82.7**	**84.7**

Conclusion

Considering the LOQ and the 10xLOQ and the mean of both fortification levels, the percentage of recovery to obtain a good accuracy of the method should be between the values 70-120 %, all values obtained are inside this range.
In both fortification levels the RSD is lower than 20 %, for which a good precision of the method is deduced.

2. Test Methodology of Clopyralid content in honey

To determine the content of Clopyralid, as the active material present in honey.

Equipment and apparatus

- Freezer, Liebherr, GN 2723 serial nr. 29.364.395.3
- Analytical balance, Ohaus Explorer e-12140 serial nr. 1121011569

Procedure

The quantification of the specimen was carried out by the described method in the Analytical Method Validation. Linearity injected in analytical method validation was used to determine specimen content from trial 01 and 02 and for specimen content from trial 03 and 04 it was prepared and injected again.

Preparation of specimens

For the analysis of the content of Clopyralid, 1 sample for each one of the specimens received was prepared, carrying one injection per sample.

In a plastic tube, approx. 5 g of specimen and 9 ml of water were added, it was dispersed. 300 □l of sodium hydroxide 5 N were added and it was let to rest for approx. 30 min at room temperature. 300 □l of sulphuric acid 5 N were added. Then 10 ml of acid acetonitrile were added. It was shaken and QUECHERS extraction reagent was added. It was stirred approx. 20 s. in the vortex and then in the agitax for approx. 5 min (see conditions in A3/2.3). It was centrifuged (5 min, 3000 rpm) at room temperature.
The upper liquid was diluted 1:1 with water and then it was filtered.

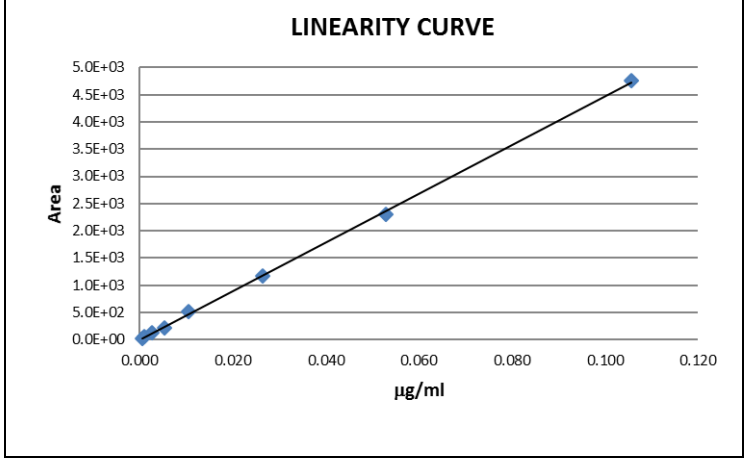
Experimental results

Specimen content from trial 01 and 02 using linearity defined in A3/4.4 (quantification trace):

Specimen	Area	Content (µg/g)
L20-01463-01-C-S1-HO-A1	15.1580	0.0012 (< LOQ)
L20-01463-01-T-S1-HO-A1	174.7040	0.0194
L20-01463-02-C-S1-HO-A1	11.9260	0.0009 (< LOQ)
L20-01463-02-T-S1-HO-A1	1089.1240	0.1232

Specimen content from trial 03, linearity was injected again:

Solution ID	Concentration (µg/ml)	Quantification trace
		Area
P1	0.0005	24.7420
P2	0.0011	50.7030
P3	0.0026	122.1310
P4	0.0053	210.7920
P5	0.0106	522.3600
P6	0.0264	1165.7450
P7	0.0528	2302.7930
P8	0.1057	4750.0630
Equation:		$y = 44558.54\,x + 1.84$
Correlation Coefficient: r		0.9994

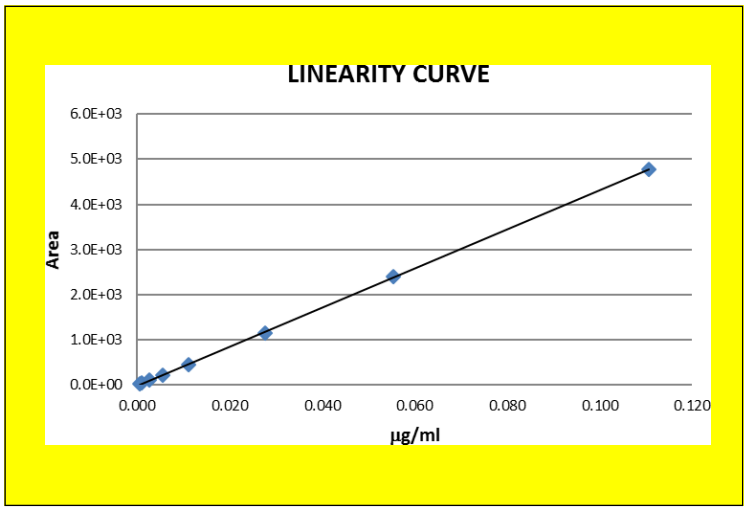


Specimen	Area	Content (µg/g)
L20-01463-03-C-S1-HO-A1	2.8270	0.0001 (< LOQ)
L20-01463-03-T-S1-HO-A1	1752.6840	0.1537

Specimen content from trial 04, linearity was injected again:

Solution ID	Concentration (µg/ml)	Quantification trace
		Area
P1	0.0006	30.5860
P2	0.0011	50.1520
P3	0.0028	121.7540
P4	0.0055	223.3290
P5	0.0111	457.5930
P6	0.0277	1154.0480
P7	0.0553	2404.0860
P8	0.1107	4780.4800

Equation:	$y = 42826.34\ x + 3.55$
Correlation Coefficient: r	0.9997



Specimen	Area	Content (µg/g)
L20-01463-04-C-S1-HO-A1	4.8790	0.0003 (< LOQ)
L20-01463-04-C-S1-3-HO-A	3.2960	0.0003 (< LOQ)
L20-01463-04-T-S1-HO-R1	3657.3720	0.3397

Conclusion

Specimen	Clopyralid Content (µg/g)
L20-01463-01-C-S1-HO-A1	< LOQ
L20-01463-01-T-S1-HO-A1	0.02
L20-01463-02-C-S1-HO-A1	< LOQ
L20-01463-02-T-S1-HO-A1	0.12
L20-01463-03-C-S1-HO-A1	< LOQ
L20-01463-03-T-S1-HO-A1	0.15
L20-01463-04-C-S1-HO-A1	< LOQ
L20-01463-04-C-S1-3-HO-A	< LOQ
L20-01463-04-T-S1-HO-R1	0.34

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

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

No new or additional studies have been submitted

A 2.1.2.1.1 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.~~1~~/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	10 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. 2.1 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 Eluent B: Methanol containing 1 % (v/v) acetic acid
Retention time(s)	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 1: 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 *m/z* 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 *m/z* 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 *m/z* 192→148 (Quantification)						
Tomato	X36538	0.01	82	4	77	8
		0.1	71	4		
Olive	X36538	0.01	107	7	98	10
		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 *m/z* 190→146 (Confirmation)						

Matrix	Analyte	Fortification level (mg/kg) (*n* = 5)	Mean recovery on levels (%)	RSD (%)	Overall Mean Recovery (%)	Overall RSD (%)
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 2: 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	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. The calibration curves obtained for both mass transitions and all matrices were linear since correlation coefficients (R) were ≥ 0.995. Linear regression was performed with 1/x-weighting.
Calibration range	<table><tr><td>Matrix</td><td>Calibration range</td><td>Corresponding fortification level</td></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>
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 A 1
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.~~1~~/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 3: 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		
Mass Transition *m/z* 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		

Matrix	Analyte	Fortification level (mg/kg) (*n* = 5)	Mean recovery on levels (%)	RSD (%)	Overall Mean Recovery (%)	Overall RSD (%)
Rice	Clopyralid	0.01	84	14	89	13
		0.1	94	11		
Mass Transition *m/z* 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 *m/z* 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 4: 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	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. 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. The calibration curves obtained for both mass transitions and all matrices were linear since correlation coefficients (R) were ≥ 0.99, 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.
Calibration range	Matrix: Tomato — Calibration range (mg/kg): 0.003 - 1.0 Matrix: Olive — Calibration range (mg/kg): 0.003 - 2.0 Matrix: Rice — Calibration range (mg/kg): 0.003 - 2.0

	<table><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></table>
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 3.
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

Reference: KCP 5.2.~~1~~/03

Report: Validation of an Analytical Method for the Determination of Clopyralid

	in Different Matrices of Animal Origin, 2019, Abe Ch. 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	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. 2.1 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 5: 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 *m/z* 190→146 (Quantification)						
Bovine	Clopyralid	0.01	88.9	2.5	85.7	4.3

Matrix	Analyte	Fortification level (mg/kg) (*n* = 5)	Mean recovery on levels (%)	RSD (%)	Overall Mean Recovery (%)	Overall RSD (%)
meat		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 *m/z* 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 6: Characteristics for the analytical method used for validation of Clopyralid residues in animal matrices.

	Clopyralid
Specificity	LC-MS/MS determination was conducted by monitoring two (2) mass transitions (*m/z* 190→146 and *m/z* 192→148). Mass transition 190→146 *m/z* is proposed to be used for quantification but both mass transitions are applicable interchangeably for quantification and confirmation. A reagent blank and two (2) control samples per matrix were extracted and analysed

<table>
<tr><td></td><td>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.</td></tr>
<tr><td>Calibration</td><td>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.
The calibration curves obtained for both ion mass transitions and all matrices were linear since coefficients of determination correlation coefficients (R) were ≥ 0.999. Linear regression was performed with 1/x-weighting.</td></tr>
<tr><td>Calibration range</td><td>
<table>
<tr><td>Matrix</td><td>Calibration range (mg/kg)</td></tr>
<tr><td colspan="2">Quantification; m/z 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; m/z 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>
</td></tr>
<tr><td>Accuracy and Precision</td><td>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).
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 5.</td></tr>
<tr><td>Assessment of matrix effects is presented</td><td>Yes
Matrix effects were ≥ ± 20 % and deemed to be significant for bovine liver and bovine kidney. Therefore, matrix-matched standards were used for quantification throughout the study.
Matrix suppression or enhancement was < 20 % 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.</td></tr>
<tr><td>Limit of determination/quantification</td><td>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.
The LOD was set at 20 % of the LOQ which is 0.002 mg/kg.</td></tr>
<tr><td>Stability of Clopyralid in Stock and Fortification Solutions</td><td>The stock solution: The mean peak area of the stored diluted stock solution was within ± 20 % 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.
The fortification solutions: The mean peak area of the stored diluted stock solution was within ± 20 % 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</td></tr>
</table>

	the dark for 21 days.
Stability of Analyte(s) in Sample Extracts	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. The mean recovery value(s) of the re-analysed extracts were in the range of 70 - 120 % and within ± 20 % 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.

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 ≤ 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

Reference:	KCP 5.2.~~1~~_/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, hydrolysation 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 ≤ 18°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 tmatrices	
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 7: 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 8: 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

<table>
<tr><td></td><td>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.</td></tr>
<tr><td>Calibration</td><td>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.</td></tr>
<tr><td>Calibration range</td><td><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></td></tr>
<tr><td>Accuracy and Precision</td><td>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 7.</td></tr>
<tr><td>Assessment of matrix effects is presented</td><td>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.</td></tr>
<tr><td>Limit of determination/quantification</td><td>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.</td></tr>
<tr><td>Stability of Clopyralid in Stock and Fortification Solutions</td><td>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.</td></tr>
<tr><td>Stability of Analyte(s) in Sample Extracts</td><td>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.</td></tr>
</table>

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 ≤ 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

Reference:	KCP 5.2.~~1~~ /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 9: Recovery results from method avalidation 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 *m/z* 192→148 (Quantification)						
Soil	Clopyralid	0.5	87	7	86	5
		5.0	85	2		
Mass Transition *m/z* 190→146 (Confirmation)						
Soil	Clopyralid	0.5	79	2	83	5
		5.0	87	2		

Table A 10: 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	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 9.
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	Clopyralid was found to be stable in stock solutions for 11 days when prepared in

Stock and Fortification Solutions	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

Reference:	KCP 5.2.~~1~~ /06
Report	Validation of an Analytical Method for the Determination of Clopyralid in Water, 2019, Knop M., 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 acid
Retention time(s)	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 11: 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 *m/z* 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 *m/z* 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 12: 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 inorder 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	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 12.
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

Reference:	KCP 5.2.~~1~~ /07
Report	Independent Laboratory Validation of an Analytical Method for the Determination of Clopyralid in Water, 2019, Richter S., 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 13: 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 *m/z* 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 *m/z* 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 14: Characteristics for the analytical method used for validation of Clopyralid residues in drinking water

	Clopyralid
Specificity	Quantification was performed by use of LC-MS/MS detection. Two (2) mass transitions were evaluated inorder to demonstrate that the method achieves a high

<table>
<tr><td></td><td>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.</td></tr>
<tr><td>Calibration</td><td>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.</td></tr>
<tr><td>Calibration range</td><td><table><tr><td>Matrix</td><td>Calibration range (µg/L)</td></tr><tr><td colspan="2">Quantification; m/z 192→148</td></tr><tr><td>Water (drinking and surface)</td><td>0.015 – 10</td></tr><tr><td colspan="2">Qualification; m/z 190→146</td></tr><tr><td>Water (drinking and surface)</td><td>0.015 – 10</td></tr></table></td></tr>
<tr><td>Accuracy and Precision</td><td>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 12.</td></tr>
<tr><td>Assessment of matrix effects is presented</td><td>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.</td></tr>
<tr><td>Limit of determination/quantification</td><td>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.</td></tr>
<tr><td>Stability of Clopyralid in Stock and Fortification Solutions</td><td>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.</td></tr>
<tr><td>Stability of Analyte(s) in Sample Extracts</td><td>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.</td></tr>
</table>

Conclusion
The method was successfully independently validated for the determination of clopyralid in drinking water from the tested LOQ of 0.05 µg/L up to 0.50 µg/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 2.1.2.4.3 Method for the determination of Clopyralid residues in water samples from aquat-ic ecotoxicological studies.

zRMS: the method is acceptable

Report:	**KCP 5.2-01; Garagna, D. and Tediosi, E., 2011**
Title:	Validation of the analytical method for the determination of Clopyralid residues in water samples from aquatic ecotoxicological studies.
Document No:	CH-606/2011
Guidelines:	EEC: SANCO/3029/99 rev 4. EEC: SANCO/825/00 rev 8.1.
GLP	Yes

INTRODUCTION

The Test Facility conducted a study to develop and validate an analytical method for the determination of Clopyralid residues in water samples from the aquatic ecotoxicological studies.
The study was performed in compliance with Study Plan CH–606/2011 and the following guidelines:

- EEC guideline SANCO/3029/99 rev. 4 dated 11/07/00: Working document "Guidance for generating and reporting methods of analysis in support of residue data requirements for Annex II (part A, Section 4) and Annex III (part A, Section 5) of the Directive 91/414".
- EEC guideline SANCO/825/00 rev. 8.1 dated 16/11/2010: "Guidance Document on Residue Analytical Methods".

and the following data requirements:

- Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009, concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC.
- Commission Regulation (EU) No 544/2011 of 10 June 2011, implementing Regulation (EC) No 1107/2009 of the European Parliament and of the Council as regards the data requirements for active substances.

Reconstituted water, algal growth medium and *Lemna* Steinberg growth medium used for method validation, were prepared in accordance with:

- OECD Guideline No. 203. "Fish, acute toxicity test", 1992.
- OECD Guideline No. 202. "*Daphnia* sp., Acute Immobilization Test", April 2004.
- OECD Guideline No. 201, "Freshwater algae and cyanobacteria growth inhibition test", 2006.
- OECD Guideline No. 221, "*Lemna* sp. Growth Inhibition Test", 2006.

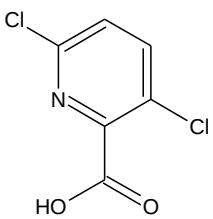
Method validation was performed as described in the "Standard Operating Procedures" in force at the involved laboratories.
The experimental phase of this study started on December 29, 2011 and was completed on January 10, 2012.

MATERIALS

Test item

A sample of Clopyralid was received by the Test Facility on October 12, 2011 (Internal code STU 452.1) as a 250 mg sample contained in an amber glass bottle.

Test item identification : Clopyralid
Common name : Clopyralid (BSI, ANSI, draft E-ISO, (m) draft F-ISO)
Chemical name (IUPAC) : 3,6-dichloro-2-pyridinecarboxylic acid
Chemical structure :



Chemical formula	:	$C_6H_3Cl_2NO_2$
Chemical Class	:	Herbicide
C.A.S. number	:	1702-17-6
Molecular weight	:	192.0
Reference material identification	:	Clopyralid PESTANAL
Internal Number	:	STU 452.1
Batch Number	:	SZBA166X
Purity	:	98.7 %
Production Date	:	June 15, 2010
Expiry Date	:	June 15, 2015
Storage conditions	:	stored at -20°C
Stability	:	stable under recommended storage conditions

To avoid degradation and assure more conservative conditions, the reference material was stored at -20°C in the freezer, Internal code No. 153, even if the relevant Certificate of Analysis recommends storage at room temperature.

Water samples

The method validation was performed on reconstituted water, on algal growth medium and *Lemna* Steinberg growth medium.

Reconstituted water was prepared as described in Annex 2 of OECD 203, 1992 (Fish, Acute toxicity test) and in Annex 3 of OECD 202, 2004 (*Daphnia* sp., Acute Immobilisation Test). The composition is as follows:

In de-ionized water (conductivity < 5 µS/cm), analytical grade, the following salts are added to a defined final nominal concentration:

$CaCl_2 \times 2H_2O$	:	2.0 mmol/L	(= 294 mg/L)
$MgSO_4 \times 7H_2O$	:	0.5 mmol/L	(= 123 mg/L)
$NaHCO_3$	:	0.75 mmol/L	(= 65 mg/L)
KCl	:	0.075 mmol/L	(= 5.8 mg/L)

Algal growth medium (OECD medium) was prepared as described in Annex 3 of OECD 201, 2006 (Freshwater Alga and Cyanobacteria, Growth Inhibition Test). The composition is as follows:

In de-ionized water (conductivity < 5 µS/cm), analytical grade, the following salts are added to a defined final nominal concentration:

Macro-nutrients:	$NaHCO_3$	50.0 mg/L
	$CaCl_2 \times 2\ H_2O$	18.0 mg/L
	NH_4Cl	15.0 mg/L
	$MgSO_4 \times 7\ H_2O$	15.0 mg/L
	$MgCl_2 \times 6\ H_2O$	12.0 mg/L
	KH_2PO_4	1.6 mg/L
Trace Elements:	$Na_2EDTA \times 2\ H_2O$	100.0 µg/L
	$FeCl_3 \times 6\ H_2O$	80.0 µg/L
	$MnCl_2 \times 4\ H_2O$	415.0 µg/L
	H_3BO_3	185.0 µg/L
	$Na_2MoO_4 \times 2\ H_2O$	7.0 µg/L
	$ZnCl_2$	3.0 µg/L
	$CoCl_2 \times 6\ H_2O$	1.5 µg/L
	$CuCl_2 \times 2\ H_2O$	0.01 µg/L

Lemna Steinberg growth medium (OECD medium) was prepared as described in OECD 221, 2006 (*Lemna* sp. Growth Inhibition Test). The composition is as follows:

Macroelements:	KNO_3	350.0 mg/L
	$Ca(NO_3)_2 \times 4\ H_2O$	295.0 mg/L
	KH_2PO_4	90.0 mg/L
	K_2HPO_4	12.6 mg/L
	$MgSO_4$ x 7 H_2O	100.0 mg/L
Microelements:	H_3BO_3	120.00 µg/L
	$ZnSO_4$ x 7 H_2O	180.00 µg/L
	Na_2MoO_4 x 2 H_2O	44.00 µg/L
	$MnCl_2$ x 4 H_2O	180.00 µg/L
	$FeCl_3$ x 6 H_2O	760.00 µg/L
	EDTA Disodiumdihydrate	1500.0 g/L

Equipment
- Analytical balance, Mettler Toledo AL204, Internal code No. 243
- HPLC/UV, Thermo Finningan SCM1000, Internal code No. 320
- HPLC/UV/PDA, Thermo Finningan UV 6000 LP, Internal code No. 276
- Laboratory Water Purification Systems, Sartorius Arium® 611, Internal code No. 355
- Refrigerator, Internal code No. 388
- Freezer, Internal code No. 153
- pHmeter WTW pH315i, Internal code No. 244
- Usual laboratory glassware
- Volumetric glassware

Reagents
- Water, HPLC grade
- Acetonitrile, HPLC grade (VWR)
- Phosphoric acid 85 % (H_3PO_4), reagent grade (Merck)
- Reconstituted water (OECD 203, 1992 and OECD 202, 2004)
- Algal growth medium (OECD 201, 2006)
- *Lemna* Steinberg growth medium (OECD 221, 2006)

EXPERIMENTAL

Specificity
The analytical conditions were suitably adapted to obtain the best results for the determination of Clopyralid residues in water samples.
The method was demonstrated to be specific for the determination of Clopyralid residues in water samples by virtue of the HPLC/UV/PDA technique.

Confirmatory test
The presence of Clopyralid in untreated and fortified matrix samples was confirmed using High Performance Liquid Chromatography with diode array detection (HPLC/PDA) by comparison of the Clopyralid UV spectrum at a particular retention time under the operating conditions described in internal Analytical Method No. 606/2011.

Linearity and System Precision
Linear regression analysis was performed using the least squares method.
The correlation coefficient was calculated using regression analysis.

Preparation of the stock solution
Using the analytical balance, a 996.87 µg/mL stock standard solution was prepared, taking into account its 98.7 % purity, by weighing 20.2 mg of Clopyralid standard into a 20 mL volumetric flask and making to volume with acetonitrile.
Using a volumetric pipette, a 498.44 µg/mL analytical standard solution was prepared diluting 1:2 the stock solution and making to volume with acetonitrile (DSS1).

Preparation of the working standard solutions
Using volumetric pipettes, five working standard solutions for linear calibration were prepared as follows:

WSS 1. 1.00 mL of the working standard solution at 1.00 µg/mL (WSS3) were transferred with a volumetric pipette into a 10 mL volumetric flask, making to volume with HPLC grade water (working standard solution at 0.10 µg/mL).
WSS 2. 1.00 mL of the working standard solution at 2.49 µg/mL (WSS4) were transferred with a volumetric pipette into a 10 mL volumetric flask, making to volume with HPLC grade water (working standard solution at 0.25 µg/mL)

WSS 3. 2.00 mL of the working standard solution at 4.98 µg /mL (WSS5) were transferred with a volumetric pipette into a 10 mL volumetric flask, making to volume with HPLC grade water (working standard solution at 1.00 µg/mL).
WSS 4. 5.00 mL of the working standard solution at 4.98 µg /mL (WSS5) were transferred with a volumetric pipette into a 10 mL volumetric flask, making to volume with HPLC grade water (working standard solution at 2.49 µg/mL).
WSS 5. 1.00 mL of the diluted standard solution at 498.44 µg/mL (DSS1) were transferred with a volumetric pipette into a 100 mL volumetric flask, making to volume with HPLC grade water (working standard solution at 4.98 µg/mL).

All the solutions were stored in a refrigerator at about 4°C.

The linearity test was performed with working standard solutions from 0.10 µg/mL to 5.00 µg/mL, nominal concentrations, corresponding to a content in water samples in the same range.
From the lowest to the highest concentration, four series of injections were performed and a solvent wash was injected after each highest standard concentration solution, in order to verify that no significant memory peak was detected.
Means and standard deviation for each concentration were calculated using the data from the four replicate injections.

Repeatability (Precision) and Recovery (Accuracy)
Both repeatability and recovery tests were performed using fortified control samples of reconstituted water (OECD 202, 2004 and OECD 203, 1992), algal growth medium (OECD 201, 2006) and *Lemna* Steinberg growth medium (OECD 221, 2006).
Each water sample was fortified at 0.15 µg/mL (L.O.Q.) and at 1.50 µg/mL (10 x L.O.Q.).
Fortified water samples were quantified using the working standard solutions prepared for the linearity test.

Preparation of the diluted standard solutions for repeatability and recovery tests:
Using a volumetric pipette, a 149.53 µg/mL diluted Clopyralid fortification solution (DSS2).was prepared transferring 1.50 mL of the stock standard solution at 996.87 µg/mL, into a 10 mL flask and making to volume with acetonitrile
A 14.95 µg/mL (ppm) diluted Clopyralid fortification solution (DSS3) was obtained transferring with a volumetric pipette 1.00 mL of the diluted standard solution DSS2 into a 10 mL volumetric flask and making a volume with acetonitrile.

Preparation and analysis of fortified water samples (reconstituted water)
Low fortification level: six 20 mL aliquots of reconstituted water were spiked, using a volumetric pipette, with 0.20 mL of the diluted fortification solution DSS3.
High fortification level: six 20 mL aliquots of reconstituted water were spiked, using a volumetric pipette, with 0.20 mL of the diluted fortification solution DSS2.

Preparation and analysis of fortified water samples (algal growth medium)
Low fortification level: six 20 mL aliquots of algal growth medium were spiked, using a volumetric pipette, with 0.20 mL of the diluted fortification solution DSS3.
High fortification level: six 20 mL aliquots of algal growth medium were spiked, using a volumetric pipette, with 0.20 mL of the diluted fortification solution DSS2.

Preparation and analysis of fortified water samples (*Lemna* growth medium)
Low fortification level: six 20 mL aliquots of Lemna growth medium were spiked, using a volumetric pipette, with 0.20 mL of the diluted fortification solution DSS3.
High fortification level: six 20 mL aliquots of Lemna growth medium were spiked, using a volumetric pipette, with 0.20 mL of the diluted fortification solution DSS2.
For each of the two fortification levels and for the three matrix, six fortified samples were processed.

The fortified samples were analysed as described in internal Analytical Method No. 606/2011 and the recovery was calculated.
These injections were alternated with those of the working standard solutions and solvent washing according to the sequence of analysis reported in Table 6.
Precision (repeatability) and accuracy (recovery) of the analytical method were assessed with the obtained data.

RESULTS

Note. Data in the tables are rounded values taken from Excel spreadsheets which will be archived together with the raw data. The use of Excel spreadsheets for calculations produces more accurate endpoints. These endpoints may slightly differ from the values derived by replacing the rounded values in the formulae given in the methods section.

Specificity and Confirmatory Test

A comparison between the chromatograms and UV spectrum (see Figures from 1 to 8) of Clopyralid test item, solvent wash, control water samples and water samples fortified at high level, did not show any significant interference.
Therefore, by using the conditions stated in the method, interferences can be avoided and Clopyralid residues can be reliably determined in the water samples.

Linearity

The stock standard solution and the working standard solutions were prepared, using volumetric glassware, as described above.
The linearity range (0.10 µg/mL to 5.00 µg/mL, nominal concentrations) was chosen to extend over a range appropriate to the quantification of analytical solutions.
From the lowest to the highest concentration, four series of injections were performed and a solvent wash was injected after each highest standard concentration, in order to verify if any memory effect occurred.
The obtained data are reported in Table 1.
No significant memory peak was detected in the washing solvent injected after each standard solution at 4.98 µg/mL (memory peak < 30 % of the L.O.Q.).
The range tested from 0.10 µg/mL to 4.98 µg/mL was found to be linear (correlation coefficient > 0.99).

TABLE 1 Linearity test with Clopyralid working standard solutions

Clopyralid	**WSS 1 0.10 µg/mL** (Peak area)	**WSS 2 0.25 µg/mL** (Peak area)	**WSS 3 1.00 µg/mL** (Peak area)	**WSS 4 2.49 µg/mL** (Peak area)	**WSS 5 4.98 µg/mL** (Peak area)
A series	161685	535857	1621764	4267611	8182914
B series	160251	538428	1622148	4270827	8241369
C series	161193	536373	1630659	4259253	8216091
D series	159804	531357	1625838	4255335	8211975
Mean	**160733**	**535504**	**1625102**	**4263257**	**8213087**
S.D.	858.9	2979.2	4134.8	7189.2	23948.2
RSD%	*0.53%*	*0.56%*	*0.25%*	*0.17%*	*0.29%*
Linear calibration: y = mx + q					
Parameter **m** (slope)	1644403	Parameter **q** (intercept)	58053	Parameter **R^2** (correlation)	0.99940

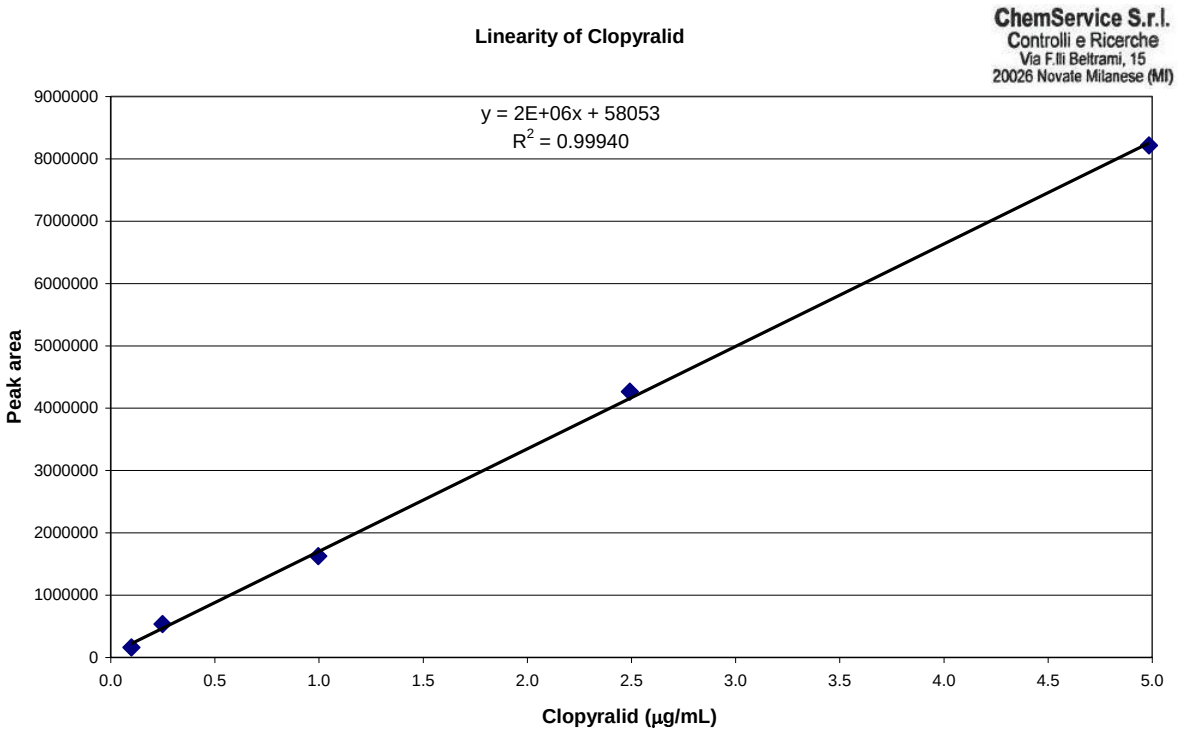


Figure 9 Linearity test chart on Clopyralid reference material

Repeatability (Precision) and Recovery (Accuracy)

Data from the injections of working standard solutions and samples for the repeatability and recovery test at two fortification levels for the three matrix (Low 0.15 µg/mL and High 1.50 µg/mL) are reported in Tables from 2 (a-b-c) to 5 (a-b); the analysis sequence is reported in Table 6.
The calibration curve was obtained using the working standard solutions prepared for the linearity test (WSS1-WSS3-WSS5).

A summary of the results is reported below.

Spike add	Mean Found	Test No.	RSD%	Recoveries
Reconstituted water				
L) 0.15 µg/mL	0.15 µg/mL	6 det.	1.75 %	96.86 % - 101.82 % ; mean 98.61 %
H) 1.50 µg/mL	1.49 µg/mL	6 det.	1.18 %	98.13 % - 101.19 % ; mean 99.61 %
Algal growth medium				
L) 0.15 µg/mL	0.15 µg/mL	6 det.	4.56 %	92.96 % - 105.52 % ; mean 100.90 %
H) 1.50 µg/mL	1.47 µg/mL	6 det.	1.58 %	96.47 % - 100.66 % ; mean 98.48 %
***Lemna* growth medium**				
L) 0.15 µg/mL	0.15 µg/mL	6 det.	4.49 %	94.45 % - 107.02 % ; mean 98.58 %
H) 1.50 µg/mL	1.49 µg/mL	6 det.	3.58 %	95.60 % - 104.04% ; mean 99.89 %

For precision, the SANCO/3029/99 rev. 4 guideline requires a RSD% lower than 20 % for each level; therefore the precision of the analytical method is considered to be acceptable.
For accuracy, SANCO/3029/99 rev. 4 guideline requires mean recovery for each level in the range from 70 % to 110%, therefore the accuracy of the analytical method is considered to be acceptable.

The SANCO/3029/99 rev. 4 guideline requires any interference from the untreated control sample to be lower than 30 % at the L.O.Q. In the sequence of analysis of fortified samples at the two fortification levels (Low at 0.15 µg/mL and High at 1.50 µg/mL), the analysis of the control samples showed no significant interference.

The limit of quantification (L.O.Q.) of this method is defined as the lowest fortification level of 0.15 µg/mL (ppm) in water matrix samples, corresponding to an injected solution at the same concentration.

The limit of detection (L.O.D.) of this method is defined as 50% of the lowest calibration level, i.e.0.05 µg/mL, corresponding to the same concentration in water matrix samples.

Residue results calculated as values < 0.05 µg/mL are classified as not detected (n.d.).
Residue results calculated as higher than the limit of detection but lower than limit of quantification, are designated as < L.O.Q. (<0.15 µg/mL).

TABLE 2a Repeatability and Recovery Tests. Calibration curve with working standard solutions (Reconstituted water)

Clopyralid	**WSS 1** **0.10 µg/mL** (Peak area)	**WSS 3** **1.00 µg/mL** (Peak area)	**WSS 5** **4.98 µg/mL** (Peak area)
1st series	158802	1628424	8192526
2nd series	162528	1625799	8246013
Mean	**160665**	**1627112**	**8219270**
Delta	3726	2625	53487
Delta %	*2.32%*	*0.16%*	*0.65%*
Y = mx + q	Parameter **m** (slope)	Parameter **q** (intercept)	Parameter **R^2** (correlation)
	1650821	-10481	1.00000

TABLE 2b Repeatability and Recovery Tests. Calibration curve with working standard solutions (Algal growth medium)

Clopyralid	**WSS 1** **0.10 µg/mL** (Peak area)	**WSS 3** **1.00 µg/mL** (Peak area)	**WSS 5** **4.98 µg/mL** (Peak area)
1st series	162528	1625799	8246013
2nd series	159936	1626435	8234253
Mean	**161232**	**1626117**	**8240133**
Delta	2592	636	11760
Delta %	*1.61%*	*0.04%*	*0.14%*
Y = mx + q	Parameter **m** (slope)	Parameter **q** (intercept)	Parameter **R^2** (correlation)
	1655379	-12908	0.99999

TABLE 2c Repeatability and Recovery Tests. Calibration curve with working standard solutions (*Lemna* growth medium)

Clopyralid	**WSS 1** **0.10 µg/mL** (Peak area)	**WSS 3** **1.00 µg/mL** (Peak area)	**WSS 5** **4.98 µg/mL** (Peak area)
1st series	159936	1626435	8234253
2nd series	160128	1625529	8230110
Mean	**160032**	**1625982**	**8232182**
Delta	192	906	4143
Delta %	*0.12%*	*0.06%*	*0.05%*
Y = mx + q	Parameter **m** (slope)	Parameter **q** (intercept)	Parameter **R^2** (correlation)
	1653821	-12846	1.00000

TABLE 3a Repeatability and Recovery Tests. Reconstituted water: Recovery at Low fortification level (0.15 µg/mL)

Vial Identification	$Area_S$	C_S (1) (µg/mL)	$Dilution_S$	Clopyralid (µg/mL)	Recovery (%)
Control rec H_2O	0	0.00	1.00	0.00	-
Spike L A rec H_2O	240852	0.15	1.00	0.15	101.82
Spike L B rec H_2O	230559	0.15	1.00	0.15	97.65
Spike L C rec H_2O	233775	0.15	1.00	0.15	98.95
Spike L D rec H_2O	231096	0.15	1.00	0.15	97.86
Spike L E rec H_2O	232785	0.15	1.00	0.15	98.55
Spike L F rec H_2O	228609	0.14	1.00	0.14	96.86
			Mean value :	**0.15**	**98.61**
			Standard deviation (S.D.) :	**0.003**	**1.73**
			Relative Standard Deviation (RSD%) :	**1.75%**	**1.75%**

(1) Quantification with the calibration curve (see Table 2a).

TABLE 3b Repeatability and Recovery Tests. Reconstituted water: Recovery at High fortification level (1.50 µg/mL)

Vial Identification	$Area_S$	C_S (1) (µg/mL)	$Dilution_S$	Clopyralid (µg/mL)	Recovery (%)
Control rec H_2O	0	0.00	1.00	0.00	-
Spike H A rec H_2O	2465787	1.50	1.00	1.50	100.32
Spike H B rec H_2O	2411829	1.47	1.00	1.47	98.13
Spike H C rec H_2O	2487315	1.51	1.00	1.51	101.19
Spike H D rec H_2O	2466639	1.50	1.00	1.50	100.35
Spike H E rec H_2O	2429205	1.48	1.00	1.48	98.83
Spike H F rec H_2O	2430132	1.48	1.00	1.48	98.87
			Mean value :	**1.49**	**99.61**
			Standard deviation (S.D.) :	**0.02**	**1.17**
			Relative Standard Deviation (RSD%) :	**1.18%**	**1.18%**

(1) Quantification with the calibration curve (see Table 2a).

TABLE 4a Repeatability and Recovery Tests. Algal growth medium: Recovery at Low fortification level (0.15 µg/mL)

Vial Identification	$Area_S$	C_S (1) (µg/mL)	$Dilution_S$	Clopyralid (µg/mL)	Recovery (%)
Control algal	0	0.00	1.00	0.00	-
Spike L A algal	243525	0.15	1.00	0.15	103.60
Spike L B algal	239241	0.15	1.00	0.15	101.87
Spike L C algal	217194	0.14	1.00	0.14	92.96
Spike L D algal	248274	0.16	1.00	0.16	105.52
Spike L E algal	230103	0.15	1.00	0.15	98.17
Spike L F algal	242814	0.15	1.00	0.15	103.31
			Mean value :	**0.15**	**100.90**
			Standard deviation (S.D.) :	**0.007**	**4.60**
			Relative Standard Deviation (RSD%) :	**4.56%**	**4.56%**

(1) Quantification with the calibration curve (see Table 2b).

TABLE 4b Repeatability and Recovery Tests. Algal growth medium: Recovery at High fortification level (1.50 µg/mL)

Vial Identification	$Area_S$	C_S (1) (µg/mL)	$Dilution_S$	Clopyralid (µg/mL)	Recovery (%)
Control algal	0	0.00	1.00	0.00	-
Spike H A algal	2441841	1.48	1.00	1.48	99.17
Spike H B algal	2478771	1.51	1.00	1.51	100.66
Spike H C algal	2416920	1.47	1.00	1.47	98.16
Spike H D algal	2446455	1.49	1.00	1.49	99.36
Spike H E algal	2374902	1.44	1.00	1.44	96.47
Spike H F algal	2389692	1.45	1.00	1.45	97.06
			Mean value :	**1.47**	**98.48**
			Standard deviation (S.D.) :	**0.02**	**1.56**
			Relative Standard Deviation (RSD%) :	**1.58%**	**1.58%**

(1) Quantification with the calibration curve (see Table 2b).

TABLE 5a Repeatability and Recovery Tests. *Lemna* growth medium: Recovery at Low fortification level (0.15 µg/mL)

Vial Identification	$Area_S$	C_S (1) (µg /mL)	$Dilution_S$	Clopyralid (µg/mL)	1) Re-covery (%)
Control *Lemna*	0	0.00	1.00	0.00	-
Spike L A *Lemna*	220728	0.14	1.00	0.14	94.45
Spike L B *Lemna*	229740	0.15	1.00	0.15	98.10
Spike L C *Lemna*	223554	0.14	1.00	0.14	95.59
Spike L D *Lemna*	251805	0.16	1.00	0.16	107.02
Spike L E *Lemna*	230811	0.15	1.00	0.15	98.53
Spike L F *Lemna*	229023	0.15	1.00	0.15	97.81
			Mean value :	**0.15**	**98.58**
			Standard deviation (S.D.) :	**0.007**	**4.43**
			Relative Standard Deviation (RSD%) :	**4.49%**	**4.49%**

(1) Quantification with the calibration curve (see Table 2c).

TABLE 5b Repeatability and Recovery Tests. *Lemna* growth medium: Recovery at High fortification level (1.50 µg/mL)

Vial Identification	$Area_S$	C_S (1) (µg /mL)	$Dilution_S$	Clopyralid (µg/mL)	Recovery (%)
Control *Lemna*	0	0.00	1.00	0.00	-
Spike H A *Lemna*	2543925	1.55	1.00	1.55	103.39
Spike H B *Lemna*	2351337	1.43	1.00	1.43	95.60
Spike H C *Lemna*	2431527	1.48	1.00	1.48	98.84
Spike H D *Lemna*	2367312	1.44	1.00	1.44	96.25
Spike H E *Lemna*	2559990	1.56	1.00	1.56	104.04
Spike H F *Lemna*	2489715	1.51	1.00	1.51	101.20
			Mean value :	**1.49**	**99.89**
			Standard deviation (S.D.) :	**0.05**	**3.58**
			Relative Standard Deviation (RSD%) :	**3.58%**	**3.58%**

(1) Quantification with the calibration curve (see Table 2c).

TABLE 6 Repeatability and Recovery tests: analysis sequence

VIAL IDENTIFICATION	Number of inj.	VIAL IDENTIFICATION	Number of inj.
WSS1 E	1	Spike H A algal	1
WSS3 E	1	Spike H B algal	1
WSS5 E	1	Spike H C algal	1
Wash	1	Spike H D algal	1
Control rec H_2O	1	Spike H E algal	1
Spike L A rec H_2O	1	Spike H F algal	1
Spike L B rec H_2O	1	Wash	1
Spike L C rec H_2O	1	WSS1 G	1
Spike L D rec H_2O	1	WSS3 G	1
Spike L E rec H_2O	1	WSS5 G	1
Spike L F rec H_2O	1	Wash	1
Control rec H_2O	1	Control *Lemna* medium	1
Spike H A rec H_2O	1	Spike L A *Lemna* medium	1
Spike H B rec H_2O	1	Spike L B *Lemna* medium	1
Spike H C rec H_2O	1	Spike L C *Lemna* medium	1
Spike H D rec H_2O	1	Spike L D *Lemna* medium	1
Spike H E rec H_2O	1	Spike L E *Lemna* medium	1
Spike H F rec H_2O	1	Spike L F *Lemna* medium	1
Wash	1	Control *Lemna* medium	1
WSS1 F	1	Spike H A *Lemna* medium	1
WSS3 F	1	Spike H B *Lemna* medium	1
WSS5 F	1	Spike H C *Lemna* medium	1
Wash	1	Spike H D *Lemna* medium	1
Control algal	1	Spike H E *Lemna* medium	1
Spike L A algal	1	Spike H F *Lemna* medium	1
Spike L B algal	1	Wash	1
Spike L C algal	1	WSS1 H	1
Spike L D algal	1	WSS3 H	1
Spike L E algal	1	WSS5 H	1
Spike L F algal	1	Wash	1
Control algal	1		

CONCLUSIONS

The analytical method was shown to be specific for Clopyralid residues in water samples from ecotoxicological tests.
The Clopyralid tested concentration in injected solutions ranged from 0.10 µg/mL to 4.98 µg/mL, corresponding to the same concentration in water samples and was found to be linear (correlation coefficient > 0.99).
The SANCO/3029/99 rev. 4 guideline requires any interference from the untreated control sample to be lower than 30 % at the L.O.Q. In the sequence of analysis of fortified samples at the two fortification levels (Low at 0.15 µg/mL and High at 1.50 µg/mL), the analysis of the control samples showed no significant interference.
The limit of quantification (L.O.Q.) of this method is defined as the lowest fortification level of 0.15 µg/mL (ppm) in water matrix samples, corresponding to an injected solution at the same concentration.
The limit of detection (L.O.D.) of this method is defined as 50% of the lowest calibration level, i.e.0.05 µg/mL, corresponding to the same concentration in water matrix samples.
Residue results calculated as values < 0.05 µg/mL are classified as not detected (n.d.).
Residue results calculated as higher than the limit of detection but less than limit of quantification, are designated as <L.O.Q. (< 0.15 µg/mL).

Spike add	Mean Found	Test No.	RSD%	Recoveries
Reconstituted water				
L) 0.15 µg/mL	0.15 µg/mL	6 det.	1.75 %	96.86 % - 101.82 % ; mean 98.61 %
H) 1.50 µg/mL	1.49 µg/mL	6 det.	1.18 %	98.13 % - 101.19 % ; mean 99.61 %
Algal growth medium				
L) 0.15 µg/mL	0.15 µg/mL	6 det.	4.56 %	92.96 % - 105.52 % ; mean 100.90 %
H) 1.50 µg/mL	1.47 µg/mL	6 det.	1.58 %	96.47 % - 100.66 % ; mean 98.48 %
***Lemna* growth medium**				
L) 0.15 µg/mL	0.15 µg/mL	6 det.	4.49 %	94.45 % - 107.02 % ; mean 98.58 %
H) 1.50 µg/mL	1.49 µg/mL	6 det.	3.58 %	95.60 % - 104.04% ; mean 99.89 %

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~~.1~~ /08
Report	Clopyralid Validation of an Analytical Method for the Determination in Air, 2019, Kirchherr M., 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 15: Recovery results from the method validation of Clopyralid using the analytical method

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

Table A 16: 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><tr><td>Matrix</td><td>Calibration range (ng/L)</td></tr><tr><td>Air</td><td>0.4 – 20</td></tr></table>
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 15.
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 Solutions	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 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/m³ Clopyralid at a total volume of 480 L air within 8 hours.

Conclusion

The method was successfully validated for Clopyralid at 4.5 µg/m3 (LOQ) and 45 µg/m3 (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/m³ (LOQ) and 45 µg/m³ (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~~.1~~_/09
Report	Development and Validation of an Analytical Method for the Determination of Clopyralid in Body Fluids, 2019, Abe Ch., 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	30 µL
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 17: 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 18: 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^2) were ≥ 0.9999. Linear regression was performed with 1/x-weighting.
Calibration range	<table><tr><td>Matrix</td><td>Calibration range (mg/L)</td></tr><tr><td colspan="2">Quantification; *m/z* 190→146</td></tr><tr><td>Urine</td><td>0.005 -0.15</td></tr><tr><td colspan="2">Qualification; *m/z* 192→148</td></tr><tr><td>Urine</td><td>0.005 -0.15</td></tr></table>
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 17.
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.