REGISTRATION REPORT

Part B

Section 5

Analytical Methods

Detailed summary of the risk assessment

Product code: **AMINO 30 SL**

Product name(s): **El Camino 30 SL, Ranchero 30 SL**

Chemical active substance:

Aminopyralid, 30 g/L

Central Zone

Zonal Rapporteur Member State: PL

CORE ASSESSMENT

(authorization)

Applicant: Innvigo Sp. z o.o.

Submission date: 01/2025

zRMS Assessment: 18/04/2025

Following commenting period/Verification of reference list: 01/07/2025

Version history

When	What
April 2025	zRMS Assessment
July 2025	Following commenting period Verification of reference list

Table of Contents

5 Analytical methods

5.1 Conclusion and summary of assessment

According to the data evaluated at EU level (EFSA Journal 2020;18(8):6229) fully validated analytical methods are available for the enforcement of the residue definition in high oil content com-modities. According to the EURLs the LOQ of 0.05 mg/kg is achievable by using the QuEChERS method (QuOil for high oil content commodities) for the routine analysis of free aminopyralid in all matrices.

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

Noticed data gaps are:
None

Commodity/crop	Supported/ Not supported
High oil content commodities/rape seed	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 aminopyralid in plant protection product is provided as follows:

Comments of zRMS:	The analytical method study code: ICB/91/2024 was fully validated in term of specificity, linearity, repeatability, accuracy according to SANCO/3030/99 rev.5. The results of analytical method validation confirm that this method is suitable for analysis the content of the active substance (aminopyralid and picloram as impurity). The method is successfully validated and accepted analysis of aminopyralid in plant protection product.

Reference:	KCP 5.1.1/01
Report	Validation of analytical method for AMINO 30 SL for determination of aminopyralid and picloram as impurity, Pniok, W., 2024, Study code: ICB/91/2024
Guideline(s):	Yes, SANCO/3030/99 rev.5 and Standard Operational Procedure SPB/179.
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

Identification of the test item.
Name of test item: AMINO 30 SL
Active ingredient: aminopyralid
Active ingredient CAS: 150114-71-9
Appearance: clear red-orange liquid
ID No: ICB/1202
Date of production: 31.07.2024
Expiration date: -
Batch No: 1/24
Number of packages used: 1 bottle (1L)
Date of receiving: 01.10.2024
Storage conditions: 18-28°C

Study methods and course of the experiment.

1. Validation of method for determination of the content of aminopyralid.
Validation was carried out according to SANCO/3030/99 rev.5 and Standard Operational Procedure SPB/179. Content of aminopyralid at a level of 30 g/L in the test item was determined accordingly by liquid chromatography with diode array detection (HPLC-DAD).

1.1. Equipment and materials.
- acetonitryle (ACN), (Merck)
- 85% phosphoric acid (Sigma-Aldrich)
- aminopyralid standard; HPC Standards GmbH; batch 814229, prod. date: November 09, 2022; exp. date: 01 November, 2027; storage conditions: 20±4°C
- standard stock solution of aminopyralid in ACN (for calibration) (Table 3)
- standard stock solution of aminopyralid in ACN (for recovery) (Table 4)
- working standard solution of aminopyralid in ACN/H_2O (1:1) mixture (for average recovery and precision for validation level LOQ) (Table 5)
- working standard solutions of aminopyralid in ACN/H_2O (1:1) mixture (for calibration) (Table 6)
- placebo
- analytical balance – accuracy 0.0001 g (Ohaus, Switzerland), WP/16
- liquid chromatograph with diode array detection (Shimadzu, Japan), WP/19, WP/42,
- chromatography column type C18, 250 mm x 4,6 mm, 5 µm (Zorbax Eclipse Plus, Agilent), K/18/HPLC
- chromatography column type C18, 250 mm x 4,6 mm, 5 µm (Zorbax Eclipse Plus, Agilent), K/11/HPLC
- chromatographic vials 1.5 mL with septa buthyl/teflon
- volumetric flasks A class 10 mL
- measuring syringes 10 µl, 50 µL, 250 µL, 500 µL, 1000 µL.

1.2. Preparation of standard solutions.

Table 3. Preparation of aminopyralid standard stock solution in ACN (for calibration).

Active ingredient	Weight [mg]	Flask volume [mL]	Concentration taking into account the purity of the standard [mg/mL]
aminopyralid	10.3	10	1.021

Table 4. Preparation of aminopyralid standard stock solution in ACN (for recovery).

Active ingredient	Weight [mg]	Flask volume [mL]	Concentration taking into account the purity of the standard [mg/mL]
aminopyralid	30.6	10	3.032

Table 5. Preparation of aminopyralid working standard solution in ACN/H_2O (1:1) mixture (for average recovery and precision for validation level LOQ).

Concentration of standard solution [mg/mL]	Volume of standard solution [µL]	Flask volume [mL]	Concentration of standard solution [µg/mL]
3.032	500	10	151.6

Table 6. Preparation of aminopyralid working standard solutions in ACN/H_2O (1:1) mixture (for calibration).

Concentration of standard solution [mg/mL]	Volume of standard solution [µL]	Flask volume [mL]	Concentration of standard solution [µg/mL]	Calibration level
1.021	10	10	1.021	Cal 1
1.021	50	10	5.105	Cal 2
1.021	250	10	25.525	Cal 3
1.021	500	10	51.05	Cal 4
1.021	1000	10	102.1	Cal 5

1. 3. Chromatography parameters.
Chromatography parameters were determined during preliminary assessment.

Primary chromatographic system.
Column: K/18/HPLC
Analysis time: 20 min
Flow: 1.4 mL/min
Column temperature: 25°C
Injection: 5 µL
Detector DAD: 220 nm
Mobile phase: A – ACN, D – 0.1% H_3PO_4
Isocratic program: phase A - 20%, D – 80%

Secondary chromatographic system.
Column: K/11/HPLC
Analysis time: 20 min
Flow: 1.4 mL/min
Column temperature: 40°C
Injection: 5 µL
Detector DAD: 220 nm
Mobile phase: A – ACN, D – 0.1% H_3PO_4
Isocratic program: phase A - 15%, D – 85%

Validation - Results and discussions

Table 5.2-1: Methods suitable for the determination of active substance in plant protection product El Camino 30 SL/ Ranchero 30 SL/ AMINO 30 SL

	Aminopyralid
Author(s), year	Pniok, W., 2024
Principle of method	HPLC-DAD
Linearity	In order to check the linearity of aminopyralid, calibration curve was prepared using standard

<table>
<tr><th></th><th>Aminopyralid</th></tr>
<tr><td>(linear between mg/L / % range of the declared content) (correlation coefficient, expressed as r)</td><td>solutions with concentrations contained in Table 6. A graph of the peak area to the concentration of aminopyralid was plotted. The resulting curve is linear in the tested concentrations. Calibration curve for aminopyralid is from 1.021 –102.1 µg/mL, which corresponding to 0.07% –6.80%. Linearity range of aminopyralid is from LOQ 1.2128 µg/mL to ULOQ 303.2 µg/mL. Correlation coefficient R^2 is 0.9999335 and the linear regression is described by equation: $f(x)=4.16627\cdot10^{-5}x-0.0362559$ for primary chromatographic system. Correlation coefficient R^2 is 0.9999894 and the linear regression is described by equation: $f(x)=4.17263\cdot10^{-5}x-0.0106102$ for secondary chromatographic system.</td></tr>
<tr><td>Precision – Repeatability Mean n = 5 (%RSD)</td><td>Table 12 shows results of precision and Horwitz ratio at 100% validation level for active ingredient for both systems.

Table 12. Results of precision and Horwitz ratio at 100% validation level for active ingredient.
<table>
<tr><th>Chromatographic system</th><th>Precision [%]</th><th>Horwitz ratio</th></tr>
<tr><td>Primary</td><td>0.28</td><td>0.12</td></tr>
<tr><td>Secondary</td><td>0.27</td><td>0.12</td></tr>
</table>
Table 17 shows results of average recovery, precision and Horwitz ratio for both systems for validation level LOQ for aminopyralid.

Table 17. Results of average recovery, precision and Horwitz ratio for active ingredient for validation level LOQ for both systems.
<table>
<tr><th>Chromatographic system</th><th>Precision [%]</th><th>Horwitz ratio</th><th>Average recovery [%]</th></tr>
<tr><td>Primary</td><td>1.36</td><td>0.35</td><td>96.2</td></tr>
<tr><td>Secendary</td><td>0.87</td><td>0.22</td><td>97.7</td></tr>
</table>
Table 19 shows results of average recovery, precision and Horwitz ratio for both systems for validation level ULOQ for aminopyralid.

Table 19. Results of average recovery, precision and Horwitz ratio for aminopyralid for validation level ULOQ for both systems.
<table>
<tr><th>Chromatographic system</th><th>Precision [%]</th><th>Horwitz ratio</th><th>Average recovery [%]</th></tr>
<tr><td>Primary</td><td>0.40</td><td>0.18</td><td>102.5</td></tr>
<tr><td>Secendary</td><td>0.25</td><td>0.12</td><td>101.6</td></tr>
</table>
Obtainted results meet the criteria: precision for aminopyralid ≤ 2.26 (validation level 100%), precision for aminopyralid ≤ 3.91(validation level LOQ), precision for aminopyralid ≤ 2.17 (validation level ULOQ), Horwitz ratio ≤ 1.0.</td></tr>
</table>

<table>
<tr><th></th><th>Aminopyralid</th></tr>
<tr><td>Accuracy
n = 5
(% Recovery)</td><td>
Table 14 shows concentrations of active ingredient and recovery for all five independent test item samples used for determination of recovery for both chromatographic systems.

Table 14. Concentrations of active ingredient and recovery for all five independent test item samples used for determination of recovery.
<table>
<tr><th>Chromatographic system</th><th>Sample</th><th>Average concentration without standard addition [µg/mL]</th><th>Standard addition [µg/mL]</th><th>Concentrations [µg/mL]</th><th>Recovery [%]</th></tr>
<tr><td rowspan="5">Primary</td><td>1</td><td rowspan="5">46.37730</td><td rowspan="5">10.6120</td><td>56.91815</td><td>99.33</td></tr>
<tr><td>2</td><td>56.77105</td><td>97.94</td></tr>
<tr><td>3</td><td>56.60026</td><td>96.33</td></tr>
<tr><td>4</td><td>56.66601</td><td>96.95</td></tr>
<tr><td>5</td><td>56.73792</td><td>97.63</td></tr>
<tr><td rowspan="5">Secondary</td><td>1</td><td rowspan="5">46.65462</td><td rowspan="5">10.6120</td><td>57.21006</td><td>99.47</td></tr>
<tr><td>2</td><td>57.44507</td><td>101.68</td></tr>
<tr><td>3</td><td>57.06956</td><td>98.14</td></tr>
<tr><td>4</td><td>57.11166</td><td>98.54</td></tr>
<tr><td>5</td><td>57.09629</td><td>98.39</td></tr>
</table>
Table 17. Results of average recovery, precision and Horwitz ratio for active ingredient for validation level LOQ for both systems.
<table>
<tr><th>Chromatographic system</th><th>Precision [%]</th><th>Horwitz ratio</th><th>Average recovery [%]</th></tr>
<tr><td>Primary</td><td>1.36</td><td>0.35</td><td>96.2</td></tr>
<tr><td>Secendary</td><td>0.87</td><td>0.22</td><td>97.7</td></tr>
</table>
Table 19 shows results of average recovery, precision and Horwitz ratio for both systems for validation level ULOQ for aminopyralid.

Table 19. Results of average recovery, precision and Horwitz ratio for aminopyralid for validation level ULOQ for both systems.
<table>
<tr><th>Chromatographic system</th><th>Precision [%]</th><th>Horwitz ratio</th><th>Average recovery [%]</th></tr>
<tr><td>Primary</td><td>0.40</td><td>0.18</td><td>102.5</td></tr>
<tr><td>Secendary</td><td>0.25</td><td>0.12</td><td>101.6</td></tr>
</table>
Obtained results meet the criteria: recovery for aminopyralid 90-110 (validation level 100%), recovery for aminopyralid 75-125 (validation level LOQ), recovery for aminopyralid 99-110 (validation level ULOQ).
</td></tr>
<tr><td>Interference/
Specificity</td><td>Specificity of the method was evaluated based on the analysis of chromatogram for placebo, sample against chromatogram of aminopyralid standard and peak purity. Analysis showed no overlapping of determined ingredient signal with the signals of matrix components under method conditions, hence method specificity criterion is fulfilled.</td></tr>
<tr><td>Comment</td><td></td></tr>
</table>

Conclusion

The analytical method meets the specificity, linearity, precision/repeatability and accuracy criteria specified in SANCO 3030 (99) rev.5.

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

An overview on the acceptable methods and possible data gaps for analysis of relevant impurities in plant protection product is provided as follows:

Comments of zRMS:	The analytical method study code: ICB/91/2024 was fully validated in term of specificity, linearity, repeatability, accuracy according to SANCO/3030/99 rev.5. The results of analytical method validation confirm that this method is suitable for analysis the content of the active substance (aminopyralid and picloram as impurity). The method is successfully validated and accepted for analysis of relevant impurities in plant protection product.

Reference:	KCP 5.1.1/02
Report	Validation of analytical method for AMINO 30 SL for determination of aminopyralid and picloram as impurity, Pniok, W., 2024, Study code: ICB/91/2024
Guideline(s):	Yes, SANCO/3030/99 rev.5 and Standard Operational Procedure SPB/179.
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

Equipment and materials.
- acetonitrile (ACN), (Merck)
- 85% phosphoric acid (Sigma-Aldrich)
- picloram standard; Sigma-Aldrich; batch BCCH2936, prod. date: March 15, 2022; exp. date: February, 2027; storage conditions: <-18°C
- standard stock solution of picloram in ACN (for calibration) (Table 7)
- working standard solution of picloram in ACN/H2O (1:1) mixture (for recovery) (Table 8)
- working standard solutions of picloram in ACN/H2O (1:1) mixture (for calibration) (Table 9)
- placebo
- analytical balance – accuracy 0.0001 g (Ohaus, Switzerland), WP/16
- liquid chromatograph with diode array detection (Shimadzu, Japan), WP/19, WP/42,
- chromatography column type C18, 250 mm x 4,6 mm, 5 µm (Zorbax Eclipse Plus, Agilent), K/18/HPLC
- chromatography column type C18, 250 mm x 4,6 mm, 5 µm (Zorbax Eclipse Plus, Agilent), K/11/HPLC
- chromatographic vials 1.5 mL with septa buthyl/teflon
- volumetric flasks A class 10 mL
- measuring syringes 10 µl, 50 µL, 100µL, 250 µL, 1000 µL.

Preparation of standard solutions

Table 7. Preparation of picloram standard stock solution in ACN (for calibration).

Active ingredient	Weight [mg]	Flask volume [mL]	Concentration taking into account the purity of the standard [mg/mL]
picloram	10.7	10	1.053

Table 8. Preparation of picloram working standard solution in ACN/H_2O (1:1) mixture (for recovery).

Concentration of standard solution [mg/mL]	Volume of standard solution [µL]	Flask volume [mL]	Concentration of standard solution [µg/mL]
1.053	1500	10	157.95

Table 9. Preparation of picloram working standard solutions in ACN/H_2O (1:1) mixture (for calibration).

Concentration of standard solution [mg/mL]	Volume of standard solution [µL]	Flask volume [mL]	Concentration of standard solution [µg/mL]	Calibration level
1.053	10	10	1.053	Cal 1
1.053	25	10	2.6325	Cal 2
1.053	50	10	5.265	Cal 3
1.053	150	10	15.795	Cal 4
1.053	250	10	26.325	Cal 5

Chromatography parameters
Chromatography parameters were determined during preliminary assessment.

Primary chromatographic system.
Column: K/18/HPLC
Analysis time: 20 min
Flow: 1.4 mL/min
Column temperature: 25°C
Injection: 10 µL
Detector DAD: 225 nm
Mobile phase: A – ACN, D – 0.1% H_3PO_4
Isocratic program: phase A - 20%, D – 80%

Secondary chromatographic system.
Column: K/11/HPLC
Analysis time: 20 min
Flow: 1.4 mL/min
Column temperature: 40°C
Injection: 10 µL
Detector DAD: 225 nm
Mobile phase: A – ACN, D – 0.1% H_3PO_4
Isocratic program: phase A - 15%, D – 85%

Validation - Results and discussions

Table 5.2-2: Methods suitable for the determination of the relevant impurities in plant protection product (PPP) El Camino 30 SL/ Ranchero 30 SL/ AMINO 30 SL

	picloram **max. 1.2 g/L**
Author(s), year	Pniok, W., 2024
Principle of method	HPLC-DAD
Linearity (linear between mg/L) (correlation coefficient, expressed as r)	In order to check the linearity of picloram, calibration curve was prepared using standard solutions with concentrations contained in Table 9. A graph of the peak area to the concentration of picloram was plotted. The resulting curve is linear in the tested concentrations. Calibration curve for picloram is from 1.053 – 26.325 µg/mL, which corresponding to 0.01% – 0.26%. Linearity range of picloram is from LOQ 1,10565 µg/mL to 26.325 µg/mL. Correlation coefficient R^2 is 0.9999588 and the linear regression is described by equation: $f(x)$=2.02465·10−5x+0.0394961 for primary chromatographic system. Correlation coefficient R^2 is 0.9998946 and the linear regression is described by equation: $f(x)$=2.04053·10−5x+0.0531710 for secondary chromatographic system.
Precision – Repeatability Mean n = 5 (%RSD)	Table 22 shows results of precision and Horwitz ratio at 100% validation level for impurity for both systems. Table 22. Results of precision and Horwitz ratio at 100% validation level for impurity. Chromatographic system / Precision [%] / Horwitz ratio Primary / 0.45 / 0.09 Secondary / 0.46 / 0.10 Table 27 shows results of average recovery, precision and Horwitz ratio for both systems for validation level LOQ for picloram. Table 27. Results of average recovery, precision and Horwitz ratio for impurity for validation level LOQ for both systems. Chromatographic system / Precision [%] / Horwitz ratio / Average recovery [%] Primary / 0.76 / 0.14 / 99.9 Secendary / 0.30 / 0.06 / 99.7 Obtained results meet the criteria: precision for picloram ≤ 4.79 (validation level 100%), precision for picloram ≤ 5.28 (validation level LOQ), Horwitz ratio ≤ 1.0.

<table>
<tr><th></th><th>picloram
max. 1.2 g/L</th></tr>
<tr><td>Accuracy
n = 5
(% Recovery)</td><td>
Table 24 shows concentrations of impurity and recovery for all five independent test item samples used for determination of recovery for both chromatographic systems.

Table 24. Concentrations of impurity and recovery for all five independent test item samples used for determination of recovery.
<table>
<tr><th>Chromatographic system</th><th>Sample</th><th>Average concentration without standard addition [µg/mL]</th><th>Standard addition [µg/mL]</th><th>Concentrations [µg/mL]</th><th>Recovery [%]</th></tr>
<tr><td rowspan="5">Primary</td><td>1</td><td rowspan="5">2.10003</td><td rowspan="5">0.5530</td><td>2.61410</td><td>92.96</td></tr>
<tr><td>2</td><td>2.62205</td><td>94.40</td></tr>
<tr><td>3</td><td>2.62255</td><td>94.49</td></tr>
<tr><td>4</td><td>2.61994</td><td>94.02</td></tr>
<tr><td>5</td><td>2.62868</td><td>95.60</td></tr>
<tr><td rowspan="5">Secondary</td><td>1</td><td rowspan="5">2.09837</td><td rowspan="5">0.5530</td><td>2.62194</td><td>94.68</td></tr>
<tr><td>2</td><td>2.61516</td><td>93.45</td></tr>
<tr><td>3</td><td>2.63431</td><td>96.92</td></tr>
<tr><td>4</td><td>2.64166</td><td>98.24</td></tr>
<tr><td>5</td><td>2.64342</td><td>98.56</td></tr>
</table>
Table 25 shows results of impurity recovery for both systems.

Table 25. Results of recovery for impurity for both systems.
<table>
<tr><th>Chromatographic system</th><th>Recovery [%]</th><th>Standard addition [%]</th></tr>
<tr><td>Primary</td><td>92.96-95.60</td><td>26.33</td></tr>
<tr><td>Secendary</td><td>93.45-98.56</td><td>26.35</td></tr>
</table>
Table 27 shows results of average recovery, precision and Horwitz ratio for both systems for validation level LOQ for picloram.

Table 27. Results of average recovery, precision and Horwitz ratio for impurity for validation level LOQ for both systems.
<table>
<tr><th>Chromatographic system</th><th>Precision [%]</th><th>Horwitz ratio</th><th>Average recovery [%]</th></tr>
<tr><td>Primary</td><td>0.76</td><td>0.14</td><td>99.9</td></tr>
<tr><td>Secendary</td><td>0.30</td><td>0.06</td><td>99.7</td></tr>
</table>
Obtained results meet the criteria: recovery for picloram 75-125 (validation level 100%), recovery for picloram 75-125 (validation level LOQ).
</td></tr>
<tr><td>Interference/
Specificity</td><td>Specificity of the method was evaluated based on the analysis of chromatogram for placebo, sample against chromatogram of picloram standard and peak purity. Analysis showed no overlapping of determined ingredient signal with the signals of matrix components under method conditions, hence method specificity criterion is fulfilled.</td></tr>
<tr><td>LOQ</td><td>1.10565 µg/mL</td></tr>
<tr><td>Comment</td><td></td></tr>
</table>

Conclusion

The analytical method meets the specificity, linearity, precision/repeatability and accuracy criteria specified in SANCO 3030 (99) rev.5.

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

Please refer to PART C – Confidential data.

5.2.1.4 Applicability of existing CIPAC methods (KCP 5.1.1)

CIPAC methods are not available.

5.2.2 Methods for the determination of residues (KCP 5.1.2)

For aminopyralid analytical method GRM 02.31 was validated for the raw agricultural commodities of oil seed rape under residue report – Devine, H.C. (2006): *Residues of Clopyralid, Picloram, Aminopyralid in Oil Seed Rape at Intervals and at Harvest following a Single Application of GF-1634*, Germany, Poland and Hungary – 2005 (Registration report of Navigator 360 SL).
We are obliged to relied upon following studies taking account that according to Regulation (EC) No 1107/2009 Article 59 Data protection: The period of data protection is 10 years starting at the date of first authorization in that Member State, except as provided in paragraph 2 of this Article or in Article 62. According to Official Journal of the European Union C 229/2 Period of protection is 10 years from date of first authorization of the product in each Member State (not the date of authorization of the new crop). First registration of the product was in 15.12.2010, therefore data protection was over on 14.12.2020, and other Applicants can refer to studies performed during authorization process.

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

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

Component of residue definition: Sum of aminopyralid and its conjugates expressed as aminopyralid				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
Oil seed rape: forage, stover & straw, grains (Residues)	Primary	0.01 mg/kg	LC/MS/MS	Devine, H.C., 2006, Devine, H.C., 2007 Report No: GHE-P-11273 Study No: CEMS-2698 EU agreed
	Confirmatory (if required)	Not required		
Artificial soil (Ecotoxicology)	Primary	4.0 mg/kg	HPLC with DAD	Czarnynoga, M., 2024 / Study code: G-56-24 / Not presented on EU level, Czarnynoga, M., 2024 / Study code: G-55-24/ Not presented on EU level, Gierbuszewska, A., 2024/ Study code: G-54-24/ Not presented on EU level,
	Confirmatory	Same as primary.		

Component of residue definition: Sum of aminopyralid and its conjugates expressed as aminopyralid				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
	(if required)			
AAP medium (Ecotoxicology)	Primary	0.1 mg/L (direct method) 0.02 mg/L (SPE method)	HPLC with DAD	Maga, D., 2024/ Study code: W-30-24 / Not presented on EU level
	Confirmatory (if required)	Same as primary.		
Elendt M7 medium (Ecotoxicology)	Primary	0.2 mg/L	HPLC with DAD	Maga, D., 2024/ Study code: W-27-24 / Not presented on EU level
	Confirmatory (if required)	Same as primary.		
20xAAP medium (Ecotoxicology)	Primary	0.2 mg/L	HPLC with DAD	Maga, D., 2024/ Study code: W-29-24/ Not presented on EU level
	Confirmatory (if required)	Same as primary.		
AAP medium (Ecotoxicology)	Primary	0.1 mg/L	HPLC with DAD	Maga, D., 2024/ Study code: W-28-24/ Not presented on EU level
	Confirmatory (if required)	Same as primary.		
50% Sucrose Solution (Eco-toxicology)	Primary	1.0 mg/kg	HPLC with DAD	Dybek, M., 2024/ Study code: B-94-24/ Not presented on EU level, Dybek, M., 2024/ Study code: B-88-24/ Not presented on EU level
	Confirmatory (if required)	Same as primary.		
0.1% Tergitol 15-S-9 Solution (Ecotoxicology)	Primary	1.0 mg/L	HPLC with DAD	Dybek, M., 2024/ Study code: B-89-24/ Not presented on EU level
	Confirmatory (if required)	Same as primary.		
Water (Ecotoxi-cology)	Primary	0.2 mg/L	HPLC with DAD	Wróbel, A., 2024/ Study code: G-59-24/ Not presented on EU level, Wróbel, A., 2024/ Study code: G-93-24/ Not presented on EU level, Czarnynoga, M., 2024/ Study code: G-58-24/ Not presented on EU level
	Confirmatory (if required)	Same as primary.		
Smart and Barko medium (Eco-toxicology)	Primary	0.02 mg/L	HPLC with DAD	Czarnecka M., 2024/ Study code: W-26-24/ Not presented on EU level
	Confirmatory (if required)	Same as primary.		

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

Data provided on Annex I inclusion is sufficient for post-authorizations methods. All data is described in EU approved documents for :

- Final addendum to the Draft Assessment Report (DAR) – Aminopyralid; July 2013; Volume 3, Annex B.5
- EFSA Journal 2013;11(9):3352

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 aminopyralid (KCP 5.2)

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

Compared to the residue definition proposed in the Draft Assessment Report (incl. its addenda) the current legal residue definition is not identical.

Current legal residue definition: Aminopyralid (sum of aminopyralid, its salts and its conjugates, expressed as aminopyralid (Reg. (EU) 2021/1841))

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

Matrix	Residue definition	MRL / limit	Reference for MRL/level Remarks
Plant, high water content	The sum of aminopyralid and its conjugates expressed as aminopyralid	LOQ: 0.01 mg/kg	EFSA Journal 2013;11(9):3352
Plant, high acid content		LOQ: 0.01 mg/kg	EFSA Journal 2013;11(9):3352
Plant, high protein/high starch content (dry commodities)		LOQ: 0.01 mg/kg	EFSA Journal 2013;11(9):3352
Plant, high oil content		LOQ: 0.01 mg/kg MRL: rapeseeds/canola seeds 0.05 mg/kg	EFSA Journal 2013;11(9):3352 Reg. (EU) 2021/1841
Muscle	Aminopyralid	LOQ: 0.01 mg/kg MRL: 0.1 mg/kg; 0.01 mg/kg (poultry only)	EFSA Journal 2013;11(9):3352 Reg. (EU) 2021/1841

Matrix	Residue definition	MRL / limit	Reference for MRL/level Remarks
Milk		LOQ: 0.01 mg/kg MRL: 0.02 mg/kg	EFSA Journal 2013;11(9):3352 Reg. (EU) 2021/1841
Eggs		LOQ: 0.01 mg/kg MRL: 0.01 mg/kg	EFSA Journal 2013;11(9):3352 Reg. (EU) 2021/1841
Fat		LOQ: 0.01 mg/kg MRL: 0.1 mg/kg; 0.01 mg/kg (poultry only)	EFSA Journal 2013;11(9):3352 Reg. (EU) 2021/1841
Liver, kidney		LOQ: 0.01 mg/kg MRL: liver: 0.05 mg/kg kidney: 1 mg/kg; 0.01 mg/kg (poultry only)	EFSA Journal 2013;11(9):3352 Reg. (EU) 2021/1841
Soil (Ecotoxicology)	Aminopyralid	LOQ: 0.001 mg/kg	EFSA Journal 2013;11(9):3352
Drinking water (Human toxicology)	Aminopyralid	LOQ: 0.05 µg/L	EFSA Journal 2013;11(9):3352
Surface water (Ecotoxicology)	Aminopyralid	LOQ: 0.05 µg/L	EFSA Journal 2013;11(9):3352
Air	Aminopyralid	LOQ: 7.7 µg/m^3	EFSA Journal 2013;11(9):3352
Tissue (meat or liver)	No residue definition provided;	not required MRL: 0.01 mg/kg	SANTE/2020/12830 Rev.2
Body fluids		not required LOQ: blood 0.025 mg/L urine 0.01 µg/mL MRL: 0.01 mg/kg	not classified as T / T+ EFSA Journal 2013;11(9):3352 SANTE/2020/12830 Rev.2

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 aminopyralid in plant matrices is given in the following tables.

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

Component of residue definition: The sum of aminopyralid and its conjugates expressed as aminopyralid				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
High water content	Primary	0.01 mg/kg	LC/MS/MS	Final addendum to the Draft Assessment Report (DAR) - Aminopyralid – July 2013; Vol. 3B.5; Wendelburg, B.M., Olberding, E.L., 2008a; Report No.: GRM 07.07, 071121 EU agreed
	ILV	0.01 mg/kg	As for primary method	Final addendum to the Draft Assessment Report (DAR) - Aminopyralid – July 2013; Vol. 3B.5; Beck, I.C., Class, T. 2008a; Report No.: P 1466 G, 080117 EU agreed
	Confirmatory (if required)	Not required		
High acid content	Primary	0.01 mg/kg	LC/MS/MS	Final addendum to the Draft Assessment Report (DAR) - Aminopyralid – July 2013; Vol. 3B.5; Wendelburg, B.M., Olberding, E.L., 2008a; Report No.: GRM 07.07, 071121 EU agreed
	ILV	Not required		
	Confirmatory (if required)	Not required		
High oil content	Primary	0.01 mg/kg	LC/MS/MS	Final addendum to the Draft Assessment Report (DAR) - Aminopyralid – July 2013; Vol. 3B.5; Wendelburg, B.M., Olberding, E.L., 2008a; Report No.: GRM 07.07, 071121 EU agreed
	ILV	Not required		
	Confirmatory (if required)	Not required		
High protein/high starch content (dry)	Primary	0.01 mg/kg		Final addendum to the Draft Assessment Report (DAR) - Aminopyralid – July 2013; Vol. 3B.5; Wendelburg, B.M., Olberding, E.L., 2008a; Report No.: GRM 07.07, 071121

Component of residue definition: The sum of aminopyralid and its conjugates expressed as aminopyralid				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
				EU agreed
	ILV	0.01 mg/kg	As for primary method	Final addendum to the Draft Assessment Report (DAR) - Aminopyralid – July 2013; Vol. 3B.5; Beck, I.C., Class, T. 2008a; Report No.: P 1466 G, 080117 EU agreed
	Confirmatory (if required)	Not required		
Difficult (if required, depends on intended use)	Primary	Not required		
	ILV			
	Confirmatory (if required)			

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

Table 5.3-3: Statement on extraction efficiency

	Method for products of plant origin
Required, available from:	-
Not required, because:	The extraction efficiency was not assessed due to following information included in SANTE/2017/10632 Rev. 4: For renewal of product authorisations or for new product authorisations or extension of uses for which no change of the MRL is needed, the data requirements used for the latest renewal or approval should be considered. This means that no additional proof of extraction efficiency is required if it had not been required in the renewal of approval/approval procedure itself. Extraction efficiency should be addressed if for a product authorization a **different** analytical methodology (in methods for monitoring) is used, compared to that of the approval/renewal procedure of the active substance, which is not the case.

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 aminopyralid in animal matrices is given in the following tables.

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

Component of residue definition: aminopyralid				
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	Final addendum to the Draft Assessment Report (DAR) - Aminopyralid – July 2013; Vol. 3B.5; Wendelburg, B.M., Olberding, E.L., 2008b; Report No.: GRM 07.08, 071121 EU agreed
	ILV	0.01 mg/kg	As for primary method	Final addendum to the Draft Assessment Report (DAR) - Aminopyralid – July 2013; Vol. 3B.5; Beck, I.C. and Class, T., 2008b; Final addendum to the Draft Report No.: P 1467 G, 080118 EU agreed
	Confirmatory (if required)	Not required		
Eggs	Primary	0.01 mg/kg	LC/MS/MS	Final addendum to the Draft Assessment Report (DAR) - Aminopyralid – July 2013; Vol. 3B.5; Wendelburg, B.M., Olberding, E.L., 2008b; Report No.: GRM 07.08, 071121 EU agreed
	ILV	Not required		
	Confirmatory (if required)	Not required		
Muscle	Primary	0.01 mg/kg	LC/MS/MS	Final addendum to the Draft Assessment Report (DAR) - Aminopyralid – July 2013; Vol. 3B.5; Wendelburg, B.M., Olberding, E.L., 2008b; Report No.: GRM 07.08, 071121 EU agreed
	ILV	Not required		
	Confirmatory (if required)	Not required		
Fat	Primary	0.01 mg/kg	LC/MS/MS	Final addendum to the Draft Assessment Report (DAR) - Aminopyralid – July 2013; Vol. 3B.5; Wendelburg, B.M., Olberding, E.L., 2008b;

Component of residue definition: aminopyralid				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (*i.e.* GC-MS or HPLC-UV)**	**Author(s), year / missing**
				Report No.: GRM 07.08, 071121 EU agreed
	ILV	Not required		
	Confirmatory (if required)	Not required		
Kidney, liver	Primary	0.01 mg/kg	LC/MS/MS	Final addendum to the Draft Assessment Report (DAR) - Aminopyralid – July 2013; Vol. 3B.5; Wendelburg, B.M., Olberding, E.L., 2008b; Report No.: GRM 07.08, 071121 EU agreed
	ILV	0.01 mg/kg	As for primary method	Final addendum to the Draft Assessment Report (DAR) - Aminopyralid – July 2013; Vol. 3B.5; Beck, I.C. and Class, T., 2008b; Report No.: P 1467 G, 080118 EU agreed
	Confirmatory (if required)	Not required		

Table 5.3-5: Statement on extraction efficiency

	Method for products of animal origin
Required, available from:	-
Not required, because:	The extraction efficiency was not assessed due to following information included in SANTE/2017/10632 Rev. 4: For renewal of product authorisations or for new product authorisations or extension of uses for which no change of the MRL is needed, the data requirements used for the latest renewal or approval should be considered. This means that no additional proof of extraction efficiency is required if it had not been required in the renewal of approval/approval procedure itself. Extraction efficiency should be addressed if for a product authorization a **different** analytical methodology (in methods for monitoring) is used, compared to that of the approval/renewal procedure of the active substance, which is not the case.

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 aminopyralid in soil is given in the following tables.

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

Component of residue definition: Aminopyralid			
Method type	**Method LOQ**	**Principle of method (*i.e.* GC-MS or HPLC-UV)**	**Author(s), year / missing**
Primary	0.0015 mg/kg	LC/MS/MS	Final addendum to the Draft Assessment Report (DAR) - Aminopyralid – July 2013; Vol. 3B.5; Wendelburg, B.M., Olberding, E.L., 2008c; Report No.: GRM 07.09, 071121 EU agreed
Confirmatory	Not required		

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 aminopyralid in surface and drinking water is given in the following tables.

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

Component of residue definition: Aminopyralid				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
Drinking water	Primary	0.05 µg/L	LC/MS/MS	Final addendum to the Draft Assessment Report (DAR) - Aminopyralid – July 2013; Vol. 3B.5; Wendelburg, B.M., Olberding, E.L., 2008d; Report No.: GRM 07.10, 071121 EU agreed
	ILV	0.05 µg/L	As for primary method	Final addendum to the Draft Assessment Report (DAR) - Aminopyralid – July 2013; Vol. 3B.5; Beck, I.C., Class, T., 2008d; Report No.: P 1464 G
	Confirmatory	Not required		
Surface water	Primary	0.05 µg/L	LC/MS/MS	Final addendum to the Draft Assessment Report (DAR) - Aminopyralid – July 2013; Vol. 3B.5; Wendelburg, B.M., Olberding, E.L., 2008d; Report No.: GRM 07.10, 071121 EU agreed
	ILV	0.05 µg/L	As for primary method	Final addendum to the Draft

Component of residue definition: Aminopyralid				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
				Assessment Report (DAR) - Aminopyralid – July 2013; Vol. 3B.5; Beck, I.C., Class, T., 2008d; Report No.: P 1464 G
	Confirmatory	Not required		

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 aminopyralid in air is given in the following tables.

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

Component of residue definition: aminopyralid			
Method type	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
Primary	Ambient air (21°C, 26% RH) LOQ: 7.7 µg/m^3 Warm, humid air (37°C, 92% RH) LOQ: 7.7 µg/m^3	LC/MS/MS	Final addendum to the Draft Assessment Report (DAR) - Aminopyralid – July 2013; Vol. 3B.5; Bacher, R., 2009; Report No.: P 1645 G, 091020
Confirmatory	Not required		

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 aminopyralid in body fluids and tissues is given in the following table.

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

Component of residue definition: No residue definition provided			
Method type	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
Primary	blood 0.025 µg/ml urine 0.01 µg/ml	LC-MS/MS	Final addendum to the Draft Assessment Report (DAR) - Aminopyralid – July 2013; Vol. 3B.5; Mollica, J.; West, S.D. 2003

Component of residue definition: No residue definition provided			
Method type	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
			Report No.: Pyxant Dow-1419/031005 EU agreed
Confirmatory	Not required		

5.3.2.8 Other studies/ information

Not required.

Appendix 1 Lists of data considered in support of the evaluation

List of data submitted by the applicant and relied on

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 5.1.1/01 KCP 5.1.1/02	Pniok, W.	2024	Validation of analytical method for AMINO 30 SL for determination of aminopyralid and picloram as impurity Study code: ICB/91/2024 ICB Pharma, 10 Lema Street 43-600, Jaworzno, Poland GLP/GEP (Y/N): Y Published (Y/N): N	N	PUH Chemirol sp. z o.o.
KCP 5.1.2/01	Czarnynoga, M.	2024	Predatory mite (*Hypoaspis (Geolaelaps) aculeifer*) reproduction test in soil Study code: G-56-24 Łukasiewicz Research Network – Institute of Industrial Organic Chemistry Branch Pszczyna Ecotoxicology Research Group Doświadczalna 27, 43 – 200 Pszczyna, Poland GLP/GEP (Y/N): Y Published (Y/N): N	N	PUH Chemirol sp. z o.o.
KCP 5.1.2/02	Czarnynoga, M.	2024	Earthworm (*Eisenia andrei*) reproduction test Study code: G-54-24 Łukasiewicz Research Network – Institute of Industrial Organic Chemistry Branch Pszczyna Ecotoxicology Research Group Doświadczalna 27, 43 – 200 Pszczyna, Poland GLP/GEP (Y/N): Y Published (Y/N): N	N	PUH Chemirol sp. z o.o.

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 5.1.2/03	Czarnynoga, M.	2024	Collembolan (*Folsomia candida*) Reproduction Test Study code: G-55-24 Łukasiewicz Research Network – Institute of Industrial Organic Chemistry Branch Pszczyna Ecotoxicology Research Group Doświadczalna 27, 43 – 200 Pszczyna, Poland GLP/GEP (Y/N): Y Published (Y/N): N	N	PUH Chemirol sp. z o.o.
KCP 5.1.2/04	Czarnynoga, M.	2024	*Anabaena flos-aquae* UTEX B 1444, Growth inhibition test Study code: W-30-24 Łukasiewicz Research Network – Institute of Industrial Organic Chemistry Branch Pszczyna Ecotoxicology Research Group Doświadczalna 27, 43 – 200 Pszczyna, Poland GLP/GEP (Y/N): Y Published (Y/N): N	N	PUH Chemirol sp. z o.o.
KCP 5.1.2/05	Maga, D.	2024	*Daphnia magna*, Acute Immobilisation Test Study code: W-27-24 Łukasiewicz Research Network – Institute of Industrial Organic Chemistry Branch Pszczyna Ecotoxicology Research Group Doświadczalna 27, 43 – 200 Pszczyna, Poland GLP/GEP (Y/N): Y Published (Y/N): N	N	PUH Chemirol sp. z o.o.
KCP 5.1.2/06	Maga, D.	2024	*Lemna gibba* CPCC 310, Growth inhibition test Study code: W-29-24 Łukasiewicz Research Network – Institute of Industrial Organic Chemistry	N	PUH Chemirol sp. z o.o.

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
			Branch Pszczyna Ecotoxicology Research Group Doświadczalna 27, 43 – 200 Pszczyna, Poland GLP/GEP (Y/N): Y Published (Y/N): N		
KCP 5.1.2/07	Maga, D.	2024	*Raphidocelis subcapitata* SAG 61.81 (formerly Pseudokirchneriella subcapitata), Growth inhibition test Study code: W-28-24 Łukasiewicz Research Network – Institute of Industrial Organic Chemistry Branch Pszczyna Ecotoxicology Research Group Doświadczalna 27, 43 – 200 Pszczyna, Poland GLP/GEP (Y/N): Y Published (Y/N): N	N	PUH Chemirol sp. z o.o.
KCP 5.1.2/08	Czarnecka, M.	2024	Water-sediment *Myriophyllum spicatum* toxicity test Study code: W-26-24 Łukasiewicz Research Network – Institute of Industrial Organic Chemistry Branch Pszczyna Ecotoxicology Research Group Doświadczalna 27, 43 – 200 Pszczyna, Poland GLP/GEP (Y/N): Y Published (Y/N): N	N	PUH Chemirol sp. z o.o.
KCP 5.1.2/09	Dybek, M.	2024	Honeybees (*Apis mellifera L.*), Chronic Oral Toxicity Test Study code: B-94-24 Łukasiewicz Research Network – Institute of Industrial Organic Chemistry Branch Pszczyna Ecotoxicology Research Group Doświadczalna 27, 43 – 200 Pszczyna, Poland	N	PUH Chemirol sp. z o.o.

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
			GLP/GEP (Y/N): Y Published (Y/N): N		
KCP 5.1.2/10	Dybek, M.	2024	Bumblebees (*Bombus* spp.), Acute Contact Toxicity Test Study code: B-89-24 Łukasiewicz Research Network – Institute of Industrial Organic Chemistry Branch Pszczyna Ecotoxicology Research Group Doświadczalna 27, 43 – 200 Pszczyna, Poland GLP/GEP (Y/N): Y Published (Y/N): N	N	PUH Chemirol sp. z o.o.
KCP 5.1.2/11	Dybek, M.	2024	Bumblebees (*Bombus* spp.), Acute Oral Toxicity Test Study code: B-88-24 Łukasiewicz Research Network – Institute of Industrial Organic Chemistry Branch Pszczyna Ecotoxicology Research Group Doświadczalna 27, 43 – 200 Pszczyna, Poland GLP/GEP (Y/N): Y Published (Y/N): N	N	PUH Chemirol sp. z o.o.
KCP 5.1.2/12	Wróbel, A.	2024	Terrestrial Plant Test: Seedling Emergence and Seedling Growth Test Study code: G-59-24 Łukasiewicz Research Network – Institute of Industrial Organic Chemistry Branch Pszczyna Ecotoxicology Research Group Doświadczalna 27, 43 – 200 Pszczyna, Poland GLP/GEP (Y/N): Y Published (Y/N): N	N	PUH Chemirol sp. z o.o.
KCP	Wróbel, A.	2024	Terrestrial Plant Test: Seedling Emergence and Seedling Growth Test	N	PUH

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
5.1.2/13			Study code: G-93-24 Łukasiewicz Research Network – Institute of Industrial Organic Chemistry Branch Pszczyna Ecotoxicology Research Group Doświadczalna 27, 43 – 200 Pszczyna, Poland GLP/GEP (Y/N): Y Published (Y/N): N		Chemirol sp. z o.o.
KCP 5.1.2/14	Czarnynoga, M.	2024	Terrestial Plant Test: Vegetative Vigour Test Study code: G-58-24 Łukasiewicz Research Network – Institute of Industrial Organic Chemistry Branch Pszczyna Ecotoxicology Research Group Doświadczalna 27, 43 – 200 Pszczyna, Poland GLP/GEP (Y/N): Y Published (Y/N): N	N	PUH Chemirol sp. z o.o.
KCP 5.1.2/15	Kanon, L.	2024	Validation of analytical method for determination of the active substance (aminopyralid) in the test item AMINO 30 SL solution in deionized water Study code: 0038/0214/FA SORBOLAB Research Laboratory LLC Zaniemyska Street 11 61-029 Poznań, Poland GLP/GEP (Y/N): Y Published (Y/N): N	N	PUH Chemirol sp. z o.o.

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

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 5.3.2.1 KCP 5.3.2.2	Wendelburg, B. M.; and Olberding, E. L.	2008a	Determination of Residues of Aminopyralid in Agricultural Commodities by Liquid Chromatography with Tandem Mass Spectrometric Detection Dow AgroSciences LLC, USA. Report No.: GRM 07.07, 071121 GLP/GEP (Y/N): Y Published (Y/N): N	N	DAS
KCP 5.3.2.1 KCP 5.3.2.2	Beck, I. C.; and Class, T.	2008a	Independent Laboratory Validation of Dow AgroSciences LLC Method GRM 07.07 - Determination of Residues of Aminopyralid in Agricultural Commodities by Liquid Chromatography with Tandem Mass Spectrometric Detection PTRL Europe GmbH, Germany Report No.: P 1466 G, 080117 GLP/GEP (Y/N): Y Published (Y/N): N	N	DAS
KCP 5.3.2.1 KCP 5.3.2.3	Wendelburg, B. M.; and Olberding, E. L.	2008b	Determination of Residues of Aminopyralid in Bovine and Poultry Tissues, Milk, and Eggs by Liquid Chromatography with Tandem Mass Spectrometric Detection Dow AgroSciences LLC, USA. Report No.: GRM 07.08, 071121 GLP/GEP (Y/N): Y Published (Y/N): N	Y	DAS
KCP 5.3.2.1 KCP 5.3.2.3	Beck, I. C. and Class, T.	2008b	Independent Laboratory Validation of Dow AgroSciences LLC Method GRM 07.08 - Determination of Residues of Aminopyralid in Bovine and Poultry Tissues, Milk, and Eggs by Liquid Chromatography with Tandem Mass Spectrometric Detection. PTRL Europe GmbH, Germany Report No.: P 1467 G, 080118 GLP/GEP (Y/N): Y Published (Y/N): N	Y	DAS
KCP 5.3.2.1 KCP	Wendelburg, B.M. and Olberding, E.L.	2008c	Determination of Residues of Aminopyralid in Soil by Liquid Chromatography with Tandem Mass Spectrometric Detection Dow AgroSciences LLC	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
5.3.2.4			Report No.: GRM 07.09, 071121 GLP/GEP (Y/N): Y Published (Y/N): N		
KCP 5.3.2.1 KCP 5.3.2.5	Wendelburg, B.M. and Olberding, E.L.	2008d	Determination of Residues of Aminopyralid in Drinking Water, Ground Water, and Surface Water by Liquid Chromatography with Tandem Mass Spectrometric Detection Dow AgroSciences LLC Report No.: GRM 07.10, 071121 GLP/GEP (Y/N): Y Published (Y/N): N	N	DAS
KCP 5.3.2.1 KCP 5.3.2.5	Beck, I.C. and Class, T.	2008d	Independent Laboratory Validation of Dow AgroSciences LLC Method GRM 07.10 - Determination of Residues of Aminopyralid in Drinking Water, Ground Water, and Surface Water by Liquid Chromatography with Tandem Mass Spectrometric Detection PTRL Europe GmbH Report No.: P 1464 G GLP/GEP (Y/N): Y Published (Y/N): N	N	DAS
KCP 5.3.2.1 KCP 5.3.2.6	Bacher, R.	2009	The Development and Validation of a Method for the Determination of Aminopyralid in Air PTRL Europe GmbH, Germany Report No.: P 1645 G, 091020 GLP/GEP (Y/N): Y Published (Y/N): N	N	DAS
KCP 5.3.2.1 KCP 5.3.2.7	Mollica, J.; West, S.D.	2003	Method Validation for the Analysis of XDE-750 (Aminopyralid) in Human Blood and Urine Pyxant Labs Inc, CO, USA. Report No.: Pyxant Dow-1419/031005 (Masterfile Number) N/A GLP/GEP (Y/N): Y Published (Y/N): N	N	DAS
KCP 5.2.2	Devine, H.C.	2006	Residues of Clopyralid, Picloram and Aminopyralid in Oil Seed Rape at Intervals and at Harvest Following a Single Application of GF-1634, Germany, Poland and Hungary – 2005 CEM Analytical Services Ltd	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 Report No.: GHE-P-11273 (Masterfile Number) N/A GLP/GEP (Y/N): Y Published (Y/N): N		
KCP 5.2.2	Devine, H.C.	2007	Residues of Clopyralid, Picloram and Aminopyralid in Oil Seed Rape at Intervals and at Harvest Following a Single Application of GF-1633 or GF-871. Nothern Europe – 2006 CEM Analytical Services Ltd DAS Report No.: GHE-P-11493 (Masterfile Number) N/A GLP/GEP (Y/N): Y Published (Y/N): N	N	DAS

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 aminopyralid

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

A 2.1.1.1.1 Analytical method 1

A 2.1.1.1.1.1 Method validation

Comments of zRMS:	Validation of the Analytical Czarnynoga, M., 2024, G-56-24) is acceptable and suitable for determination of aminopyralid in artificial soil.

Reference:	KCP 5.1.2/01
Report	Predatory mite (Hypoaspis (Geolelaps) aculeifer) reproduction test in soil, Czarnynoga, M., 2024, Study code: G-56-24
Guideline(s):	Yes OECD Guideline No. 226; SANTE/2020/12830, Rev. 2; Standard Operating Procedure SOP/C/9
Deviations:	No
GLP:	Yes
Acceptability:	Yes

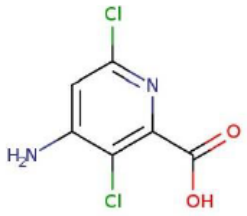
Materials and methods

Analytical standard of aminopyralid was used to method validation.

Aminopyralid

IUPAC Name:	4-amino-3,6-dichloropyridine-2-carboxylic acid (IUPAC)
Molecular Formula:	$C_6H_4Cl_2N_2O_2$
Cas No.:	150114-71-9
Purity:	97.99 % ± 0.31% (g/g)
Series No:	G1239715
Valid to:	22 March 2028
Molecular weight:	207.01 g/mol

Structural [2]:



Stock and standard solutions

The stock solution with a concentration of 1.0 mg/mL was prepared by weighting 10.0 mg of standard into a volumetric flask with a capacity of 10.0 mL, dissolving in acetonitrile for HPLC, and next the volume was made up to 10.0 mL with the same solvent. The working solutions were prepared by diluting

standard solutions of higher concentrations.

Fortification of samples

For the preparation of procedural recoveries and validation experiments, fortified samples were prepared from standard solution. The appropriate amount of spiking solutions was added to the matrix to prepare LOQ and 10xLOQ.

Sample preparation for the chromatographic analysis

10 g of artificial soil sample was weighted. The sample was shaken with 10 mL of mixture of acetonitrile for HPLC and deionized water (50:50 *v/v*) for 5 minutes. Next the sample was placed in an ultrasonic bath for 10 minutes, then centrifuge (5 minutes, 3800 rpm) and the extract collected. The extraction repeated with 10 mL of mixture of acetonitrile for HPLC and deionized water (50:50 *v/v*). The extracts were combined together. The sample was diluted with mixture of acetonitrile for HPLC and deionized water (*v/v*) (50:50; *v/v*), if necessary An aliquot of the final volume was transferred into a HPLC vial for further quantification using HPLC-DAD.

2.5.1. Schematic diagram of the analytical method

Overview of the fortification of samples work-up by a flow chart / picture is presented below:

D2- mixture of acetonitrile for HPLC and deionized water (*v/v*) (50:50; *v/v*),

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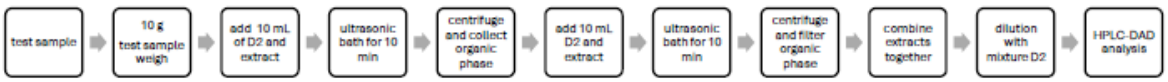

D2 – mixture of acetonitrile for HPLC and deionized water (50:50, *v/v*).

2.6. Instrumentation and conditions

The chromatographic systems and conditions used for the analysis are shown in the table below [SOP/C/328, SOP/C/592, SOP/C/607].

	Parameter
Chromatographic System	High Performance Liquid Chromatography (HPLC)
Chromatograph	Shimadzu, Prominence - *i* (Shimadzu Corporation Japan)
Analytical Column	Luna 5µm C18 (2) 100Å (2) 250x4.6 mm
Oven temperature	35°C
Injection Volume	10 µL
Mobile Phase	acetonitrile HPLC : ortho-phosphoric acid solution 0.05 % (65 : 35, *v/v*)
Flow Rate	0.6 mL/min
Wave length	240 nm
Retention time	approx. 5. min
Detection System	Diode Array Detector

Results and discussions

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

Matrix	Analyte	Fortification level (mg/kg) (*n* = x)	Mean recovery (%)	RSD (%)	Comments
Artificial soil	Aminopyralid	Control – 0.0 mg/kg– two replicates LOQ – 4.0 mg/kg – five replicates 10xLOQ – 40.0 mg/kg – five replicates	87.7 – 98.3 %	0.5– 0.7 %	-

Table A 2: Characteristics for the analytical method used for validation of aminopyralid residues in artificial soil

	Aminopyralid residues
Author(s), year	M. Czarnynoga, 2024
Principle of method	HPLC-DAD
Linearity (linear be-tween mg/L) (correlation coefficient,	Working solutions at all calibration levels were injected successively to the chromatographic column and the chromatograms were recorded. The range of linearity of the analytical graph and the ranges of calibration curve corresponding to the real samples level are presented in the table below.

<table>
<tr><td></td><td>Aminopyralid residues</td></tr>
<tr><td>expressed as r)</td><td>
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<tr><th>Analyte</th><th>Working solution concentrations [µg/mL]</th><th>Range of linearity of calibration curve [mg/L]</th><th>Equivalent calibration range of linearity [mg/kg]</th></tr>
<tr><td>Aminopyralid</td><td>0.5, 1.0, 2.0, 5.0, 10.0, 20.0, 50.0</td><td>0.5 – 50.0</td><td>1.0 – 100.0</td></tr>
</table>
The equation of the calibration line is presented as the linear equation; y = ax + b (a – slope, b – intercept). The linear coefficient r^2 must be higher than 0.99. Range of the linearity was given in µg/mL (equal to mg/L).
<table>
<tr><th>Range of linearity of calibration curve [mg/L]</th><th>Analyte</th><th>Slope</th><th>Intercept</th><th>Coefficient r²</th></tr>
<tr><td>0.5 – 50.0</td><td>Aminopyralid</td><td>46376.1</td><td>950.036</td><td>0.9991894</td></tr>
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Linear weighted calibration (1/C weighting) is used for calibration curve. The suitability of the chosen function was demonstrated as the regression residuals (d_i). The regression residuals are presented in a residual plot in range equal to range of linearity of calibration curve.</td></tr>
<tr><td>Precision, accuracy and uncer-tainty</td><td>Precision of a particular methods is defined as its repeatability (RSD – relative standard deviation [%]). The repeatability for detected substances analysed are presented in table below. The RSD is ≤ 20% per each level.
The accuracy of the method is reported as mean recovery ± relative standard deviation. Recovery was reported as mean recovery ± relative standard deviation (RSD). Mean recoveries for each level is in the range 70-120%.
A summary of the recovery data of control and fortified samples are presented in the table below.
<table>
<tr><th>Method</th><th>Analyte</th><th>Fortification Level [mg/kg]</th><th>Number of Replicates</th><th>Mean Recovery [%]</th><th>RSD [%]</th></tr>
<tr><td rowspan="2">dilution method</td><td rowspan="2">Aminopyralid</td><td>4.0</td><td>5</td><td>87.7</td><td>0.7</td></tr>
<tr><td>40.0</td><td>5</td><td>98.3</td><td>0.5</td></tr>
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In order to study the recovery level, the solution of the detected substance was added to non-treated control sample and then analysed using the methods described above.</td></tr>
<tr><td>Specificity</td><td>The analytical method specificity was estimated on the basis of the analysis of the chromatograms obtained for the control, and fortified samples of matrix. Considering the results of the analysis, no signal of detected substance was overlapping with matrix signal of the control samples in the experiments conditions. Therefore, the specificity of the method was demonstrated. Furthermore, any interference are directly apparent and would be observed in the chromatograms of the matrix control sample.</td></tr>
<tr><td>Matrix Ef-fects</td><td>Matrix effect for each method was checked during the method validation. Assessment of matrix effects was performed by comparing the standard prepared in solution to standard prepared in blank matrix at appropriate concentration.
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<tr><th>Method</th><th>Analyte</th><th>Concentration [mg/L]</th><th>Matrix effect [%]</th></tr>
<tr><td>extraction method</td><td>Aminopyralid</td><td>2.0</td><td>-7.2</td></tr>
</table></td></tr>
<tr><td>LOQ
LOD</td><td>Limit of Quantification was estimated as the lowest concentration of a detected substance at which an acceptable mean recovery and precision (normally 70 – 120% with a relative standard deviation of preferably ≤ 20%).
The limit of detection (LOD) is defined as the lowest detectable concentration or amount of an analyte in a sample. LOD is expressed as the lowest calibration standard. LOD is less than or equal to 30% of LOQ.</td></tr>
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<tr><td>extraction method</td><td>Aminopyralid</td><td>4.0</td><td>2.0</td><td>1.0</td><td>0.5</td></tr>
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<tr><td>Extraction stability</td><td>Final extract stability was not determined. The final extracts were analysed within 24 h.</td></tr>
<tr><td>Comment</td><td>The validation parameters are within the acceptance range and fulfil EU requirements given in SANTE/2020/12830 Rev.2.</td></tr>
</table>

Conclusion

The method was successfully validated for determination of aminopyralid in artificial soil with an LOQ of 4.0 mg/kg according to the guidance document(s) SANTE/2020/12830 Rev.2. With regard to selectivity, accuracy and precision, the analytical methods were applied successfully for each analytical set when analysing the specimens of the study.

A 2.1.1.1.2 Analytical method 2

A 2.1.1.1.2.1 Method validation

Comments of zRMS:	Validation of the Analytical Gierbuszewska, A., 2024, G-54-24) is acceptable and suitable for determination of aminopyralid in artificial soil.

Reference:	KCP 5.1.2/02
Report	Earthworm (Eisenia Andrei), Reproduction Test, Gierbuszewska, A., 2024, Study code: G-54-24
Guideline(s):	Yes OECD Guideline No. 222 (2016); SANTE/2020/12830, Rev. 2; Standard Operating Procedure SOP/C/9.
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

Analytical standard of aminopyralid was used to method validation.

Aminopyralid

IUPAC Name:	4-amino-3,6-dichloropyridine-2-carboxylic acid (IUPAC)	Structural [2]:
Molecular Formula:	$C_6H_4Cl_2N_2O_2$	
Cas No.:	150114-71-9	
Purity:	97.99 % ± 0.31% (g/g)	
Series No:	G1239715	
Valid to:	22 March 2028	
Molecular weight:	207.01 g/mol	

Stock and standard solutions

The stock solution with a concentration of 1.0 mg/mL was prepared by weighting 10.0 mg of standard into a volumetric flask with a capacity of 10.0 mL, dissolving in acetonitrile for HPLC, and next the volume was made up to 10.0 mL with the same solvent. The working solutions were prepared by diluting standard solutions of higher concentrations.

Fortification of samples

For the preparation of procedural recoveries and validation experiments, fortified samples were prepared from standard solution. The appropriate amount of spiking solutions was added to the matrix to prepare LOQ and 10xLOQ.

Sample preparation for the chromatographic analysis

10 g of artificial soil sample was weighted. The sample was shaken with 10 mL of mixture of acetonitrile for HPLC and deionized water (50:50 *v/v*) for 5 minutes. Next the sample was placed in an ultrasonic bath for 10 minutes, then centrifuge (5 minutes, 3800 rpm) and the extract collected. The extraction repeated with 10 mL of mixture of acetonitrile for HPLC and deionized water (50:50 *v/v*). The extracts were combined together. The sample was diluted with mixture of acetonitrile for HPLC and deionized water (*v/v*) (50:50; *v/v*), if necessary An aliquot of the final volume was transferred into a HPLC vial for further quantification using HPLC-DAD.

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Overview of the fortification of samples work-up by a flow chart / picture is presented below:

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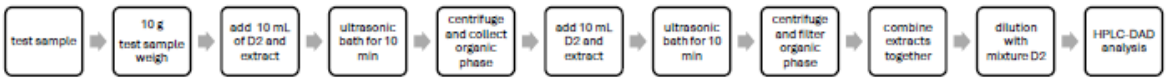

D2 – mixture of acetonitrile for HPLC and deionized water (50:50, *v/v*).

2.6. Instrumentation and conditions

The chromatographic systems and conditions used for the analysis are shown in the table below [SOP/C/328, SOP/C/592, SOP/C/607].

	Parameter
Chromatographic System	High Performance Liquid Chromatography (HPLC)
Chromatograph	Shimadzu, Prominence - *i* (Shimadzu Corporation Japan)
Analytical Column	Luna 5µm C18 (2) 100Å (2) 250x4.6 mm
Oven temperature	35°C
Injection Volume	10 µL
Mobile Phase	acetonitrile HPLC : ortho-phosphoric acid solution 0.05 % (65 : 35, *v/v*)
Flow Rate	0.6 mL/min
Wave length	240 nm
Retention time	approx. 5. min
Detection System	Diode Array Detector

Results and discussions

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

Matrix	Analyte	Fortification level (mg/kg) (*n* = x)	Mean recovery (%)	RSD (%)	Comments
Artificial soil	Aminopyralid	Control – 0.0 mg/kg– two replicates LOQ – 4.0 mg/kg – five replicates 10xLOQ – 40.0 mg/kg – five replicates	81.1 – 95.5 %	0.4 – 0.8 %	-

Table A 2: Characteristics for the analytical method used for validation of aminopyralid residues in artificial soil

	Aminopyralid residues
Author(s), year	A. Gierbuszewska, 2024
Principle of method	HPLC-DAD
Linearity (linear be-tween mg/L) (correlation coefficient,	Working solutions at all calibration levels were injected successively to the chromatographic column and the chromatograms were recorded. The range of linearity of the analytical graph and the ranges of calibration curve corresponding to the real samples level are presented in the table below.

Aminopyralid residues

expressed as r)

Analyte	Working solution concentrations [µg/mL]	Range of linearity of calibration curve [mg/L]	Equivalent calibration range of linearity [mg/kg]
Aminopyralid	0.5, 1.0, 2.0, 5.0, 10.0, 20.0, 50.0	0.5 – 50.0	1.0 – 100.0

The equation of the calibration line is presented as the linear equation; y = ax + b (a – slope, b – intercept). The linear coefficient r^2 must be higher than 0.99. Range of the linearity was given in µg/mL (equal to mg/L).

Range of linearity of calibration curve [mg/L]	Analyte	Slope	Intercept	Coefficient r^2
0.5 – 50.0	Aminopyralid	46376.1	950.036	0.9991894

Linear weighted calibration (1/C weighting) is used for calibration curve. The suitability of the chosen function was demonstrated as the regression residuals (d_i). The regression residuals are presented in a residual plot in range equal to range of linearity of calibration curve.

Precision, accuracy and uncertainty

Precision of a particular methods is defined as its repeatability (RSD – relative standard deviation [%]). The repeatability for detected substances analysed are presented in table below. The RSD is ≤ 20% per each level.
The accuracy of the method is reported as mean recovery ± relative standard deviation. Recovery was reported as mean recovery ± relative standard deviation (RSD). Mean recoveries for each level is in the range 70-120%.
A summary of the recovery data of control and fortified samples are presented in the table below.

Method	Analyte	Fortification Level [mg/kg]	Number of Replicates	Mean Recovery [%]	RSD [%]
extraction method	Aminopyralid	4.0	5	81.1	0.4
		40.0	5	95.5	0.8

In order to study the recovery level, the solution of the detected substance was added to non-treated control sample and then analysed using the methods described above.

Specificity

The analytical method specificity was estimated on the basis of the analysis of the chromatograms obtained for the control, and fortified samples of matrix. Considering the results of the analysis, no signal of detected substance was overlapping with matrix signal of the control samples in the experiments conditions. Therefore, the specificity of the method was demonstrated. Furthermore, any interference are directly apparent and would be observed in the chromatograms of the matrix control sample.

Matrix Effects

Matrix effect for each method was checked during the method validation. Assessment of matrix effects was performed by comparing the standard prepared in solution to standard prepared in blank matrix at appropriate concentration.
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Method	Analyte	Concentration [mg/L]	Matrix effect [%]
extraction method	Aminopyralid	2.0	-10.5

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A 2.1.1.1.3 Analytical method 3

A 2.1.1.1.3.1 Method validation

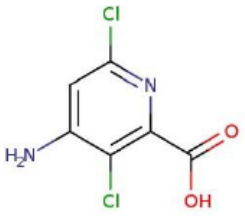
Comments of zRMS:	Validation of the Analytical Czarnynoga, M., 2024, G-55-24) is acceptable and suitable for determination of aminopyralid in artificial soil.

Reference:	KCP 5.1.2/03
Report	Collembolan (Folsomia candida), Reproduction Test, Czarnynoga, M., 2024, Study code: G-55-24
Guideline(s):	Yes according to the OECD Guideline No. 232 (2016); SANTE/2020/12830, Rev. 2; Standard Operating Procedure SOP/C/9.
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

Analytical standard of aminopyralid was used to method validation.

Aminopyralid

IUPAC Name:	4-amino-3,6-dichloropyridine-2-carboxylic acid (IUPAC)	Structural [2]: 
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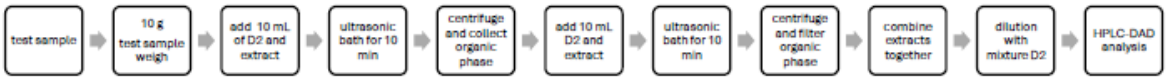

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Matrix	Analyte	Fortification level (mg/kg) (*n* = x)	Mean recovery (%)	RSD (%)	Comments
		LOQ – 4.0 mg/kg – five replicates 10xLOQ – 40.0 mg/kg – five replicates			

Table A 2: Characteristics for the analytical method used for validation of aminopyralid residues in artificial soil

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<tr><td>Linearity (linear between mg/L) (correlation coefficient, expressed as r)</td><td>Working solutions at all calibration levels were injected successively to the chromatographic column and the chromatograms were recorded. The range of linearity of the analytical graph and the ranges of calibration curve corresponding to the real samples level are presented in the table below.
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The matrix effect did not exceed ± 20 %. The matrix effect and concentration are presented in table below.
<table>
<tr><th>Method</th><th>Analyte</th><th>Concentration [mg/L]</th><th>Matrix effect [%]</th></tr>
<tr><td>extraction method</td><td>Aminopyralid</td><td>2.0</td><td>-7.2</td></tr>
</table></td></tr>
<tr><td>LOQ
LOD</td><td>Limit of Quantification was estimated as the lowest concentration of a detected substance at which an acceptable mean recovery and precision (normally 70 – 120% with a relative standard deviation of preferably ≤ 20%).
The limit of detection (LOD) is defined as the lowest detectable concentration or amount of an analyte in a sample. LOD is expressed as the lowest calibration standard. LOD is less than or equal to 30% of LOQ.
Limit of quantification (LOQ) and a limit of detection (LOD) are presented in the table below.
<table>
<tr><th>Method</th><th>Analyte</th><th>LOQ [mg analyte /kg]</th><th>equivalent calibration level [mg/L]</th><th>LOD [mg analyte/kg]</th><th>equivalent calibration level [mg/L]</th></tr>
<tr><td>extraction method</td><td>Aminopyralid</td><td>4.0</td><td>2.0</td><td>1.0</td><td>0.5</td></tr>
</table></td></tr>
<tr><td>Extraction stability</td><td>Final extract stability was not determined. The final extracts were analysed within 24 h.</td></tr>
<tr><td>Comment</td><td>The validation parameters are within the acceptance range and fulfil EU requirements given in SANTE/2020/12830 Rev.2.</td></tr>
</table>

Conclusion

The method was successfully validated for determination of aminopyralid in artificial soil with an LOQ of 4.0 mg/kg according to the guidance document(s) SANTE/2020/12830 Rev.2. With regard to selectivity, accuracy and precision, the analytical methods were applied successfully for each analytical set when analysing the specimens of the study.

A 2.1.1.1.4 Analytical method 4

A 2.1.1.1.4.1 Method validation

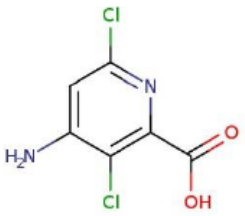
Comments of zRMS:	Validation of the Analytical Maga, D., 2024, W-30-24) is acceptable and suitable for determination of aminopyralid in AAP medium.

Reference:	KCP 5.1.2/04
Report	Anabaena flos-aquae UTEX B 1444, Growth inhibition test, Maga, D., 2024, Study code: W-30-24
Guideline(s):	Yes according to the OECD Guideline No. 201 (2006)/ EU method C.3.; SANTE/2020/12830, Rev. 2; Standard Operating Procedure SOP/C/9.
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

Analytical standard of aminopyralid was used to method validation.

Aminopyralid

IUPAC Name:	4-amino-3,6-dichloropyridine-2-carboxylic acid (IUPAC)	Structural [2]: 
Molecular Formula:	$C_6H_4Cl_2N_2O_2$	
Cas No.:	150114-71-9	
Purity:	97.99 % ± 0.31% (g/g)	
Series No:	G1239715	
Valid to:	22 March 2028	
Molecular weight:	207.01 g/mol	

Stock and standard solutions

The stock solution with a concentration of 1.0 mg/mL was prepared by weighting 10.0 mg of standard into a volumetric flask with a capacity of 10.0 mL, dissolving in acetonitrile for HPLC, and next the volume was made up to 10.0 mL with the same solvent. The working solutions were prepared by diluting standard solutions of higher concentrations.

Fortification of samples

For the preparation of procedural recoveries and validation experiments, fortified samples were prepared from standard solution. The appropriate amount of spiking solutions was added to the matrix to prepare LOQ and 10xLOQ.

Sample preparation for the chromatographic analysis

Direct method

1 mL of AAP medium sample was measured. The sample was diluted with mixture of acetonitrile for HPLC and deionized water (50:50, *v/v*), if necessary. An aliquot of the final volume was transferred into a HPLC vial for further quantification using HPLC-DAD.

2.5.1.1. Schematic diagram of the analytical method

Overview of the fortification of samples work-up by a flow chart / picture is presented below:

Overview of the test samples work-up by a flow chart / picture is presented below:

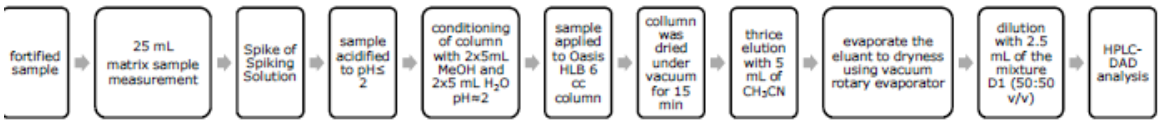
D1 – mixture of acetonitrile for HPLC and deionized water (50:50, *v/v*)

SPE method

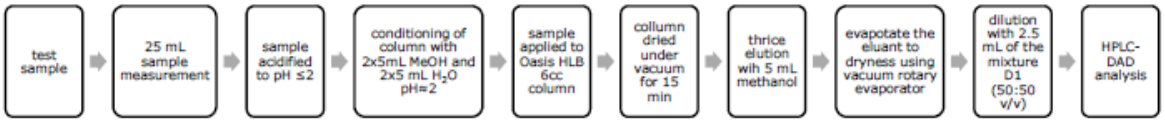
Each sample of volume 25 mL was acidified by hydrochloric acid to pH≤2 and applied to Oasis HLB 6 cc (500 mg) column conditioned previously by sequential washing once with 5 mL of methanol, twice with 5 mL of deionised water pH≈2. Following the sample introduction the column was dried under vacuum for 15 minutes. The active substance was eluted thrice with 5 mL of acetonitrile for HPLC. The eluate was evaporated to dryness using vacuum rotary evaporator. The dry residue was redissolved in 2.5 mL of mixture acetonitrile and deionized water (50:50; *v/v*). The sample was diluted with mixture of acetonitrile for HPLC and deionized water (*v/v*) (50:50; *v/v*), if necessary. An aliquot of the final volume was transferred into a HPLC vial for further quantification using HPLC-DAD.

2.5.2.1. Schematic diagram of the analytical method

Overview of the fortification samples work-up by a flow chart / picture is presented below:



Overview of the test samples work-up by a flow chart / picture is presented below:



D1 – mixture of acetonitrile for HPLC and deionized water (50:50, *v/v*)

2.6. Instrumentation and conditions

The chromatographic systems and conditions used for the analysis are shown in the table below [SOP/C/328, SOP/C/592, SOP/C/607].

	Parameter
Chromatographic System	High Performance Liquid Chromatography (HPLC)
Chromatograph	Shimadzu, Prominence - *i* (Shimadzu Corporation Japan)
Analytical Column	Luna 5µm C18 (2) 100Å (2) 250x4.6 mm
Oven temperature	35°C
Injection Volume	10 µL
Mobile Phase	acetonitrile HPLC : ortho-phosphoric acid solution 0.05 % (65 : 35, *v/v*)
Flow Rate	0.6 mL/min
Wave length	240 nm
Retention time	approx. 5. min
Detection System	Diode Array Detector

Results and discussions

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

Matrix	**Analyte**	**Fortification level (mg/kg) (*n* = x)**	**Mean recovery (%)**	**RSD (%)**	**Comments**
AAP medium	Aminopyralid	Direct method: Control – 0.0 mg/L – two replicates LOQ – 0.1 mg/L – five replicates 10xLOQ – 1.0 mg/L – five replicates SPE method: Control – 0.0 mg/L – two replicates LOQ – 0.02 mg/L – five replicates 10xLOQ – 0.2 mg/L – five replicates	89 – 104 % (direct method) 85 - 93.5 % (SPE method)	2.0 – 5.6 % (direct method) 1.1 – 5.9 % (SPE method)	-

Table A 2: Characteristics for the analytical method used for validation of aminopyralid residues in AAP medium

<table>
<tr><th></th><th>Aminopyralid residues</th></tr>
<tr><td>Author(s), year</td><td>D. Maga, 2024</td></tr>
<tr><td>Principle of method</td><td>HPLC-DAD</td></tr>
<tr><td>Linearity (linear be-tween mg/L) (correlation coefficient, expressed as r)</td><td>Working solutions at all calibration levels were injected successively to the chromatographic column and the chromatograms were recorded. The range of linearity of the analytical graph and the ranges of calibration curve corresponding to the real samples level are presented in the table below.
<table>
<tr><th>Analyte</th><th>Working solution concentrations [µg/mL]</th><th>Range of linearity of calibration curve [mg/L]</th><th>Equivalent calibration range of linearity [mg/L]</th><th>Method</th></tr>
<tr><td rowspan="2">Aminopyralid</td><td rowspan="2">0.02, 0.05, 0.1, 0.2, 0.5, 1.0, 2.0, 5.0</td><td rowspan="2">0.02 – 5.0</td><td>0.02 – 5.0</td><td>Direct method</td></tr>
<tr><td>0.002 – 0.5</td><td>SPE method</td></tr>
</table>
The equation of the calibration line is presented as the linear equation; y = ax + b (a – slope, b – intercept). The linear coefficient r2 must be higher than 0.99. Range of the linearity was given in µg/mL (equal to mg/L).
<table>
<tr><th>Range of linearity of calibration curve [mg/L]</th><th>Analyte</th><th>Slope</th><th>Intercept</th><th>Coefficient r²</th></tr>
<tr><td>0.02 – 5.0</td><td>Aminopyralid</td><td>46551.5</td><td>194.143</td><td>0.9999782</td></tr>
</table>
Linear weighted calibration (1/C weighting) is used for calibration curve. The suitability of the chosen function was demonstrated as the regression residuals (di). The regression residuals are presented in a residual plot in range equal to range of linearity of calibration curve.</td></tr>
<tr><td>Precision, accuracy and uncer-tainty</td><td>Precision of a particular methods is defined as its repeatability (RSD – relative standard deviation [%]). The repeatability for detected substances analysed are presented in table below. The RSD is ≤ 20% per each level.
The accuracy of the method is reported as mean recovery ± relative standard deviation. Recovery was reported as mean recovery ± relative standard deviation (RSD). Mean recoveries for each level is in the range 70-120%.
A summary of the recovery data of control and fortified samples are presented in the table below.
<table>
<tr><th>Method</th><th>Analyte</th><th>Fortification Level [mg/L]</th><th>Number of Replicates</th><th>Mean Recovery [%]</th><th>RSD [%]</th></tr>
<tr><td rowspan="2">direct method</td><td rowspan="4">Aminopyralid</td><td>0.1</td><td>5</td><td>89.0</td><td>5.6</td></tr>
<tr><td>1.0</td><td>5</td><td>104</td><td>2.0</td></tr>
<tr><td rowspan="2">SPE method</td><td>0.02</td><td>5</td><td>85.0</td><td>5.9</td></tr>
<tr><td>0.2</td><td>5</td><td>93.5</td><td>1.1</td></tr>
</table>
In order to study the recovery level, the solution of the detected substance was added to non-treated control sample and then analysed using the methods described above.</td></tr>
<tr><td>Specificity</td><td>The analytical method specificity was estimated on the basis of the analysis of the chromatograms obtained for the control, and fortified samples of matrix. Considering the results of the analysis, no signal of detected substance was overlapping with matrix signal of the control samples in the experiments conditions. Therefore, the specificity of the method was demonstrated. Furthermore, any interference are directly apparent and would be observed in the chromatograms of the matrix control sample.</td></tr>
</table>

<table>
<tr><th></th><th>Aminopyralid residues</th></tr>
<tr><td>Matrix Effects</td><td>Matrix effect for each method was checked during the method validation. Assessment of matrix effects was performed by comparing the standard prepared in solution to standard prepared in blank matrix at appropriate concentration.
The matrix effect did not exceed ± 20 %. The matrix effect and concentration are presented in table below
<table>
<tr><th>Method</th><th>Analyte</th><th>Concentration [mg/L]</th><th>Matrix effect [%]</th></tr>
<tr><td>direct method</td><td rowspan="2">Aminopyralid</td><td>0.1</td><td>-6.6</td></tr>
<tr><td>SPE method</td><td>0.2</td><td>10.0</td></tr>
</table></td></tr>
<tr><td>LOQ
LOD</td><td>Limit of Quantification was estimated as the lowest concentration of a detected substance at which an acceptable mean recovery and precision (normally 70 – 120% with a relative standard deviation of preferably ≤ 20%).
The limit of detection (LOD) is defined as the lowest detectable concentration or amount of an analyte in a sample. LOD is expressed as the lowest calibration standard. LOD is less than or equal to 30% of LOQ.
Limit of quantification (LOQ) and a limit of detection (LOD) are presented in the table below.
<table>
<tr><th>Analyte</th><th>Method</th><th>LOQ [mg analyte /L]</th><th>Equivalent calibration level [mg/L]</th><th>LOD [mg analyte/L]</th><th>Equivalent calibration level [mg/L]</th></tr>
<tr><td rowspan="2">Aminopyralid</td><td>direct method</td><td>0.1</td><td>0.1</td><td>0.02</td><td>0.02</td></tr>
<tr><td>SPE method</td><td>0.02</td><td>0.2</td><td>0.002</td><td>0.02</td></tr>
</table></td></tr>
<tr><td>Extraction stability</td><td>Final extract stability was not determined. The final extracts were analysed within 24 h.</td></tr>
<tr><td>Comment</td><td>The validation parameters are within the acceptance range and fulfil EU requirements given in SANTE/2020/12830 Rev.2.</td></tr>
</table>

Conclusion

The method was successfully validated for determination of aminopyralid in AAP medium with an LOQ of 0.1 mg/L for direct method and LOQ of 0.02 mg/L for SPE method according to the guidance document(s) SANTE/2020/12830 Rev.2. With regard to selectivity, accuracy and precision, the analytical methods were applied successfully for each analytical set when analysing the specimens of the study.

A 2.1.1.1.5 Analytical method 5

A 2.1.1.1.5.1 Method validation

Comments of zRMS:	Validation of the Analytical Maga, D., 2024, W-27-24) is acceptable and suitable for determination of aminopyralid in Elendt M7 medium.

Reference: KCP 5.1.2/05

Report: Daphnia magna, Acute immobilisation test, Maga, D., 2024, Study code: W-27-24

Guideline(s):	Yes according to the OECD Guideline No. 202 (2004)/ EU method C.2; SANTE/2020/12830, Rev. 2; Standard Operating Procedure SOP/C/9.
Deviations:	No
GLP:	Yes
Acceptability:	Yes

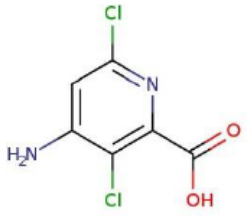
Materials and methods

Analytical standard of aminopyralid was used to method validation.

Aminopyralid

IUPAC Name:	4-amino-3,6-dichloropyridine-2-carboxylic acid (IUPAC)
Molecular Formula:	$C_6H_4Cl_2N_2O_2$
Cas No.:	150114-71-9
Purity:	97.99 % ± 0.31% (g/g)
Series No:	G1239715
Valid to:	22 March 2028
Molecular weight:	207.01 g/mol

Structural [2]:



Stock and standard solutions

The stock solution with a concentration of 1.0 mg/mL was prepared by weighting 10.0 mg of standard into a volumetric flask with a capacity of 10.0 mL, dissolving in acetonitrile for HPLC, and next the volume was made up to 10.0 mL with the same solvent. The working solutions were prepared by diluting standard solutions of higher concentrations.

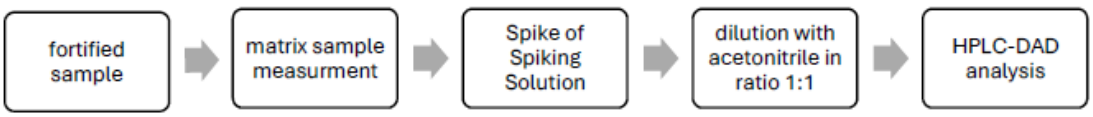
Fortification of samples

For the preparation of procedural recoveries and validation experiments, fortified samples were prepared from standard solution. The appropriate amount of spiking solutions was added to the matrix to prepare LOQ and 10xLOQ.

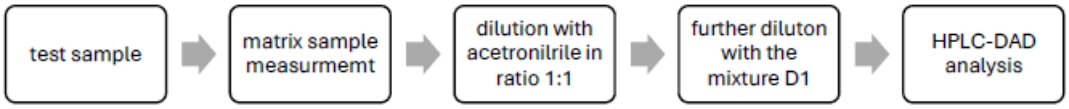
Sample preparation for the chromatographic analysis

Each sample in a volume of 1.0 mL was diluted with acetonitrile for HPLC in a ration 1:1. The sample was diluted with mixture of acetonitrile for HPLC and deionized water (50:50, v/v), if necessary. An aliquot of the final volume was transferred into a HPLC vial for further quantification using HPLC-DAD.

Overview of the fortification of samples work-up by a flow chart / picture is presented below:



Overview of the test samples work-up by a flow chart / picture is presented below:



D1 – mixture of acetonitrile for HPLC and deionized water (50:50, *v/v*)

2.6. Instrumentation and conditions

The chromatographic systems and conditions used for the analysis are shown in the table below [SOP/C/328, SOP/C/592, SOP/C/607].

	Parameter
Chromatographic System	High Performance Liquid Chromatography (HPLC)
Chromatograph	Shimadzu, Prominence - *i* (Shimadzu Corporation Japan)
Analytical Column	Luna 5µm C18 (2) 100Å (2) 250x4.6 mm
Oven temperature	35°C
Injection Volume	10 µL
Mobile Phase	acetonitrile HPLC : ortho-phosphoric acid solution 0.05 % (65 : 35, *v/v*)
Flow Rate	0.6 mL/min
Wave length	240 nm
Retention time	approx. 5.1 min
Detection System	Diode Array Detector

Results and discussions

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

Matrix	**Analyte**	**Fortification level (mg/kg) (*n* = x)**	**Mean recovery (%)**	**RSD (%)**	**Comments**
Elendt M7 medium	Aminopyralid	Control – 0.0 mg/L – two replicates LOQ – 0.2 mg/L – five replicates 10xLOQ – 2.0 mg/L – five replicates	100 – 104 %	0.1– 1.0 %	-

Table A 2: Characteristics for the analytical method used for validation of aminopyralid residues in Elendt M7 medium

	Aminopyralid residues
Author(s),	D. Maga, 2024

<table>
<tr><th></th><th>Aminopyralid residues</th></tr>
<tr><td>year</td><td></td></tr>
<tr><td>Principle of method</td><td>HPLC-DAD</td></tr>
<tr><td>Linearity (linear between mg/L) (correlation coefficient, expressed as r)</td><td>Working solutions at all calibration levels were injected successively to the chromatographic column and the chromatograms were recorded. The range of linearity of the analytical graph and the ranges of calibration curve corresponding to the real samples level are presented in the table below.
<table>
<tr><th>Analyte</th><th>Working solution concentrations [µg/mL]</th><th>Range of linearity of calibration curve [mg/L]</th><th>Equivalent calibration range of linearity [mg/L]</th></tr>
<tr><td>Aminopyralid</td><td>0.02, 0.05, 0.1, 0.2, 0.5, 1.0, 2.0, 5.0</td><td>0.02 – 5.0</td><td>0.04 – 10.0</td></tr>
</table>
The equation of the calibration line is presented as the linear equation; y = ax + b (a – slope, b – intercept). The linear coefficient r2 must be higher than 0.99. Range of the linearity was given in µg/mL (equal to mg/L).
<table>
<tr><th>Range of linearity of calibration curve [mg/L]</th><th>Analyte</th><th>Slope</th><th>Intercept</th><th>Coefficient r²</th></tr>
<tr><td>0.02 – 5.0</td><td>Aminopyralid</td><td>46551.5</td><td>194.143</td><td>0.9999782</td></tr>
</table>
Linear weighted calibration (1/C weighting) is used for calibration curve. The suitability of the chosen function was demonstrated as the regression residuals (d_i). The regression residuals are presented in a residual plot in range equal to range of linearity of calibration curve.</td></tr>
<tr><td>Precision, accuracy and uncertainty</td><td>Precision of a particular methods is defined as its repeatability (RSD – relative standard deviation [%]). The repeatability for detected substances analysed are presented in table below. The RSD is ≤ 20% per each level.
The accuracy of the method is reported as mean recovery ± relative standard deviation. Recovery was reported as mean recovery ± relative standard deviation (RSD). Mean recoveries for each level is in the range 70-120%.
A summary of the recovery data of control and fortified samples are presented in the table below.
<table>
<tr><th>Method</th><th>Analyte</th><th>Fortification Level [mg/L]</th><th>Number of Replicates</th><th>Mean Recovery [%]</th><th>RSD [%]</th></tr>
<tr><td rowspan="2">dilution method</td><td rowspan="2">Aminopyralid</td><td>0.2</td><td>5</td><td>100</td><td>1.0</td></tr>
<tr><td>2.0</td><td>5</td><td>104</td><td>0.1</td></tr>
</table>
In order to study the recovery level, the solution of the detected substance was added to non-treated control sample and then analysed using the methods described above.</td></tr>
<tr><td>Specificity</td><td>The analytical method specificity was estimated on the basis of the analysis of the chromatograms obtained for the control, and fortified samples of matrix. Considering the results of the analysis, no signal of detected substance was overlapping with matrix signal of the control samples in the experiments conditions. Therefore, the specificity of the method was demonstrated. Furthermore, any interference are directly apparent and would be observed in the chromatograms of the matrix control sample.</td></tr>
<tr><td>Matrix Effects</td><td>Matrix effect for each method was checked during the method validation. Assessment of matrix effects was performed by comparing the standard prepared in solution to standard prepared in blank matrix at appropriate concentration.
The matrix effect did not exceed ± 20 %. The matrix effect and concentration are presented in table below
<table>
<tr><th>Method</th><th>Analyte</th><th>Concentration [mg/L]</th><th>Matrix effect [%]</th></tr>
<tr><td>dilution method</td><td>Aminopyralid</td><td>0.1</td><td>-2.7</td></tr>
</table></td></tr>
</table>

<table>
<tr><th></th><th>Aminopyralid residues</th></tr>
<tr><td>LOQ
LOD</td><td>Limit of Quantification was estimated as the lowest concentration of a detected substance at which an acceptable mean recovery and precision (normally 70 – 120% with a relative standard deviation of preferably ≤ 20%).
The limit of detection (LOD) is defined as the lowest detectable concentration or amount of an analyte in a sample. LOD is expressed as the lowest calibration standard. LOD is less than or equal to 30% of LOQ.
Limit of quantification (LOQ) and a limit of detection (LOD) are presented in the table below.
<table>
<tr><th>Method</th><th>Analyte</th><th>LOQ [mg analyte /L]</th><th>equivalent calibration level [mg/L]</th><th>LOD [mg analyte/L]</th><th>equivalent calibration level [mg/L]</th></tr>
<tr><td>dilution method</td><td>Aminopyralid</td><td>0.2</td><td>0.1</td><td>0.04</td><td>0.02</td></tr>
</table></td></tr>
<tr><td>Extraction stability</td><td>Final extract stability was not determined. The final extracts were analysed within 24 h.</td></tr>
<tr><td>Comment</td><td>The validation parameters are within the acceptance range and fulfil EU requirements given in SANTE/2020/12830 Rev.2.</td></tr>
</table>

Conclusion

The method was successfully validated for determination of aminopyralid in Elendt M7 medium with an LOQ of 0.2 mg/L according to the guidance document(s) SANTE/2020/12830 Rev.2. With regard to selectivity, accuracy and precision, the analytical methods were applied successfully for each analytical set when analysing the specimens of the study.

A 2.1.1.1.6 Analytical method 6

A 2.1.1.1.6.1 Method validation

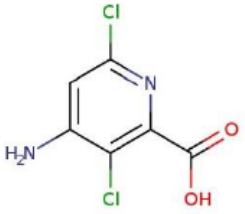
Comments of zRMS:	Validation of the Analytical Maga, D., 2024, W-29-24) is acceptable and suitable for determination of aminopyralid in 20xAAP medium.

Reference:	KCP 5.1.2/06
Report	Lemna gibba CPCC 310, Growth inhibition test, Maga, D., 2024, Study code: W-29-24
Guideline(s):	Yes according to the OECD Guideline No. 211 (2006)/ EU method C.26.; SANTE/2020/12830, Rev. 2; Standard Operating Procedure SOP/C/9.
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

Analytical standard of aminopyralid was used to method validation.

Aminopyralid

IUPAC Name:	4-amino-3,6-dichloropyridine-2-carboxylic acid (IUPAC)	Structural [2]: 
Molecular Formula:	$C_6H_4Cl_2N_2O_2$	
Cas No.:	150114-71-9	
Purity:	97.99 % ± 0.31% (g/g)	
Series No:	G1239715	
Valid to:	22 March 2028	
Molecular weight:	207.01 g/mol	

Stock and standard solutions

The stock solution with a concentration of 1.0 mg/mL was prepared by weighting 10.0 mg of standard into a volumetric flask with a capacity of 10.0 mL, dissolving in acetonitrile for HPLC, and next the volume was made up to 10.0 mL with the same solvent. The working solutions were prepared by diluting standard solutions of higher concentrations.

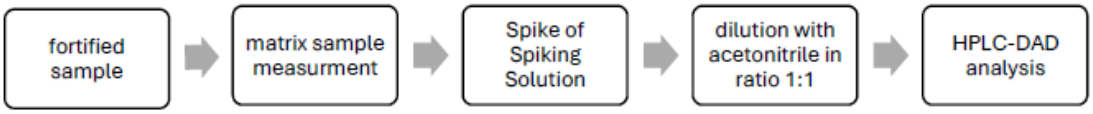
Fortification of samples

For the preparation of procedural recoveries and validation experiments, fortified samples were prepared from standard solution. The appropriate amount of spiking solutions was added to the matrix to prepare LOQ and 10xLOQ.

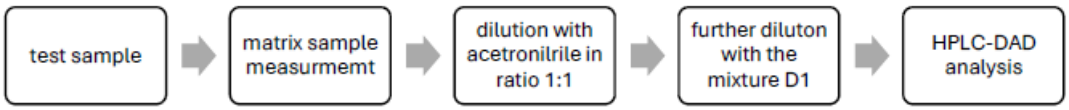
Sample preparation for the chromatographic analysis

Each sample in a volume of 1.0 mL was diluted with acetonitrile for HPLC in a ration 1:1. The sample was diluted with mixture of acetonitrile for HPLC and deionized water (50:50, v/v), if necessary. An aliquot of the final volume was transferred into a HPLC vial for further quantification using HPLC-DAD.

Overview of the fortification of samples work-up by a flow chart / picture is presented below:



Overview of the test samples work-up by a flow chart / picture is presented below:



D1 – mixture of acetonitrile for HPLC and deionized water (50:50, *v/v*)

2.6. Instrumentation and conditions

The chromatographic systems and conditions used for the analysis are shown in the table below [SOP/C/328, SOP/C/592, SOP/C/607].

	Parameter
Chromatographic System	High Performance Liquid Chromatography (HPLC)
Chromatograph	Shimadzu, Prominence - *i* (Shimadzu Corporation Japan)
Analytical Column	Luna 5µm C18 (2) 100Å (2) 250x4.6 mm
Oven temperature	35°C
Injection Volume	10 µL
Mobile Phase	acetonitrile HPLC : ortho-phosphoric acid solution 0.05 % (65 : 35, *v/v*)
Flow Rate	0.6 mL/min
Wave length	240 nm
Retention time	approx. 5.1 min
Detection System	Diode Array Detector

Results and discussions

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

Matrix	Analyte	Fortification level (mg/kg) (*n* = x)	Mean recovery (%)	RSD (%)	Comments
20xAAP medium	Aminopyralid	Control – 0.0 mg/L – two replicates LOQ – 0.2 mg/L – five replicates 10xLOQ – 2.0 mg/L – five replicates	93.0 – 99.0 %	1.8– 2.2 %	-

Table A 2: Characteristics for the analytical method used for validation of aminopyralid residues in 20xAAP medium

	Aminopyralid residues
Author(s), year	D. Maga, 2024
Principle of method	HPLC-DAD
Linearity (linear between mg/L) (correla-tion coeffi-cient, ex-	Working solutions at all calibration levels were injected successively to the chromatographic column and the chromatograms were recorded. The range of linearity of the analytical graph and the ranges of calibration curve corresponding to the real samples level are presented in the table below.

<table>
<tr><th></th><th>Aminopyralid residues</th></tr>
<tr><td>pressed as r)</td><td>
<table>
<tr><th>Analyte</th><th>Working solution concentrations [µg/mL]</th><th>Range of linearity of calibration curve [mg/L]</th><th>Equivalent calibration range of linearity [mg/L]</th></tr>
<tr><td>Aminopyralid</td><td>0.02, 0.05, 0.1, 0.2, 0.5, 1.0, 2.0, 5.0</td><td>0.02 – 5.0</td><td>0.04 – 10.0</td></tr>
</table>
The equation of the calibration line is presented as the linear equation; y = ax + b (a – slope, b – intercept). The linear coefficient r2 must be higher than 0.99. Range of the linearity was given in µg/mL (equal to mg/L).
<table>
<tr><th>Range of linearity of calibration curve [mg/L]</th><th>Analyte</th><th>Slope</th><th>Intercept</th><th>Coefficient r^2</th></tr>
<tr><td>0.02 – 5.0</td><td>Aminopyralid</td><td>46551.5</td><td>194.143</td><td>0.9999782</td></tr>
</table>
Linear weighted calibration (1/C weighting) is used for calibration curve. The suitability of the chosen function was demonstrated as the regression residuals (d_i). The regression residuals are presented in a residual plot in range equal to range of linearity of calibration curve.</td></tr>
<tr><td>Precision, accuracy and uncer-tainty</td><td>Precision of a particular methods is defined as its repeatability (RSD – relative standard deviation [%]). The repeatability for detected substances analysed are presented in table below. The RSD is ≤ 20% per each level.
The accuracy of the method is reported as mean recovery ± relative standard deviation. Recovery was reported as mean recovery ± relative standard deviation (RSD). Mean recoveries for each level is in the range 70-120%.
A summary of the recovery data of control and fortified samples are presented in the table below.
<table>
<tr><th>Method</th><th>Analyte</th><th>Fortification Level [mg/L]</th><th>Number of Replicates</th><th>Mean Recovery [%]</th><th>RSD [%]</th></tr>
<tr><td rowspan="2">dilution method</td><td rowspan="2">Aminopyralid</td><td>0.2</td><td>5</td><td>93.0</td><td>2.2</td></tr>
<tr><td>2.0</td><td>5</td><td>99.0</td><td>1.8</td></tr>
</table>
In order to study the recovery level, the solution of the detected substance was added to non-treated control sample and then analysed using the methods described above.</td></tr>
<tr><td>Specificity</td><td>The analytical method specificity was estimated on the basis of the analysis of the chromatograms obtained for the control, and fortified samples of matrix. Considering the results of the analysis, no signal of detected substance was overlapping with matrix signal of the control samples in the experiments conditions. Therefore, the specificity of the method was demonstrated. Furthermore, any interference are directly apparent and would be observed in the chromatograms of the matrix control sample.</td></tr>
<tr><td>Matrix Effects</td><td>Matrix effect for each method was checked during the method validation. Assessment of matrix effects was performed by comparing the standard prepared in solution to standard prepared in blank matrix at appropriate concentration.
The matrix effect did not exceed ± 20 %. The matrix effect and concentration are presented in table below
<table>
<tr><th>Method</th><th>Analyte</th><th>Concentration [mg/L]</th><th>Matrix effect [%]</th></tr>
<tr><td>dilution method</td><td>Aminopyralid</td><td>0.1</td><td>-3.1</td></tr>
</table></td></tr>
<tr><td>LOQ
LOD</td><td>Limit of Quantification was estimated as the lowest concentration of a detected substance at which an acceptable mean recovery and precision (normally 70 – 120% with a relative standard deviation of preferably ≤ 20%).
The limit of detection (LOD) is defined as the lowest detectable concentration or amount of an analyte in a sample. LOD is expressed as the lowest calibration standard. LOD is less than or equal to 30% of LOQ.
Limit of quantification (LOQ) and a limit of detection (LOD) are presented in the table below.</td></tr>
</table>

	Aminopyralid residues
	<table><tr><th>Method</th><th>Analyte</th><th>LOQ [mg analyte /L]</th><th>equivalent calibration level [mg/L]</th><th>LOD [mg analyte/L]</th><th>equivalent calibration level [mg/L]</th></tr><tr><td>dilution method</td><td>Aminopyralid</td><td>0.2</td><td>0.1</td><td>0.04</td><td>0.02</td></tr></table>
Extraction stability	Final extract stability was not determined. The final extracts were analysed within 24 h.
Comment	The validation parameters are within the acceptance range and fulfil EU requirements given in SANTE/2020/12830 Rev.2.

Conclusion

The method was successfully validated for determination of aminopyralid in 20xAAP medium with an LOQ of 0.2 mg/L according to the guidance document(s) SANTE/2020/12830 Rev.2. With regard to selectivity, accuracy and precision, the analytical methods were applied successfully for each analytical set when analysing the specimens of the study.

A 2.1.1.1.7 Analytical method 7

A 2.1.1.1.7.1 Method validation

Comments of zRMS:	Validation of the Analytical Maga, D., 2024, W-28-24) is acceptable and suitable for determination of aminopyralid in AAP medium.

Reference:	KCP 5.1.2/07
Report	Algae, growth inhibition toxicity (Raphidocelis subcapitata), Maga, D., 2024, Study code: W-28-24
Guideline(s):	Yes according to the OECD Guideline No. 201 (2006)/ EU method C.3.; SANTE/2020/12830, Rev. 2; Standard Operating Procedure SOP/C/9.
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

Analytical standard of aminopyralid was used to method validation.

Aminopyralid

IUPAC Name:	4-amino-3,6-dichloropyridine-2-carboxylic acid (IUPAC)	Structural [2]: 
Molecular Formula:	$C_6H_4Cl_2N_2O_2$	
Cas No.:	150114-71-9	
Purity:	97.99 % ± 0.31% (g/g)	
Series No:	G1239715	
Valid to:	22 March 2028	
Molecular weight:	207.01 g/mol	

Stock and standard solutions

The stock solution with a concentration of 1.0 mg/mL was prepared by weighting 10.0 mg of standard into a volumetric flask with a capacity of 10.0 mL, dissolving in acetonitrile for HPLC, and next the volume was made up to 10.0 mL with the same solvent. The working solutions were prepared by diluting standard solutions of higher concentrations.

Fortification of samples

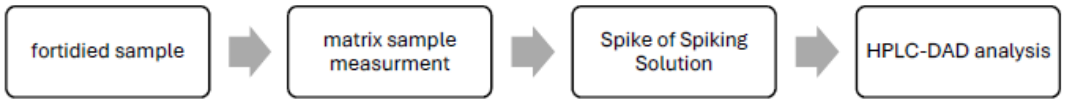
For the preparation of procedural recoveries and validation experiments, fortified samples were prepared from standard solution. The appropriate amount of spiking solutions was added to the matrix to prepare LOQ and 10xLOQ.

Sample preparation for the chromatographic analysis

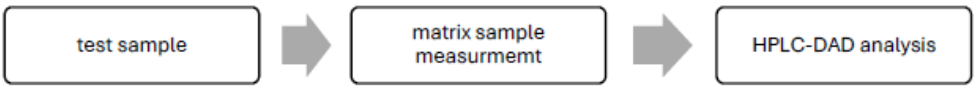
1 mL of AAP medium sample was measured. The sample was diluted with mixture of acetonitrile for HPLC and deionized water (50:50, v/v), if necessary. An aliquot of the final volume was transferred into a HPLC vial for further quantification using HPLC-DAD

2.5.1. Schematic diagram of the analytical method

Overview of the fortification of samples work-up by a flow chart / picture is presented below:



Overview of the test samples work-up by a flow chart / picture is presented below:



2.6. Instrumentation and conditions

The chromatographic systems and conditions used for the analysis are shown in the table below [SOP/C/328, SOP/C/592, SOP/C/607].

	Parameter
Chromatographic System	High Performance Liquid Chromatography (HPLC)
Chromatograph	Shimadzu, Prominence - *i* (Shimadzu Corporation Japan)
Analytical Column	Luna 5µm C18 (2) 100Å (2) 250x4.6 mm
Oven temperature	35°C
Injection Volume	10 µL
Mobile Phase	acetonitrile HPLC : ortho-phosphoric acid solution 0.05 % (65 : 35, *v/v*)
Flow Rate	0.6 mL/min
Wave length	240 nm
Retention time	approx. 5.1 min
Detection System	Diode Array Detector

Results and discussions

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

Matrix	Analyte	Fortification level (mg/kg) (*n* = x)	Mean recovery (%)	RSD (%)	Comments
AAP medium	Aminopyralid	Control – 0.0 mg/L – two replicates LOQ – 0.1 mg/L – five replicates 10xLOQ – 1.0 mg/L – five replicates	89 – 104 %	2.0 – 5.6 %	-

Table A 2: Characteristics for the analytical method used for validation of aminopyralid residues in AAP medium

	Aminopyralid residues
Author(s), year	D. Maga, 2024
Principle of method	HPLC-DAD
Linearity (linear between mg/L) (correlation coefficient, expressed as	Working solutions at all calibration levels were injected successively to the chromatographic column and the chromatograms were recorded. The range of linearity of the analytical graph and the ranges of calibration curve corresponding to the real samples level are presented in the table below.

<table>
<tr><th></th><th>Aminopyralid residues</th></tr>
<tr><td>r)</td><td>
<table>
<tr><th>Analyte</th><th>Working solution concentrations [µg/mL]</th><th>Range of linearity of calibration curve [mg/L]</th><th>Equivalent calibration range of linearity [mg/L]</th></tr>
<tr><td>Aminopyralid</td><td>0.02, 0.05, 0.1, 0.2, 0.5, 1.0, 2.0, 5.0</td><td>0.02 – 5.0</td><td>0.02 – 5.0</td></tr>
</table>
The equation of the calibration line is presented as the linear equation; y = ax + b (a – slope, b – intercept). The linear coefficient r2 must be higher than 0.99. Range of the linearity was given in µg/mL (equal to mg/L).
<table>
<tr><th>Range of linearity of calibration curve [mg/L]</th><th>Analyte</th><th>Slope</th><th>Intercept</th><th>Coefficient r²</th></tr>
<tr><td>0.02 – 5.0</td><td>Aminopyralid</td><td>46551.5</td><td>194.143</td><td>0.9999782</td></tr>
</table>
Linear weighted calibration (1/C weighting) is used for calibration curve. The suitability of the chosen function was demonstrated as the regression residuals (d_i). The regression residuals are presented in a residual plot in range equal to range of linearity of calibration curve.</td></tr>
<tr><td>Precision, accuracy and uncer-tainty</td><td>Precision of a particular methods is defined as its repeatability (RSD – relative standard deviation [%]). The RSD is ≤ 20% per each level.
The accuracy of the method is reported as mean recovery ± relative standard deviation. Recovery was reported as mean recovery ± relative standard deviation (RSD). Mean recoveries for each level is in the range 70-120%.
A summary of the precision and recovery data of control and fortified samples are presented in the table below:
<table>
<tr><th>Method</th><th>Analyte</th><th>Fortification Level [mg/L]</th><th>Number of Replicates</th><th>Mean Recovery [%]</th><th>RSD [%]</th></tr>
<tr><td rowspan="2">direct method</td><td rowspan="2">Aminopyralid</td><td>0.1</td><td>5</td><td>89.0</td><td>5.6</td></tr>
<tr><td>1.0</td><td>5</td><td>104</td><td>2.0</td></tr>
</table>
In order to study the recovery level, the solution of the detected substance was added to non-treated control sample and then analysed using the methods described above.</td></tr>
<tr><td>Specificity</td><td>The analytical method specificity was estimated on the basis of the analysis of the chromatograms obtained for the control, and fortified samples of matrix. Considering the results of the analysis, no signal of detected substance was overlapping with matrix signal of the control samples in the experiments conditions. Therefore, the specificity of the method was demonstrated. Furthermore, any interference are directly apparent and would be observed in the chromatograms of the matrix control sample.</td></tr>
<tr><td>Matrix Ef-fects</td><td>Matrix effect for each method was checked during the method validation. Assessment of matrix effects was performed by comparing the standard prepared in solution to standard prepared in blank matrix at appropriate concentration.
The matrix effect did not exceed ± 20 %. The matrix effect and concentration are presented in table below
<table>
<tr><th>Method</th><th>Analyte</th><th>Concentration [mg/L]</th><th>Matrix effect [%]</th></tr>
<tr><td>direct method</td><td>Aminopyralid</td><td>0.1</td><td>-6.6</td></tr>
</table></td></tr>
<tr><td>LOQ
LOD</td><td>Limit of Quantification was estimated as the lowest concentration of a detected substance at which an acceptable mean recovery and precision (normally 70 – 120% with a relative standard deviation of preferably ≤ 20%).
The limit of detection (LOD) is defined as the lowest detectable concentration or amount of an analyte in a sample. LOD is expressed as the lowest calibration standard. LOD is less than or equal to 30% of LOQ.</td></tr>
</table>

<table>
<tr><td></td><td>Aminopyralid residues</td></tr>
<tr><td></td><td>Limit of quantification (LOQ) and a limit of detection (LOD) are presented in the table below.
<table>
<tr><th>Method</th><th>Analyte</th><th>LOQ [mg analyte /L]</th><th>Equivalent calibration level [mg/L]</th><th>LOD [mg analyte/L]</th><th>Equivalent calibration level [mg/L]</th></tr>
<tr><td>direct method</td><td>Aminopyralid</td><td>0.1</td><td>0.1</td><td>0.02</td><td>0.02</td></tr>
</table></td></tr>
<tr><td>Extraction stability</td><td>Final extract stability was not determined. The final extracts were analysed within 24 h.</td></tr>
<tr><td>Comment</td><td>The validation parameters are within the acceptance range and fulfil EU requirements given in SANTE/2020/12830 Rev.2.</td></tr>
</table>

Conclusion

The method was successfully validated for determination of aminopyralid in AAP medium with an LOQ of 0.1 mg/L according to the guidance document(s) SANTE/2020/12830 Rev.2. With regard to selectivity, accuracy and precision, the analytical methods were applied successfully for each analytical set when analysing the specimens of the study.

A 2.1.1.1.8 Analytical method 8

A 2.1.1.1.8.1 Method validation

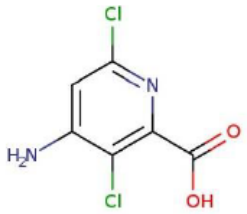
Comments of zRMS:	Validation of the Analytical Czarnecka, M., 2024, W-26-24) is acceptable and suitable for determination of aminopyralid in Smart and Barko medium.

Reference:	KCP 5.1.2/08
Report	Water-Sediment Myriophyllum Spicatum Toxicity Test, Czarnecka, M., 2024, Study code: W-26-24
Guideline(s):	Yes according to the OECD Guideline No. 239/ EU method C.3.; SANTE/2020/12830, Rev. 2; Standard Operating Procedure SOP/C/9.
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

Analytical standard of aminopyralid was used to method validation.

Aminopyralid

IUPAC Name:	4-amino-3,6-dichloropyridine-2-carboxylic acid (IUPAC)	Structural [2]: 
Molecular Formula:	$C_6H_4Cl_2N_2O_2$	
Cas No.:	150114-71-9	
Purity:	97.99 % ± 0.31% (g/g)	
Series No:	G1239715	
Valid to:	22 March 2028	
Molecular weight:	207.01 g/mol	

Stock and standard solutions

The stock solution with a concentration of 1.0 mg/mL was prepared by weighting 10.0 mg of standard into a volumetric flask with a capacity of 10.0 mL, dissolving in acetonitrile for HPLC, and next the volume was made up to 10.0 mL with the same solvent. The working solutions were prepared by diluting standard solutions of higher concentrations.

Fortification of samples

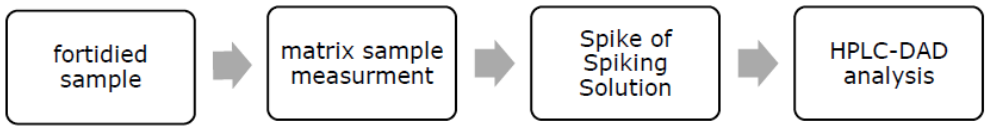
For the preparation of procedural recoveries and validation experiments, fortified samples were prepared from standard solution. The appropriate amount of spiking solutions was added to the matrix to prepare LOQ and 10xLOQ.

Sample preparation for the chromatographic analysis

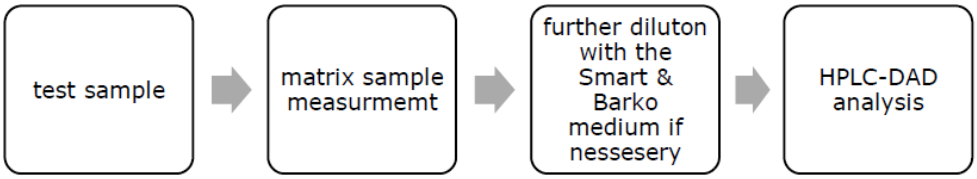
1.0 mL of sample was measured. The sample was diluted with the Smart & Barko medium, if necessary. An aliquot of the final volume was transferred into a HPLC vial for further quantification using HPLC-DAD.

2.5.1. Schematic diagram of the analytical method

Overview of the fortification of samples work-up by a flow chart / picture is presented below:



Overview of the test samples work-up by a flow chart / picture is presented below:



2.6. Instrumentation and conditions

The chromatographic systems and conditions used for the analysis are shown in the table below [SOP/C/328, SOP/C/592, SOP/C/607].

	Parameter
Chromatographic System	High Performance Liquid Chromatography (HPLC)
Chromatograph	Shimadzu, Prominence - *i* (Shimadzu Corporation Japan)
Analytical Column	Synergi 4µm Fusion-RP 80Å (2) 150x4.6 mm
Oven temperature	35°C
Injection Volume	30 µL
Mobile Phase	acetonitrile HPLC : ortho-phosphoric acid solution 0.05 % (45 : 55, *v/v*)
Flow Rate	0.6 mL/min
Wave length	225 nm
Retention time	approx. 4.2 min
Detection System	Diode Array Detector

Results and discussions

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

Matrix	Analyte	Fortification level (mg/kg) (*n* = x)	Mean recovery (%)	RSD (%)	Comments
Smart and Barko medium	Aminopyralid	Control – 0.0 mg/L – two replicates LOQ – 0.02 mg/L – five replicates 10xLOQ – 0.2 mg/L – five replicates	99.4 - 100 %	0.4 – 2.3 %	-

Table A 2: Characteristics for the analytical method used for validation of aminopyralid residues in AAP medium

	Aminopyralid residues
Author(s), year	M. Czarnecka, 2024
Principle of method	HPLC-DAD
Linearity (linear between mg/L) (correlation coefficient,	Working solutions at all calibration levels were injected successively to the chromatographic column and the chromatograms were recorded. The range of linearity of the analytical graph and the ranges of calibration curve corresponding to the real samples level are presented in the table below.

	Aminopyralid residues
expressed as r)	

Analyte	Working solution concentrations [µg/mL]	Range of linearity of calibration curve [mg/L]	Equivalent calibration range of linearity [mg/L]
Aminopyralid	0.005, 0.01, 0.05, 0.1, 0.5	0.005 – 0.5	0.005 – 0.5

The equation of the calibration line is presented as the linear equation; y = ax + b (a – slope, b – intercept). The linear coefficient r2 must be higher than 0.99. Range of the linearity was given in µg/mL (equal to mg/L).

Range of linearity of calibration curve [mg/L]	Analyte	Slope	Intercept	Coefficient r^2
0.005 – 0.5	Aminopyralid	279555	1326.21	0.9998411

Linear weighted calibration (1/C weighting) is used for calibration curve. The suitability of the chosen function was demonstrated as the regression residuals (di). The regression residuals are presented in a residual plot in range equal to range of linearity of calibration curve.

Precision, accuracy and uncer-tainty

Precision of a particular methods is defined as its repeatability (RSD – relative standard deviation [%]). The RSD is ≤ 20% per each level.
The accuracy of the method is reported as mean recovery ± relative standard deviation. Recovery was reported as mean recovery ± relative standard deviation (RSD). Mean recoveries for each level is in the range 70-120%.
A summary of the precision and recovery data of control and fortified samples are presented in the table below:

Method	Analyte	Fortification Level [mg/L]	Number of Replicates	Mean Recovery [%]	RSD [%]
Direct method	Aminopyralid	0.02	5	100	2.3
		0.2	5	99.4	0.4

In order to study the recovery level, the solution of the detected substance was added to non-treated control sample and then analysed using the methods described above.

Specificity

The analytical method specificity was estimated on the basis of the analysis of the chromatograms obtained for the control, and fortified samples of matrix. Considering the results of the analysis, no signal of detected substance was overlapping with matrix signal of the control samples in the experiments conditions. Therefore, the specificity of the method was demonstrated. Furthermore, any interference are directly apparent and would be observed in the chromatograms of the matrix control sample.

Matrix Effects

In accordance with the study report: *Since potential matrix effects were compensated by using matrix matched calibration standards (same matrix load), no instrument recovery samples were prepared and analysed in addition.*

~~Matrix effect for each method was checked during the method validation. Assessment of matrix effects was performed by comparing the standard prepared in solution to standard prepared in blank matrix at appropriate concentration.~~
~~The matrix effect did not exceed ± 20 %. The matrix effect and concentration are presented in table below~~

Method / ~~Analyte~~	Concentration [mg/L]	Matrix effect [%]
direct method	Aminopyralid	0.1	~~-6.6~~

	Aminopyralid residues
LOQ **LOD**	Limit of Quantification was estimated as the lowest concentration of a detected substance at which an acceptable mean recovery and precision (normally 70 – 120% with a relative standard deviation of preferably ≤ 20%). The limit of detection (LOD) is defined as the lowest detectable concentration or amount of an analyte in a sample. LOD is expressed as the lowest calibration standard. LOD is less than or equal to 30% of LOQ. Limit of quantification (LOQ) and a limit of detection (LOD) are presented in the table below.

Method	Analyte	LOQ [mg analyte /L]	equivalent calibration level [mg/L]	LOD [mg analyte/L]	equivalent calibration level [mg/L]
direct method	Aminopyralid	0.02	0.02	0.005	0.005

Extraction stability	Final extract stability was not determined. The final extracts were analysed within 24 h.
Comment	The validation parameters are within the acceptance range and fulfil EU requirements given in SANTE/2020/12830 Rev.2.

Conclusion

The method was successfully validated for determination of aminopyralid in Smart and Barko medium medium with an LOQ of 0.02 mg/L according to the guidance document(s) SANTE/2020/12830 Rev.2. With regard to selectivity, accuracy and precision, the analytical methods were applied successfully for each analytical set when analysing the specimens of the study.

A 2.1.1.1.9 Analytical method 9

A 2.1.1.1.9.1 Method validation

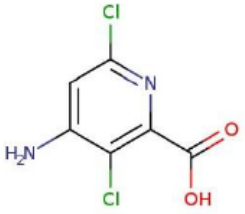
Comments of zRMS:	Validation of the Analytical Dybek M. , 2024, B-94-24) is acceptable and suitable for determination of aminopyralid in 50% Sucrose Solution.

Reference:	KCP 5.1.2/09
Report	Heoneybees (Apis melifera L.) Chronic Oral Toxicity Test, ~~Maga, D.,~~ Dybek M. 2024, Study code: B-94-24
Guideline(s):	Yes according to the OECD Guideline No. 245 (2017); SANTE/2020/12830, Rev. 2; Standard Operating Procedure SOP/C/9.
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

Analytical standard of aminopyralid was used to method validation.

Aminopyralid

IUPAC Name:	4-amino-3,6-dichloropyridine-2-carboxylic acid (IUPAC)	Structural [2]: 
Molecular Formula:	$C_6H_4Cl_2N_2O_2$	
Cas No.:	150114-71-9	
Purity:	97.99 % ± 0.31% (g/g)	
Series No:	G1239715	
Valid to:	22 March 2028	
Molecular weight:	207.01 g/mol	

Stock and standard solutions

The stock solution with a concentration of 1.0 mg/mL was prepared by weighting 10.0 mg of standard into a volumetric flask with a capacity of 10.0 mL, dissolving in acetonitrile for HPLC, and next the volume was made up to 10.0 mL with the same solvent. The working solutions were prepared by diluting standard solutions of higher concentrations.

Fortification of samples

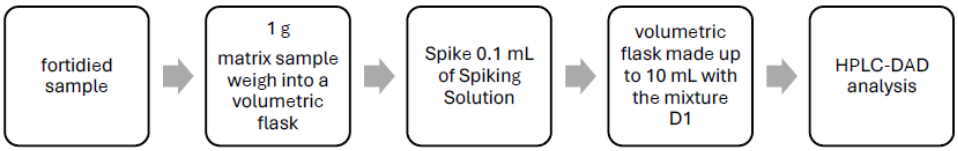
For the preparation of procedural recoveries and validation experiments, fortified samples were prepared from standard solution. The appropriate amount of spiking solutions was added to the matrix to prepare LOQ and 10xLOQ.

Sample preparation for the chromatographic analysis

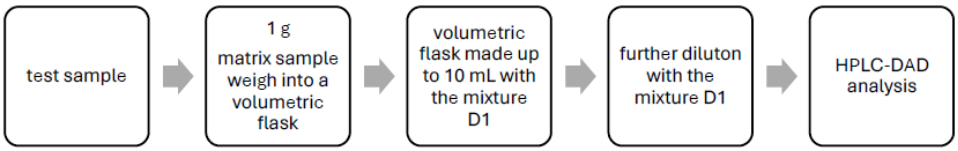
The weight of 1 g of the sample was weigh into a volumetric flask with a capacity of 10 mL and made up to 10 mL with mixture of acetonitrile for HPLC and deionized water (50:50; v/v). The sample was diluted with mixture of 50% acetonitrile, if necessary. An aliquot of the final volume was transferred into a HPLC vial for further quantification using HPLC-DAD.

2.5.1. Schematic diagram of the analytical method

Overview of the fortification of samples work-up by a flow chart / picture is presented below:



Overview of the test samples work-up by a flow chart / picture is presented below:



D1 – mixture of acetonitrile for HPLC and deionized water (50:50, *v/v*)

2.6. Instrumentation and conditions

The chromatographic systems and conditions used for the analysis are shown in the table below [SOP/C/328, SOP/C/592, SOP/C/607].

	Parameter
Chromatographic System	High Performance Liquid Chromatography (HPLC)
Chromatograph	Shimadzu, Prominence - *i* (Shimadzu Corporation Japan)
Analytical Column	Luna 5µm C18 (2) 100Å (2) 250x4.6 mm
Oven temperature	35°C
Injection Volume	10 µL
Mobile Phase	acetonitrile HPLC : ortho-phosphoric acid solution 0.05 % (65 : 35, *v/v*)
Flow Rate	0.6 mL/min
Wave length	240 nm
Retention time	approx. 5.1 min
Detection System	Diode Array Detector

Results and discussions

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

Matrix	Analyte	Fortification level (mg/kg) (*n* = x)	Mean recovery (%)	RSD (%)	Comments
50% Sucrose Solution	Aminopyralid	Control – 0.0 mg/L – two replicates LOQ – 1.0 mg/L – five replicates 10xLOQ – 10.0 mg/L – five replicates	94.8 – 107 %	0.5– 2.8 %	-

Table A 2: Characteristics for the analytical method used for validation of aminopyralid residues in 50% Sucrose Solution

	Aminopyralid residues
Author(s), year	M. Dybek, 2024
Principle of method	HPLC-DAD
Linearity (linear be-tween mg/L) (correlation coefficient,	Working solutions at all calibration levels were injected successively to the chromatographic column and the chromatograms were recorded. The range of linearity of the analytical graph and the ranges of calibration curve corresponding to the real samples level are presented in the table below.

<table>
<tr><th></th><th>Aminopyralid residues</th></tr>
<tr><td>expressed as r)</td><td>
<table>
<tr><th>Analyte</th><th>Working solution concentrations [µg/mL]</th><th>Range of linearity of calibration curve [mg/L]</th><th>Equivalent calibration range of linearity [mg/kg]</th></tr>
<tr><td>Aminopyralid</td><td>0.02, 0.05, 0.1, 0.2, 0.5, 1.0, 2.0, 5.0</td><td>0.02 – 5.0</td><td>0.2 – 50.0</td></tr>
</table>
The equation of the calibration line is presented as the linear equation; y = ax + b (a – slope, b – intercept). The linear coefficient r2 must be higher than 0.99. Range of the linearity was given in µg/mL (equal to mg/L).
<table>
<tr><th>Range of linearity of calibration curve [mg/L]</th><th>Analyte</th><th>Slope</th><th>Intercept</th><th>Coefficient r^2</th></tr>
<tr><td>0.02 – 5.0</td><td>Aminopyralid</td><td>46551.5</td><td>194.143</td><td>0.9999782</td></tr>
</table>
Linear weighted calibration (1/C weighting) is used for calibration curve. The suitability of the chosen function was demonstrated as the regression residuals (d_i). The regression residuals are presented in a residual plot in range equal to range of linearity of calibration curve.</td></tr>
<tr><td>Precision, accuracy and uncertainty</td><td>Precision of a particular methods is defined as its repeatability (RSD – relative standard deviation [%]). The repeatability for detected substances analysed are presented in table below. The RSD is ≤ 20% per each level.
The accuracy of the method is reported as mean recovery ± relative standard deviation. Recovery was reported as mean recovery ± relative standard deviation (RSD). Mean recoveries for each level is in the range 70-120%.
A summary of the recovery data of control and fortified samples are presented in the table below.
<table>
<tr><th>Method</th><th>Analyte</th><th>Fortification Level [mg/kg]</th><th>Number of Replicates</th><th>Mean Recovery [%]</th><th>RSD [%]</th></tr>
<tr><td rowspan="2">dilution method</td><td rowspan="2">Aminopyralid</td><td>1.0</td><td>5</td><td>107</td><td>0.5</td></tr>
<tr><td>10.0</td><td>5</td><td>94.8</td><td>2.8</td></tr>
</table>
In order to study the recovery level, the solution of the detected substance was added to non-treated control sample and then analysed using the methods described above.</td></tr>
<tr><td>Specificity</td><td>The analytical method specificity was estimated on the basis of the analysis of the chromatograms obtained for the control, and fortified samples of matrix. Considering the results of the analysis, no signal of detected substance was overlapping with matrix signal of the control samples in the experiments conditions. Therefore, the specificity of the method was demonstrated. Furthermore, any interference are directly apparent and would be observed in the chromatograms of the matrix control sample</td></tr>
<tr><td>Matrix Effects</td><td>Matrix effect for each method was checked during the method validation. Assessment of matrix effects was performed by comparing the standard prepared in solution to standard prepared in blank matrix at appropriate concentration.
The matrix effect did not exceed ± 20 %. The matrix effect and concentration are presented in table below
<table>
<tr><th>Method</th><th>Analyte</th><th>Concentration [mg/L]</th><th>Matrix effect [%]</th></tr>
<tr><td>dilution method</td><td>Aminopyralid</td><td>0.1</td><td>-4.9</td></tr>
</table></td></tr>
<tr><td>LOQ
LOD</td><td>Limit of Quantification was estimated as the lowest concentration of a detected substance at which an acceptable mean recovery and precision (normally 70 – 120% with a relative standard deviation of preferably ≤ 20%).
The limit of detection (LOD) is defined as the lowest detectable concentration or amount of an analyte in a sample. LOD is expressed as the lowest calibration standard. LOD is less than or equal to 30% of LOQ.
Limit of quantification (LOQ) and a limit of detection (LOD) are presented in the table below.</td></tr>
</table>

	Aminopyralid residues

Method	Analyte	LOQ [mg analyte /kg]	equivalent calibration level [mg/L]	LOD [mg analyte/kg]	equivalent calibration level [mg/L]
dilution method	Aminopyralid	1.0	0.1	0.2	0.02

Extraction stability	Final extract stability was not determined. The final extracts were analysed within 24 h.
Comment	The validation parameters are within the acceptance range and fulfil EU requirements given in SANTE/2020/12830 Rev.2.

Conclusion

The method was successfully validated for determination of aminopyralid in 50% Sucrose Solution with an LOQ of 1.0 mg/L according to the guidance document(s) SANTE/2020/12830 Rev.2. With regard to selectivity, accuracy and precision, the analytical methods were applied successfully for each analytical set when analysing the specimens of the study.

A 2.1.1.1.10 Analytical method 10

A 2.1.1.1.10.1 Method validation

Comments of zRMS:	Validation of the Analytical Dybek M., 2024, B-89-24) is acceptable and suitable for determination of aminopyralid in 0.1% Tergitol 15-S-9 Solu-tion.

Reference:	KCP 5.1.2/10
Report	Bumblebee (Bombus spp.) Acute Contact Toxicity Test, ~~Maga, D.,~~ Dybek M. 2024, Study code: B-89-24
Guideline(s):	according to the OECD Guideline No. 246; SANTE/2020/12830, Rev. 2; Standard Operating Procedure SOP/C/9.
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

Analytical standard of aminopyralid was used to method validation.

Aminopyralid

IUPAC Name:	4-amino-3,6-dichloropyridine-2-carboxylic acid (IUPAC)	Structural [2]: 
Molecular Formula:	$C_6H_4Cl_2N_2O_2$	
Cas No.:	150114-71-9	
Purity:	97.99 % ± 0.31% (g/g)	
Series No:	G1239715	
Valid to:	22 March 2028	
Molecular weight:	207.01 g/mol	

Stock and standard solutions

The stock solution with a concentration of 1.0 mg/mL was prepared by weighting 10.0 mg of standard into a volumetric flask with a capacity of 10.0 mL, dissolving in acetonitrile for HPLC, and next the volume was made up to 10.0 mL with the same solvent. The working solutions were prepared by diluting standard solutions of higher concentrations.

Fortification of samples

For the preparation of procedural recoveries and validation experiments, fortified samples were prepared from standard solution. The appropriate amount of spiking solutions was added to the matrix to prepare LOQ and 10xLOQ.

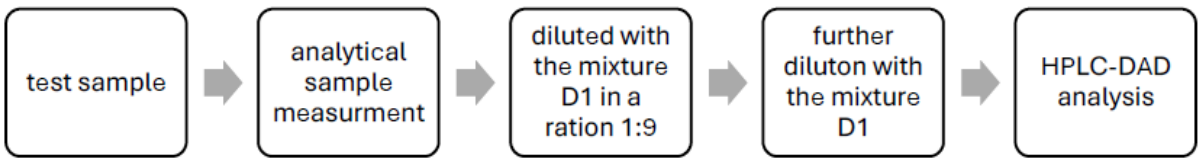
Sample preparation for the chromatographic analysis

Each sample in a volume of 1 mL was diluted with mixture of acetonitrile for HPLC and deionized water (50:50; v/v) in a ration 1:9. The sample was diluted with mixture of acetonitrile for HPLC and deionised water (50:50; v/v), if necessary. An aliquot of the final volume was transferred into a HPLC vial for further quantification using HPLC-DAD.

2.5.1. Schematic diagram of the analytical method

Overview of the fortification of samples work-up by a flow chart / picture is presented below:

Overview of the test samples work-up by a flow chart / picture is presented below:



D1 – mixture of acetonitrile for HPLC and deionized water (50:50, *v/v*)

2.6. Instrumentation and conditions

The chromatographic systems and conditions used for the analysis are shown in the table below [SOP/C/328, SOP/C/592, SOP/C/607].

	Parameter
Chromatographic System	High Performance Liquid Chromatography (HPLC)
Chromatograph	Shimadzu, Prominence - *i* (Shimadzu Corporation Japan)
Analytical Column	Luna 5µm C18 (2) 100Å (2) 250x4.6 mm
Oven temperature	35°C
Injection Volume	10 µL
Mobile Phase	acetonitrile HPLC : ortho-phosphoric acid solution 0.05 % (65 : 35, *v/v*)
Flow Rate	0.6 mL/min
Wave length	240 nm
Retention time	approx. 5.1 min
Detection System	Diode Array Detector

Results and discussions

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

Matrix	Analyte	Fortification level (mg/kg) (*n* = x)	Mean recovery (%)	RSD (%)	Comments
0.1% Tergitol 15-S-9 Solu-tion	Aminopyralid	Control – 0.0 mg/L – two replicates LOQ – 1.0 mg/L – five replicates 10xLOQ – 10.0 mg/L – five replicates	95.8 – 99.3 %	0.3– 1.3 %	-

Table A 2: Characteristics for the analytical method used for validation of aminopyralid residues in 0.1% Tergitol 15-S-9 Solution

	Aminopyralid residues
Author(s), year	M. Dybek, 2024
Principle of method	HPLC-DAD
Linearity (linear be-tween mg/L) (correlation coefficient, expressed as	Working solutions at all calibration levels were injected successively to the chromatographic column and the chromatograms were recorded. The range of linearity of the analytical graph and the ranges of calibration curve corresponding to the real samples level are presented in the table below.

<table>
<tr><th></th><th>Aminopyralid residues</th></tr>
<tr><td>r)</td><td>
<table>
<tr><th>Analyte</th><th>Working solution concentrations [µg/mL]</th><th>Range of linearity of calibration curve [mg/L]</th><th>Equivalent calibration range of linearity [mg/kg]</th></tr>
<tr><td>Aminopyralid</td><td>0.02, 0.05, 0.1, 0.2, 0.5, 1.0, 2.0, 5.0</td><td>0.02 – 5.0</td><td>0.2 – 50.0</td></tr>
</table>
The equation of the calibration line is presented as the linear equation; y = ax + b (a – slope, b – intercept). The linear coefficient r2 must be higher than 0.99. Range of the linearity was given in µg/mL (equal to mg/L).
<table>
<tr><th>Range of linearity of calibration curve [mg/L]</th><th>Analyte</th><th>Slope</th><th>Intercept</th><th>Coefficient r²</th></tr>
<tr><td>0.02 – 5.0</td><td>Aminopyralid</td><td>46551.5</td><td>194.143</td><td>0.9999782</td></tr>
</table>
Linear weighted calibration (1/C weighting) is used for calibration curve. The suitability of the chosen function was demonstrated as the regression residuals (d_i). The regression residuals are presented in a residual plot in range equal to range of linearity of calibration curve.</td></tr>
<tr><td>Precision, accuracy and uncer-tainty</td><td>Precision of a particular methods is defined as its repeatability (RSD – relative standard deviation [%]). The repeatability for detected substances analysed are presented in table below. The RSD is ≤ 20% per each level.
The accuracy of the method is reported as mean recovery ± relative standard deviation. Recovery was reported as mean recovery ± relative standard deviation (RSD). Mean recoveries for each level is in the range 70-120%. A summary of the recovery data of control and fortified samples are presented in the table below.
<table>
<tr><th>Method</th><th>Analyte</th><th>Fortification Level [mg/L]</th><th>Number of Replicates</th><th>Mean Recovery [%]</th><th>RSD [%]</th></tr>
<tr><td rowspan="2">dilution method</td><td rowspan="2">Aminopyralid</td><td>1.0</td><td>5</td><td>95.8</td><td>1.3</td></tr>
<tr><td>10.0</td><td>5</td><td>99.3</td><td>0.3</td></tr>
</table>
In order to study the recovery level, the solution of the detected substance was added to non-treated control sample and then analysed using the methods described above.</td></tr>
<tr><td>Specificity</td><td>The analytical method specificity was estimated on the basis of the analysis of the chromatograms obtained for the control, and fortified samples of matrix. Considering the results of the analysis, no signal of detected substance was overlapping with matrix signal of the control samples in the experiments conditions. Therefore, the specificity of the method was demonstrated. Furthermore, any interference are directly apparent and would be observed in the chromatograms of the matrix control sample</td></tr>
<tr><td>Matrix Ef-fects</td><td>Matrix effect for each method was checked during the method validation. Assessment of matrix effects was performed by comparing the standard prepared in solution to standard prepared in blank matrix at appropriate concentration.
The matrix effect did not exceed ± 20 %. The matrix effect and concentration are presented in table below
<table>
<tr><th>Method</th><th>Analyte</th><th>Concentration [mg/L]</th><th>Matrix effect [%]</th></tr>
<tr><td>dilution method</td><td>Aminopyralid</td><td>0.1</td><td>-4.0</td></tr>
</table></td></tr>
<tr><td>LOQ
LOD</td><td>Limit of Quantification was estimated as the lowest concentration of a detected substance at which an acceptable mean recovery and precision (normally 70 – 120% with a relative standard deviation of preferably ≤ 20%).
The limit of detection (LOD) is defined as the lowest detectable concentration or amount of an analyte in a sample. LOD is expressed as the lowest calibration standard. LOD is less than or equal to 30% of LOQ.
Limit of quantification (LOQ) and a limit of detection (LOD) are presented in the table below.</td></tr>
</table>

	Aminopyralid residues
	<table><tr><th>Method</th><th>Analyte</th><th>LOQ [mg analyte /kg]</th><th>equivalent calibration level [mg/L]</th><th>LOD [mg analyte/kg]</th><th>equivalent calibration level [mg/L]</th></tr><tr><td>dilution method</td><td>Aminopyralid</td><td>1.0</td><td>0.1</td><td>0.2</td><td>0.02</td></tr></table>
Extraction stability	Final extract stability was not determined. The final extracts were analysed within 24 h.
Comment	The validation parameters are within the acceptance range and fulfil EU requirements given in SANTE/2020/12830 Rev.2.

Conclusion

The method was successfully validated for determination of aminopyralid in 0.1% Tergitol 15-S-9 Solution with an LOQ of 1.0 mg/L according to the guidance document(s) SANTE/2020/12830 Rev.2. With regard to selectivity, accuracy and precision, the analytical methods were applied successfully for each analytical set when analysing the specimens of the study.

A 2.1.1.1.11 Analytical method 11

A 2.1.1.1.11.1 Method validation

Comments of zRMS:	Validation of the Analytical Dybek M., 2024, B-88-24) is acceptable and suitable for determination of aminopyralid in 50% Sucrose Solution.

Reference: KCP 5.1.2/11

Report: Bumblebee (Bombus spp.) Acute Oral Toxicity Test ~~Maga, D.,~~ Dybek M. 2024, Study code: B-88-24

Guideline(s): according to the OECD Guideline No. 247; SANTE/2020/12830, Rev. 2; Standard Operating Procedure SOP/C/9.

Deviations: No

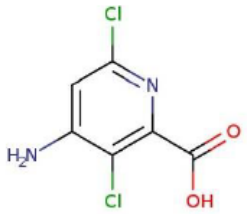
GLP: Yes

Acceptability: Yes

Materials and methods

Analytical standard of aminopyralid was used to method validation.

Aminopyralid

IUPAC Name:	4-amino-3,6-dichloropyridine-2-carboxylic acid (IUPAC)	Structural [2]: 
Molecular Formula:	$C_6H_4Cl_2N_2O_2$	
Cas No.:	150114-71-9	
Purity:	97.99 % ± 0.31% (g/g)	
Series No:	G1239715	
Valid to:	22 March 2028	
Molecular weight:	207.01 g/mol	

Stock and standard solutions

The stock solution with a concentration of 1.0 mg/mL was prepared by weighting 10.0 mg of standard into a volumetric flask with a capacity of 10.0 mL, dissolving in acetonitrile for HPLC, and next the volume was made up to 10.0 mL with the same solvent. The working solutions were prepared by diluting standard solutions of higher concentrations.

Fortification of samples

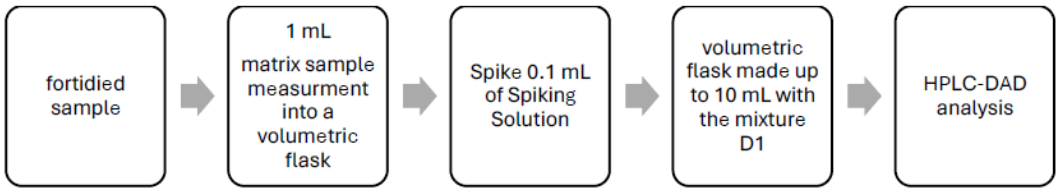
For the preparation of procedural recoveries and validation experiments, fortified samples were prepared from standard solution. The appropriate amount of spiking solutions was added to the matrix to prepare LOQ and 10xLOQ.

Sample preparation for the chromatographic analysis

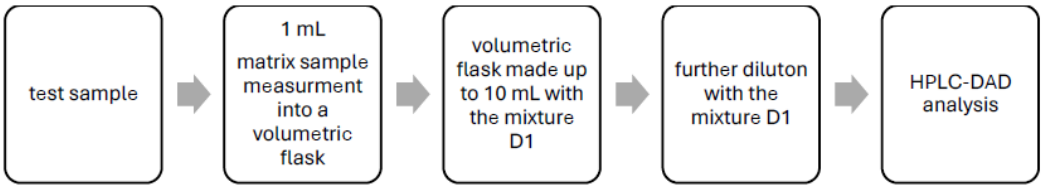
The volume of 1 mL of the sample was measured into a volumetric flask with a capacity of 10 mL and made up to 10 mL with mixture of acetonitrile for HPLC and deionized water (50:50; v/v). The sample was diluted with mixture of 50% acetonitrile, if necessary. An aliquot of the final volume was transferred into a HPLC vial for further quantification using HPLC-DAD

2.5.1. Schematic diagram of the analytical method

Overview of the fortification of samples work-up by a flow chart / picture is presented below:



Overview of the test samples work-up by a flow chart / picture is presented below:



D1 – mixture of acetonitrile for HPLC and deionized water (50:50, *v/v*)

2.6. Instrumentation and conditions

The chromatographic systems and conditions used for the analysis are shown in the table below [SOP/C/328, SOP/C/592, SOP/C/607].

	Parameter
Chromatographic System	High Performance Liquid Chromatography (HPLC)
Chromatograph	Shimadzu, Prominence - *i* (Shimadzu Corporation Japan)
Analytical Column	Luna 5µm C18 (2) 100Å (2) 250x4.6 mm
Oven temperature	35°C
Injection Volume	10 µL
Mobile Phase	acetonitrile HPLC : ortho-phosphoric acid solution 0.05 % (65 : 35, *v/v*)
Flow Rate	0.6 mL/min
Wave length	240 nm
Retention time	approx. 5.1 min
Detection System	Diode Array Detector

Results and discussions

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

Matrix	Analyte	Fortification level (mg/kg) (*n* = x)	Mean recovery (%)	RSD (%)	Comments
50% Sucrose Solution	Aminopyralid	Control – 0.0 mg/L – two replicates LOQ – 1.0 mg/L – five replicates 10xLOQ – 10.0 mg/L – five replicates	100 – 109 %	1.3– 1.5 %	-

Table A 2: Characteristics for the analytical method used for validation of aminopyralid residues in 50% Sucrose Solution

	Aminopyralid residues
Author(s), year	M. Dybek, 2024
Principle of method	HPLC-DAD
Linearity (linear between mg/L) (correlation coefficient, expressed as	Working solutions at all calibration levels were injected successively to the chromatographic column and the chromatograms were recorded. The range of linearity of the analytical graph and the ranges of calibration curve corresponding to the real samples level are presented in the table below.

Aminopyralid residues

r)

Analyte	Working solution concentrations [µg/mL]	Range of linearity of calibration curve [mg/L]	Equivalent calibration range of linearity [mg/kg]
Aminopyralid	0.02, 0.05, 0.1, 0.2, 0.5, 1.0, 2.0, 5.0	0.02 – 5.0	0.2 – 50.0

The equation of the calibration line is presented as the linear equation; y = ax + b (a – slope, b – intercept). The linear coefficient r2 must be higher than 0.99. Range of the linearity was given in µg/mL (equal to mg/L).

Range of linearity of calibration curve [mg/L]	Analyte	Slope	Intercept	Coefficient r^2
0.02 – 5.0	Aminopyralid	46551.5	194.143	0.9999782

Linear weighted calibration (1/C weighting) is used for calibration curve. The suitability of the chosen function was demonstrated as the regression residuals (d_i). The regression residuals are presented in a residual plot in range equal to range of linearity of calibration curve.

Precision, accuracy and uncertainty

Precision of a particular methods is defined as its repeatability (RSD – relative standard deviation [%]). The repeatability for detected substances analysed are presented in table below. The RSD is ≤ 20% per each level.
The accuracy of the method is reported as mean recovery ± relative standard deviation. Recovery was reported as mean recovery ± relative standard deviation (RSD). Mean recoveries for each level is in the range 70-120%. A summary of the recovery data of control and fortified samples are presented in the table below.

Method	Analyte	Fortification Level [mg/L]	Number of Replicates	Mean Recovery [%]	RSD [%]
dilution method	Aminopyralid	1.0	5	109	1.5
		10.0	5	100	1.3

In order to study the recovery level, the solution of the detected substance was added to non-treated control sample and then analysed using the methods described above.

Specificity

The analytical method specificity was estimated on the basis of the analysis of the chromatograms obtained for the control, and fortified samples of matrix. Considering the results of the analysis, no signal of detected substance was overlapping with matrix signal of the control samples in the experiments conditions. Therefore, the specificity of the method was demonstrated. Furthermore, any interference are directly apparent and would be observed in the chromatograms of the matrix control sample

Matrix Effects

Matrix effect for each method was checked during the method validation. Assessment of matrix effects was performed by comparing the standard prepared in solution to standard prepared in blank matrix at appropriate concentration.
The matrix effect did not exceed ± 20 %. The matrix effect and concentration are presented in table below

Method	Analyte	Concentration [mg/L]	Matrix effect [%]
dilution method	Aminopyralid	0.1	-8.8

LOQ
LOD

Limit of Quantification was estimated as the lowest concentration of a detected substance at which an acceptable mean recovery and precision (normally 70 – 120% with a relative standard deviation of preferably ≤ 20%).
The limit of detection (LOD) is defined as the lowest detectable concentration or amount of an analyte in a sample. LOD is expressed as the lowest calibration standard. LOD is less than or equal to 30% of LOQ.
Limit of quantification (LOQ) and a limit of detection (LOD) are presented in the table below.

	Aminopyralid residues
	<table><tr><th>Method</th><th>Analyte</th><th>LOQ [mg analyte /kg]</th><th>equivalent calibration level [mg/L]</th><th>LOD [mg analyte/kg]</th><th>equivalent calibration level [mg/L]</th></tr><tr><td>dilution method</td><td>Aminopyralid</td><td>1.0</td><td>0.1</td><td>0.2</td><td>0.02</td></tr></table>
Extraction stability	Final extract stability was not determined. The final extracts were analysed within 24 h.
Comment	The validation parameters are within the acceptance range and fulfil EU requirements given in SANTE/2020/12830 Rev.2.

Conclusion

The method was successfully validated for determination of aminopyralid in 50% Sucrose Solution with an LOQ of 1.0 mg/L according to the guidance document(s) SANTE/2020/12830 Rev.2. With regard to selectivity, accuracy and precision, the analytical methods were applied successfully for each analytical set when analysing the specimens of the study.

A 2.1.1.1.12 Analytical method 12

A 2.1.1.1.12.1 Method validation

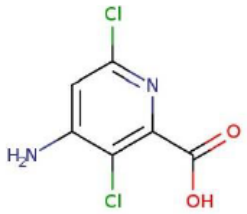
Comments of zRMS:	Validation of the Analytical Wróbel, A., 2024, G-59-24) is acceptable and suitable for determination of aminopyralid in water.

Reference:	KCP 5.1.2/12
Report	Terrestrial Plant Test: Seedling Emergence and Seedling Growth Test, Wróbel, A., 2024, Study code: G-59-24
Guideline(s):	according to the OECD Guideline No. 208 (2006); SANTE/2020/12830, Rev. 2; Standard Operating Procedure SOP/C/9.
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

Analytical standard of aminopyralid was used to method validation.

Aminopyralid

IUPAC Name:	4-amino-3,6-dichloropyridine-2-carboxylic acid (IUPAC)	Structural [2]: 
Molecular Formula:	$C_6H_4Cl_2N_2O_2$	
Cas No.:	150114-71-9	
Purity:	97.99 % ± 0.31% (g/g)	
Series No:	G1239715	
Valid to:	22 March 2028	
Molecular weight:	207.01 g/mol	

Stock and standard solutions

The stock solution with a concentration of 1.0 mg/mL was prepared by weighting 10.0 mg of standard into a volumetric flask with a capacity of 10.0 mL, dissolving in acetonitrile for HPLC, and next the volume was made up to 10.0 mL with the same solvent. The working solutions were prepared by diluting standard solutions of higher concentrations.

Fortification of samples

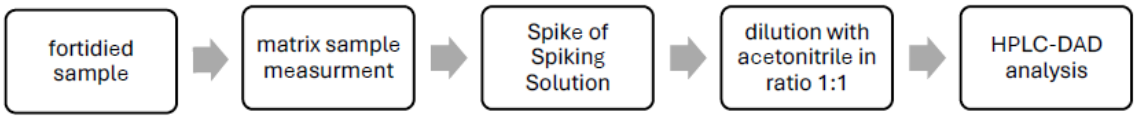
For the preparation of procedural recoveries and validation experiments, fortified samples were prepared from standard solution. The appropriate amount of spiking solutions was added to the matrix to prepare LOQ and 10xLOQ.

Sample preparation for the chromatographic analysis

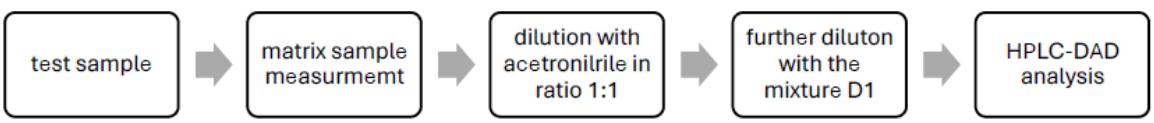
Each sample in a volume of 1.0 mL was diluted with acetonitrile for HPLC in a ration 1:1. The sample was diluted with mixture of acetonitrile for HPLC and deionized water (50:50, v/v), if necessary. An aliquot of the final volume was transferred into a HPLC vial for further quantification using HPLC-DAD.

2.5.1. Schematic diagram of the analytical method

Overview of the fortification of samples work-up by a flow chart / picture is presented below:



Overview of the test samples work-up by a flow chart / picture is presented below:



D1 – mixture of acetonitrile for HPLC and deionized water (50:50, *v/v*)

2.6. Instrumentation and conditions

The chromatographic systems and conditions used for the analysis are shown in the table below [SOP/C/328, SOP/C/592, SOP/C/607].

	Parameter
Chromatographic System	High Performance Liquid Chromatography (HPLC)
Chromatograph	Shimadzu, Prominence - *i* (Shimadzu Corporation Japan)
Analytical Column	Luna 5µm C18 (2) 100Å (2) 250x4.6 mm
Oven temperature	35°C
Injection Volume	10 µL
Mobile Phase	acetonitrile HPLC : ortho-phosphoric acid solution 0.05 % (65 : 35, *v/v*)
Flow Rate	0.6 mL/min
Wave length	240 nm
Retention time	approx. 5.1 min
Detection System	Diode Array Detector

Results and discussions

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

Matrix	Analyte	Fortification level (mg/kg) (*n* = x)	Mean recovery (%)	RSD (%)	Comments
Water	Aminopyralid	Control – 0.0 mg/L – two replicates LOQ – 0.2 mg/L – five replicates 10xLOQ – 2.0 mg/L – five replicates	92.5 – 96.8 %	1.3– 1.6 %	-

Table A 2: Characteristics for the analytical method used for validation of aminopyralid residues in water

	Aminopyralid residues
Author(s), year	~~M. Dybek~~ Wróbel, A.,, 2024
Principle of method	HPLC-DAD
Linearity (linear between mg/L) (correlation coefficient, expressed as	Working solutions at all calibration levels were injected successively to the chromatographic column and the chromatograms were recorded. The range of linearity of the analytical graph and the ranges of calibration curve corresponding to the real samples level are presented in the table below.

Aminopyralid residues

r)

Analyte	Working solution concentrations [µg/mL]	Range of linearity of calibration curve [mg/L]	Equivalent calibration range of linearity [mg/L]
Aminopyralid	0.02, 0.05, 0.1, 0.2, 0.5, 1.0, 2.0, 5.0	0.02 – 5.0	0.04 – 10.0

The equation of the calibration line is presented as the linear equation; y = ax + b (a – slope, b – intercept). The linear coefficient r2 must be higher than 0.99. Range of the linearity was given in µg/mL (equal to mg/L).

Range of linearity of calibration curve [mg/L]	Analyte	Slope	Intercept	Coefficient r^2
0.02 – 5.0	Aminopyralid	46551.5	194.143	0.9999782

Linear weighted calibration (1/C weighting) is used for calibration curve. The suitability of the chosen function was demonstrated as the regression residuals (d_i). The regression residuals are presented in a residual plot in range equal to range of linearity of calibration curve.

Precision, accuracy and uncer-tainty

Precision of a particular methods is defined as its repeatability (RSD – relative standard deviation [%]). The repeatability for detected substances analysed are presented in table below. The RSD is ≤ 20% per each level.
The accuracy of the method is reported as mean recovery ± relative standard deviation. Recovery was reported as mean recovery ± relative standard deviation (RSD). Mean recoveries for each level is in the range 70-120%. A summary of the recovery data of control and fortified samples are presented in the table below.

Method	Analyte	Fortification Level [mg/L]	Number of Replicates	Mean Recovery [%]	RSD [%]
dilution method	Aminopyralid	0.2	5	92.5	1.6
		2.0	5	96.8	1.3

In order to study the recovery level, the solution of the detected substance was added to non-treated control sample and then analysed using the methods described above.

Specificity

The analytical method specificity was estimated on the basis of the analysis of the chromatograms obtained for the control, and fortified samples of matrix. Considering the results of the analysis, no signal of detected substance was overlapping with matrix signal of the control samples in the experiments conditions. Therefore, the specificity of the method was demonstrated. Furthermore, any interference are directly apparent and would be observed in the chromatograms of the matrix control sample

Matrix Effects

Since potential matrix effects were compensated by using matrix matched calibration standards (same matrix load), no instrument recovery samples were prepared and analysed in addition.

LOQ LOD

Limit of Quantification was estimated as the lowest concentration of a detected substance at which an acceptable mean recovery and precision (normally 70 – 120% with a relative standard deviation of preferably ≤ 20%).
The limit of detection (LOD) is defined as the lowest detectable concentration or amount of an analyte in a sample. LOD is expressed as the lowest calibration standard. LOD is less than or equal to 30% of LOQ.
Limit of quantification (LOQ) and a limit of detection (LOD) are presented in the table below.

Method	Analyte	LOQ [mg analyte /L]	equivalent calibration level [mg/L]	LOD [mg analyte/L]	equivalent calibration level [mg/L]
dilution method	Aminopyralid	0.2	0.1	0.04	0.02

	Aminopyralid residues
Extraction stability	Final extract stability was not determined. The final extracts were analysed within 24 h.
Comment	The validation parameters are within the acceptance range and fulfil EU requirements given in SANTE/2020/12830 Rev.2.

Conclusion

The method was successfully validated for determination of aminopyralid in water with an LOQ of 0.2 mg/L according to the guidance document(s) SANTE/2020/12830 Rev.2. With regard to selectivity, accuracy and precision, the analytical methods were applied successfully for each analytical set when analysing the specimens of the study.

A 2.1.1.1.13 Analytical method 13

A 2.1.1.1.13.1 Method validation

Comments of zRMS:	Validation of the Analytical Wróbel, A., 2024, G-93-24) is acceptable and suitable for determination of aminopyralid in water.

Reference:	KCP 5.1.2/13
Report	Terrestrial Plant Test: Seedling Emergence and Seedling Growth Test-Wróbel, A., 2024, Study code: G-93-24
Guideline(s):	according to the OECD Guideline No. 208 (2006); SANTE/2020/12830, Rev. 2; Standard Operating Procedure SOP/C/9.
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

Analytical standard of aminopyralid was used to method validation.

Aminopyralid

IUPAC Name:	4-amino-3,6-dichloropyridine-2-carboxylic acid (IUPAC)	Structural [2]: 
Molecular Formula:	$C_6H_4Cl_2N_2O_2$	
Cas No.:	150114-71-9	
Purity:	97.99 % ± 0.31% (g/g)	
Series No:	G1239715	
Valid to:	22 March 2028	
Molecular weight:	207.01 g/mol	

Stock and standard solutions

The stock solution with a concentration of 1.0 mg/mL was prepared by weighting 10.0 mg of standard into a volumetric flask with a capacity of 10.0 mL, dissolving in acetonitrile for HPLC, and next the volume was made up to 10.0 mL with the same solvent. The working solutions were prepared by diluting standard solutions of higher concentrations.

Fortification of samples

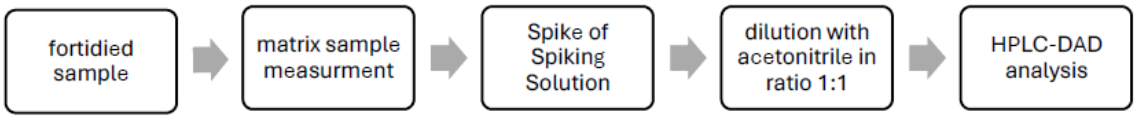
For the preparation of procedural recoveries and validation experiments, fortified samples were prepared from standard solution. The appropriate amount of spiking solutions was added to the matrix to prepare LOQ and 10xLOQ.

Sample preparation for the chromatographic analysis

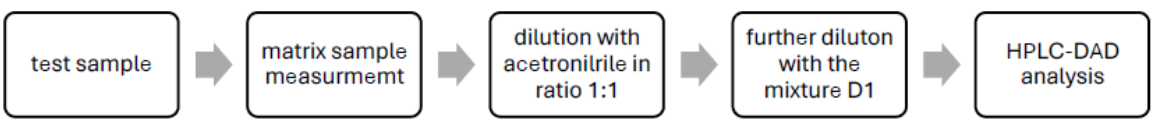
Each sample in a volume of 1.0 mL was diluted with acetonitrile for HPLC in a ration 1:1. The sample was diluted with mixture of acetonitrile for HPLC and deionized water (50:50, v/v), if necessary. An aliquot of the final volume was transferred into a HPLC vial for further quantification using HPLC-DAD.

2.5.1. Schematic diagram of the analytical method

Overview of the fortification of samples work-up by a flow chart / picture is presented below:



Overview of the test samples work-up by a flow chart / picture is presented below:



D1 – mixture of acetonitrile for HPLC and deionized water (50:50, *v/v*)

2.6. Instrumentation and conditions

The chromatographic systems and conditions used for the analysis are shown in the table below [SOP/C/328, SOP/C/592, SOP/C/607].

	Parameter
Chromatographic System	High Performance Liquid Chromatography (HPLC)
Chromatograph	Shimadzu, Prominence - *i* (Shimadzu Corporation Japan)
Analytical Column	Luna 5µm C18 (2) 100Å (2) 250x4.6 mm
Oven temperature	35°C
Injection Volume	10 µL
Mobile Phase	acetonitrile HPLC : ortho-phosphoric acid solution 0.05 % (65 : 35, *v/v*)
Flow Rate	0.6 mL/min
Wave length	240 nm
Retention time	approx. 5.1 min
Detection System	Diode Array Detector

Results and discussions

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

Matrix	Analyte	Fortification level (mg/kg) (*n* = x)	Mean recovery (%)	RSD (%)	Comments
Water	Aminopyralid	Control – 0.0 mg/L – two replicates LOQ – 0.2 mg/L – five replicates 10xLOQ – 2.0 mg/L – five replicates	92.5 – 96.8 %	1.3– 1.6 %	-

Table A 2: Characteristics for the analytical method used for validation of aminopyralid residues in water

	Aminopyralid residues
Author(s), year	A. Wróbel, 2024
Principle of method	HPLC-DAD
Linearity (linear between mg/L) (correlation coefficient, expressed as	Working solutions at all calibration levels were injected successively to the chromatographic column and the chromatograms were recorded. The range of linearity of the analytical graph and the ranges of calibration curve corresponding to the real samples level are presented in the table below.

Aminopyralid residues

r)

Analyte	Working solution concentrations [µg/mL]	Range of linearity of calibration curve [mg/L]	Equivalent calibration range of linearity [mg/L]
Aminopyralid	0.02, 0.05, 0.1, 0.2, 0.5, 1.0, 2.0, 5.0	0.02 – 5.0	0.04 – 10.0

The equation of the calibration line is presented as the linear equation; y = ax + b (a – slope, b – intercept). The linear coefficient r2 must be higher than 0.99. Range of the linearity was given in µg/mL (equal to mg/L).

Range of linearity of calibration curve [mg/L]	Analyte	Slope	Intercept	Coefficient r^2
0.02 – 5.0	Aminopyralid	46551.5	194.143	0.9999782

Linear weighted calibration (1/C weighting) is used for calibration curve. The suitability of the chosen function was demonstrated as the regression residuals (d_i). The regression residuals are presented in a residual plot in range equal to range of linearity of calibration curve.

Precision, accuracy and uncer-tainty

Precision of a particular methods is defined as its repeatability (RSD – relative standard deviation [%]). The repeatability for detected substances analysed are presented in table below. The RSD is ≤ 20% per each level.
The accuracy of the method is reported as mean recovery ± relative standard deviation. Recovery was reported as mean recovery ± relative standard deviation (RSD). Mean recoveries for each level is in the range 70-120%. A summary of the recovery data of control and fortified samples are presented in the table below.

Method	Analyte	Fortification Level [mg/L]	Number of Replicates	Mean Recovery [%]	RSD [%]
dilution method	Aminopyralid	0.2	5	92.5	1.6
		2.0	5	96.8	1.3

In order to study the recovery level, the solution of the detected substance was added to non-treated control sample and then analysed using the methods described above.

Specificity

The analytical method specificity was estimated on the basis of the analysis of the chromatograms obtained for the control, and fortified samples of matrix. Considering the results of the analysis, no signal of detected substance was overlapping with matrix signal of the control samples in the experiments conditions. Therefore, the specificity of the method was demonstrated. Furthermore, any interference are directly apparent and would be observed in the chromatograms of the matrix control sample

Matrix Effects

Since potential matrix effects were compensated by using matrix matched calibration standards (same matrix load), no instrument recovery samples were prepared and analysed in addition.

LOQ LOD

Limit of Quantification was estimated as the lowest concentration of a detected substance at which an acceptable mean recovery and precision (normally 70 – 120% with a relative standard deviation of preferably ≤ 20%).
The limit of detection (LOD) is defined as the lowest detectable concentration or amount of an analyte in a sample. LOD is expressed as the lowest calibration standard. LOD is less than or equal to 30% of LOQ.
Limit of quantification (LOQ) and a limit of detection (LOD) are presented in the table below.

Method	Analyte	LOQ [mg analyte /L]	equivalent calibration level [mg/L]	LOD [mg analyte/L]	equivalent calibration level [mg/L]
dilution method	Aminopyralid	0.2	0.1	0.04	0.02

	Aminopyralid residues
Extraction stability	Final extract stability was not determined. The final extracts were analysed within 24 h.
Comment	The validation parameters are within the acceptance range and fulfil EU requirements given in SANTE/2020/12830 Rev.2.

Conclusion

The method was successfully validated for determination of aminopyralid in water with an LOQ of 0.2 mg/L according to the guidance document(s) SANTE/2020/12830 Rev.2. With regard to selectivity, accuracy and precision, the analytical methods were applied successfully for each analytical set when analysing the specimens of the study.

A 2.1.1.1.14 Analytical method 14

A 2.1.1.1.14.1 Method validation

Comments of zRMS:	Validation of the Analytical Czarnynoga, M., 2024, G-58-24) is acceptable and suitable for determination of aminopyralid in water.

Reference:	KCP 5.1.2/14
Report	Terrestial Plant Test: Vegetative Vigour Test, Czarnynoga, M., 2024, Study code: G-58-24
Guideline(s):	according to the OECD Guideline No. 227; SANTE/2020/12830, Rev. 2; Standard Operating Procedure SOP/C/9.
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

Analytical standard of aminopyralid was used to method validation.

Aminopyralid

IUPAC Name:	4-amino-3,6-dichloropyridine-2-carboxylic acid (IUPAC)	Structural [2]: 
Molecular Formula:	$C_6H_4Cl_2N_2O_2$	
Cas No.:	150114-71-9	
Purity:	97.99 % ± 0.31% (g/g)	
Series No:	G1239715	
Valid to:	22 March 2028	
Molecular weight:	207.01 g/mol	

Stock and standard solutions

The stock solution with a concentration of 1.0 mg/mL was prepared by weighting 10.0 mg of standard into a volumetric flask with a capacity of 10.0 mL, dissolving in acetonitrile for HPLC, and next the volume was made up to 10.0 mL with the same solvent. The working solutions were prepared by diluting standard solutions of higher concentrations.

Fortification of samples

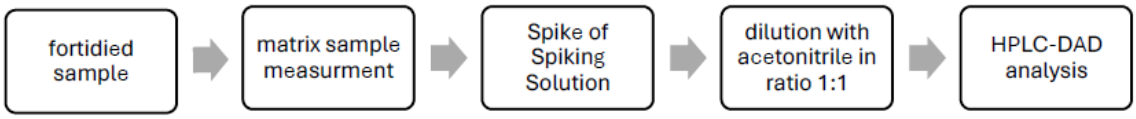
For the preparation of procedural recoveries and validation experiments, fortified samples were prepared from standard solution. The appropriate amount of spiking solutions was added to the matrix to prepare LOQ and 10xLOQ.

Sample preparation for the chromatographic analysis

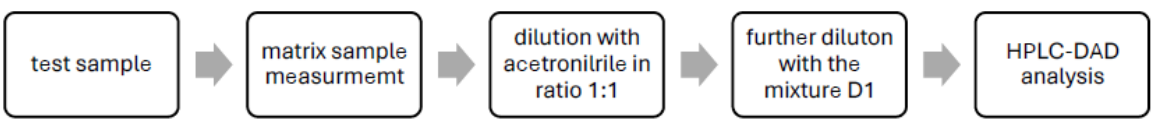
Each sample in a volume of 1.0 mL was diluted with acetonitrile for HPLC in a ration 1:1. The sample was diluted with mixture of acetonitrile for HPLC and deionized water (50:50, v/v), if necessary. An aliquot of the final volume was transferred into a HPLC vial for further quantification using HPLC-DAD.

2.5.1. Schematic diagram of the analytical method

Overview of the fortification of samples work-up by a flow chart / picture is presented below:



Overview of the test samples work-up by a flow chart / picture is presented below:



D1 – mixture of acetonitrile for HPLC and deionized water (50:50, *v/v*)

2.6. Instrumentation and conditions

The chromatographic systems and conditions used for the analysis are shown in the table below [SOP/C/328, SOP/C/592, SOP/C/607].

	Parameter
Chromatographic System	High Performance Liquid Chromatography (HPLC)
Chromatograph	Shimadzu, Prominence - *i* (Shimadzu Corporation Japan)
Analytical Column	Luna 5µm C18 (2) 100Å (2) 250x4.6 mm
Oven temperature	35°C
Injection Volume	10 µL
Mobile Phase	acetonitrile HPLC : ortho-phosphoric acid solution 0.05 % (65 : 35, *v/v*)
Flow Rate	0.6 mL/min
Wave length	240 nm
Retention time	approx. 5.1 min
Detection System	Diode Array Detector

Results and discussions

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

Matrix	Analyte	Fortification level (mg/kg) (*n* = x)	Mean recovery (%)	RSD (%)	Comments
Water	Aminopyralid	Control – 0.0 mg/L – two replicates LOQ – 0.2 mg/L – five replicates 10xLOQ – 2.0 mg/L – five replicates	92.5 – 96.8 %	1.3– 1.6 %	-

Table A 2: Characteristics for the analytical method used for validation of aminopyralid residues in water

	Aminopyralid residues
Author(s), year	M. Czarnynoga, 2024
Principle of method	HPLC-DAD
Linearity (linear between mg/L) (correlation coefficient, expressed as	Working solutions at all calibration levels were injected successively to the chromatographic column and the chromatograms were recorded. The range of linearity of the analytical graph and the ranges of calibration curve corresponding to the real samples level are presented in the table below.

Aminopyralid residues

r)

Analyte	Working solution concentrations [µg/mL]	Range of linearity of calibration curve [mg/L]	Equivalent calibration range of linearity [mg/L]
Aminopyralid	0.02, 0.05, 0.1, 0.2, 0.5, 1.0, 2.0, 5.0	0.02 – 5.0	0.04 – 10.0

The equation of the calibration line is presented as the linear equation; y = ax + b (a – slope, b – intercept). The linear coefficient r2 must be higher than 0.99. Range of the linearity was given in µg/mL (equal to mg/L).

Range of linearity of calibration curve [mg/L]	Analyte	Slope	Intercept	Coefficient r^2
0.02 – 5.0	Aminopyralid	46551.5	194.143	0.9999782

Linear weighted calibration (1/C weighting) is used for calibration curve. The suitability of the chosen function was demonstrated as the regression residuals (d_i). The regression residuals are presented in a residual plot in range equal to range of linearity of calibration curve.

Precision, accuracy and uncer-tainty

Precision of a particular methods is defined as its repeatability (RSD – relative standard deviation [%]). The repeatability for detected substances analysed are presented in table below. The RSD is ≤ 20% per each level.
The accuracy of the method is reported as mean recovery ± relative standard deviation. Recovery was reported as mean recovery ± relative standard deviation (RSD). Mean recoveries for each level is in the range 70-120%. A summary of the recovery data of control and fortified samples are presented in the table below.

Method	Analyte	Fortification Level [mg/L]	Number of Replicates	Mean Recovery [%]	RSD [%]
dilution method	Aminopyralid	0.2	5	92.5	1.6
		2.0	5	96.8	1.3

In order to study the recovery level, the solution of the detected substance was added to non-treated control sample and then analysed using the methods described above.

Specificity

The analytical method specificity was estimated on the basis of the analysis of the chromatograms obtained for the control, and fortified samples of matrix. Considering the results of the analysis, no signal of detected substance was overlapping with matrix signal of the control samples in the experiments conditions. Therefore, the specificity of the method was demonstrated. Furthermore, any interference are directly apparent and would be observed in the chromatograms of the matrix control sample.

Matrix Effects

Since potential matrix effects were compensated by using matrix matched calibration standards (same matrix load), no instrument recovery samples were prepared and analysed in addition.

LOQ LOD

Limit of Quantification was estimated as the lowest concentration of a detected substance at which an acceptable mean recovery and precision (normally 70 – 120% with a relative standard deviation of preferably ≤ 20%).
The limit of detection (LOD) is defined as the lowest detectable concentration or amount of an analyte in a sample. LOD is expressed as the lowest calibration standard. LOD is less than or equal to 30% of LOQ.
Limit of quantification (LOQ) and a limit of detection (LOD) are presented in the table below.

Method	Analyte	LOQ [mg analyte /L]	equivalent calibration level [mg/L]	LOD [mg analyte/L]	equivalent calibration level [mg/L]
dilution method	Aminopyralid	0.2	0.1	0.04	0.02

	Aminopyralid residues
Extraction stability	Final extract stability was not determined. The final extracts were analysed within 24 h.
Comment	The validation parameters are within the acceptance range and fulfil EU requirements given in SANTE/2020/12830 Rev.2.

Conclusion

The method was successfully validated for determination of aminopyralid in water with an LOQ of 0.2 mg/L according to the guidance document(s) SANTE/2020/12830 Rev.2. With regard to selectivity, accuracy and precision, the analytical methods were applied successfully for each analytical set when analysing the specimens of the study.

A 2.1.1.1.15 Analytical method 15

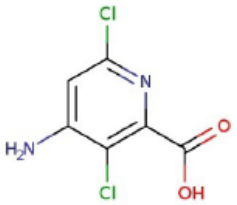
A 2.1.1.1.15.1 Method validation

Comments of zRMS:	Validation of the Analytical Kanon, L., 2024, 0038/0214/FA) is acceptable and suitable for determination of the active substance (aminopyralid) in the test item AMINO 30 SL solution in deionized water with an limit of detection (LOD) equals 0.20 mg as/L limit of quantification (LOQ) equals 1.01 mg as/L

Reference:	KCP 5.1.1/15
Report	Validation of analytical method for determination of the active substance (aminopyralid) in the test item AMINO 30 SL solution in deionized water, Kanon, L., 2024, Study code: 0038/0214/FA
Guideline(s):	Yes, experimental procedure SPB-FA/11 and guideline SANTE/2020/12830, rev. 2.
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

Test item
Name: AMINO 30 SL
Test item description: liquid
Type of packaging material: HDPE
Date of delivery: 30.07.2024
Batch No.: 1/24
Name of active substance: aminopyralid
IUPAC name: 4-Amino-3,6-dichloro-2-pyridinecarboxylic acid
Molar mass: 207.01 g/mol
Molecular formula: $C_6H_4Cl_2N_2O_2$



Structural formula:
CAS: 150114-71-9
Content: 29.67 g/L
Density of the test item: 1.0220 g/mL
Date of production: 07.2024
Expiry date: -
Storage conditions temperature: 10-30°C
Reference number: LBS/58/24
Certificate of analysis: Appendix 1

Test method
Validation of method was based on experimental procedure SPB-FA/11 and guideline SANTE/2020/12830, rev. 2. During analytical method validation the following parameters: selectivity, linearity, accuracy, precision (repeatability), limit of detection and limit of quantification were determined. Determination of the active substances was performed by high performance liquid chromatography with PDA detection on the basis of signals from the active substances. Identification of the active substances was performed by comparing the UV spectra and retention times of the active substances standards solutions and the test item solution. In the study manual integration of chromatograms was used.

Reagents and solutions
- aminopyralid standard (HPC Standards, lot number 814229, certificate of analysis – Appendix 2)
- acetonitrile, HPLC grade (VWR, lot number 24F044015)
- methanol, HPLC grade (VWR, lot number 24A104015)
- orthophosphoric acid, 85.1% p.a. (Chempur, lot number 231212505)
- deionized water
- ultrapure water
- 0.1% (v/v) orthophosphoric acid solution (prepared by transferring 1.18 mL of orthophosphoric acid into a flask and filling with ultrapure water up to 1000 mL)

Equipment
- high performance liquid chromatography Shimadzu Nexera X3 series LC-40 with PDA detector
- analytical balance Radwag XA 82/220.4Y.A
- automatic pipettes: Transferpette S 5 mL, Transferpette S 1000 µL, Transferpette S 200 µL
- deionizer SolPure 78
- system for obtaining ultrapure water Millipore Synergy UV
- volumetric flasks class A
- ultrasonic cleaner Sonic 10
- syringe filters 0.22 µm

Chromatographic conditions
Column: Gemini 3 µm C_{18} 110 Å 150 x 4.6 mm
Detection: 250 nm
Injection volume: 20 µL
Column oven temperature: 40°C
Mobile phase acetonitrile (B) : 0.1% (v/v) H3PO4 (A)
Mobile phase composition 70% (B): 30% (A)
Mobile phase flow: 1 mL/min
Time of analysis: 3 min

Validation - Results and discussions

Table 5.2-1: Methods suitable for the determination of active substances in plant protection product El Camino 30 SL/ Ranchero 30 SL/ AMINO 30 SL solution in deionized water

	Aminopyralid
Author(s), year	Kanon, L., 2024
Principle of method	HPLC-DAD
Linearity (linear between mg/L / % range of the declared content) (correlation coefficient, expressed as r)	The active substances standards solutions were used to determine the linearity of the method. By dilution deionized water, solutions of aminopyralid at concentrations: 0.20 mg as/L; 2.00 mg as/L; 5.00 mg as/L; 10.00 mg as/L and 20.00 mg as/L were obtained. After the performed chromatographic analysis, the plots of peaks areas and concentrations of the active substances were prepared. Calibration curves are described by equation: $$f(x) = 52796.7 \cdot x + 3358.8$$ where: f(x) - chromatographic peak area x - concentration of active substance [mg as/L] For each concentration level, the regression residuals (d_i) were determined and the dependence of the regression residuals on the level of the calibration curve was plotted. Regression residuals were calculated according to the formula: $$d_i = y_i - yy_i$$ where: d_i - regression residuals at the level i [mg as/L] y_i . determined active substance concentration at level i [mg as/L] yy_i - theoretical active substance concentration at level i [mg as/L] i - level of the calibration curve Correlation coefficients equals: r= 1.000 Criteria of acceptance were fulfilled: - r≥0.99 - random distribution of regression residues (d_i) was obtained. Range of linearity should ensure the possibility of determining concentrations in the range ≥30% of LOQ value ≤20% above highest, nominal concentration of the analyzed substance.
Precision – Repeatability Mean n = 10 (%RSD)	To determine precision of the method, the results of sample determinations prepared during the determination of accuracy were used. Precision (repeatability) was calculated by determination the value of the relative standard deviation (RSD [%]) for each of concentration level according to equation: $$RSD = 100 \cdot \frac{s}{\bar{x}}$$ where: RSD - relative standard deviation [%] s - standard deviation of repeatability [mg as/L] x - arithmetic mean of the obtained results of the active substance concentration in the test item solution [mg as/L] 100 - unit conversion factor [%] Precision of method equals: level I (mean determined concentration 0.91 mg as/L): 1.10%, level II (mean determined concentration 9.59 mg as/L): 0.31%. Criteria of acceptance were fulfilled: - RSD [%] ≤20% at each fortification level

	Aminopyralid
	- no more than one outlier was identified from the obtained results using the Q-Dixon test at each fortification level. Table 7. The results of precision determination

Sample labeling	Aminopyralid concentration in the test item solution in deionized water [mg/L]	Mean value of concentration [mg/L]	Standard deviation [mg/L]	Precision [% RSD]
0038/0214/FA ap 1_1	0.91	0.91	0.01	1.10
0038/0214/FA ap 1_2	0.91			
0038/0214/FA ap 1_3	0.90			
0038/0214/FA ap 1_4	0.90			
0038/0214/FA ap 1_5	0.91			
0038/0214/FA ap 2_1	9.55	9.59	0.03	0.31
0038/0214/FA ap 2_2	9.62			
0038/0214/FA ap 2_3	9.61			
0038/0214/FA ap 2_4	9.60			
0038/0214/FA ap 2_5	9.58			

Accuracy n = 10 (% Recovery)

Determination of the method accuracy was performed by analysis:
- deionized water (in duplicate)
- the test item solution in deionized water prepared at concentration of aminopyralid: level I: 1.01 mg as/L (in five repetitions),
- the test item solution in deionized water prepared at concentration of aminopyralid: level II: 10.01 mg as/L (in five repetitions).

After determination, recovery (accuracy) of method was calculated by comparing determination results with theoretical concentration of each active substance in the test item solutions in deionized water according to formula:

$$R = \frac{C_{det}}{C_{theo}} \cdot 100$$

where:
R - recovery [%]
C_{det} - determined concentration of the active substance [mg as/L]
C_{theo} - theoretical concentration of the active substance [mg as/L]
100 - unit conversion factor [%]

The theoretical concentration of the active substances in the test item solution was calculated according to formula:

$$C_{theo} = \frac{C_{Ti} \cdot C\%}{100}$$

where:
C_{theo} - theoretical concentration of the active substance [mg as/L]
C_{Ti} - concentration of the test item solution [mg Ti/L]
C% - percentage content of active substance in the test item (calculated according to point 4.5) [% (w/w)]
100 - unit conversion factor [% (w/w)]
Accuracy of method equals: 92.76% (level I: 89.70%, level II: 95.82%)
Criteria of acceptance were fulfilled:
- mean recovery in the range of 70-120% at each fortification level
- no more than one outlier was identified from the obtained results using the Q-Dixon test at each fortification level.

The results of accuracy determination are shown in Table 6.

	Aminopyralid

Table 6. The results of accuracy determination

Sample labeling	Determined concentration of aminopyralid [mg/L]	Theoretical concentration of aminopyralid [mg/L]	Recovery [%]	Mean recovery on levels [%]	Mean method recovery [%]
0038/0214/FA ap 1_1	0.91	1.01	90.10	89.70	92.76
0038/0214/FA ap 1_2	0.91		90.10		
0038/0214/FA ap 1_3	0.90		89.11		
0038/0214/FA ap 1_4	0.90		89.11		
0038/0214/FA ap 1_5	0.91		90.10		
0038/0214/FA ap 2_1	9.55	10.01	95.40	95.82	
0038/0214/FA ap 2_2	9.62		96.10		
0038/0214/FA ap 2_3	9.61		96.00		
0038/0214/FA ap 2_4	9.60		95.90		
0038/0214/FA ap 2_5	9.58		95.70		
0038/0214/FA ap 0_1	0.00	-	-	-	-
0038/0214/FA ap 0_2	0.00				

Interference/ Specificity	For selectivity analysis the following analysis were performed: - mobile phase - deionized water - the active substances standard solution in deionized water at the LOD level, - the test item solution in deionized water at the LOQ level. Retention times and UV spectra of active substances in the standards solutions and the test item solution were compared. Acceptance criteria were fulfilled: - at the retention times of active substances signals on the chromatogram, there were no signals originating from other substances of area exceeding 30% of active substances areas in the test item solution at the LOQ level - comparison of the UV spectra in the standards solutions and the test item solution allowed the identification of the active substances in the test item solution.
Comment	

Conclusion

The analytical method meets the specificity, linearity, precision/repeatability and accuracy criteria specified in SANCO 3030 (99) rev.5.

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.