

REGISTRATION REPORT

Part B

Section 5

Analytical Methods

Detailed summary of the risk assessment

Product code: Salaman 510

Product name(s): **SAVIAL FORTE**

Chemical active substance:

potassium phosphonates (510 g/L, expr. as phosphorous acid)

Central Zone

Zonal Rapporteur Member State: Poland

CORE ASSESSMENT

(authorization)

Applicant: Lainco, S.A. /Exclusivas Sarabia S.A / Biovert S.L.

Submission date: October 2021

MS Evaluation date: July 2022

MS Finalisation date: dd/mm/yyyy

Version history

When	What
October 2021	Application for the first approval of the product's code SALAMAN 510 in Poland.
July 2022	MS-PL evaluation

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zRMS's comments or conclusions are highlighted in grey colour.

5 Analytical methods

5.1 Conclusion and summary of assessment

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

Noticed data gaps are: none

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

Noticed data gaps are: none

Commodity/crop	Supported/ Not supported
Pome fruits	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)

Comments of zRMS:	The presented, below, ionic HPLC analytical method has been validated according to EU Guidance SANCO/3030/99 rev.4, applicable when the test was performed. However the method is acceptable and suitable for the determination of phosphonic acid in the formulation Salaman 510.
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An overview on the acceptable methods and possible data gaps for analysis of potassium phosphonates in the plant protection product Salaman 510 is provided as follows:

Reference:	KCP 5.1/01
Report	“Salaman 510. Content analysis and stability in accelerated storage conditions.” Romo, S. (2012) Cambium Report No. E12079
Guideline(s):	SANCO/3029/99 rev.4 (11 July 2000)
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Method Outline

The content of the active ingredient phosphonic acid (H_3PO_3) in Salaman 510 formulation was determined by dilution of the test item in acidic water and determination by chromatography HPLC with ionic detection.

Validation - Results and discussions

Table 5.2-1: Methods suitable for the determination of active substance potassium phosphonates in the plant protection product Salaman 510

	potassium phosphonates
Author(s), year	Romo, S. (2012)
Principle of method	HPLC with ionic detection
Linearity (linear between mg/L / % range of the declared content) (correlation coefficient, expressed as r)	A calibration curve of the first degree was found. The calibration curve was calculated on three standards, injected by duplicate containing from 0.514 mg/mL to 1.55 mg/ml (0.5089-1.5345 % w/v) of phosphorous acid. The correlation coefficient (r) was found to be 0.9999.
Precision – Repeatability Mean (%RSD)	Overall precision (method precision): the relative standard deviation (RSD) for the whole procedure of sample preparation and measurement of five extracts was found to be 0.68 % which complies with the Horwitz parameter for this concentration (1.48 %). (method precision) The analysis of five injections of the same sample give an RSD value of 0.03% (instrumental precision).
Accuracy (% Recovery)	The accuracy of the method was determined with the standard addition method. Two samples (0.9707 and 1.0808 % w/v Phosphorous acid) were prepared with addition of the known quantity of the standard, with a concentration of phosphonic acid. The two solutions prepared to assay the accuracy correspond to a 13 % and 25 % of the nominal concentration of the test item. Two injection were done with the three samples (test item solution, accuracy solution 1 and accuracy solution 2). The recovery was of 100% from the first preparation and 99.7 of the second, giving a mean average of 99.9%. Therefore, the analytical method presents an acceptable accuracy for the determination of the phosphonic acid in Salaman 510.
Interference/ Specificity	Solutions of blank sample, standard and test item samples were injected in the chromatographic system. No peaks at the retention time of the active ingredient were detected in blank samples.
Acceptance Criteria under San-co 3030/99 rev. 4	zRMS comment
Linearity Range appropriate to the nominal concentration of the analyte \pm 20%. Duplicate determinations at 3 concentrations or single determination at 5 conc.; $R^2 > 0.98$ ($r > 0.99$)	the method is linear between $\pm >> 20\%$ the nominal concentration of phosphonic acid. Duplicate determinations (injections) at 3 concentrations. correlation coefficient $R^2 > 0.98$ ($r > 0.99$) Acceptable
Precision – repeatability n = or > 5 replicate sample determinations; (%RSD)	5 independently weighted formulation sample determinations %RSD < 1.48 (%RSDr) Horrat ratio: Hr < 1 Acceptable

	potassium phosphonates
Accuracy – recovery At least 2 independent recovery determinations the mean recovery should be in the range required by SAN-CO/3030/99	2 independent recovery determinations using the standard addition method. Recovery is between 99.7% – 100% thus between 98-102% Acceptable
Interference/ Specificity interference from other substances in the blank formulation should not contribute more than 3% of the response measured for the target analyte	Specificity chromatograms are provided by the applicant in study report. These demonstrate no analyte interferences and that method is specific to phosphite. HPLC is considered highly specific Acceptable

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

Not relevant, during the formulation process, no impurities of toxicological, ecotoxicological or environmental concern are formed.

zRMS:

Based on Peer Review of the pesticide risk assessment of the active substance potassium phosphonates (EFSA Journal 2012;10(12):2963) no relevant impurities of toxicological, ecotoxicological and/or environmental concern were identified in the active substance as manufactured

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

Since the formulants or other constituents in Salaman 510 are not considered to be of particular toxicological, ecotoxicological or environmental concern, corresponding analytical methods are not considered to be required.

5.2.1.4 Applicability of existing CIPAC methods (KCP 5.1.1)

No CIPAC method is available for the determination of phosphonic acid.

5.2.2 Methods for the determination of residues (KCP 5.1.2)

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

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

Component of residue definition: potassium phosphonates (as phosphonic acid)				
Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing / EU agreed
Plant: high water content and acidic commodities	Primary	0.5 mg/kg	GC/FPD	Guillet, M. & Simonin, B., 1998 (DAR/Monograph) EU agreed
	ILV	0.5 mg/kg	GC/FPD	Wais,A., 1999 (DAR/Monograph) EU agreed
	Confirmatory	0.2 mg/kg	GC/NPD	Mende, 2011 2001 Addendum 1 of the dAR (2005) EU agreed
Plants: high acid content and high water content (<i>citrus, grapes</i>) (residues)	Primary	1.0 mg/kg	Modified QuEChERS multi-residue method of highly polar pesticides with subsequent LC/MS-MS analysis	Sahvorest, N., Gimeno Martos, C (2014a) (KCP 5.2/01a) Sahvorest, N., Gimeno Martos, C 2014b (KCP 5.2/02b)
Plants: high water, high acid and high oil crops (<i>citrus, grapes, apples and olives</i>) (residues)	ILV	1.0 mg/kg	Highly polar pesticides in plant material (QuPPE-Method) with subsequent LC/MS-MS analysis	Martinez, S., 2017 (KCP 5.2/02)
Plants: high water content (<i>pome fruits</i>)	Primary	0.1 mg/kg	LC-MS/MS	Blanco, J., 2020 Report No. S19-03964 (KCP 5.2/03)
Soil (Environmental fate)	Primary	0.1 mg/kg	GC/FPD, wax column	Kieken J.L., 1999 Report 99-135 (AR 214-99) EU agreed
Soil (Environmental fate)	Primary	0.1 mg/kg	GC/FPD, wax column	Barbier G., 2004 Report 04-20 EU agreed
Drinking water (Environmental fate)	Primary	2.0 µg/kg	GC/FPD, wax column	Kieken J.L., 2000 Report 99-211 (AR 231-99) EU agreed
Drinking water Surface water (Environmental fate)	Primary	2.0 µg/kg 4.0 µg/kg	HPLC/ICP/MS, ion Pak column	Diot R., Guyot C., Kieken J.L., 2001 Report R&D/CRLD/an/0015845 EU agreed
Water (ecotoxicological)	Primary	5.0 mg/L 10.0 mg/L	HPLC/IC	Pupp, A. and Wydra, V., 2012a Report IBACON 65673230 (KCP 5.2/04) Pupp, A. and Wydra, V., 2012a Report IBACON 65672220 (KCP 5.2/05) Pupp, A. and Wydra, V., 2012a Report IBACON 65671210 (KCP 5.2/06) Kuhl, R. and Wydra, V., 2014 Report IBACON 65675231 (KCP 5.2/07)

Analytical methods for the determination of residues of phosphonic acid (H₃PO₃) in high water matrices are submitted for citrus (Gimeno Martos, C., 2014a) and grapes (Gimeno Martos, C., 2014b). The

analytical methods have been performed and validated in accordance to SANCO/3030/99 rev.4 with LOQ=1.0 mg/kg and they are acceptable for pre-registration. These methods are also accepted for monitoring purposes. ILV method are provided. Please refer to Table ~~5.3-2~~ below 5.2-2 above.

Analytical method for the determination of residues of potassium phosphonate (as phosphorous acid) in the raw agricultural commodity apple is submitted (Blanco, J., 2020). This LC-MS/MS analytical method has been performed and validated in accordance to SANCO/3030/99 rev.4 with LOQ=0.1 mg/kg and it is acceptable for pre-registration. The ILV method is provided for apples (Martinez, S., 2017), however it based on different method.

The analytical methods proposed for the determination of residues of phosphonic acid (H_3PO_3) in soil and water are the same methods considered for Annex I inclusion of Fosetyl-Al and were considered acceptable.

Analytical methods for determination of phosphonic acid in water have been submitted for ecotoxicological studies in aquatic organisms. The analytical methods are considered in accordance to SANCO/3029/99 rev.4 and were considered acceptable for pre-registration.

Analytical methods for the determination of residues in food of animal origin, air and body fluids and tissues are not required.

The analytical method for determination of potassium phosphonate (expressed as phosphonate acid) in samples from ecotoxicological studies on honeybees was submitted and fully validated according to the requirements of guideline SANCO/3029/99 rev.4 for pre-registration and also according to SANCO/825/00 rev.8.1.

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

5.3.1 Analysis of the plant protection product (KCP 5.2)

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

5.3.2 Description of analytical methods for the determination of residues of potassium phosphonates (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. While the current legal residue definition according to regulation (EU) No 2021/1807 is “fosetyl-Al (sum fosetyl, phosphonic acid and their salts, expressed as fosetyl)”, in the Conclusion on the peer review of the pesticide risk assessment of the active substance potassium phosphonate (EFSA Journal 2012;10(12):2963) “phosphonic acid and its salts, expressed as phosphonic acid” is defined as the relevant residue for monitoring. The EU agreed residue definition was used for the present assessment.

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 Pome fruits (apple and pear)	Phosphonic acid and its salts expressed as phosphonic acid	150 mg/kg	RG(EU) 2019/552 of 04/04/19
Muscle	Not relevant	Not required	EFSA conclusion, EFSA Journal 2012;10(12):2963
Milk		Not required	EFSA conclusion, EFSA Journal 2012;10(12):2963
Eggs		Not required	EFSA conclusion, EFSA Journal 2012;10(12):2963
Fat		Not required	EFSA conclusion, EFSA Journal 2012;10(12):2963
Liver, kidney		Not required	EFSA conclusion, EFSA Journal 2012;10(12):2963
Soil (Ecotoxicology)	Phosphonic acid and its salts expressed as phosphonic acid	0.05 mg/kg	common limit
Drinking water (Human toxicology)	Phosphonic acid and its salts expressed as phosphonic acid	0.1 µg/kg	General limit for drinking water
Surface water (Ecotoxicology)	Phosphonic acid and its salts expressed as phosphonic acid	> 11800 µg/L	LC ₅₀ O. mykiss (96h) EC ₅₀ Daphnia magna (48h) EFSA conclusion, EFSA Journal 2012;10(12):2963
Air	Phosphonic acid and its salts expressed as phosphonic acid	Not required	AOEL: 5 mg/kg bw/d EFSA Journal 2012;10(12):2963
Tissues (meat or liver)	Phosphonic acid and its salts expressed as phosphonic acid	0.1 mg/kg	SANCO/825/00 rev. 8.1
Body fluids		0.05 mg/L	SANCO/825/00 rev. 8.1

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

An analytical method (Guillet & Simonin, 1998) and its ILV (Wais, 1999), using GC/FPD for the determination of Fosetyl-Al residues in plants have already been provided by the notifier (Bayer) in the DAR/Monograph and were considered as validated with **LOQ = 0.5 mg/kg** for phosphonic acid (H₃PO₃) in acidic commodities and in high water content commodities.

A confirmatory method (Mende, [20112001](#)) using GC/NPD was presented and validated in the Addendum 1 of the DAR (2005) with **LOQ=0.2mg/kg** for phosphonic acid separately in high water content commodities. As the extraction is performed at controlled pH, the method is also suitable for acidic crops.

It has been also provided a method for risk assessment which can be used for monitoring. The analytical method (Sahvorost, N. (2014)) with its ILV (Martinez. S, 2017) for the determination of phosphonic acid in high water content, acidic and fatty crops has been provided and validated by LC-MS/MS at **LOQ=1mg/kg** in olives, citrus, grapes and apples. As data have been provided for two mass transitions for the ILV, the method is highly specific.

Please refer to the Table 5.2-2.

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

Analytical methods for the determination of residues of potassium phosphonate in animal matrices are not required.

An overview on the acceptable methods and possible data gaps for analysis of potassium phosphonate in animal matrices is given in the following tables.

Table 5.3-2: Validated methods for food and feed of animal origin (if appropriate)

Component of residue definition: phosphonic acid and its salts, expressed as phosphonic acid				
Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing
Kidney, liver, fat, muscle, milk and eggs	Primary	2.0 mg/kg	LC-MS/MS	...
	ILV	2.0 mg/kg	LC-MS/MS
	Primary	- mg/kg	--	...
	ILV	- mg/kg	--	...

Conclusion on analytical methods for the determination of phosphonic acid in foodstuff of animal origin (DAR from fosetyl-AL, data not anymore protected)

An analytical method AR 172-98 (Wair, 1999) with its ILV (Le Brun, 1999) has been provided in the first monograph of Fosetyl-Al and is not anymore protected. The method has been validated by GC-FPD with **LOQ=0.1mg/L** for phosphonic acid in milk and with **LOQ=0.5mg/kg** for phosphonic acid in egg, meat, liver and kidney. The method is not highly specific.

However, the LOQ is too high in milk, muscle, liver, kidney and egg when compared with the current MRL set for phosphonic acid (0.5mg/kg* in meat, fat, liver, kidney and 0.1mg/kg* in milk and eggs). It should also be noticed that in a previous Reasoned opinion (EFSA Journal 2012;10(11):2961), it has already been accepted to set specific MRL only for phosphonic acid in crops and foodstuff of animal origin. Since the current residue definition for potassium phosphonates is phosphonic acid only, MRLs of the current regulation 2016/1003 can be applied for phosphonic acid. The method AR 172-98 is therefore considered suitable for the determination of phosphonic acid in foodstuff of animal origin.

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

The analytical methods proposed for the determination of residues of phosphonic acid (H₃PO₃) in soil are the same methods considered for Annex I inclusion of fosetyl-Al and were considered acceptable.

No additional data is presented.

Table 5.3-3: Validated methods for soil (if appropriate)

Component of residue definition: phosphonic acid and its salts, expressed as phosphonic acid			
Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing
Primary & confirmatory	0.1 mg/kg	GC/FPD, wax column	Kieken J.L., 1999 Report 99-135 (AR 214-99) EU agreed

Component of residue definition: phosphonic acid and its salts, expressed as phosphonic acid			
Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing
ILV	0.2 mg/kg	LC-MS/MS	Sala,A., 2015c Report RAU-037-15 Perboni, A., 2016d Amendment Report RAU-037-15

Conclusion on analytical methods for the determination of phosphonic acid in soil (DAR of fosetyl-Al, data not anymore protected)

An analytical method (Kieken, 1999), using GC/FPD for the determination of Foseyl-Al residues in soil has already been provided by the notifier (Bayer) in the DAR/Monograph and was considered as validated with **LOQ = 0.1 mg/kg** for Fosetyl-Al and **LOQ = 0.1 mg/kg** for phosphonic acid (H₃PO₃). A confirmatory method is missing for the determination of fosetyl al residue (fosetyl al and phosphonic acid) in soil.

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 potassium phosphonate in surface and drinking water is given in the following table. For the detailed valuation of new studies, it is referred to Appendix 2.

The analytical methods proposed for the determination of residues of phosphonic acid (H₃PO₃) in water are the same methods considered for Annex I inclusion of fosetyl-Al and were considered acceptable.

No additional data is presented.

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

Component of residue definition: phosphonic acid and its salts, expressed as phosphonic acid				
Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing
Drinking water	Primary	2.0 µg/kg	GC/FPD, wax column	Kieken J.L., 2000 Report 99-211 (AR 231-99) EU agreed
	Primary	2.0 µg/kg	HPLC/ICP/MS, ion Pak column	Diot R., Guyot C., Kieken J.L., 2001 Report R&D/CRLD/an/0015845 EU agreed
	Primary	2.0 µg/kg	LC-MS/MS	Sala,A., 2015d Report RAU-038-15
	ILV	2.0 µg/kg	LC-MS/MS	Fifi, A.P., 2015d Report BT147/15
Surface water	Primary	4 µg/L	HPLC/ICP/MS, ion Pak column	Diot R., Guyot C., Kieken J.L., 2001 Report R&D/CRLD/an/0015845 EU agreed
	Primary	2.0 µg/kg	LC-MS/MS	Sala,A., 2015d Report RAU-038-15
	ILV	2.0 µg/kg	LC-MS/MS	Fifi, A.P., 2015d Report BT147/15
	Primary	5 and 10 µg/L	HPLC/IC	Pupp, A. and Wydra, V., 2012a

Component of residue definition: phosphonic acid and its salts, expressed as phosphonic acid				
Matrix type	Method type	Method LOQ	Principle of method (i.e. GC-MS or HPLC-UV)	Author(s), year / missing
				Report IBACON 65673230 (KCP 5.2/04) Pupp, A. and Wydra, V., 2012a Report IBACON 65672220 (KCP 5.2/05) Pupp, A. and Wydra, V., 2012a Report IBACON 65671210 (KCP 5.2/06) Kuhl, R. and Wydra, V., 2014 Report IBACON 65675231 (KCP 5.2/07) Note: method used in the ecotoxicological studies in aquatic organism.

The applicant has no access to the methods from DAR of potassium phosphonates. However, methods from the DAR of fosetyl-Al can be used since they are not anymore under protection.

Conclusion on analytical methods for the determination of phosphonic acid in water (DAR of fosetyl-Al, data not anymore protected)

An analytical method (Kieken, 2000), using GC/FPD for the determination of Fosetyl-Al and HPLC-ICP/MS for H₃PO₃ in surface and drinking water has already been provided in the DAR/Monograph and was considered as validated with the following LOQ:

	Surface water	Drinking Water
phosphonic acid	4 µg/L	2 µg/L

The LOQ determined is also too high for phosphonic acid in drinking water.

A new analytical method completely validated (with LOQ ≤ 0.1 µg/L) for the determination of H₃PO₃ in drinking water and a confirmatory method in surface water is missing.

An ILV for drinking water should be provided at the renewal of the active substance.

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

According to the EFSA conclusion on pesticide peer review (*EFSA Journal 2012;10(12):2963*), “An analytical method for monitoring the active substance in air is not required.”

Potassium Phosphonate is an inorganic substance. The vapour pressure is considered negligible and no volatilization of the technical product potassium phosphite is expected. Therefore, an analytical method is not required.

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

Analytical method for the determination of residues of potassium phosphonate in body fluids and tissues is not required as the active substance is not classified as “toxic” or “highly toxic”. Furthermore, Potassium phosphonate has no metabolites classified as toxic or highly toxic. Therefore, an analytical method is not required.

5.3.2.8 Other studies/ information

A study to determinate the potassium phosphonate (as phosphorous acid) in samples from ecotoxicological studies on honeybees is presented. A summary of the analytical method is presented in Appendix 2 (A.2.A.9 *Other Studies/Information*).

Reference	KCP 5.2/08
Report	Hernández, S. (2017)
Title	“Determination of potassium phosphonate (expressed as phosphonate acid) in samples from ecotoxicological studies on honeybees.”
Document N°	E16145
Guidelines	SANCO/3029/99 Rev.4 SANCO/825/00 rev. 8.1
GLP	Yes

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 5.1/01	Romo, S.	2012	“Salaman 510. Content analysis and stability in accelerated storage conditions.” Report No. E12079 Cambium GLP: yes Unpublished	N	Lainco S.A. Exc.Sarabia S.A. Biovert S.L.
KCP 5.2/01a	Sahvorost, N Gimeno Martos, C	2014a	ANALYTICAL REPORT.- Magnitude of residues in Citrus following three applications with the formulated product SALAMAN 510 (Potassium Phosphite 510 g/L SL) PTRL Europe ID P2743 G (Analytical part of the study TRC12-090) GLP: Yes Unpublished	N	Lainco S.A. Exc.Sarabia S.A. Biovert S.L.
KCP 5.2/01b	Sahvorost, N Gimeno Martos, C	2014b	ANALYTICAL REPORT.- Magnitude of residues in Grapevine following three applications with the formulated product SALAMAN 510 (Potassium Phosphite 510 g/L SL) PTRL Europe ID P2744 G (Analytical part of the study TRC12-244) GLP: Yes Unpublished	N	Lainco S.A. Exc.Sarabia S.A. Biovert S.L.
KCP 5.2/02	Martinez, S.	2017	“Determination of phosphonate residues in plant matrices (citrus, grapes, olives and apples). Independent laboratory validation.” Report No. E15011 Cambium, S.L. GLP: yes Unpublished	N	Lainco S.A. Exc.Sarabia S.A. Biovert S.L.

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 5.2/03	Blanco, J.	2020	Analytical phase report of study: “Determination of residues of potassium phosphonate (as phosphorous acid) after three applications of SALAMAN 510 in apple (outdoor) at 3 sites in Poland, 2019.” Report No. S19-03964 Eurofins AgroSciences Services GLP: yes Unpublished	N	Lainco S.A. Exc.Sarabia S.A. Biovert S.L.
KCP 5.2/04	Pupp and Wydra	2012a	“Acute Toxicity of Salaman 510 (510 g/L phosphorous acid) to Rainbow Trout (<i>Oncorhynchus mykiss</i>) in a 96-hour Static Limit Test.” Analytical phase of the study Nr.- IBACON 65673230 GLP: Yes Published	N	Lainco S.A. Exc.Sarabia S.A. Biovert S.L.
KCP 5.2/05	Pupp and Wydra	2012b	“Acute Toxicity of Salaman 510 (510 g/L phosphorous acid) to Daphnia magna in a Static 48-hour Immobilisation Limit-Test.” Analytical phase of the study Nr.- IBACON 65672220 GLP: Yes Published	N	Lainco S.A. Exc.Sarabia S.A. Biovert S.L.
KCP 5.2/06	Pupp and Wydra	2012c	“Toxicity of Salaman 510 (510 g/L phosphorus acid) to Pseudokirchneriella subcapitata in an Algal Growth Inhibition Test.” Analytical phase of the study Nr.- IBACON 65671210 GLP: Yes Published	N	Lainco S.A. Exc.Sarabia S.A. Biovert S.L.
KCP 5.2/07	Pupp and Wydra	2013	“Toxicity of Salaman 510 (510 g/L phosphorous acid) to Rainbow Trout (<i>Oncorhynchus mykiss</i>) in a Prolonged Semi Static Test over 28 Days.” Analytical phase of the study Nr.- IBACON 65675231 GLP: Yes Published	N	Lainco S.A. Exc.Sarabia S.A. Biovert S.L.
KCP 5.2/08	Hernández, S.	2017	“Determination of potassium phosphonate (expressed as phosphonate acid) in samples from ecotoxicological studies on honeybees.” Report No. E16145 Cambium, S.L. GLP: yes Unpublished	N	Lainco S.A. Exc.Sarabia S.A. Biovert S.L.

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
Annex II 4.2.1.1	Guillet, M., Simonis B	1998	Fosetyl-al and its metabolites (phosphorous acid): Analytical method for the determination of residues in plant products. Report number R003461 (Bayer (former Aventis CropScience)) Rhone-Polenc Secteur Agro R&D/CRDL/AN/9746801 GLP: no Not published.	N	Bayer
Annex II 4.2.1.2	Wais, A	1999	Fosetyl-al nd its metabolite (phosphorous acid) – Validation of an analytical method for the determination of residues in plant products (banana, grape, lettuce, hop) Report number R004565 (Bayer (former Aventis CropScience)) Rhone-Polenc Secteur Agro (Study: nr. 98-125) GLP: no Not published.	N	Bayer
HA 4.2.1/3	Mende, P.	2011	Independent laboratory validation (ILV) of an analytical method for determination of residue of phosphonic acid in food of plant origin. Report nr: S11_03203 (Eurofins Agroscience Services EcoChem GmbH Luxembourg Industries Ltd GLP: yes Not published	Y	LBG
Annex II Point 4.2.1	Mende, P	2001	Analytical method for determination of residues of fosetyl and phosphorous acid in plant material Source: Bayer Cropscience Document No: C011966 GLP : yes Not published	N	Bayer

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
Annex II 4.2.2	Kieken J.L.	1999	“Fosetyl-Al and its metabolite (phosphorous acid). Analytical method for the determination of residues in soil. Method AR 214-99.” R&D/CRLD/AN/9916463 Study n° 99-135; Report Nr.-R011736 GLP: No Published	N	
Annex II, 4.2.3	Kieken J.L.	2000	“Fosetyl-Al and its metabolite (phosphorous acid). Analytical method for the determination of residues in drinking water and surface water. Method AR 231-99.” R&D/CRLD/AN/0015093 Study n° 99-211; Report Nr.-R011760 GLP: No Published	N	
Annex II, 4.2.3	Diot R., Guyot C., Kieken J.L.	2001	“Phosphorous acid. Evaluation of the HPLC/ICP/MS technique for the determination of residue in drinking water and surface water.” R&D/CRLD/AN/0015845 Study n° 448163; Report Nr.-R013051 GLP: No Published	N	

List of data submitted by the applicant and relied on, but already evaluated at EU peer review for the renewal of Fosetyl-Al a.s.

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCA 4.2/04	...	2015	“Validation of the analytical method to determine residue of Phosphorous Acid in different matrices of animal origin (kidney, liver, fat, muscle, milk and eggs)” Report No.... ... GLP: yes Unpublished	N	Sipcam Oxon*
-	...	2016	“Amendment No. 1 to the Final Report RAU-059-15: Validation of the analytical method to determine residue of Phosphorous Acid in different matrices of animal origin (kidney, liver, fat, muscle, milk and eggs)” ... GLP: yes Unpublished	N	Sipcam Oxon*
KCA 4.2/06	...	2015c	“Independent Laboratory Validation of the analytical method to determine residue of Phosphorous acid in different animal origin matrices (kidney, liver, fat, muscle, milk and eggs)” Report No. GLP: yes Unpublished	N	Sipcam Oxon*
-	...	2016c	“Amendment No. 1 to the Final Report ...: Independent Laboratory Validation of the analytical method to determine residue of Phosphorous acid in different animal origin matrices (kidney, liver, fat, muscle, milk and eggs)” GLP: yes Unpublished	N	Sipcam Oxon*
KCA 4.2/07	Sala, A.	2015c	“Validation of the analytical method to determine residues of Fosetyl-Aluminium and Phosphorous acid residues in soil (sandy loam)” Report No. RAU-037-15 Research Center BioSphereS, Lodi, Italy GLP: yes Unpublished	N	Sipcam Oxon*

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
-	Perboni, A.	2016d	“Amendment No. 1 to the Final Report RAU-037-15: Validation of the analytical method to determine residues of Fosetyl-Aluminium and Phosphorous acid residues in soil (sandy loam)” Research Center BioSphereS, Lodi, Italy GLP: yes Unpublished	N	Sipcam Oxon*
KCA 4.2/08	Sala, A.	2015d	“Validation of the analytical method to determine residues of Fosetyl-Aluminium and Phosphorous acid residues two kinds of water matrices (drinking water and surface water)” Report No. RAU-038-15 Research Center BioSphereS, Lodi, Italy GLP: yes Unpublished	N	Sipcam Oxon*
BT147/09	Fifi, A.P.	2015d	“Independent Laboratory Validation of the analytical method to determine residue of Fosetyl-Al and Phosphorous acid in water (drinking and surface)” Report No. BT147/15 BioTecnologie BT S.r.l., Todi, Italy GLP: yes Unpublished	N	Sipcam Oxon*
-	...	2016	“Validation of the analytical method to determine residues of Phosphorous acid in different animal origin matrices (kidney, liver, fat, muscle, milk and eggs)” Report No.. ... GLP: yes Unpublished	N	Sipcam Oxon*
-	...	2016a	“Independent Laboratory Validation of the analytical method for the determination of Phosphorous acid in different matrices of animal origin (kidney, liver, fat, muscle, milk and eggs) validated in the Study .. by ... GLP: yes Unpublished	N	Sipcam Oxon*

** The Potassium Phosphonates Task Force (Lainco, S.A., Biovert, S.L., Exclusivas Sarabia, S.A) has access to these studies. Refer to the Letter of Access submitted.

The following tables are to be completed by MS

List of data submitted by the applicant and not relied on

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

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

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

Appendix 2 Detailed evaluation of submitted analytical methods

A 2.1 Analytical methods for potassium phosphonate

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

Comments of zRMS:	The presented, below, ionic HPLC analytical method has been validated according to EU Guidance SANCO/3030/99 rev.4, applicable when the test was performed. However the method is acceptable and suitable for the determination of phosphonic acid in the formulation Salaman 510.
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Reference:	KCP 5.1/01
Report:	“Salaman 510. Content analysis and stability in accelerated storage conditions.” Romo, S. (2012) Cambium Report No. E12079
Guideline(s):	SANCO/3029/99 rev.4 (11 July 2000)
Deviations:	--
GLP:	Yes
Acceptability:	Yes

Method Outline

The content of the active ingredient phosphonic acid (H_3PO_3) in Salaman 510 formulation was determined by dilution of the test item in acidic water and determination by chromatography HPLC with ionic detection.

Validation of the method

An analytical method for the determination of phosphonic acid (H_3PO_3) in Salaman 510 was validated with respect to specificity, linearity of detector response, precision and accuracy. The method is characterized with the follows:

Specificity

Solutions of blank sample, standard and test item samples were injected in the chromatographic system. No peaks at the retention time of the active ingredient were detected in blank samples.

Linearity

A calibration curve of the first degree was found. The calibration curve was calculated on three standards, injected by duplicate containing from 0.514 mg/ml to 1.55 mg/ml of phosphorous acid. The correlation coefficient (r) was found to be 0.9999

Precision

Overall precision (method precision): the relative standard deviation (RSD) for the whole procedure of sample preparation and measurement of five extracts was found to be 0.68% which complies with the Horwitz parameter for this concentration (1.48%). (method precision)

The analysis of five injections of the same sample gave a RSD value of 0.03% (instrumental precision)

Accuracy

The accuracy of the method was determined with the standard addition method. Two samples were prepared with addition of the known quantity of the standard, with a concentration of phosphonic acid. The two solutions prepared to assay the accuracy correspond to a 13% and 25% of the nominal concentration of the test item. Two injection were done with the three samples (test item solution, accuracy solution 1 and accuracy solution 2). The recovery was of 100% from the first preparation and 99.7 of the second, giving a mean average of 99.9%. Therefore, the analytical method presents an acceptable accuracy for the determination of the phosphonic acid in Salaman 510.

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

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

A 2.1.2.1.1 Analytical method for the determination of residues of potassium phosphonate in POME FRUITS

Comments of zRMS:	The method was fully validated for the determination of the residues of phosphonic acid and its salts in citrus samples and processed matrices with a LOQ = 1.0 mg/kg (high acid and high water content commodities) according to the guideline SANCO/3029/99 rev.4. Mean recovery values obtained by LC-MS/MS for both fortification levels and for both MRN transition (quantification and confirmation) comply with the standard acceptance criteria of guideline SANCO/3029/99 rev.4, (the mean recovery at each fortification level in the range of 70-110% and the RSD ≤20%). It is therefore concluded, that the method is applicable on citrus samples and processed matrices using HPLC with MS-MS detection. This method can be used for pre-registration purposes.
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Reference:	KCP 5.2/01a; Gimeno Martos, C, 2014a
Report:	“Magnitude of residues in Citrus Following three applications with the formulated product SALAMAN 510 (Potassium Phosphite 510 g/L SL).” Savorost, N Gimeno Martos, C (2014a) PTL Europe Report No.: ID P2743 G (Analytical part of the study TRC12-090)
Guideline(s):	SANCO/3029/99 rev.4
Deviations:	--
GLP:	Yes
Acceptability:	Yes

Method Outline

Residues of phosphonic acid and its salts were determined using a method developed for the quick determination of highly polar pesticides in plant material (QuPPE-Method). Specimens were homogenized, extracted with methanol containing 1 % formic acid and phosphonic acid was determined by LC-MS/MS. The analytical method as applied to phosphonic acid achieves a limit of quantification (LOQ) of 1.0 mg/kg per matrix group (matrices with high and low water content, fluid matrices).

Two MRN transitions were monitored, one for primary quantification and one for quantitative confirmation.

Method validation

Specificity

Highly specific LC-MS/MS uses the MRM transition at 81 m/z → 79 m/z for primary quantification of phosphonic acid. The 2nd MRM transition was employed for qualitative confirmation of residue results. LC-MS/MS allows detection of phosphonic acid concentrations of as low as 0.50 ng/mL with 50 µL injections, therefore providing sufficient sensitivity to determine and to confirm residues of the analyte in the final extracts.

Linearity

Linearity of the method for the determination of phosphoric acid was studied in the range of nominal concentrations between 0.5 and 50 ng/mL, including five standard solutions. The lower margin of the linearity test was 20 % of the LOQ.

Calibration

Calibration standards in solvent were injected for the determination of the retention time and for preparing the standard calibration curves. The calibration curves were obtained by correlation of the peak area of the analytical standards with their corresponding concentration in ng/mL.

The calibration functions were calculated by linear regression analysis. The correlation coefficients (r) of the 1/x weighted linear regression for both MS/MS transitions monitored were always > 0.99. Example of the calibration data and calibration curve are given in the following tables.

Linearity study for phosphonic acid in solvent.

MS/MS transition 81 → 79 m/z (quantification)	
Conc. (ng/mL)	Average response
0.50	1.55E+05
0.50	1.63E+05
2.50	6.61E+05
5.00	1.31E+06
10.00	2.83E+06
25.00	7.01E+06
50.00	1.39E+07
Y = 2.78 x 10 ⁵ x X + 1.24 x 10 ⁴ (r=0.9998)	

MS/MS transition 81 → 63 m/z (confirmation)	
Conc. (ng/mL)	Average response
0.50	5.48E+04
0.50	6.42E+04
2.50	2.95E+05
5.00	5.18E+05
10.00	1.15E+06
25.00	2.84E+06
Y = 1.12 x 10 ⁵ x X + 2.99 x 10 ³ (r=0.9997)	

Matrix effects

Matrix effects (i.e. response of analyte in calibration solutions in solvent versus response in matrix matched calibration solutions) were not significant (i.e. < 20%).

LC-MS/MS allows the detection of phosphonic acid at concentrations as low as 0.50 ng/mL with 50-µL injections, therefore providing sufficient sensitivity to determine and to confirm residues of the analyte in the final extracts. Highly specific LC-MS/MS uses the MRM transition at 81 → 79 m/z for primary quantification and 81 → 63 m/z for quantitative confirmation of residue results. Thus, no additional confirmatory method is required for the unambiguous detection and determination of phosphonic acid results.

Precision

Accuracy of the analytical method for citrus specimens was studied by means of recovery experiments with blank samples fortified at two concentration levels with phosphonic acid, at the LOQ level (1.0 mg/kg) and at a higher level (50xLOQ) which covers the highest residues found. Precision and repeatability were also estimated from these experiments. Fortified specimens were processed and analyzed concurrently with the field specimens.

LOQ: 1.0 mg/kg for phosphonic acid.

LOD: 0.3 mg/kg for phosphonic acid (30% of the LOQ)

Acceptable average recoveries ranging from 70 to 110 % with relative standard deviations ≤ 20 % were observed for phosphonic acid, as summarized below:

Fortification in fruit, flesh, puree and wet pomace (matrices with high level water)

Fortification level		Recoveries			No. of analysis	Overall recovery	
Level	[mg/kg]	Singles values [%]	Mean	RSD		Mean	RSD
Phosphonic acid 81 m/z ->79 m/z (quantification)							
LOQ	1.0	105, 109, 103, 110, 108, 97(2), 106	104%	5%	8	101%	8%
50xLOQ	50	96, 104, 84, 81, 109, 99, 101, 98, 104, 102, 108	99%	9%	11		
Phosphonic acid 81 m/z ->63 m/z (confirmation)							
LOQ	1.0	106, 110, 104, 109, 108, 100, 107, 97	105%	4%	8	102%	8%
50xLOQ	50	97, 104, 84, 79, 109, 100(2), 98, 104(2), 107	99%	9%	11		

Fortification in water and juice (fluid matrices)

Fortification level		Recoveries			No. of analysis	Overall recovery	
Level	[mg/kg]	Singles values [%]	Mean	RSD		Mean	RSD
Phosphonic acid 81 m/z ->79 m/z (quantification)							
LOQ	1.0	111, 105, 98, 104, 110	105%	5%	5	103%	6%
50xLOQ	50	92, 106(2), 96, 101	100%	6%	5		
Phosphonic acid 81 m/z ->63 m/z (confirmation)							
LOQ	1.0	108, 105, 101, 110, 112	107%	4%	5	103%	7%
50xLOQ	50	89, 105, 104, 96, 102	97%	7%	5		

Fortification in peel, dry pomace, wastes and marmalade (matrices with low water content)

Fortification level		Recoveries			No. of analysis	Overall recovery	
Level	[mg/kg]	Singles values [%]	Mean	RSD		Mean	RSD
Phosphonic acid 81 m/z ->79 m/z (quantification)							
LOQ	1.0	104, 99, 90, 110(2), 105, 98, 100, 108	103%	6%	9	99%	10%
50xLOQ	50	84, 89, 97, 80, 96, 109, 113	95%	13%	7		
Phosphonic acid 81 m/z ->63 m/z (confirmation)							
LOQ	1.0	104, 103, 90, 108(2), 110, 105, 100, 102	103%	6%	9	100%	10%
50xLOQ	50	83, 90, 93, 82, 96, 109, 111	95%	12%	7		

Conclusion:

The method is acceptable for the analysis of phosphonic acid in citrus in field applications.

Comments of zRMS:	<p>The method was fully validated for the determination of the residues of phosphonic acid and its salts in grapes samples and processed matrices with a LOQ = 1.0 mg/kg, according to the guideline SANCO/3029/99 rev.4.</p> <p>Mean recovery values obtained by LC-MS/MS for both fortification levels and for both MRN transition (quantification and confirmation) comply with the standard acceptance criteria of guideline SANCO/3029/99 rev.4, (the mean recovery at each fortification level in the range of 70-110% and the RSD ≤20%). It is therefore concluded, that the method is applicable on grapevine samples and processed matrices using HPLC with MS-MS detection.</p> <p>This method can be used for pre-registration purposes.</p>
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Reference:	KCP 5.2/01b (Sahvorost, N-Gimeno Martos, C, 2014b)
Report:	<p>“Magnitude of residues in Grapevine Following three applications with the formulated product SALAMAN 510 (Potassium Phosphite 510 g/L SL).”</p> <p>Sahvorost, N-Gimeno Martos, C, (2014b)</p> <p>PTRL Europe ID P2744 G</p> <p>Analytical part of the study TRC12-244)</p>
Guideline(s):	SANCO/3029/99 rev.4
Deviations:	--
GLP:	Yes
Acceptability:	Yes

Method Outline

Residues of phosphonic acid and its salts were determined using a method developed for the quick determination of highly polar pesticides in plant material (QuPPE-Method). Specimens were homogenized, extracted with methanol containing 1 % formic acid and phosphonic acid was determined by LC-MS/MS. The analytical method as applied to phosphonic acid achieves a limit of quantification (LOQ) of 1.0 mg/kg per matrix group (matrices with high and low water content, fluid matrices).

Two MRN transitions were monitored, one for primary quantification and one for quantitative confirmation.

Method validation

Specificity

Highly specific LC-MS/MS uses the MRM transition at 81 m/z → 79 m/z for primary quantification of phosphonic acid. The 2nd MRM transition is employed for qualitative confirmation of residue results. LC-MS/MS allows detection of phosphonic acid concentrations of as low as 0.50 ng/mL with 50 µL injections, therefore providing sufficient sensitivity to determine and to confirm residues of the analyte in the final extracts.

Linearity

Linearity of the method for the determination of phosphoric acid is studied in the range of nominal concentrations between 0.5 and 50 ng/mL, including five standard solutions. The lower margin of the linearity test is 20 % of the LOQ.

Calibration

Calibration standards in solvent were injected for the determination of the retention time and for preparing the standard calibration curves. The calibration curves were obtained by correlation of the peak area of the analytical standards with their corresponding concentration in ng/mL.

The calibration functions were calculated by linear regression analysis. The correlation coefficients (r) of the 1/x weighted linear regression for both MS/MS transitions monitored were always > 0.99. Example of the calibration data and calibration curve are given in the following tables.

Linearity study for phosphonic acid in solvent.

MS/MS transition 81 → 79 m/z (quantification)		MS/MS transition 81 → 63 m/z (confirmation)	
Conc. (ng/mL)	Average response	Conc. (ng/mL)	Average response
0.50	1.23E+05	0.50	5.14E+04
1.00	1.59E+05	1.00	1.04E+05
1.00	1.64E+05	1.00	1.07E+05
5.00	1.23E+06	5.00	5.13E+05
10.00	2.52E+06	10.00	1.05E+06
10.00	2.48E+06	10.00	1.02E+06
25.00	6.77E+06	25.00	2.82E+06
25.00	6.71E+06	25.00	2.78E+06
50.00	1.28E+07	50.00	5.32E+06
Y = 2.6 x 10 ⁵ x X - 6.74 x 10 ³ (r=0.9994)		Y = 1.08 x 10 ⁵ x X - 4.88 x 10 ³ (r=0.9994)	

Matrix effects

Matrix effects (i.e., response of analyte in calibration solutions in solvent versus response in matrix matched calibration solutions) were not significant (i.e. < 20%).

Precision

Accuracy of the analytical method for grapes specimens is studied by means of recovery experiments with blank samples fortified at two concentration levels with phosphonic acid, at the LOQ level (1.0 mg/kg) and at a higher level (50xLOQ) which covers the highest residues found. Precision and repeatability are also estimated from these experiments. Fortified specimens are processed and analyzed concurrently with the field specimens.

LOQ: 1.0 mg/kg for phosphonic acid.

LOD: 0.3 mg/kg for phosphonic acid (30% of the LOQ)

Acceptable average recoveries ranging from 70 to 110 % with relative standard deviations ≤ 20 % were observed for phosphonic acid, as summarized below:

Fortification in bunches, fruit, berries, washed berries, wet pomace and must (matrices with high water content)

Fortification level		Recoveries			No. of analysis	Overall recovery	
Level	[mg/Kg]	Singles values [%]	Mean	RSD		Mean	RSD
Phosphonic acid 81 m/z ->79 m/z (quantification)							
LOQ	1.0	107, 109, 96, 106, 101	104%	5%	5	105%	4%
50xLOQ	50	109, 110, 109, 99, 106, 108	107%	4%			
Phosphonic acid 81 m/z ->63 m/z (confirmation)							
LOQ	1.0	106, 107, 96, 106, 102	104%	4%	5	105%	4%
50xLOQ	50	108, 110, 108, 100, 107, 110	107%	4%			

Fortification in AF wine, PF wine, MF wine, lees, wine, sediments and aged wine (fluid matrices)

Fortification level		Recoveries			No. of analysis	Overall recovery	
Level	[mg/Kg]	Singles values [%]	Mean	RSD		Mean	RSD

		<i>Phosphonic acid 81 m/z ->79 m/z (quantification)</i>					
LOQ	1.0	106, 100, 102, 110(2), 108, 109	106%	4%	7	104%	4%
50xLOQ	50	105, 97, 103, 104, 106, 108, 97	103%	4%	7		
		<i>Phosphonic acid 81 m/z ->63 m/z (confirmation)</i>					
LOQ	1.0	107, 100(2), 109, 105, 104, 110	105%	4%	7	104%	4%
50xLOQ	50	106, 96, 105, 108(2), 106, 100	104%	4%	7		

Fortification in stems and dry pomace (matrices with low water content)

Fortification level		Recoveries			No. of analysis	Overall recovery	
Level	[mg/Kg]	Singles values [%]	Mean	RSD		Mean	RSD
		<i>Phosphonic acid 81 m/z ->79 m/z (quantification)</i>					
LOQ	1.0	76, 109	93%	--	2	92%	19%
50xLOQ	50	78, 106	92%	--	2		
		<i>Phosphonic acid 81 m/z ->63 m/z (confirmation)</i>					
LOQ	1.0	78, 113	95%	--	2	94%	19%
50xLOQ	50	80, 107	93%	--	2		

Conclusion:

The method is acceptable.

* * * * *

Comments of zRMS:	<p>The method was fully validated for the determination of the residues of phosphonic acid and its salts in apple samples with a LOQ = 0.1 mg/kg, according to the guideline SANCO/3029/99 rev.4.</p> <p>Mean recovery values obtained by LC-MS/MS for both fortification levels (of 0.1 mg/kg (LOQ) and 1.0 mg/kg (10x LOQ)) and for one mass transition (quantification) comply with the standard acceptance criteria of guideline SANCO/3029/99 rev.4, (the mean recovery at each fortification level in the range of 70-110% and the RSD ≤20%). Matrix effects on LC-MS/MS detection were investigated and found to be insignificant. It is therefore concluded, that the method is applicable on apple samples and processed matrices using HPLC with MS-MS detection.</p> <p>This method can be used for pre-registration purposes.</p>
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Reference:	KCP 5.2/03 (Blanco, J., 2020)
Report:	<p>“Determination of residues of potassium phosphonate (as phosphorous acid) after three applications of SALAMAN 510 in apple (outdoor) at 3 sites in Poland, 2019.”</p> <p>Blanco, J. (2020)</p> <p>Eurofins AgroSciences Services</p> <p>Report No. S19-03964</p>
Guideline(s):	SANCO/3029/99, rev. 4.
Deviations:	--
GLP:	Yes
Acceptability:	Yes

Method outline

The aim of the study was to determine residue levels of potassium phosphonate (as phosphorous acid) in the raw agricultural commodity apple.

The residue analytical method was based on LC-MS/MS detection.

The Limit of quantification (LOQ) of the analytical method was 0.1 mg/kg with a limit of detection (LOD) set at 0.03 mg/kg (30% of the LOQ).

Method summary

In brief, 10 g of apple samples were extracted in 50 mL centrifugation tubes by adding 20 mL methanol/water (1:1 v/v) and shaking by a horizontal flatbed shaker for 10 min at maximal speed; then centrifuged for 5 minutes at 4000 rpm. The upper layer was decanted into 50 mL volumetric flask. These process was repeated once more, the second extract was also decanted into the same flask. The combined extracts were adjusted to 50 mL with extraction solution and mixed well. An aliquot of about 1.5 mL of the extract was filtered through a 0.45 µm single-use syringe-top regenerated cellulose filter. 50 µL of obtained each sample filtrated extract was transferred into HPLC vials with 950 µL extraction solution for analysis.

Method conditions

A summary of the typical chromatographic and mass spectrometric conditions used for quantification is included in the following table.

Chromatographic conditions						
HPLC system	Shimadzu HPLC system, Software: Analyst 1.6.3					
Pre-column	HPLC guard column (KJO-4282, Phenomenex) wit 4 m C18 cartridge (AJO-4287, Phenomenex)					
Column	Thermo Hypercarb, 100 mm x 4 mm, 5 µm (Part No. 35005-104030)					
Column oven temperature	30 °C					
Injection volume	40 µL					
Mobile phases	Eluent A: water + 0.5% formic acid Eluent B: methanol					
Isometric flow	% Eluent A	% Eluent B		Flow (µL/min)		
	85	15		800		
Divert valve	0.0 min to 2.7 min to waste; 2.7 min to 4.6 min to MS; 4.6 min to 8 min to waste					
Retention time	Approx. 3.1 min (phosphonic acid)					
Mass spectrometric conditions for Phosphonic acid						
MS system	SCIEX 5500 System, SCIEX (Triple quadrupol mass spectrometer)					
Ionisation type	Electrospray ionization (ESI, Turbolon Spray)					
Polarity	Negative ion mode					
Scan type	MS/MS, Multiple Reaction Monitoring (MRM)					
Capillary voltage (IS)	-4500 V		Ionspray turbo heater (TEM)		550 °C	
Curtain gas (CUR)	30 (arbitrary units)		Gas flow 1 (GS1)		70 (arbitrary units)	
Collision gas (CAD)	8 (arbitrary units)		Gas flow 2 (GS2)		40 (arbitrary units)	
Analyte monitored	Ion mass transition monitored (m/z)	Declustering potential (DP) [V]	Entrance potential (EP) [V]	Collision energy (CE) [V]	Cell exit potential (CXP) [V]	Dwell time [ms]
	Phosphonic acid	81 → 79*	-75	-10	-22	-7

	81 → 63	-75	-10	-35	-7	500
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* Proposed (and/or used) for quantification but both of the mass transitions listed can be used for quantification.

METHOD VALIDATION

Selectivity

The analyte was determined in the final sample extracts by use of LC-MS/MS detection.

One (1) mass transition was evaluated. A second mass transition was monitored for confirmation of peak identity but was not used for quantification of samples.

Untreated samples for accompanying control sample work up, for determination of (procedural) recoveries and, if needed, for preparation of matrix-matched calibration standards originated from the current study.

At least one (1) control sample per analytical set was analysed to investigate the residue level of the analyte and to check for any background interferences at the expected retention time of the analyte.

The blank values at the expected retention times of the analyte of the control sample material that were used for determinations of the recoveries did not exceed 30 % of the LOQ.

Since blank peaks were not observed blank correction was not necessary.

Furthermore, at least one (1) reagent blank sample, which is a sample work up without matrix present, was conducted with each analytical set. Reagent blank values did not exceed 30 % of the LOQ.

Matrix Effects

The effect of matrix on the LC-MS/MS response was assessed by comparing peak areas of matrix-matched standards with solvent standards at identical nominal concentrations. Matrix effects were calculated as follows:

$$\text{Matrix effect (\%)} = [(100 \cdot A_{\text{Matrix-Std}}) / (A_{\text{Solv-Std}})] - 100$$

$A_{\text{Matrix-Std}}$: Peak area of solvent standard

$A_{\text{Solv-Std}}$: Peak area of matrix-matched standard

The matrix effects are summarised in the table below:

Matrix / Commodity	Standard Concentration (ng/mL)	Matrix effect for phosphonic acid (%)	
		Quantification (m/z 81 → 79)	Confirmation (m/z 81 → 63)
Study No. S19-03964			
Apple (fruits)	1	-3.3	-2.7
	2.5	-4.4	-4.4
	5	-6.5	-7.6
	10	-12	-13
	15	-13	-13
	20	-10	-12
	30	-4.8	-6.2
	Mean	-7.9	-8.3

(+) matrix enhancement; (-) matrix suppression

Matrix effects were $\geq \pm 20$ % and deemed to be significant. However, matrix-matched standards were used for quantification throughout the analytical phase.

Linearity

The linearity of the detector response was demonstrated by single determination of matrix-matched standards at eight concentration levels ranging from 0.30 ng/mL to 30 ng/mL. This range corresponds to a for-

tification level of 0.03 mg/kg to 3 mg/kg and thus covers the range from no more than 30 % of the LOD and at least + 20 % of the highest analyte concentration detected in any (diluted) sample extract.

The calibration curves obtained for both mass transitions/ions/analytes and all matrices were linear since coefficients of determination (R^2) were ≥ 0.999 . Linear regression was performed with 1/x-weighting.

Quantification

Quantification was performed using a calibration curve that fulfilled the above given criteria. The injection of standard solutions was spread evenly over the whole analytical sequence. The linear regression equation was used for calculation of the analyte concentrations.

If necessary, sample extracts and extracts from high level recovery samples were diluted with control matrix extract at least by a factor of 20 to be within the calibration range.

Procedural Recoveries

The method's applicability in terms of accuracy and repeatability was assessed for each analytical set by fortification of control (untreated) test portions of the respective matrix and subsequent determination of the procedural recoveries upon applying the analytical method.

Procedural recoveries were handled and stored in the same way and for the same time period as the analytical sample extracts that we prepared within the same analytical set.

At least one (1) procedural recovery was performed at the level of LOQ and one (1) at the level of 10x LOQ per analytical set.

Higher residues were confirmed by one (1) recovery determination in the range of the level or higher than the level of the highest residues found in a sample.

The following procedural recoveries were obtained:

Phosphonic acid (validation)							
Matrix	Fortification Level (mg/kg)	Procedural Recovery (%)	Mean Recovery (%)	Rel. Std. Dev. (%)	Replicates	Overall Mean Recovery (%)	Overall Rel. Std. Dev. (%)
Study No. S19-03964							
Mass transition m/z 81 \rightarrow 79 (quantification)							
Apple (fruit)	0.1 (LOQ)	92.9, 98.1, 97.6	96.2	3.0	3	92.4	5.1
	1	90.9, 88.4, 86.5	88.6	2.5	3		
Mass transition m/z 81 \rightarrow 63 (quantification)							
Apple (fruit)	0.1 (LOQ)	96, 98.4, 93.7	96.0	2.5	3	92.5	4.7
	1	91.2, 88.3, 87.5	89.0	2.0	3		

No observable peak was detected in any control sample extract.

Recoveries are without any blank correction.

Single recoveries were in the range of 60 - 120 % each, while the mean recoveries at each fortification level were in the range of 70 - 110 %. Wherever applicable ($n \geq 3$), the relative standard deviation was ≤ 20 % for each level.

LOQ

The limit of quantification is 0.1 mg/kg for phosphonic acid in apple.

Conclusion:

The method is acceptable for the analysis of phosphonic acid in apples.

A 2.1.2.1.1 Independent laboratory validation

Comments of zRMS:	<p>The independent laboratory validation of an analytical method for the analysis of phosphonate residues in plant matrices of citrus, grapes, olives and apples was provided.</p> <p>The method was fully validated for the determination of the residues of phosphonic acid in each matrix with a LOQ = 1.0 mg/kg, according to requirements of the guideline SANCO/825/00 rev.8.1.</p> <p>The method can be regarded as an ILV for the determination of phosphonic acid in citrus and grapes (high acid content commodity) of the Gimeno Martos, C. (2014a) and Gimeno Martos, C. (2014b) method.</p>
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Reference:	KCP 5.2/02 (Martinez, S., 2017)
Report	<p>“Determination of phosphonate residues in plant matrices (citrus, grapes, olives and apples). Independent laboratory validation.”</p> <p>Martínez, S. (2017). Cambium, S.L. Report No. E15011</p>
Test facility (analytical part)	CAMBIUM S.L.
Guideline(s):	SANCO/3029/99 rev.4
Deviations:	--
GLP:	Yes
Acceptability:	Yes

Method outline

The aim of the study was to provide an independent laboratory validation of an analytical method for the analysis of phosphonate residues in plant matrices (citrus, grapes, olives and apples).

The residue analytical method was derived from the method developed for the quick determination of highly polar pesticides in plant material of phosphonic acid (QuPpe-Method). It is based on extraction procedure with subsequent UPLC-MS/MS determination of Phosphonic acid.

The specimens were extracted with Methanol containing 1 % of formic acid and the mixture was shaken intensively and homogenized. Subsequently the samples were centrifuged for phase separation. After that, the extract obtained from step one was dilute using a mixture of Methanol/Millipore water (1/1, v/v) containing 0.1 % formic acid. Finally, the diluted extracts were analyzed by UPLC-MS/MS.

This report is an ILV of method (Sahvorost, N. (2014)), already assessed for the first authorisation of this preparation.

Method conditions

The final extracts were analysed by liquid chromatography with tandem mass spectrometric detection (UPLC-MS/MS):

LC System	Waters ACQUITY UPLC H-Class		
LC Column	Thermo Hypercarb column (Length 100 mm, i.d.: 4.6 mm, particle size: 5 µm). Pre column: Waters C18. Temp 40 °C		
LC Method	Solvent A : Millipore water + 0.5 % formic acid		
	Solvent B : MeOH + 0.1 % formic acid		
	Flow : 0.6 ml/min		
	Injection Volume : 50 µL		
	Analysis time : 10 min		
	Sample Temp. : 12° C		
	Time (min)	A (%)	B (%)
	0	70	30

		10	70	30	
MS/MS System	Waters Xevo TQD	LM1 Resolution:	5.56		
		HM1 Resolution:	14.24		
		Ion Energy 1:	0.40		
		LM2 Resolution:	11.00		
		HM2 Resolution:	14.49		
		Ion Energy 2:	0.50		
Ion Source Conditions ESI Negative Polarity	Source Temperature :	150 °C			
	Desolvation temperature :	600 °C			
	Source Voltages				
	Capillary :	0.6 (kV)			
	Cone :	35 (V)			
	Source Gas Flow				
	Desolvation :	1000 (L/Hr)			
	Cone :	50 (L/Hr)			
MS/MS Conditions for phosphonic acid in negative mode	Span (Da) :	0.7			
	MS/MS transition for quantification:	80.98 m/z > 79.11 m/z			
	Cone potential (kV) :	35			
	Collision potential (V) :	10			
	Dwell time (s) :	0.3			
	MS/MS transition for confirmation :	80.98 m/z > 63.22 m/z			
	Cone potential (kV) :	36			
	Collision potential (V) :	15			
Dwell time (s) :	0.3				

Method validation

Specificity

Chromatograms were provided for matrix matched calibration standards (grapes, apples, citrus, olives) control and fortified samples at LOQ (grapes, apples, citrus, olives). Data were submitted for both transitions. The results obtained for the blank were below the 30 % of the LOQ determined ensuring the specificity of the method.

Matrix effects

To check possible ion enhancement or suppression effects in UPLC/MS-MS analysis, a linearity without adding matrix extract (blank) was prepared, using only dilution solvent to make the final volume. This linearity was injected and used to quantify the recovery levels (LOQ and 10xLOQ) prepared in each matrix validation and to compare the results.

Significant matrix effects were sometimes observed.

However, this matrix effect has no impact on the study since all groups validations were prepared using matrix extract (blank).

Linearity

Citrus, grapes and apple:

Sample preparation:

10 g of specimen is extracted with 10 ml of methanol (1% formic acid). Total volume considered: 20 ml (aprox).

After this, 100µL of the extract is diluted with 900 µL of dilution solvent (methanol/water 1:1) and injected in LC-MS/MS.

Therefore, the extracts are prepared at an equivalent concentration of 50 g of specimen per 1 L of solvent.

According to this, the linearity expressed in the mass fraction of the original sample is presented in the following table:

Solution	Theoretical concentration in the vial	Theoretical concentration of the mass fraction of the specimen
P1	10 µg/L	0.2 mg phosphonic acid/ Kg
P2	25 µg/L	0.5 mg phosphonic acid/ Kg
P3	50 µg/L	1.0 mg phosphonic acid/ Kg
P4	100 µg/L	2.0 mg phosphonic acid/ Kg
P5	250 µg/L	5.0 mg phosphonic acid/ Kg
P6	500 µg/L	10.0 mg phosphonic acid/ Kg
P7	1000 µg/L	20.0 mg phosphonic acid/ Kg

Olive:

Sample preparation:

5 g of specimen is mixed with 7.9 g of water and extracted with 10 ml of methanol (1% formic acid). Total volume considered: 20 ml (aprox), considering a water content for the olives of 40-45%

After this, 100µL of the extract is diluted with 900 µL of dilution solvent (methanol/water 1:1) and injected in LC-MS/MS.

Therefore, the extracts are prepared at an equivalent concentration of 25 g of specimen per 1 L of solvent.

According to this, the linearity expressed in the mass fraction of the original sample is presented in the following table:

Solution	Theoretical concentration in the vial	Theoretical concentration of the mass fraction of the specimen
P1	10 µg/L	0.4 mg phosphonic acid/ Kg
P2	25 µg/L	1.0 mg phosphonic acid/ Kg
P3	50 µg/L	2.0 mg phosphonic acid/ Kg
P4	100 µg/L	4.0 mg phosphonic acid/ Kg
P5	250 µg/L	10.0 mg phosphonic acid/ Kg
P6	500 µg/L	20.0 mg phosphonic acid/ Kg
P7	1000 µg/L	40.0 mg phosphonic acid/ Kg

For Phosphonic acid the evaluation was done by external standard calibrations. Standard curves were obtained by injections of 7 calibration solutions in matrix. The dynamic range of the calibration function ranges from 0.011 to 1.1 µg/ml. Correlation coefficients *r* were always > 0.99. Regressions were provided for citrus, grapes, apples and olives and for both transitions.

Recoveries

The analytical method for Phosphonic acid achieves a limit of quantification (LOQ) of 1 mg/kg and a limit of detection (LOD) of 0.3 mg/kg (30 % of LOQ). For concurrent method and result validation, specimens fortified at LOQ (1 mg/kg) were processed concurrently with the field specimens and examined for recoveries by UPLC-MS/MS. Mean recoveries for each level are in the acceptable range of 70 to 110 % with relative standard deviations ≤ 20 %.

Fortification in citrus

Fortification level		Recoveries			No. of analysis
Level	[mg/kg]	Singles values [%]	Mean	RSD	
<i>Phosphonic acid 81 m/z ->79 m/z (quantification)</i>					
LOQ	1.0	100, 102, 97, 103, 101	101%	2.3%	5
10xLOQ	10	100, 97, 103, 104, 101	101%	2.6%	5
<i>Phosphonic acid 81 m/z ->63 m/z (confirmation)</i>					

LOQ	1.0	99, 102, 98, 99, 100	100%	1.2%	5
10xLOQ	10	100, 95, 102, 102, 101	100%	2.9%	5

Fortification in grapes

Fortification level		Recoveries			No. of analysis
Level	[mg/kg]	Singles values [%]	Mean	RSD	
<i>Phosphonic acid 81 m/z ->79 m/z (quanification)</i>					
LOQ	1.0	91, 85, 87, 91, 91	89%	3.1%	5
10xLOQ	10	98, 96, 95, 92, 88	94%	4.1%	5
<i>Phosphonic acid 81 m/z ->63 m/z (confirmation)</i>					
LOQ	1.0	93, 92, 91, 89, 95	92.2%	2.4%	5
10xLOQ	10	97, 98, 96, 94, 92	95.5%	2.6%	5

Fortification in apples

Fortification level		Recoveries			No. of analysis
Level	[mg/kg]	Singles values [%]	Mean	RSD	
<i>Phosphonic acid 81 m/z ->79 m/z (quanification)</i>					
LOQ	1.0	101, 101, 102, 104, 102	102%	1.4%	5
10xLOQ	10	93, 99, 106, 102, 105	101%	5.1%	5
<i>Phosphonic acid 81 m/z ->63 m/z (confirmation)</i>					
LOQ	1.0	91, 98, 100, 101, 97	97.5%	3.8%	5
10xLOQ	10	94, 96, 104, 101, 103	100%	4.3%	5

Fortification in olive

Fortification level		Recoveries			No. of analysis
Level	[mg/kg]	Singles values [%]	Mean	RSD	
<i>Phosphonic acid 81 m/z ->79 m/z (quanification)</i>					
LOQ	1.0	105, 109, 101, 101, 106	104%	3.3%	5
10xLOQ	10	92, 86, 80, 70, 67	79%	13.5%	5
<i>Phosphonic acid 81 m/z ->63 m/z (confirmation)</i>					
LOQ	1.0	105, 106, 101, 92, 110	103%	6.7%	5
10xLOQ	10	90, 88, 81, 73, 71	81%	10.2%	5

Precision

According to the previous table, RSD were in acceptable limits.

LOQ

The limit of quantification is 1.0mg/kg for phosphonic acid in citrus, grapes, olives and apples.

Conclusion:

The method is acceptable for the analysis of phosphonic acid in citrus, grapes, olives and apples.

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

No new or additional studies have been submitted.

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

No new or additional studies have been submitted.

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

Comments of zRMS:	The analytical methods (Pupp and Wydra, 2012a,b,c) provided to verify the concentrations of the phosphonic acid in water media using ion chromatography with conductivity detection (IC-DC) can be considered suitable for this purpose. Specificity, linearity accuracy and repeatability are found acceptable for phosphonic acid with a LOQ = 10 mg/L in water.
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Reference:	KCP 5.2/04 (Pupp and Wydra, 2012a)
Report	Analytical phase of study: “Acute Toxicity of Salaman 510 (510 g/L phosphorous acid) to Rainbow Trout (<i>Oncorhynchus mykiss</i>) in a 96-hour Static Limit Test.” Pupp, A. (2012) Report No. 65673230 Institut für Biologische Analytik und Consulting IBACON GmbH
Guideline(s):	Not stated in the report
Deviations:	--
GLP:	Yes
Acceptability:	Yes

Method outline

Ion Chromatography with Conductivity Cell.

Limit of Detection: 0.48 mg a.i./L

Limit of Quantification: 10 mg test item/L 108 % (n = 5, RSD 3 %)

Mean Recovery in the Fortified Samples: 104 % (n = 10, RSD 4 %)

Mean Recovery in the Test Samples: Freshly prepared: 101 % (n = 2, RSD 1 %)
Aged: 99 % (n = 2, RSD 1 %)

IC-Conditions:

IC-System: Dionex DX-120

Column: Phenomenex Star Anion A300 (100 * 4.6 mm)

Oven Temperature: 40 °C

Detector: Conductivity cell

Suppressor: ASRS ultra 4 mm

Mobile Phase: Carbonat-Buffer (700 mg disodiumcarbonate and 951 mg sodiumhydrogencarbonate were dissolved in 5 L pure water)

Flow Rate: 1.0 mL / min

Injection Volume: 50 µL

Validity criteria of the method

Determination of the Test Item

Based on the results of IC-DC measurements the concentration of the test item was determined using a calibration curve.

Calibration Range

0.5 mg to 30 mg reference item/L

Linearity of Response

Correlation of peak area of different standard solutions with their corresponding concentrations, using a linear regression.

Regression Coefficient (r^2)

At least 0.9994

Typical Calibration Curve

$$y = 0.0417 * x - 0.0200$$

Limit of Detection: 0.48 mg a.s./L

Limit of Quantification

The Limit of Quantification (LOQ) was determined as the lowest fortification level at which an acceptable mean recovery (70 to 110 % of nominal) with a relative standard deviation (RSD) < 20 % was obtained.

10 mg test item/L

108 % (n = 5, RSD 3 %)

Mean Recovery in the Fortified Samples:

104 % (n = 10, RSD 4 %)

Mean Recovery in the Test Samples:

Freshly prepared: 101 % (n = 2, RSD 1 %)

Aged: 99 % (n = 2, RSD 1 %)

Summary of analytical results

sample description [mg test item/L]	% of Nominal ¹	RSD (%)	n
Control	n.a.	n.a.	2
100	100	1	4

¹ mean value of all measured samples per treatment group

RSD: relative standard deviation per treatment group

n: number of analysed samples

n.a.: not applicable

Conclusion: the method was fully validated.

* * * * *

Reference:	KCP 5.2/05 (Pupp and Wydra, 2012b)
Report	Analytical phase of study: "Acute Toxicity of Salaman 510 (510 g/L phosphorous acid) to <i>Daphnia magna</i> in a Static 48-hour Immobilisation Limit-Test." Pupp, A. (2012) Report No. 65672220 Institut für Biologische Analytik und Consulting IBACON GmbH

Guideline(s):	Not stated in the report
Deviations:	--
GLP:	Yes
Acceptability:	Yes

Method outline

Ion Chromatography with Conductivity Cell.

Limit of Detection: 0.38 mg a.i./L

Limit of Quantification: 10 mg test item/L
89 % (n = 5, RSD 4 %)

Mean Recovery in the Fortified Samples: 94 % (n = 10, RSD 6 %)

Mean Recovery in the Test Samples:

Freshly prepared: 102 % (n = 2, RSD 1 %)

Aged test media: 102 % (n = 2, RSD 2 %)

IC-Conditions:

IC-System: Dionex DX-120

Column: Phenomenex Star Anion A300 (100 * 4.6 mm)

Oven Temperature: 40 °C

Detector: Conductivity cell

Suppressor: ASRS ultra 4 mm

Mobile Phase: Carbonat-Buffer (700 mg disodiumcarbonate and 951 mg sodiumhydrogencarbonate were dissolved in 5 L pure water)

Flow Rate: 1.0 mL / min

Injection Volume: 50 µL

Validity criteria of the method

Determination of the Test Item

Based on the results of IC-DC measurements the concentration of the test item was determined using a calibration curve.

Calibration Range

0.5 mg to 30 mg test item/L

Linearity of Response

Correlation of peak area of different standard solutions with their corresponding concentrations, using a linear regression.

Regression Coefficient (r²)

At least 0.9994

Typical Calibration Curve

$$y = 0.0417 * x - 0.02$$

Limit of Detection: 0.38 mg test item/L

Limit of Quantification

The Limit of Quantification (LOQ) was determined as the lowest fortification level at which an acceptable mean recovery (70 to 110 % of nominal) with a relative standard deviation (RSD) < 20 % was obtained.

10 mg test item/L
 89 % (n = 5, RSD 4 %)

Mean Recovery in the Fortified Samples:

Freshly prepared: 102 % (n = 2, RSD 1 %)

Aged test media: 102 % (n = 2, RSD 2 %)

Summary of analytical results

sample description [mg test item/L]	% of Nominal ¹	RSD (%)	n
Control	n.a.	n.a.	2
100	102	1	4

¹ mean value of all measured samples per treatment group

RSD: relative standard deviation per treatment group

n: number of analysed samples

n.a.: not applicable

Conclusion: the method was fully validated.

* * * * *

Reference:	KCP 5.2/06 ((Pupp and Wydra, 2012c)
Report	“Toxicity of Salaman 510 (510 g/L phosphorus acid) to <i>Pseudokirchneriella subcapitata</i> in an Algal Growth Inhibition Test.” Pupp and Wydra (2012) Analytical phase of the study Nr. - IBACON 65671210
Guideline(s):	SANCO 3029/99 rev.4
Deviations:	--
GLP:	Yes

Method Outline

Water specimens were diluted with water (if necessary), and analysed by HPLC/IC with suppressor with an anionic column.

The same analytical method has been used for all studies. The validation data are presented below.

Validation

Specificity

The specificity was tested with injections of blank samples. No peaks at the retention time of phosphonic acid were found.

Linearity

Linearity of the method for the determination of phosphoric acid was studied in the range of nominal concentrations between 0.5 and 30 mg/L, including five standards solutions. The correlation coefficient (r^2) was 0.9994.

Precision

Accuracy of the analytical method for water specimens was studied by means of recovery experiments with blank samples fortified at two concentration levels with phosphonic acid, at the LOQ level (10.0 mg/L) and at a higher level (10xLOQ, 100 mg/L).

Study IBACON 65673230

Fortification level	Matrix	Recoveries	No. of	Overall recovery
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Level	[mg/kg]		Singles values [%]	Mean	RSD	analysis	Mean	RSD
LOQ	10.0	Water	107, 104, 106, 112 ,109	108%	3%	5	104%	4%
10xLOQ	100.0	water	100, 103, 100, 100, 100	101%	1%	5		
LOQ		10.0 mg/L for phosphonic acid. (n=5; RSD= 3%)						
LOD		0.48 mg/L for phosphonic acid (30% of the LOQ)						

Study IBACON 65672220

Fortification level		Matrix	Recoveries			No. of analysis	Overall recovery	
Level	[mg/kg]		Singles values [%]	Mean	RSD		Mean	RSD
LOQ	10.0	Water	87, 93, 94, 88, 85	90%	4%	5	94%	6%
10xLOQ	100.0	water	98, 98, 98, 94, 100	98%	2%	5		
LOQ		10.0 mg/L for phosphonic acid. (n=5; RSD= 3%)						
LOD		0.38 mg/L for phosphonic acid (30% of the LOQ)						

Study IBACON 65671210

Fortification level		Matrix	Recoveries			No. of analysis	Overall recovery	
Level	[mg/kg]		Singles values [%]	Mean	RSD		Mean	RSD
LOQ	10.0	Water	120, 131, 125, 126, 126	126%	4%	5	113%	12%
10xLOQ	100.0	water	100, 100, 102, 96, 101	100%	2%	5		
LOQ		10.0 mg/L for phosphonic acid. (n=5; RSD= 3%)						
LOD		0.42 mg/L for phosphonic acid (30% of the LOQ)						

Conclusion: The methods are considered adequate.

Comments of zRMS:	The analytical method (Pupp and Wydra, 2013) provided to verify the concentrations of the phosphonic acid in water media using ion chromatography with conductivity detection (IC-DC) can be considered suitable for this purpose. Specificity, linearity accuracy and repeatability are found acceptable for phosphonic acid with a LOQ = 5 mg/L in water.
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Reference:	KCP 5.2/07 (Pupp and Wydra, 2013)
Report	“Toxicity of Salaman 510 (510 g/L phosphorous acid) to Rainbow Trout (<i>Oncorhynchus mykiss</i>) in a Prolonged Semi Static Test over 28 Days.” Pupp and Wydra (2013) Analytical phase of the study Nr.- IBACON 65675231
Guideline(s):	SANCO 3029/99 rev.4
Deviations:	--
GLP:	Yes

Method Outline:

Water specimens were analyzed with no dilution, by HPLC/IC with suppressor with an anionic column. The same analytical method has been used for all studies. The validation data are presented below.

Validation

Specificity:

The specificity was tested with injections of blank samples. No peaks at the retention time of phosphonic acid were found.

Linearity:

Linearity of the method for the determination of phosphoric acid was studied in the range of nominal concentrations between 1.0 and 30 mg/L, including five standards solutions. The correlation coefficient (r^2) was 0.9980.

Precision:

Accuracy of the analytical method for water specimens was studied by means of recovery experiments with blank samples fortified at two concentration levels with phosphonic acid, at the LOQ level (5.0 mg/L) and at a higher levels (15 mg/L and 40 mg/L).

Fortification level		Matrix	Recoveries			No. of analysis	Overall recovery	
Level	[mg/kg]		Singles values [%]	Mean	RSD		Mean	RSD
LOQ	5.0	Water	73, 73, 93, 76, 79	80	9%	5	90%	11%
	15.0	Water	89, 91, 89, 89, 93	90	2%	5		
	40.0	water	101, 101, 100, 100, 101	101	0.5%	5		
LOQ		5.0 mg/L for phosphonic acid. (n=5; RSD= 11%)						
LOD		0.1 mg/L for phosphonic acid						

Conclusion: The method is considered adequate.

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

No new or additional studies have been submitted.

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

No new or additional studies have been submitted.

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

Comments of zRMS:	The method was fully validated for the determination of phosphonate acid in samples from ecotoxicological studies on honeybees (water, sucrose, diet matrix), according to the guideline SANCO/3029/99 rev.4 and SANCO/825/00 rev.8.1.
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Reference:	KCP 5.2/08 (Hernández, S, 2017)
Report	“Determination of potassium phosphonate (expressed as phosphonate acid) in samples from ecotoxicological studies on honeybees.” Report No. E16145 Cambium, S.L.
Guideline(s):	SANCO/3029/99 Rev.4

	SANCO/825/00 rev. 8.1
Deviations:	- Study plan deviation relating to sucrose solution preparation. 50 % w/v solution was prepared instead of 50 % w/w. - Study plan deviation relating to MC1 and MC2 solutions preparation for sucrose solution matrix. Correct preparation was defined in this final report. Deviations of the study present a nil impact in the study since they do not affect to the integrity of the data.
GLP:	Yes
Acceptability:	Yes

Method outline

The technique applied for Potassium Phosphonate content determination is ionic HPLC.

Water matrix	LOQ = 10.72 µg/mL	Mean Recovery 99.7 %	Recovery 70-110 %
		RSD = 1.69 %	RSD ≤ 20 %
	LOD = 3.22 µg/mL		
	10 × LOQ = 107.18 µg/mL	Mean Recovery 95.5 %	Recovery 70-110%
		RSD = 1.67 %	RSD ≤ 20 %
Sucrose matrix	LOQ = 8.28 µg/mL	Mean Recovery 99.7 %	Recovery 70-110 %
		RSD = 1.67 %	RSD ≤ 20 %
	LOD = 2.48 µg/mL		
	10 × LOQ = 107.18 µg/mL	Mean Recovery 97.2 %	Recovery 70-110%
		RSD = 2.05 %	RSD ≤ 20 %
Diet matrix	LOQ = 8.28 µg/mL	Mean Recovery 99.1 %	Recovery 70-110 %
		RSD = 0.72 %	RSD ≤ 20 %
	LOD = 2.48 µg/mL		
	10 × LOQ = 85.74 µg/mL	Mean Recovery 104.1 %	Recovery 70-110%
		RSD = 0.73 %	RSD ≤ 20 %

Method conditions

Column : Shodex IC Ni-424 anion exchange with suppressor
 (100 × 4.6 mm dimensions)
 Eluent : 100 % eluent
 Flow : 1 mL/min
 Analysis Time : 10 minutes
 Polarity : Negative
 Recorder Range : 0.5 µS/cm
 Range : × 100
 Response : SLOW
 Zero suppress : 0 µS/cm
 Injection Volume : 25 µL
 Oven Temperature : 30 °C

Method validation

Summary of the analytical method validation for potassium phosphonate quantification in stock solutions

• Water matrix:

PARAMETER TYPE	RESULTS	ACCEPTANCE/REFUSAL CRITERION
	PARAMETER	
SELECTIVITY	R _t reference item: 6.093 min R _t fortified sample of test item (MC1): 6.080 min R _t matrix: --	Not overlapping of peaks is observed. Interference < 30 % of the LOQ

	R _t solvent: -- The matrix interferences contribute in a 0.0 %		
LINEARITY	The calibration curve was calculated by one injection of six standards from 5.26 µg/mL to 152.83 µg/mL		
	Correlation coefficient	r = 0.9999	r ≥ 0.99
ACCURACY PRECISION RECOVERY	LOQ = 10.72 µg/mL	Mean Recovery 99.7 % RSD = 1.69 %	Recovery 70-110 % RSD ≤ 20 %
	LOD = 3.22 µg/mL	LOD is defined as 30 % of the LOQ	
	10 × LOQ = 107.18 µg/mL	Mean Recovery 101.7 % RSD = 0.99 %	Recovery 70-110 % RSD ≤ 20 %
	SOLUTION RECOVERY (MC)	Recovery = 101.1 % RSD = 1.13 %	Recovery 70-110 % RSD ≤ 20 %

• Sucrose matrix:

PARAMETER TYPE	RESULTS		ACCEPTANCE/REFUSAL CRITERION
	PARAMETER		
SELECTIVITY	R reference item: 6.057 min R fortified sample of test item (MC1): 6.057 min R matrix: -- R solvent: -- The matrix interferences contribute in a 0.0 %		Not overlapping of peaks is observed. Interference < 30 % of the LOQ
LINEARITY	The calibration curve was calculated by one injection of seven standards from 2.07 µg/mL to 200.23 µg/mL		
	Correlation coefficient	r = 0.9999	r ≥ 0.99
ACCURACY PRECISION RECOVERY	LOQ = 8.28 µg/mL	Mean Recovery 95.5 % RSD = 1.67 %	Recovery 70-110 % RSD ≤ 20 %
	LOD = 2.48 µg/mL	LOD is defined as 30 % of the LOQ	
	10 × LOQ = 85.74 µg/mL	Mean Recovery 97.2 % RSD = 2.05 %	Recovery 70-110 % RSD ≤ 20 %
	SOLUTION RECOVERY (MC)	Recovery = 98.4 % RSD = 0.52 %	Recovery 70-110 % RSD ≤ 20 %

• Diet matrix:

PARAMETER TYPE	RESULTS		ACCEPTANCE/REFUSAL CRITERION
	PARAMETER		
SELECTIVITY	R reference item: 6.027 min R fortified sample of test item (MC1): 6.033 min R matrix: -- R solvent: -- The matrix interferences contribute in a 0.0 %		Not overlapping of peaks is observed. Interference < 30 % of the LOQ
LINEARITY	The calibration curve was calculated by one injection of seven standards from 2.07 µg/mL to 152.83 µg/mL		
	Correlation coefficient	r = 0.9998	r ≥ 0.99
ACCURACY PRECISION RECOVERY	LOQ = 8.28 µg/mL	Mean Recovery 99.1% RSD = 0.72 %	Recovery 70-110 % RSD ≤ 20 %
	LOD = 2.48 µg/mL	LOD is defined as 30 % of the LOQ	
	10 × LOQ = 85.74 µg/mL	Mean Recovery 104.1% RSD = 0.73 %	Recovery 70-110 % RSD ≤ 20 %
	SOLUTION RECOVERY (MC)	Recovery = 103.1 % RSD = 0.55 %	Recovery 70-110 % RSD ≤ 20 %