EURL-SRM - Analytical Observations Report

Concerning the following…

- **Compound(s)**: Propineb, 1,2-Diaminopropane (a.k.a. propylene diamine)
- **Commodities**: Plant origin
- **Extraction Method(s)**: SRM-14 method involving reductive cleavage with HCl/$SnCl_2$ followed by alkaline QuEChERS-type partitioning
- **Instrumental analysis**: LC-MS/MS (ion-pair chromatography)

Intermediate analytical observations as regards the analysis of propineb as propylenediamine following reductive cleavage with HCl/SnCl2 and measurement via ion-pair LC-MS/MS

Version 1 (December 2021)

Background information

Propineb is a polymeric complex between zinc cations and anionic propylene-bis-dithiocarbamate ligands, that acts as a foliar fungicide against various types of fungi, such as blight and downy mildew[1]. Propineb used to be approved in the EU but the approval was not renewed and expired in March 2018[2]. In other parts of the world, such as in Asia (e.g. India, Malaysia, Turkey), South America (e.g. Chile, Brazil) and in Australia, propineb is still approved and in fact quite popular, as it is cheap and relatively easy to handle. In these countries plant protection products - formulated as wettable powders or granules – contain propineb either as the only active substance, or in combination with other fungicides, such as carbendazim, copper oxychloride, iprovalicarb, triadimefon oxadixyl,and cymoxanil. Among the crops treated are fruiting vegetables (e.g. tomatoes, sweet peppers, aubergines, melons, green beans), leafy vegetables (e.g. lettuce), tree fruits and berries (e.g. pome fruit, grapes), bulb and tuber vegetables (e.g. onions and potatoes) as well as hops.

In Reg. 396/2005/EC, propineb residues are currently regulated in two residue definitions, one focusing on the carbon disulfide (CS_2) and the other on the propylene diamine moiety: a) Sum of "*Dithiocarbamates (including maneb, mancozeb, metiram, **propineb**, thiram and ziram), expressed as CS_2)*"[3]; b) "*Propineb (expressed as propilendiamine)*" [4].

[1] https://www.fao.org/fileadmin/templates/agphome/documents/Pests_Pesticdes/JMPR/Evaluation93/propineb.pdf

2 The maximum period of grace ended in June 2019

[3] COMMISSION REGULATION (EU) 2017/171 of 30 January 2017 amending Annexes II, III and IV to Regulation (EC) No 396/2005 of the European Parliament and of the Council as regards maximum residue levels for aminopyralid, azoxystrobin, cyantraniliprole, cyflufenamid, cyproconazole, diethofencarb, dithiocarbamates, fluazifop-P, fluopyram, haloxyfop, isofetamid, metalaxyl, prohexadione, propaquizafop, pyrimethanil, Trichoderma atroviride strain SC1 and zoxamide in or on certain products

[4] COMMISSION REGULATION (EU) 2021/1864 of 22 October 2021 amending Annexes II, III and V to Regulation (EC) No 396/2005 of the European Parliament and of the Council as regards maximum residue levels for amisulbrom, flubendiamide, meptyldinocap, metaflumizone and propineb in or on certain products.

In the past, a separate residue definition :("Propineb/propylenethiourea (sum of propineb and propylenethiourea") used to additionally apply for infant and follow-on formulae[5] with an MRL of 0.006 mg/kg being in force for the sum. This residue definition created some confusion, as it was not clear how the residues were to be expressed. With the recently introduced Regulations 1040/2021/EC and 1041/2021/EC, the residue definition was harmonized with that of Reg. 396/2005/EC (with the MRL of 0.006 mg/kg still applying)[6].

To analytically cover the residue definition focusing on the CS_2 common moiety, numerous methods have been developed. Virtually all these methods involve reductive cleavage with $HCl/SnCl_2$, which results in the formation of CS_2 and the corresponding amine, i.e. ethylenediamine in the case of ethylene-bis-dithiocarbamates and 1,2-diaminopropane (a.k.a. **p**ropylene-1,2-**di**a**m**ine, **PDA**) in the case of propineb. The analysis of CS_2 is either achieved photometrically, following derivatization, or directly via GC. GC-methods involve for example direct headspace sampling, headspace SPME enrichment and liquid-liquid partitioning to a non-polar solvent, such as isooctane. Judging on information collected during proficiency tests, the latter approach is meanwhile the most commonly used one by official laboratories. In 2009, the EURL-SRM published a method based on this approach[7].

To cover the residue definition based on PDA, the EURL-SRM presented a method in 2014[8]. The method was based on the aforementioned approach for the analysis of CS_2, with PDA being analyzed from the aqueous phase, which was neutralized, and diluted 5-fold prior to analysis of PDA via HILIC chromatography on a pentafluorophenyl column from Restek. PDA-D_6 was used as an internal standard to correct for matrix effects and volumetric errors. Unfortunately, the use of this method appeared to affect the LC-injector system and the measurement of other compounds. As this was attributed to the high ion-load in the extracts, the method was modified in two different ways: a) A dispersive solid phase cleanup with metal scavengers (e.g. Sigma QuadraPure IDA), was introduced,which considerably reduced the Sn-load in the diluted aqueous hydrolysates, and b) PDA was partitioned from an aliquot of the aqueous hydrolysate into acetonitrile (using a QuEChERS-like partitioning under alkaline conditions). In the latter case, tin and other ions largely remained in the aqueous phase and were thus effectively separated. Due to the relatively poor partitioning behavior of PDA into acetonitrile, results had to be corrected for recovery, which was also achieved by the use of PDA-D_6 as internal standard. Despite the poor QuEChERS-recoveries this approach, overall, allowed for a more sensitive and less matrix-effect-prone analysis of PDA and was thus further optimized as shown in the following.

[5] Regulation (EU) No 2016/127- Article 4(3) in conjunction with Annex IV and Regulation (EU) No 2016/128 Article 3(3) in conjunction with Annex II

[6] It is assumed that the more specific residue definition of Reg 396/2005/EC ("*Propineb (expressed as propilendiamine)*" applies.

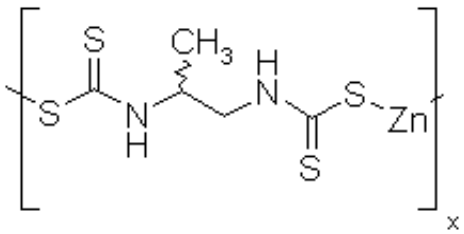
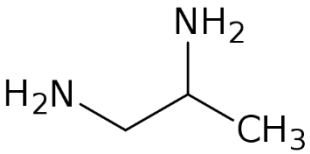
[7] EURL-SRM, Analysis of Dithiocarbamate Residues in Foods of Plant Origin involving Cleavage into Carbon Disulfide, Partitioning into Isooctane and Determinative Analysis by GC-ECD (Method "SRM-14"), Version 2, Last update: 18.12.2009, published on https://www.eurl-pesticides.eu/library/docs/srm/meth_DithiocarbamatesCs2_EurlSrm.pdf

[8] Analysis of Propineb as Propylenediamine via LC-MS/MS in Fruit and Vegetables https://www.eurl-pesticides.eu/userfiles/file/EurlSRM/EPRW2014_Benkenstein_Analysis-of-Propineb-as-Propylendiamine-via-LC-MS-MS_114.pdf

Analyte properties and analytical strategies

The physicochemical properties and additional information on propineb and PDA are given in *Table 1*.

Table 1: Chemical Properties of propineb and 1,2-diaminopropane (1,2-Propylene diamine).

Propineb (CAS: 12071-83-9 (Monomer); 9016-72-2 (Homopolymer)**)** Other names: polymeric zinc propylenebis(di-thiocarbamate)		
Parameter	Value	Notes
Molecular Mass	289.79 g/mol of Zn salt/complex of monomer 226,39 g/mol of monomeric acid = Propylene 1,2-bis(dithio-carbamic acid)	
Formula	$C_5H_8N_2S_4Zn$ (Zn salt/complex of monomer) $[C_5H_8N_2S_4Zn]_x$ (homopolymer)	
Boiling point	Degradation at 160 °C	
pKa	Monomer: 1,73 (acidic; computed by chemicalize.com) *Note: In presence of metal ions (in this case Zn) protonation/deproto-nation equilibria are shifted*	
LogP	Variable depending on polymerization/complexation grade and pH. (largely irrelevant due to poor solubility in most solvents). Monomer: -0.1 (pH >4.5), 0.8 (pH3), 1.9 (pH2), 2.3 (pH1)	
Stability	Dissociates in water. Presence of chelating agents speeds up dissociating. Monomers are highly instable in aqueous solution	
Residue definition (EU)	1) "*Dithiocarbamates (including maneb, mancozeb, metiram, propineb, thiram and ziram), expressed as CS_2*"; Reg. (EU) 2017/171	
	2) "*Propineb (expressed as propilendiamine)*"; Reg. (EU) 2021/1864	
Approved in …	No authorization in place within the EU (Approval expired 22 March 2018, authorizations withdrawn 22 June 2018, max. period of grace: 22 June 2019 Still in use in various countries in Asia, South-America and elsewhere	
Toxicity	According to Reg. 1272/2008, propineb is classified in cat. 4 as regards its acute toxicity, in cat. 2 as regards its specific toxicity to target organs at repeated exposure, in cat. 1 as regards its skin sensitizing properties and in cat. 1 as regards its acute hazard to the aquatic environment[9]. The ARfD is set at 0.1 and the ADI at 0.007 mg/kg bw (corresponds to approx. 0.025 and 0.0018 mg/kg bw respectively when expressed as PDA).	
Propane-1,2-diamine (CAS: 78-90-0) Other names: 1,2-diaminopropane, propylene-1,2-diamine, 1,2-Propylenediamine, PDA		
Parameter	Value	Notes
Molecular Mass	74.13 g/mol	
Formula	$C_3H_{10}N_2$	
Boiling point	119.6°C	
pKa	pKa: 9.83 (strongest basic), 7.12 (second strongest basic)	
LogP	-7.1 at pH <3, -6.8 at pH 4, -6 at pH 5, -5.1 at pH 6 -1.0 at pH >11	(pH dependent); computed by chemicalize.org
Water solubility	miscible	pH independent
Stability	-	
Residue definition EU	"*Propineb (expressed as propilendiamine)*"; Reg. (EU) 2021/1864	

[9] Reg. (EC) No1272/2008 of 16 December 2008 on classification, labelling and packaging of substances and mixtures, amending and repealing Directives 67/548/EEC and 1999/45/EC, and amending Reg. (EC) No 1907/2006, amended by Commission Delegated Regulation (EU) 2021/1962 of 12 August 2021

Toxicity	According to Reg. 1272/2008, Propane-1,2-diamin is classified in cat. 4 as regards its acute toxicity and in cat 1A as regards its skin corroding properties[7]

PDA, is a highly polar compound throughout the entire pH range, and exhibits the highest polarity under acidic to neutral conditions, see logD vs. pH plot in *Figure 1*.

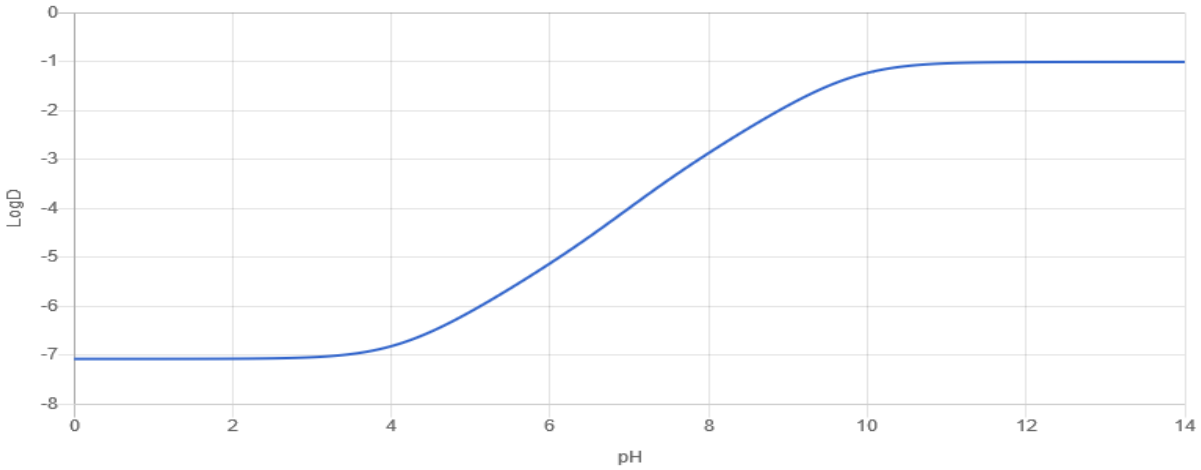


Figure 1: pH-dependence of PDA lipophilicity[10]. Note: Lower logD value =higher hydrophilicity.

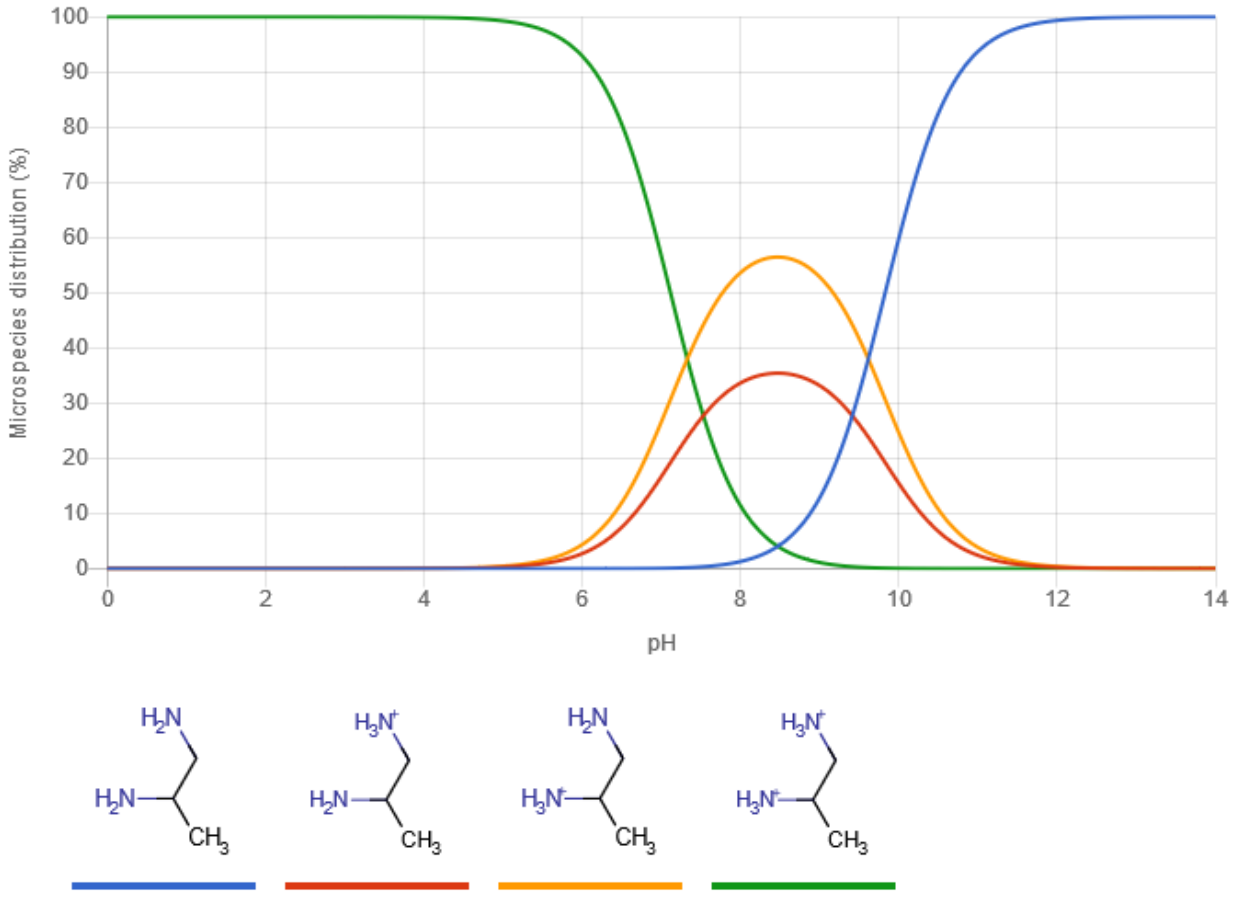


Figure 2: pH-dependent of 1,2-diaminopropane[11].

[10] *Figure computed by chemicalize.com*
[11] Figure computed by chemicalize.com

To improve PDA-partitioning into the organic phase during the QuEChERS approach, its lipophilicity (= affinity for the organic phase), needs to be increased. This can be achieved e.g. by derivatization with a lipophilic moiety, or by raising the pH to values >11, where the molecule is virtually entirely present in its uncharged form (both amino groups of PDA deprotonated, see Figure 2), in which the logP is still quite low (-1,0), but much higher than at the normal pH range of QuEChERS.

Extraction and partitioning

As the aqueous phase of the traditional CS_2-analysis approach is highly acidic an extreme pH shift is necessary prior to the QuEChERS-like approach. This is achieved by adding a saturated sodium hydroxide solution to an aliquot of the aqueous phase. The volatility of amines increases dramatically in the deprotonated form (noticeable by an ammonia-like/fishy odor). Appropriate measures thus need to be taken to minimize these losses, including the following:

a) Add acetonitrile (and internal standard) prior to adding the base,
b) Preferably cool-down the mixture of hydrolysate aliquot and acetonitrile before starting with the partitioning procedure,
c) Add the base at once and close the extraction vessel immediately after adding the base,
d) Make sure that the vessel is cooled down prior reopening it to transfer any aliquot for storage or measurement (e.g. cool down the vessel prior, during[12] or after centrifugation)
e) Quickly transfer the final extract in vessels containing a sufficient amount of acid to shift the pH to lower pH values and therefore minimize PDA volatility[13].
f) Generally sustain the extraction time as short as possible.

Chromatographic separation

Being a highly polar compound, PDA is only insufficiently retained on reversed-phase columns. Hydrophilic interaction liquid chromatography (HILIC) generally provides better retention but experiments involving injection of the acetonitrile extracts onto two HILIC columns tested (BEH-Amide- and PFP phase) showed a rather poor chromatographic resolution and/or sensitivity. To improve chromatographic separation and sensitivity of detection, a completely different approach was introduced, involving ion pairing with heptafluorobutyric acid to obtain retention of PDA (in for of a HFBA-ion-pair) on the C_{18} column. More details on this approach are given below.

Specificity

Various polyamines including diamines occur naturally in all eukaryotes where they assume various functions e.g. in cell growth, cellular translation and biosynthesis[14]. One of those naturally occurring diamines is 1,3-diaminopropane, which is an isomer of the propineb degradant 1,2-diaminopropane (PDA). LC-MS(/MS) fragmentation experiments revealed that the two isomeric compounds show similar mass transitions. Sufficient chromatographic separation of both substances is thus deemed paramount for being able to analyze PDA at low levels.

[12] A coolable centrifuge may be used here
[13] Extract acidification was also shown being beneficial for the peak form of PDA in subsequent ion-pair chromatography
[14] A. J. Michael, Department of Pharmacology, University of Texas Southwestern, Polyamines in Eukaryotes, Bacteria and Archaea, The Journal of Biological Chemistry, Vol 291, NO 29 pp, 14896-14903, July 15 2016

Apparatus, Chemicals and Consumables

Chemicals and Materials

The apparatus and materials needed for the traditional acidic CS_2-hydrolysis can be found in method "SRM-14" on the EURL website[6]. The used materials and apparatuses are mostly listed in the QuEChERS standard procedure (EN-15662). Additionally used chemicals are listed in Table 2.

Table 2: Used chemicals for the alkaline QuEChERS approach and the measurement by LC-MS/MS.

Chemical/Reagent	Purity, Assay	Brand/Source	Article No.
Sodium hydroxide pellets	EMSURE®, for analysis (≥ 99.0 %)	Supelco ®/Merck	1.06498.1000
Acetonitrile (gradient grade for liquid chromatography)	LiChrosolv® Reag. Ph Eur. (≥ 99.9 %)	Supelco ®/Merck	1.00030.2500
Heptafluorobutyric acid	suitable for ion chromatography (≥99.5%)	Supelco ®/Merck	52411-5mL-F
Ammonium formate (Eluent additive for LC-MS)	99.0 - 101.0 %	Fluka/Honeywell	55674-50G
Methanol (for UHPLC-MS)	Chemsolute® (min. 99.97 %)	Th. Geyer	1485.2500
Ultra purity water	Obtained by a Milli-Q IQ7000 water purification system from Merck		

Disclaimer: Names of companies are given for the convenience of the reader and do not indicate any preference by the EURL-SRM towards these companies and their products

Analytical standards of analytes and isotopically labeled internal standards (ILISs)

The suppliers of the used analytical standards and some notes are shown in **Fehler! Verweisquelle konnte nicht gefunden werden.**.

Table 3: Sources of Analytical standards.

Compounds	Details on standards used		Provider	Other providers, notes
Propineb	Purity: Supplier Code:	101.7 % 45643-250MG	Fluka	Please be aware, that propineb standards available on the market, may differ considerably. In some cases (CS_2) recovery rates obtained using the above-mentioned hydrolysis conditions were <20 %, even though a higher purity was reported
	Purity: Supplier Code:	71.5 % (w/w) - (friendly donation)	Bayer	
1,2-diaminopropane	Purity: Supplier Code:	99,9 % 117498-100G	Aldrich	
1,2-diaminopropane -D_6 (ILIS)	Purity: Supplier Code:	98 % 701408-100mg	Sigma	

Disclaimer: Names of companies are given for the convenience of the reader and do not indicate any preference by the EURL-SRM towards these companies and their products

Stock and working "solutions"[15] are prepared as follows[16].:

[15] Chemically, these "solutions" are rather suspensions of small propineb particles in solvent, resulting in turbid stock suspensions, which are currently deemed being stable for up to one hour

[16] Zipper, H. et al., Facing Analytical Challenges of Dithiocarbamate Analysis - Step-by-Step, presentation presented on the Joint Workshop of the EURLs, 20.-22.October 2021, Almeria, Spain; available at https://www.eurl-pesticides.eu/userfiles/file/EurlFV/Joint2021/Zipper2.pdf

Propineb: Stock suspensions for propineb at e.g. 1 mg/mL are prepared daily in a water/acetonitrile/xanthan gum mixture (95/5/0.2 v/v/w) taking the purity of the standard substance into account. The stock suspensions should not be sonicated as degradation of propineb was observed. Working suspensions may be also prepared as necessary by diluting the stock dispersion with the water/acetonitrile/xanthan gum mixture (95/5/0.2 v/v/w) immediately after the preparation of the stock suspension and preferably used within 30 minutes after preparation.

1,2-diaminopropane (native substance and ILIS):
Stock solutions of both the native substance and the ILIS (both e.g. 1 mg/mL) are prepared in acetonitrile taking the purity of the standard substance into account and stored in a refrigerator for up to 30 months. Working solutions are prepared as necessary in acetonitrile and may be stored in the refrigerator until use.

Sample Preparation

Homogenization: The samples are homogenized by cryogenic milling using dry ice.

Sample preparation: The samples are subjected to the traditional reductive cleavage of dithiocarbamates to CS_2 as stated in the method "SRM-14" on the EURL website[6]. The aqueous phase ("aqueous CS_2-hydrolysate") is then subjected to a QuEChERS-like partitioning step under alkaline conditions, as follows:
A reasonable amount (e.g. > 30 mL) of the "aqueous CS_2-hydrolysate" is transferred into a falcon tube and centrifuged for e.g. 5 min at 5000 rpm. A 10 mL aliquot of the supernatant (corresponding to a 2.5 g sample portion[17]) is transferred into another falcon tube and 100 µL of PDA-D_6 (20 µg/mL) is added. After adding 10 mL of acetonitrile and preferably cooling down the vessel (e.g. in a freezer for approx. 30 min) before the addition of 3 mL of sodium hydroxide solution (25 N), the vessel is closed and shaken for 1 min to allow PDA to partition into the acetonitrile phase[18]. After centrifugation for e.g. 5 min at 5,000 rpm, the vial is re-opened (preferably cool down the vessel before opening[19]) and 0.5 mL of the upper acetonitrile phase is swiftly transferred into a vial containing 1 mL of eluent A[20] (see Table 4) for LC-MS/MS analysis.

A brief flow-chart of the QuChERS-based sample preparation procedure is shown in Figure 3.

[17] 10 mL hydrolysate correspond to 2.5 g sample where 50 g sample are employed and the total volume of the aqueous phase is 200 mL.If 20 g sample are used only 1 g sample are represented in 1 mL hydrolysate
[18] The high salt load in the aqueous solution, resulting from the high content of hydrochloric acid in the hydrolysate and the sodium hydroxide added, induces partitioning without the need of adding additional partitioning salts
[19] Preferably, cool down the extract prior to opening the vessel. This may be achieved by placing the vial in a freezer prior or after centrifugation or by using a coolable centrifuge so far available.
[20] Eluent A contains sufficient acidity to keeps ensure low pH in the mixture, thus ensuring that PDA in a protonated and thus less volatile form.

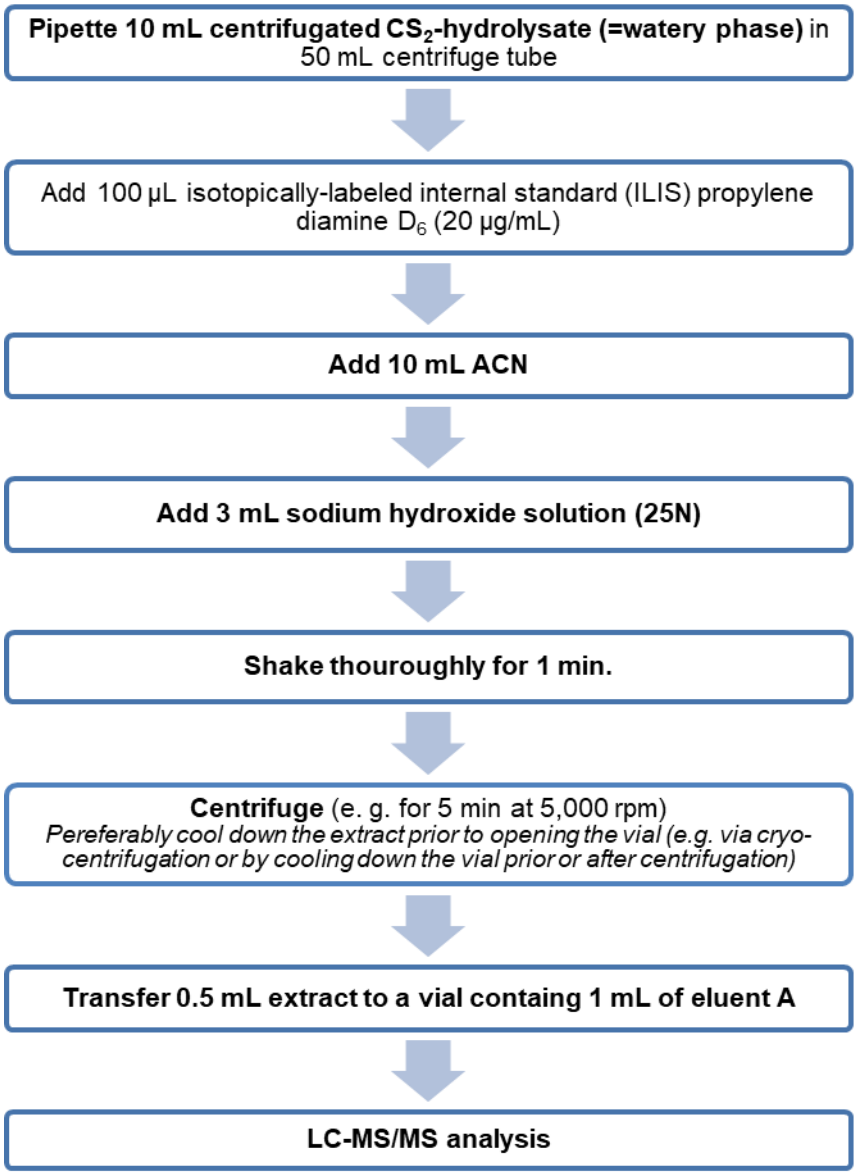


Figure 3: Method at a glance - modified alkaline QuEChERS for the determination of propylene diamine following traditional reductive DTC cleavage with HCl/$SnCl_2$ ("CS_2-hydrolysis"); eluent A: see Table 4.

Measurement:

The diluted extract is directly subjected to LC-MS/MS separation and measurement. Exemplary LC-MS/MS conditions are given in Table 4. Experience has shown that the column needs to be pre-conditioned thoroughly with the ion-pairing agent heptafluorobutyric acid before the first injection of a sequence, in order to obtain a stable condition. For this, you may flush the column with 40 % eluent A at a flow rate 0.3 mL/min for at least 20 min. A good indicator for sufficient column conditioning is the stabilization of the system pressure under the mentioned conditions. Following the above pre-conditioning step, the column can be equilibrated with the starting solvent composition of the gradient.

Table 4: Instrumentation details (LC: Shimadzu Nexera LC-40-system; MS: Sciex QTrap 5500+)

Instrument parameters	Conditions					
Column/temperature	Agilent ZORBAX Eclipse Plus C18 RRHD, 2.1 x 50 mm, 1.8µm; 40 °C					
Pre-column	Agilent ZORBAX Eclipse Plus C18 2.1x5mm, 1.8µm					
Eluent A	4 mM heptafluorobutyric acid +2 mM ammonium formate in water[21]					
Eluent B	4 mM heptafluorobutyric acid +2 mM ammonium formate in methanol					
Gradient	%A	Flow [mL/min]	Time [min]			
	97.5	0.2	0			
	97.5	0.2	1.0			
	60.0	0.2	7.0			
	30.0	0.3	7.1			
	10.0	0.3	8.0			
	10.0	0.3	12.0			
	97.5	0.2	12.1			
	97.5	0.2	14.0			
Injection volume	4 µL					
Acquired mass transitions (m/z)	Compound	Mass transitions and their MS-parameters				
		Q 1 (m/z)	Q 3 (m/z)	DP[1)] (V)	CE[2)] (V)	CXP[3)] (V)
	1,2-diaminopropane (PDA)	75	58	44	13	10
		58	41	81	21	8
	1,2-diaminopropane-D_6 (PDA-D_6)	81	64	36	15	8
		81	46	36	27	6
Ionisation mode	ESI Positive					
Ion Source Parameters	Curtain Gas Flow	25 psi				
	Ion Spray Voltage	4500 V				
	Temperature	550 °C				
	Nebulizer Gas Flow	60 psi				
	Heater Gas Flow	60 psi				

1) DP: Declustering Potential
2) CE: Collission Energy
3) CXP: Cell Exit Potential

Calibration and Matrix Effects

As PDA still exhibits a very low lipophilicity at alkaline conditions, partitioning yields into the acetonitrile phase remain quite low at approx. 20 %. The ILIS (PDA-D_6) typically corrects very well for these losses as well as for any evaporation losses, volumetric errors and matrix effects. In case of a non-availability of PDA-D_6 the following alternative approaches may be followed:

a) **Procedural calibration:** A suitable number of 10 mL-portions of a matrix or solvent blank[22] of the aqueous CS_2-hydrolysate are spiked with PDA and the alkaline QuEChERS approach described above is conducted.
b) **Standard additions approach:** Four 10 mL-portions of the aqueous CS_2-hydrolysate of the sample are transferred into separate falcon tubes. One of them is not spiked and the other ones are spiked with suitable increasing amounts of PDA. All vessels are subjected to the alkaline QuEChERS approach described above.

[21] *Add 200 µL HFBA solution (2N) and 200 µL ammonium formate solution (1N) to 100 mL of purified water.*
[22] Preferably, use matrix of the same type. The use of a reagent blank is also a viable alternative if matrix effects are insignificant.

The use of ILIS in combination with the abovementioned procedural calibration or in combination with the standard additions calibration approach are also possible and typically lead to the most accurate results.

Experiments and observations:

Adjusting the post-extraction dilution factor in combination with the injection volume

When optimizing the post-extraction (pre-measurement) steps the main focus was at reducing evaporation losses, at minimizing matrix effects, at ensuring a good compatibility of the injected extract with the chromatographic system, at obtaining acceptable separation of PDA from its naturally occurring isomer 1,3-diaminopropane and at still achieving sufficient sensitivity. As signal intensity is also a function of the injection volume, extract dilution factor and injection volume were optimized simultaneously, always keeping an eye on the other criteria (chromatographic separation, peak width and matrix effects). Without dilution of the final extract, PDA eluted as a broad double-peak compromising detection sensitivity and selectivity. The higher PDA volatility (due to the high extract pH) additionally affected long-time stability and therefore accuracy under these conditions (data not shown).
Diluting the final extract with eluent A (see Table 4) at a 1:2 ratio combined with an injection of 4 µL extract was deemed a suitable compromise, ensuring acceptable PDA sensitivity and the best signal ratio of PDA against 1,3-diaminopropane (v:v; see Figure 4), which could not be sufficiently separated at this stage of the method development process. Increasing the injection volume was also not favorable, as the signal intensity of 1,3-diaminopropane increased at a higher rate compared to that of PDA with the 1,3-diaminopropane peak broadening thus, affecting quantification and identification of PDA. Increasing the dilution factor further caused a drop in PDA signal intensity, thus compromising signal-to-noise ratio and method sensitivity overall (data not shown).

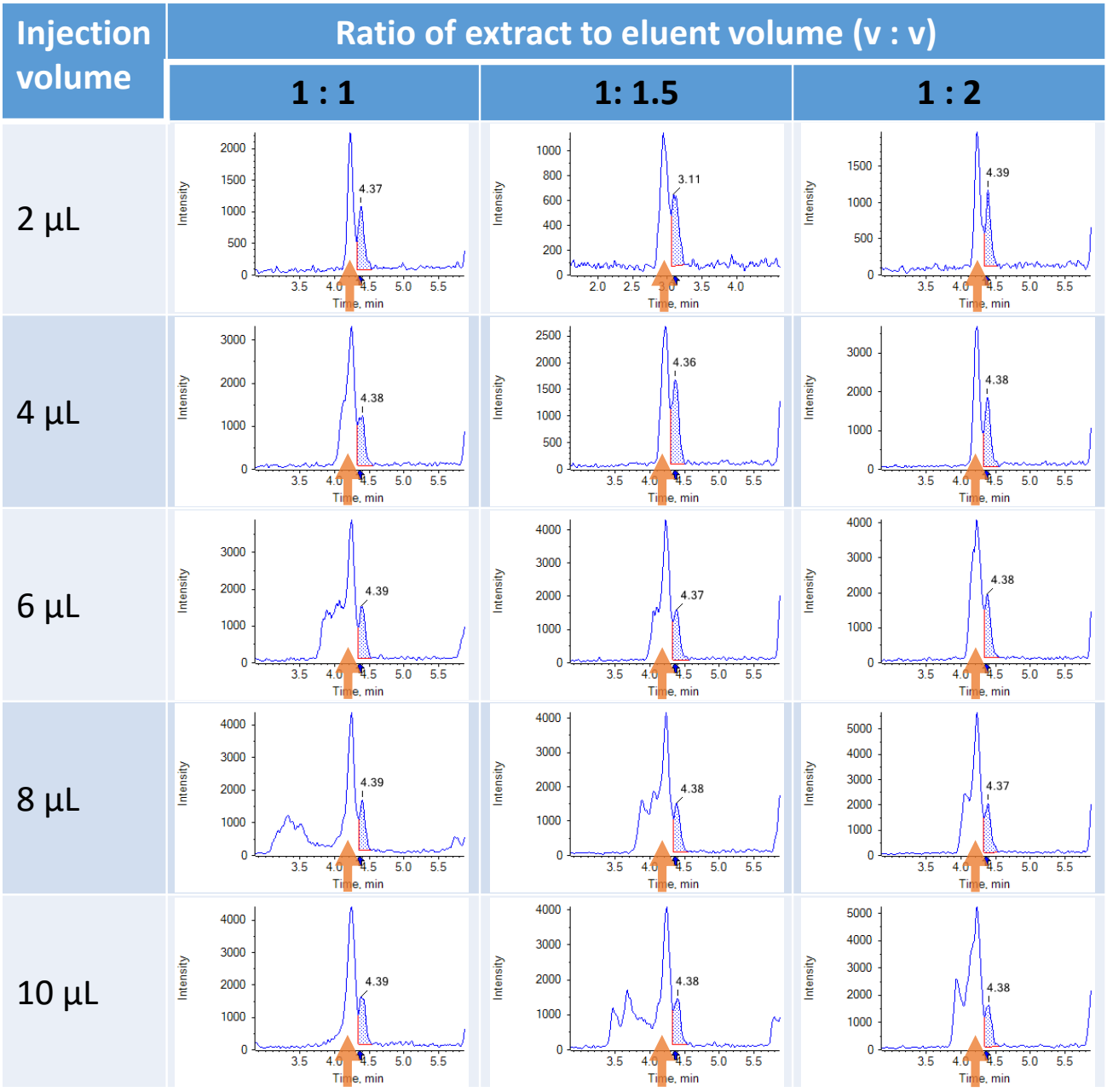


Figure 4: Optimization of the injection volume and the extract dilution with eluent A. Quantifier mass trace m/z 75/58, matrix: cucumber, PDA-concentration: 0.05 mg/kg (LOQ). 1,3-diaminopropane (naturally present) is marked with an orange arrow.

Influence of the type of calibration on results:

As the partitioning yields of PDA into the acetonitrile phase during the QuEChERS approach are quite low (~20 %), a calibration approach correcting for recovery such as the use of an ILIS (e.g. PDA-D_6) or a procedural calibration is needed.
Both solvent-based calibration using ILIS and procedural matrix-matched calibration (with or without ILIS) may be used to obtain acceptable results. This is exemplarily shown in Figure 5. Using an external solvent-based calibration and ILIS the recovery rate is corrected for the abovementioned PDA partitioning losses as well as for the matrix effects, but not for any poor conversion yield of propineb to PDA during the acidic hydrolysis. Disregarding the ILIS and calculating via the peak-areas, a recovery rate of 22% is obtained, which is neither corrected for poor conversion yields nor for matrix

effects (in this case negligible). This figure reflects the approximate recovery of the QuEChERS partitioning step.
Matrix-based procedural calibration with ILIS resulted in an apparent recovery rate of 85%, which matches well with the 87% of the ILIS-corrected external solvent calibration and more or less reflects the conversion yield of propineb to PDA in the hydrolysis step. Matrix-based procedural calibration without using ILIS for calculation more or less corrected the partitioning losses but obviously introduced a positive bias (101 % vs. 85 %).

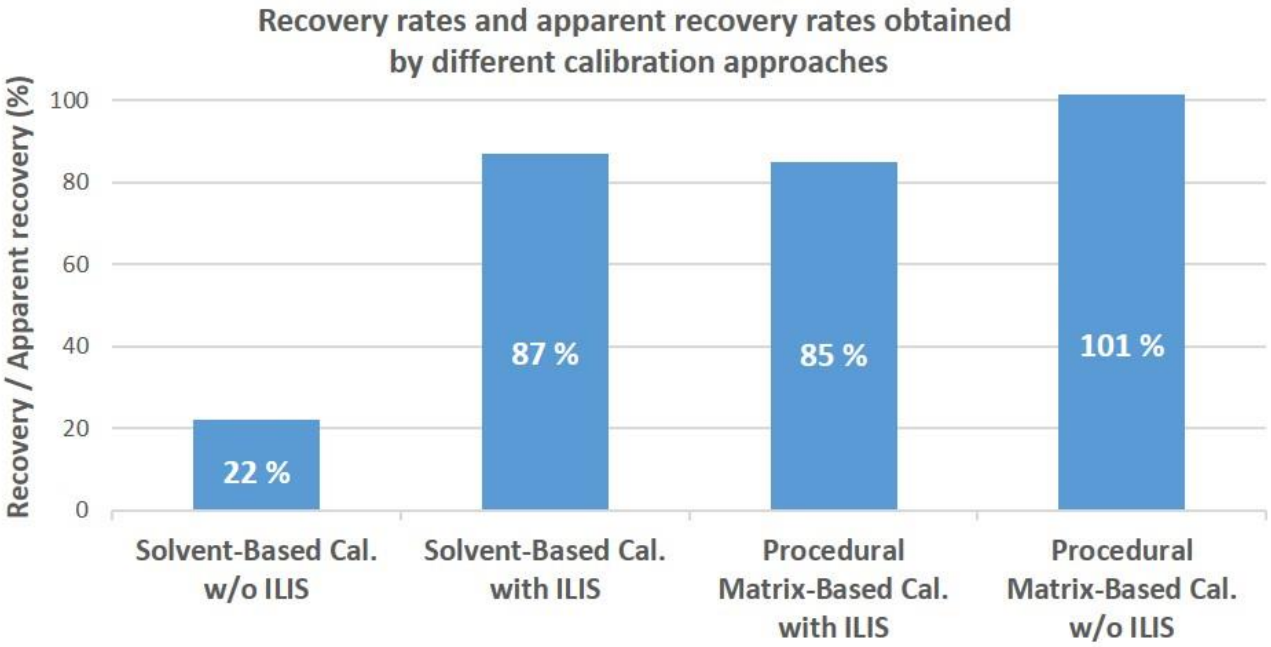


Figure 5: Recovery rates obtained/calculated depending on the calibration type used (0.05 mg/kg, Matrix: fruit)

Validation data:

Validation experiments for propineb determined as PDA were conducted on kiwi fruit homogenate with propineb being spiked in quintuplicate to 50 g portions (at the beginning of the procedure). The propineb standard suspension used for spiking was prepared as described above. The obtained recovery rates and the observed matrix effects are shown in Table 5. Matrix effects were calculated by comparing the PDA signal intensities obtained from a standard solution prepared in blank extract with the signal intensities obtained from an equally concentrated standard solution prepared in a whole processed reagent blank. The corresponding chromatograms are shown in Figure 6.

Validation experiments with matrices belonging to other matrix groups are pending. Based on the experiences gained with the acidic matrix kiwi fruit, an LOQ of 0.05 ppm in high water content commodities is deemed achievable.

Table 5 Recoveries, relative standard variations (RSD) and the matrix effect for the validation of PDA in kiwi fruit at 0.05 mg/kg (expressed as propineb), n = 5.

Matrix	Sample weight	Spiking level of propineb expressed as PDA[1] (mg/kg)	Mass trace	Calculation using[2] …						Matrix effect[3]
				ILIS PDA-D_6 and **matrix-based** procedural calibration		Matrix-based procedural calibration (via area)		ILIS PDA-D_6 and solvent-based procedural calibration		
				Mean Rec.	RSD	Mean Rec.	RSD	Mean Rec.	RSD	
Kiwi fruit	50 g	0.05	75/58	79 %	5 %	88 %	4 %	82 %	5 %	+6 %
			58/41	73 %	8 %	83 %	7 %	77 %	5 %	

1) *Propineb was spiked to analytical portions of kiwi homogenate for the recovery experiments. A level of 0.05 mg/kg PDA corresponds to 0.196 mg/kg propineb for cucumber and kiwi fruit, based on a conversion factor of 3.91 (see molecular weights in Table 1).*
2) *Calculated using mean peak areas of matrix-based semi-procedural calibration standard vs. mean peak areas of solvent-based semi-procedural calibration standard of PDA-D_6. Semi-Procedural calibration standards were prepared as follows:*
*a) **Solvent-based**: by spiking a number of 10 mL aliquots of "aqueous CS_2 hydrolysates" of solvent (=reagent blank) with PDA and subjecting them to alkaline QuEChERS*
*b) **Matrix-based**: by spiking a number of 10 mL aliquots of "aqueous CS_2 hydrolysates" of matrix (=matrix blank) with PDA and subjecting them to alkaline QuEChERS*

Chromatograms of the validation experiments:

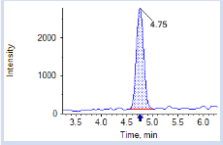
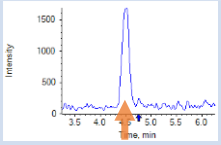
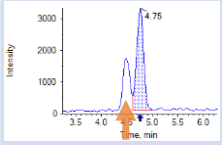
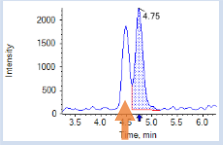
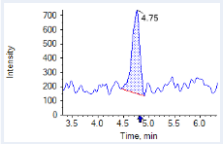
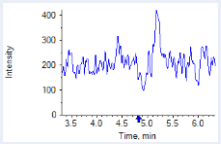
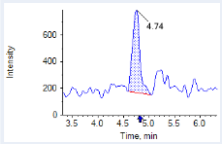
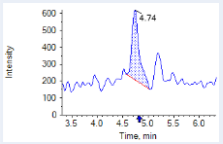
Matrix	Level of PDA spiked	Mass transition	Solvent calibration (120 % level)	Matrix blank	Matrix calibration (120 % level)	Recovery
Kiwi fruit	0.05 ppm	75/58				
		58/41				

Figure 6 Selected chromatograms of the conducted validation of PDA in kiwi fruit at 0.05 ppm (LOQ); the matrix interference by 1,3-diaminopropane is marked as orange arrow.

Intermediate Conclusions and Outlook:

A method for the analysis of propineb residues was developed, which starts with the traditional reductive cleavage with HCl/$SnCl_2$ to CS_2 and 1,2-diaminopropane (PDA), followed by a QuEChERS-like step under alkaline conditions. PDA measurement involves ion-pair LC separation on a C_{18} column followed by electrospray ionization in the positive mode and MS/MS analysis.
Chromatographic separation of PDA from the naturally occurring isomer 1,3-diaminopropane proved challenging, with the PDA signal being increasingly interfered at high 1,3-diaminopropane concentrations. Validation of propineb was successful in kiwi at 0.05 mg/kg (expressed as PDA), which corresponds to the current MRL of 0.05* mg/kg according to Regulation 396/2005/EC. In cucumber, recovery rates were sufficiently consistent but rather low, which raises the need of optimizing the reductive cleavage procedure. Further validation experiments on other types of commodities and the optimization of the reductive cleavage are planned. The use of derivatization to improve the analysis of PDA is also planned to be checked.

For labs planning to routinely apply this method, a suitable screening of propineb metabolites as trigger for this method is deemed recommendable, even though the determination of CS_2 can be conducted simultaneously. Measurement of many samples from the market suggests PTU (propylene thiourea), one of the main metabolites and reaction products of propineb, is a suitable marker for propineb. PTU can be determined by the QuPPe method[23]. The suitability of additional metabolites for propineb as marker substances is currently being investigated by the EURL-SRM and will be reported elsewhere.

History

Action	When	Document Version
Experiments	H2 2013 and H1 2014 (analysis of PDA directly from the aqueous CS2 hydrolysate) – Poster at EPRW in 2014. Q1 2018 and H2 , further experiments involving analysis of PDA using modified QuEChERS. 2020, development of the ion-pairing LC-method, final adjustments of the QuEChERS-procedure and validation experiments.	
Observation document placed online	January 2022	V1

[23] https://www.eurl-pesticides.eu/docs/public/tmplt_article.asp?CntID=887&LabID=200&Lang=EN