EURL-SRM - Analytical Observations Report

Concerning the following…

- **Compound(s)**: Phthalimide (PI), Tetrahydrophthalimide (THPI)
- **Commodities**: Plant origin
- **Extraction Method(s)**: CEN-QuEChERS
- **Instrumental analysis**: LC-MS/MS

Analysis of the folpet degradant phthalimide and the captan degradant tetrahydrophthalimide by QuEChERS and LC-MS/MS

Version 2 (16.03.2023)

Background information

In 2016, the legal residue definitions for captan and folpet were modified to include their respective degradants tetrahydrophthalimide (THPI) and phthalimide (PI)[1,2]. This inclusion has lessened the need for labs to take measures to minimize the degradation of captan and folpet during the various stages of analysis (sample comminution, extraction, cleanup, extract storage, see Figure 1), but has also created new challenges for the labs, as GC-analysis of PI and THPI is tricky. This is because the THPI and PI signals obtained in GC originate partly from the THPI and PI amounts originally present in the extracts and partly from those amounts formed from captan and folpet within the hot GC injector. Using ILIS or other suitable calibration techniques, the thermal losses of captan and folpet within the GC-injector are corrected. The degradants formed, however, add up to the existing THPI and PI signals and are thus overestimated. Summing up an already corrected GC-result of the parent compound to the overestimated GC-result of the degradation product (expressed as parent) will lead to overestimated results for the sum.

A procedure, in which the parts of THPI and PI formed from parent breakdown during GC-injection are deducted from the respective detected signals, has been elaborated by the EURL-SRM and published in an analytical observations report[3]. This approach can deliver sufficiently accurate results but it has limitations when it comes to routine applicability. Issues concerning extraction and cleanup of captan and folpet are also discussed in the said document.
LC-MS/MS offers a possibility to circumvent the problems and errors associated with direct GC analysis of PI and THPI. In the case of PI, analysis via LC-MS/MS additionally circumvents the risk

[1] For Captan: Reg. (EU) 2016/452 of 29 March 2016; latest amendment Reg. (EU) 2019/1015
[2] For Folpet: Reg. (EU) 2016/156 of 18 January 2016; latest amendment Reg. (EU) 2022/93
[3] **http://www.eurl-pesticides.eu/userfiles/file/EurlSRM/meth_CaptanFolpet_EurlSRM.pdf**

of false positives, resulting when compounds other than folpet (e.g. phthalanhydrite) thermally decompose to PI during GC-injection.
In this context, it should be kept in mind, that PI levels, unrelated to folpet, may be also formed during sample processing (mainly drying), for example when phthalates or phthalanhydrite react with nitrogen-containing compounds[4,5]. The LC-MS/MS approach cannot distinguish between the PI levels originating from folpet and those levels of other origin. It is further worthwhile noticing, that THPI is also not fully specific to captan, as it is also formed from captafol, which was, however, internationally banned many years ago and thus unlikely to be used[6].

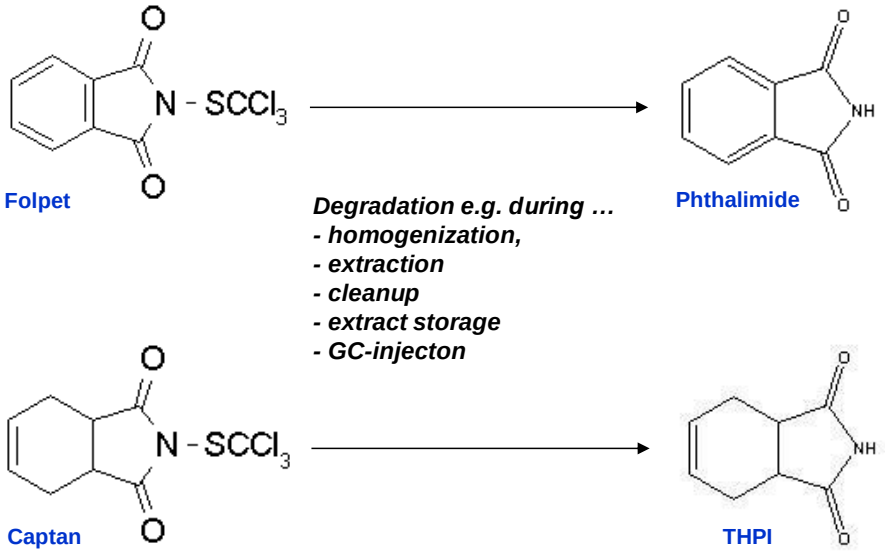


Figure 1: **Degradation of captan and folpet to THPI and PI and critical steps during analysis**

Brief trials in 2016-19, for direct LC-MS/MS analysis of THPI and PI and of captan and folpet (as such or as in-source fragments) in one single run, were rather dissatisfying in terms of sensitivity. However, as GC-analysis suffers from difficulties to distinguish between the parts of THPI and PI originally present in the sample and those parts generated within the GC-injector, it was decided to give LC-MS/MS measurement another try in the hope of achieving the required sensitivity at least for the metabolites PI and THPI. A method using QuEChERS and LC-ESI (neg)-MS/MS was therefore published by the EURL[7]. However, a lack of sensitivity during routine analysis was observed for PI and THPI with this approach. Additionally, the method lacked of specifity as for PI just one "real" mass transition was obtained, while the other mass trace was a pseudo-MRM (parent mass/parent mass, m/z 146/146).

[4] Relana (2016/07/22): http://www.relana-online.de/wp-content/uploads/2016/07/PP_16-03_Folpet-PI_vers20160722.pdf
[5] Maximilian Wittig, Julia Biller, Athanasios Nitsopoulos, Albrecht Friedle; Food Chemistry; Volume 374, 16 April 2022, 131544; De novo formation of phthalimide from ubiquitous phthalic acid derivatives during the drying process of tea (Camellia sinensis) and selected herbal infusions
[6] Captafol was identified as a carcinogen and is was withdrawn from the German market in 1986. It is not approved in the EU and according to **https://ntp.niehs.nih.gov/ntp/roc/content/profiles/captafol.pdf** "… *by 2010, no countries were identified that still allowed the use of captafol on food crops.*" Furthermore, captafol is among the compounds the trade of which is regulated by the Rotterdam convention.
[7] https://www.eurl-pesticides.eu/library/docs/srm/EurlSrm_Observation_Captan_Folpet_LC-V1.pdf

A new attempt was thus made to check whether good sensitivity can also be achieved in the ESI positive mode. Both PI and THPI showed better fragmentation patterns in the positive mode, resulting in several useful mass transitions, see Figure 2.

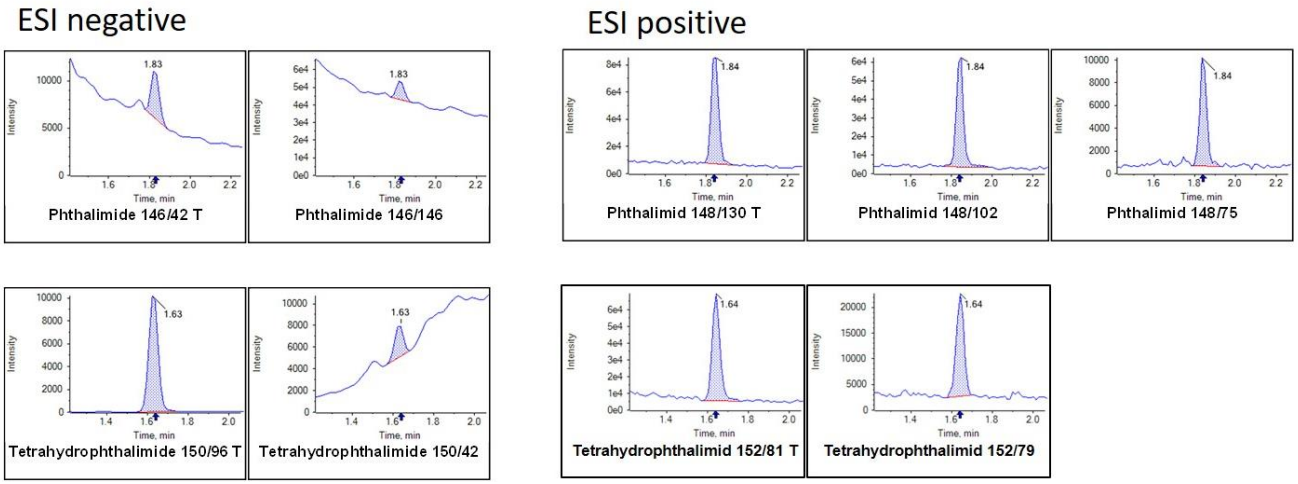


Figure 2: Exemplary comparison of ESI modes using the same eluent conditions for a solvent standard of 0.01 µg/mL in acetonitrile.

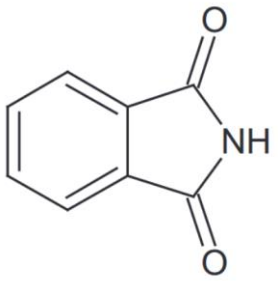
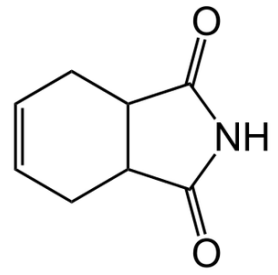
Using this approach, PI and THPI were analyzed by LC-MS/MS in the ESI (pos) mode from QuEChERS extracts. The procedure is straightforward and sensitive, and does not require high-end instrumentation as both PI and THPI are measured sensitively in the ESI positive mode. The procedure has the potential for being incorporated into the multiresidue scheme of labs. If not incorporated it may also run standalone and employed in case of a detection of a marker compound by a routinely employed method (e.g. detection of captan and/or tetrahydrophthalimid by a GC-based method).

Analyte properties

The physicochemical properties and additional information on phthalimid and tetradhydropthalimid are given in Table 1. The data on their respective parent compounds and additional analytical strategies and information can be found in the observations on “Quantification of Residues of Folpet and Captan in QuEChERS Extracts” (Report SRM-07) [8] and “Analysis of Captan, Folpet and their respective metabolites Phthalimide and Tetrahydrophthalimide via LC-MS/MS either directly or following hydrolysis” (Report SRM-42)[5].

[8] https://www.eurl-pesticides.eu/userfiles/file/EurlSRM/meth_CaptanFolpet_EurlSRM.pdf

Table 1: Chemical Properties of PI and THPI

Phthalimide (CAS: 85-41-6) Other names: 1,2-benzenedicarboximide, 1H-isoindole-1,3(2H)-dione		
Parameter	Value/Notes	
Molecular Mass	147.133 g/mol	
Formula	$C_8H_5NO_2$	
Boiling point	366 °C	
pKa	8.4 moderately acidic (computed by chemicalize.com)	
LogP	• Chemicalize.com (computed): ~ 0.68 up to pH 7; drops dramatically from pH 8 onwards; 0 at pH 9; -0.75 at pH 10	
Water solubility	• Chemicalize.com (computed): ~1.8 mg/mL at pH up to 8; increases dramatically from pH 9 onwards • ECHA: 360 mg/l at 25°C	
Stability	Hydrolysis to phthalic acid via phthalamic acid as an intermediate	
Residue definition (EU)	Folpet (sum of folpet and phtalimide, expressed as folpet); Reg. (EU) 2018/832	
Approved in …	**Folpet**: AT, BE, BG, CY, CZ, DE, DK, EL, ES, FR, HR, HU, IE, IT, LU, MT, NL, PL, PT, RO, SE, SI, SK	
Toxicity	Phthalimide itself is not classified according to Reg. 1272/2008 due to its low toxicity in general [9,10]. No ARfD or ADI is set for phthalimide. EFSA (2017): The toxicological reference values of the parent apply to the metabolite phthalimide for the consumer risk assessment.	
Other sources	Possible metabolite from phosmet and ditalimphos; rarely found among a few burning coal fire sites as the natural mineral kladnoite[11]. In presence of compounds with primary amino groups and preferably anhydric conditions it is also formed from phthalic acid and phthalic anhydride. This may explain the high presence of phthalimide in dry products, see also[4,5]. A formation of phthalimide from phthalic anhydride and phthalic acid in the hot GC-injector also takes place.	
Tetrahydropthalimde (CAS: 1469-48-3) Other names: (3aR,7aS)-2,3,3a,4,7,7a-hexahydro-1H-isoindole-1,3-dione, 4-Cyclohexene-1,2-dicarboximide		
Parameter	Value / Notes	
Molecular Mass	151.165 g/mol	
Formula	$C_8H_9NO_2$	
Boiling point	337 °C	
pKa	10.4 slightly acidic (computed by chemicalize.com)	
LogP	• Chemicalize.com (computed): ~ 0.16 up to pH 9; drops dramatically from pH 10 onwards; -0.5 at pH 11	
Water solubility	• Chemicalize.com (computed): ~30 mg/mL at pH up to 9; increases dramatically from pH 10 onwards • ECHA: 12.2 g/l at 20 ± 0.5 °C at pH 3.4	
Stability	Hydrolytically quite stable	
Residue definition EU	Captan (Sum of captan and THPI, expressed as captan); Reg. (EU) 2019/1015	
Approved in …	**Captan**: AT, BE, BG, CY, CZ, DE, EE, EL, ES, FR, HR, HU, IE, IT, LT, LU, LV, NL, PL, PT, RO, SI, SK	
Toxicity	Tetrahydrophthalimide itself is not classified according to Reg. 1272/2008 due to its low toxicity in general [9,10]. No ARfD or ADI is set for tetrahydrophthalimide. "… THPI, 3-OH THPI and 5-OH THPI were demonstrated to be of lower toxicity compared to captan but data were not sufficient to derive specific reference values … it was concluded that the reference values for captan would also apply…" (EFSA Reasoned Opinion 2014)	
Other sources	Possible metabolite of captafol	

[9] Reg. (EC) No 1272/2008 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

[10] https://echa.europa.eu/de/registration-dossier/-/registered-dossier/13146/7/11/6

[11] https://www.mindat.org/min-2222.html

Apparatus, Chemicals and Consumables

Chemicals and Materials

The used materials and apparatuses are listed in the QuEChERS standard procedure (EN-15662).

Analytical standards of the analytes

The suppliers of the used analytical standards are shown in Table 2.

Table 2: Sources of Analytical standards (exemplary).

Compounds	Details on standards used		Provider
Phthalimide	Supplier Code / Purity	674338 (99.7 %)	HPC
Tetrahydrophthalimde	Supplier Code / Purity	DRE-C17406500 (99.0 %)	Dr. Ehrenstorfer GmbH

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 solutions of both substances (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 48 months. Working solutions, e.g. in terms of a mix of both substances, are prepared as necessary in acetonitrile and may be stored in the refrigerator for many months until use.

Sample Preparation

Homogenization:

Samples are homogenized by cryogenic milling using dry ice according to Document Nº SANTE/12682/2019.

Sample preparation:

The samples are extracted according to the QuEChERS-CEN (citrate-buffered; EN-15662) method without applying dSPE-cleanup. High oil content commodities are extracted according to the QuOil method (CEN/TS 17062:2019) instead of the QuEChERS method. As internal standards chlorpyrifos-D_{10} and propyzamide-D_3 (e.g. 100 µL of a mixture in acetonitrile at 10 µg/mL each) may be used. The internal standards are added to the sample portion before extraction in case of QuEChERS extraction and to an aliquot of the final extract in case of QuOil extraction. Isotope labelled PI and THPI may also be used to correct for matrix effects even when using a calibration standard based on a different matrix or based on solvent[12].

[12] In this case keep in mind that captan D6 and folpet D4 may also degrade to PI-D4 and THPI-D4 thus influencing the signals.

Measurement

The extract is directly subjected to LC-MS/MS separation and measurement. Exemplary LC-MS/MS conditions are given in Table 3.

Table 3: Instrumentation and method details (LC: Agilent 1290 Infinity II; MS: Sciex QTrap 5500+)

Instrument parameters	Conditions					
Column/temperature	Waters Acquity BEH C_{18}, 2.1x100 mm, 1.7 µm; 40 °C					
Pre-column	Van Guard BEH C_{18} 1.7um					
Eluent A	0.01% acetic acid in Water + 5 % acetonitrile					
Eluent B	0.01% acetic acid in acetonitrile					
Gradient	**%A**	**Flow [mL/min]**	**Time [min]**			
	95	0.4	0			
	10	0.4	3.00			
	10	0.4	6.00			
	95	0.4	6.10			
	95	0.4	10.00			
Injection volume	2 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)**
	Phthalimde	148	130	66	23	10
		148	102	66	35	10
		148	75	66	37	12
	Tetrahydrophthalimde	152	81	101	19	10
		152	79	101	33	12
	Chlorpyrifos-D_{10} (internal standard)	360	199	95	23	12
	Propyzamid-D_3 (internal standard)	259	193	61	21	10
Ionisation mode	ESI Positive					
Ion Source Parameters	**Curtain Gas Flow**	40 psi				
	Ion Spray Voltage	4500 V				
	Temperature	550 °C				
	Nebulizer Gas Flow	60 psi				
	Heater Gas Flow	70 psi				

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

Validation data:

Validation experiments for PI and THPI were conducted on matrices representing all main commodi-tiy groups of plant origin according Document Nº SANTE/12682/2019. Both substances were spiked in quintuplicate to sample homogenates as described in the particular QuEChERS-CEN and QuOil procedures using a mixture of both substances in acetonitrile prepared as described above. The obtained recovery rates and the observed matrix effects are shown in Table 4 and Table 5. Matrix effects were calculated by comparing the respective signal intensities obtained from a standard solu-tion prepared in extract of the respective blank matrix with the signal intensities obtained from an equally concentrated standard solution prepared in acetonitrile. Exemplary chromatograms are shown in Figure 3 and Figure 4.

Table 4: Validation data of phthalimide (PI) using QuEChERS and QuOil methods. Spiked at 0.005 mg/kg and 0.010 mg/kg, each n = 5.

Matrix	Method	Spiking level (mg/kg)	Mass transition trace	Calculation using matrix-matched calibration		Matrix effect[1)]
				Mean Rec.	RSD	
Cucumber	QuEChERS	0.005	148/130	92 %	3 %	-25 %
			148/102	90 %	6 %	
			148/75	93 %	4 %	
		0.010	148/130	96 %	3 %	
			148/102	97 %	3 %	
			148/75	98 %	5 %	
Grapes		0.005	148/130	91 %	10 %	-62 %
			148/102	92 %	7 %	
			148/75	85 %	14 %	
		0.010	148/130	88 %	6 %	
			148/102	97 %	5 %	
			148/75	99 %	4 %	
Wheat flour		0.005	148/130	98 %	3 %	-37 %
			148/102	96 %	2 %	
			148/75	97 %	5 %	
		0.010	148/130	90 %	2 %	
			148/102	93 %	3 %	
			148/75	89 %	4 %	
Peanut butter	QuOil	0.005	148/130	96 %	5 %	-13 %
			148/102	96 %	4 %	
			148/75	106 %	7 %	
		0.010	148/130	101 %	4 %	
			148/102	96 %	6 %	
			148/75	98 %	4 %	

1) *Based signals obtained in mass trace 148/130 at a spiking level corresponding to 0.012 mg/kg. Calculated using the mean peak area of matrix-matched standard (A_M) and the mean peak areas of solvent-based standard (A_S), with the formula: $((A_M / A_A) - 1)*100$*

Table 5: Validation data of THPI using QuEChERS and QuOil methods. Spiked at 0.005 mg/kg and 0.010 mg/kg, each n = 5.

Matrix	Method	Spiking level (mg/kg)	Mass transition trace	Calculation using matrix-matched calibration		Matrix effect[1)]
				Mean Rec.	RSD	
Cucumber	QuEChERS	0.005	152/81	94 %	2 %	-19 %
			152/79	97 %	5 %	
		0.010	152/81	97 %	3 %	
			152/79	96 %	4 %	
Grapes		0.005	152/81	91 %	5 %	-71 %
			152/79	86 %	15 %	
		0.010	152/81	91 %	8 %	
			152/79	89 %	14 %	
Wheat flour		0.005	152/81	95 %	2 %	-21 %
			152/79	n.d.[2]	-	
		0.010	152/81	80 %	3 %	
			152/79	n.d.[2]	-	
Peanut butter	QuOil	0.005	152/81	98 %	1 %	-12 %
			152/79	n.d.[3]	-	
		0.010	152/81	98 %	2 %	
			152/79	n.d.[3]	-	

1) *Based signals obtained in mass trace 152/81 at a spiking level corresponding to 0.012 mg/kg. Calculated using the mean peak area of matrix-matched standard (A_M) and the mean peak areas of solvent-based standard (A_S), with the formula: $((A_M / A_A)- 1)*100$*

2) *In the given mass-trace, the blank wheat extract showed signals ("blank values") well exceeding 30 % of the expected peak signal. The validation was therefore considered invalid as the method criteria of the Document Nº SANTE/11312/2021 were not met.*

3) *Not detectable, as mass trace is severely interfered by matrix.*

Exemplary chromatograms of validation experiments:

Figure 3: Selected chromatograms obtained from injection during the validation of phthalimide (PI) at 0.005 mg/kg (LOQ).

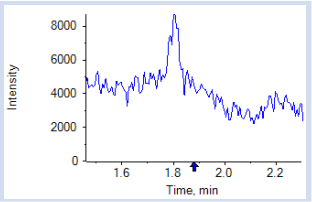
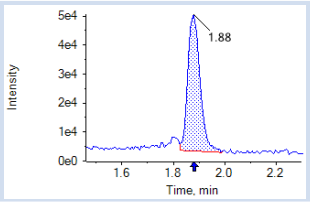
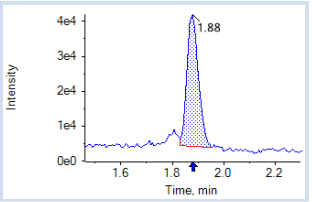
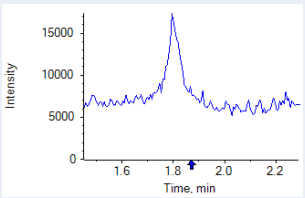
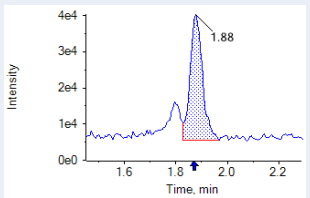
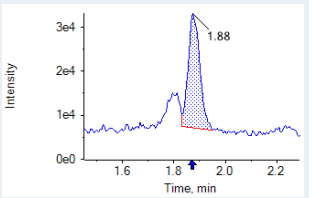
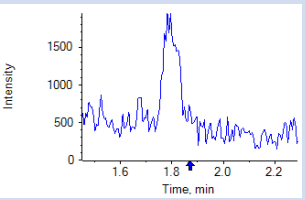
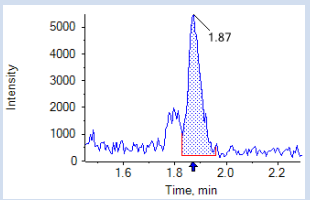
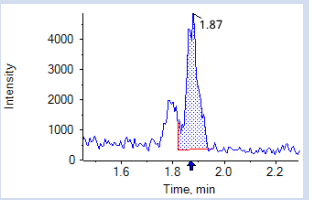
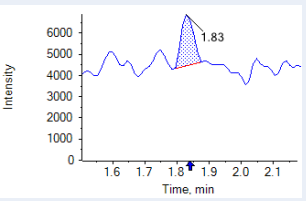
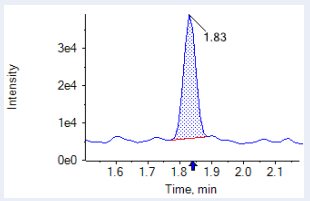
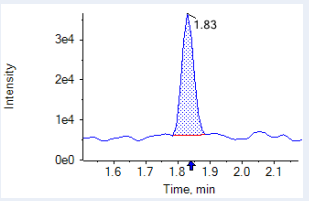
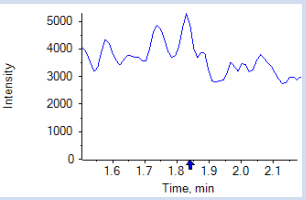
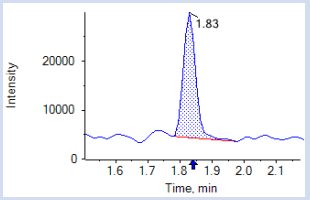
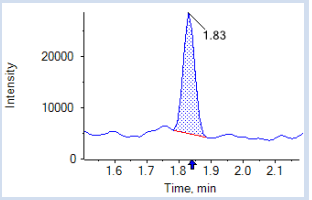
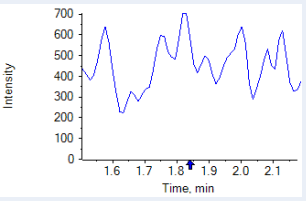
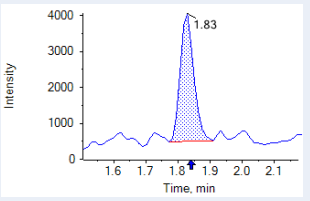
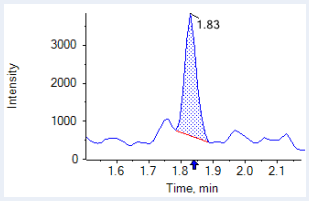
Matrix	Mass transition	Matrix blank	Matrix calibration (120 % level)	Recovery
cucumber	148/130			
	148/102			
	148/75			
grape	148/130			
	148/102			
	148/75			

Figure 3, cont.

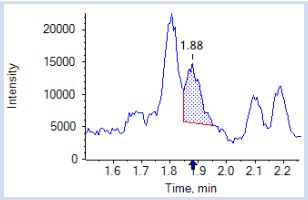
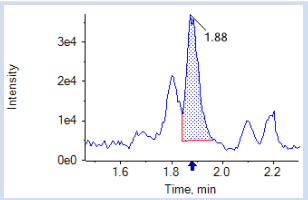
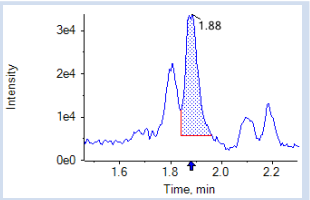
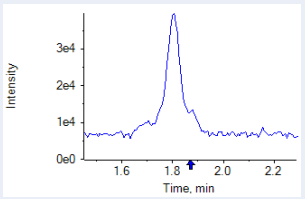
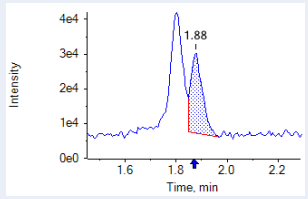
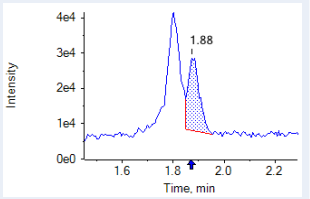
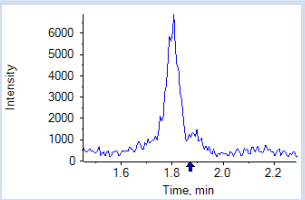
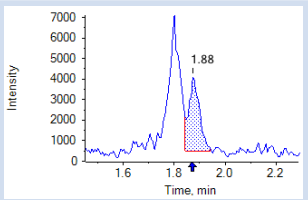
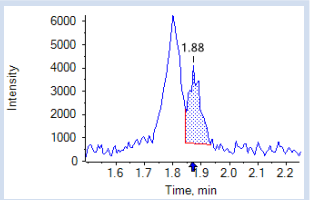
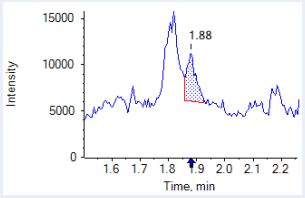
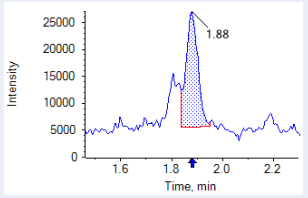
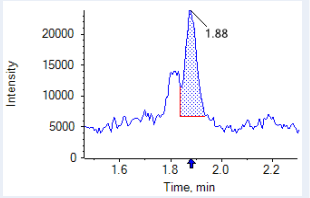
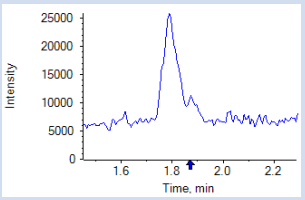
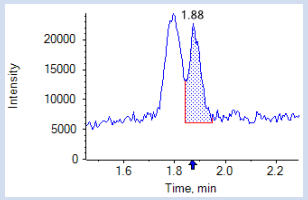
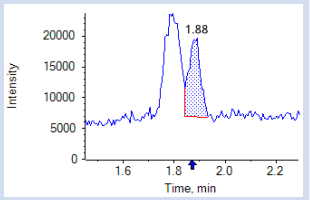
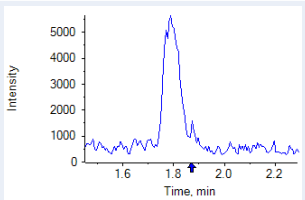
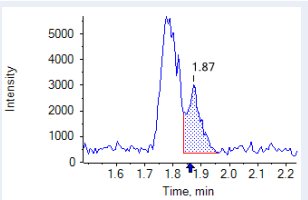
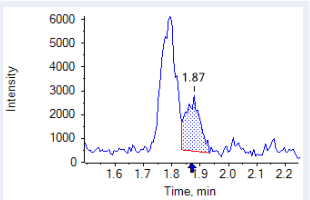
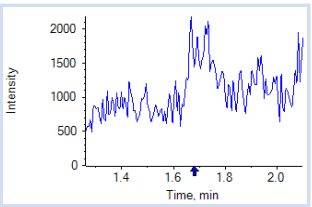
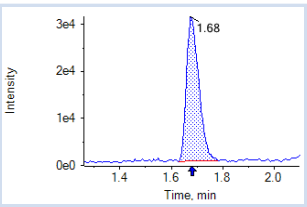
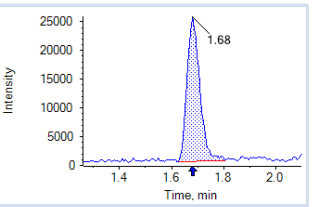
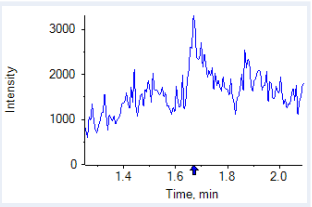
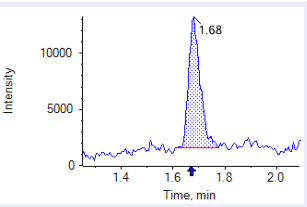
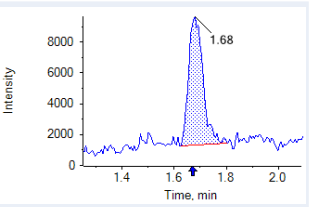
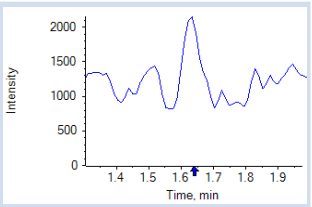
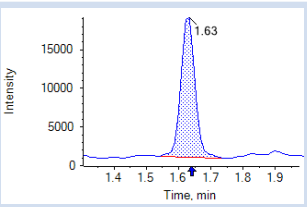
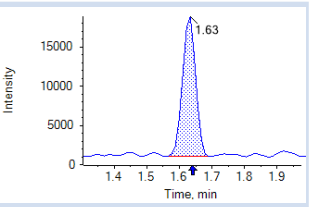
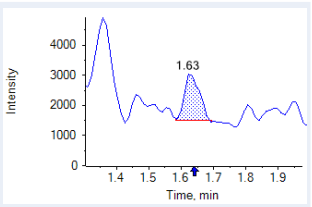
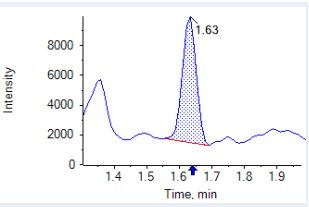
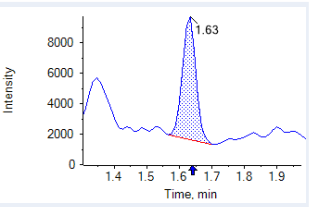
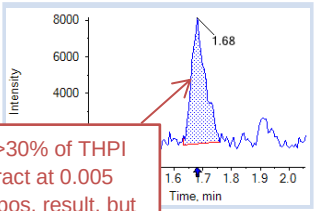
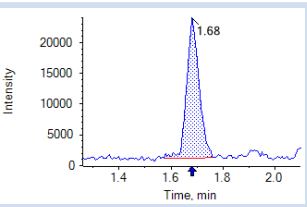
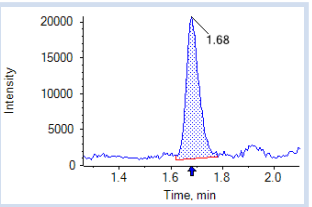
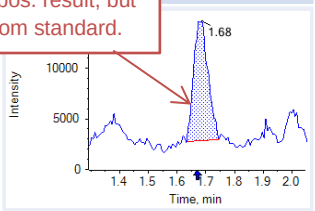
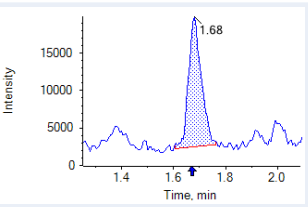
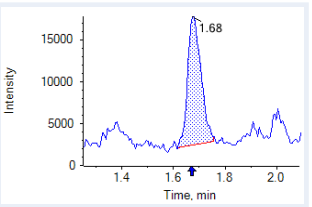
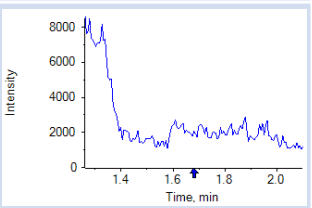
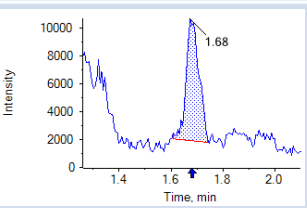
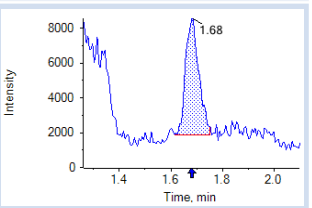
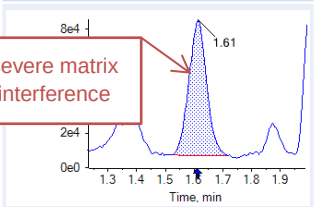
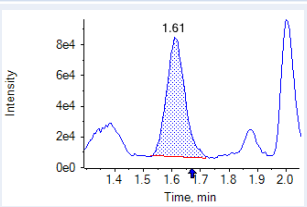
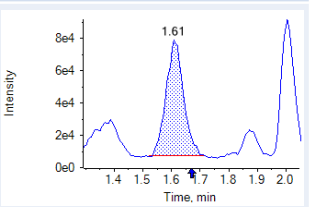
Matrix	Mass transition	Matrix blank	Matrix calibration (120 % level)	Recovery
wheat flour	148/130			
	148/102			
	148/75			
peanut butter	148/130			
	148/102			
	148/75			

Figure 4:: Selected chromatograms of the conducted validation of THPI at 0.005 mg/kg (LSVL).

Matrix	Mass transition	Matrix blank	Matrix calibration (120 % level)	Recovery
cucumber	152/81			
	152/79			
grape	152/81			
	152/79			
wheat flour	152/81			
	152/79			
peanut butter	152/81			
	152/79			

Area of blank extr. >30% of THPI area in spiked extract at 0.005 mg/kg. Risk of false pos. result, but ion ratio deviates from standard.

severe matrix interference

Other Experiments and Observations:

Conversion of Captan and Captafol to THPI and of Folpet to PI, during extraction / cleanup:

To study the stability of captan, captafol and folpet prior as well as during QuEChERS extraction and cleanup, a small scale experiment was conducted using homogenates of cucumber (neutral pH) and orange (acidic pH). Captafol was also considered in this experiment as it is known to degrade to THPI as well.

Matrix homogenates were spiked with captan, captafol and folpet under different conditions and following different approaches as follows:

a) Spiked in frozen condition followed by immediate extraction;
   i. No clean-up
   ii. cleanup with PSA and immediate acidification after clean-up
   iii. cleanup with PSA, followed by a standing time of 2h at RT before acidification
b) Spiked in thawed condition (at RT), followed by a standing time of 2h (at RT) before extraction

All experiments were conducted in duplicate. Homogenate portions were spiked with a mixture of folpet and captan at 0.2 mg/kg each. Different homogenate portions were spiked with captafol at 0.2 mg/kg. The parents (Captan/Folpet and Captafol) and their respective metabolites (THPI/PI and THPI respectively) were measured via LC-MS/MS in the ESI-pos. mode (details of method for parents not shown here).

Degradation of both, captan, folpet and captafol was negligible when freshly spiked samples were extracted immediately and measured directly from the raw extract (without PSA clean-up being conducted), see Figure 5, Figure 6 and Figure 7.

When these raw extracts were subjected to PSA clean-up, captan/captafol as well as folpet degraded slightly, leading to the formation of THPI and PI, respectively. Interestingly, the degradation rates of captan and captafol only increased slightly (slight increase of THPI) while folpet was not affected at all when the PSA-cleaned-up extract was left standing for 2 h at RT prior to re-acidification. In past experiments, a more pronounced degradation of captan and folpet in non-acidified extracts following PSA-cleanup was observed.

Most critical was the degradation of captan, captafol and folpet when spiked onto thawed sample homogenates and left standing for 2 h at RT (“worst-case”). In the case of cucumber homogenates, which have a higher pH, this resulted in a nearly complete conversion of captan and captafol to THPI and an extensive conversion of folpet to PI. In all three cases transformation yields were high. In the more acidic orange homogenates, degradation was expectedly slower, yet still significant, with roughly 40% of the captan and captafol transforming to THPI. Folpet didn’t notably transform to PI in orange representing acidic homogenates. The higher stability of these compounds under acidic condition is in agreement with hydrolysis data from literature, and with previous observations regarding the degradation behavior of captan and folpet[13]. Nevertheless, it is to be assumed, that degradation of captan, captafol and folpet does not only depend on pH, as it seems to be strongly driven also by other factors, such as enzymes that could catalyze the degradation within the homogenates.

No enzymatic activity is expected in QuEChERS extracts. The water content is low in QuEChERS raw extracts and very low in cleaned-up QuEChERS extracts. This results in a higher stability of captan, captafol and folpet even at the relatively high extract pH after PSA cleanup.

[13] https://www.eurl-pesticides.eu/userfiles/file/EurlSRM/meth_CaptanFolpet_EurlSRM.pdf

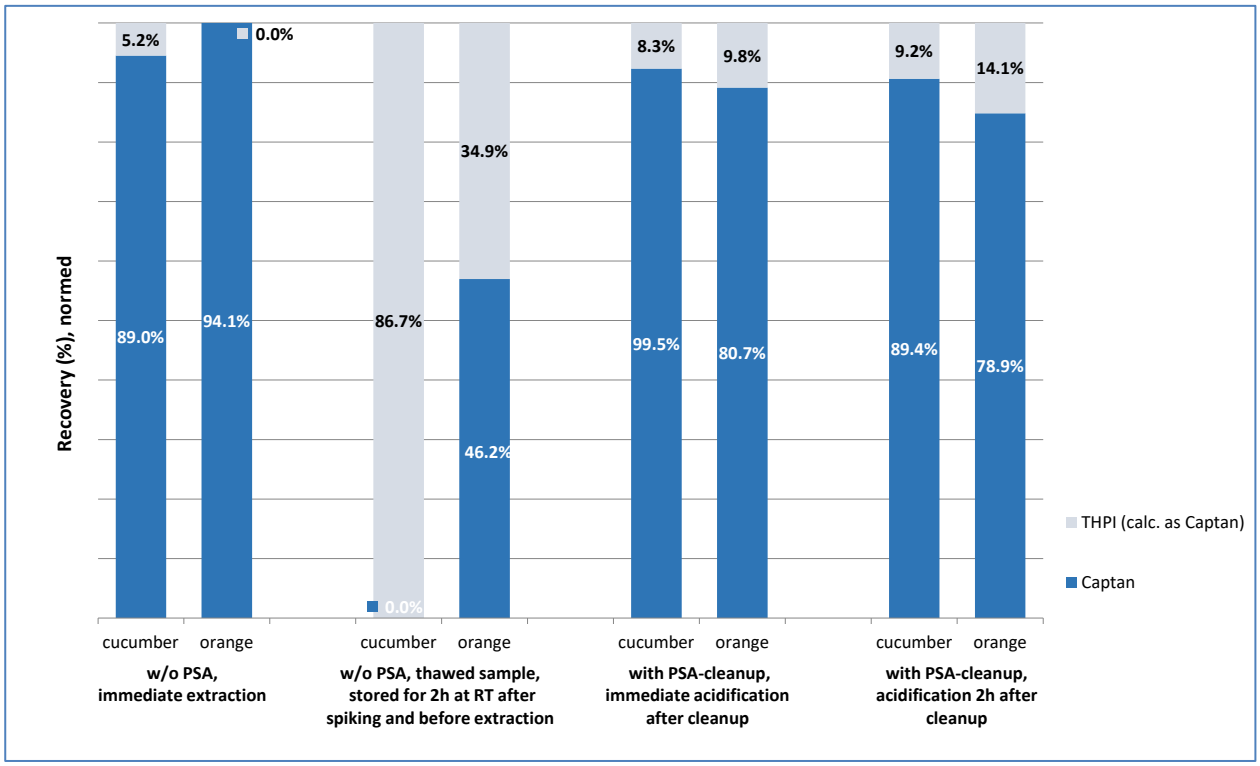


Figure 5: Degradation of Captan to THPI during sample preparation (each n= 2); summed recovery normed to 100 % for each approach. The percentages of Captan reflect the recovery rates, the percentages of THPI are expressed as Captan

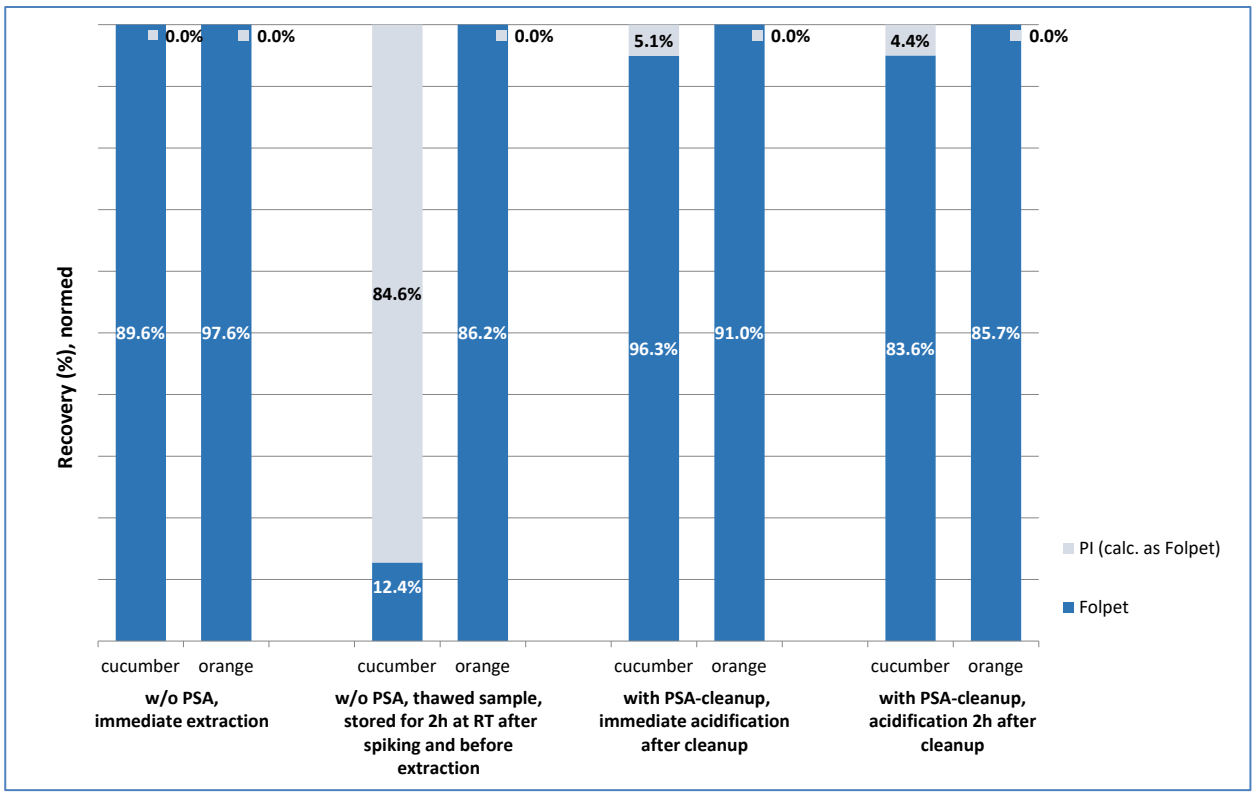


Figure 6: Degradation of Folpet to PI during sample preparation (each n= 2); summed recovery normed to 100 % for each approach. The percentages of Folpet reflect the recovery rates, the percentages of PI are expressed as Folpet

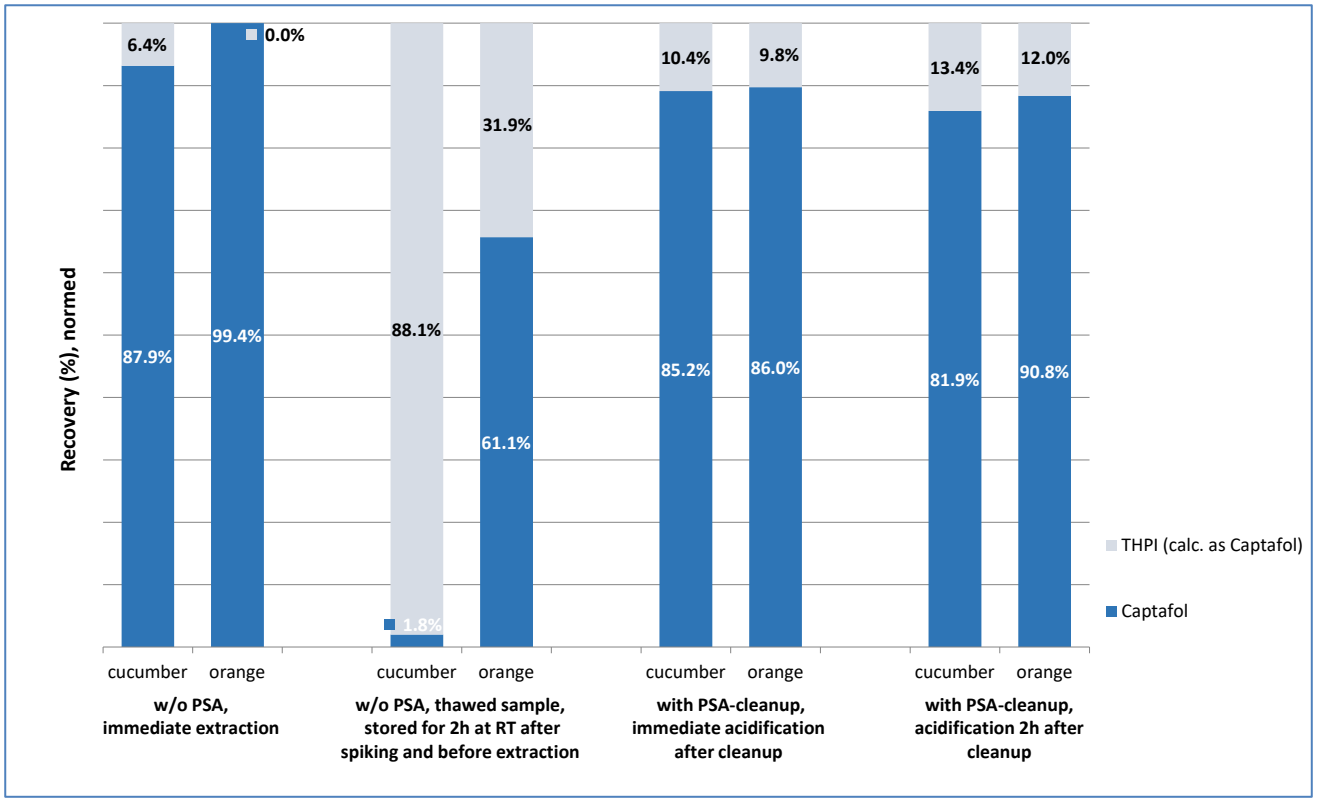


Figure 7: Degradation of Captafol to THPI during sample preparation (each n= 2); summed recovery normed to 100 % for each approach. The percentages of Captafol reflect the recovery rates, the percentages of THPI are expressed as Captafol

Discussion, intermediate conclusions and outlook:

A simple and sensitive method for the analysis of PI and THPI was developed based on QuEChERS extraction and LC-MS/MS determination in the ESI-pos. mode using a C18 column for separation.

Validations of THPI and PI were conducted on various commodities at 0.005 and 0.010 mg/kg, which correspond to ~0.01 and ~0.02 mg/kg when expressed as Folpet and Captan respectively.

PI-validation at these levels was successful for three mass-transitions (m/z 148/130 and 148/102 and 148/75) in cucumber, grapes, wheat flour and peanut butter. Validation of THPI was successful for both measured mass-transitions (m/z 152/81 and 152/79) in cucumber and grapes at both tested levels (0.005 mg/kg and 0.010 mg/kg). However, in wheat flour and peanut butter, THPI validation at 0.005 mg/kg and 0.010 mg/kg was only successful at one single mass-transition (m/z 152/81). The second mass transition (m/z 152/79) showed an MS-interference both in wheat flour extracts (considerably) and peanut butter (very strongly), thus not allowing proper measurement of THPI at low levels. Interestingly, signal suppression on THPI was moderate to negligible in these two commodities. The interference in the case of wheat even showed the same retention time as THPI, but the deviating ion ratio could help to avid a false positive result. Further experiments are planned to increase selectivity and enable identification of THPI at low levels, both at the sample preparation (i.e. cleanup) and at the measurement stage.

The parent compounds of THPI and PI (Captan and Folpet) show a rather poor sensitivity in LC-MS/MS not allowing accurate analyses at low levels. For such analyses the well-established GC methodology is thus recommended (see SRM-07). As Captan and Folpet may degrade to THPI and PI at various stages of the procedure, it is important to analyse THPI/PI and Captan/Folpet from the same extract and within a reasonably short time distance.

Based on validation experiments conducted by the EURL-SRM, for Captan and Folpet using GC, and for THPI and PI via the present procedure, the lowest MRLs for Captan (Sum) and Folpet (Sum) in acidic and non-acidic commodities of high water content (at 0.02* and 0.03* mg/kg- expressed as parent - respectively), are considered well enforceable. In dry commodities of low or high fat content the MRLs at 0.07* seem well achievable for PI whereas more experiment are required for THPI.

Overall, it could be shown, that THPI and PI can be potentially incorporated into the multiresidue scheme of labs. If not incorporated, the presented approach may also run as a standalone procedure that is employed following detection of a marker compound during routinely analysis (e.g. detection of Captan and/or THPI by a GC-based method).

It was observed that measurement sensitivity significantly decreases in the presence of ammonium buffers in the LC-MS/MS mobile phase. As the gradient used allows sensitive analysis of many pesticides and metabolites, THPI and PI can be easily integrated into an efficient multiresidue analysis scheme. If not, the presented approach may also run as a standalone procedure triggered upon the detection of a marker compound in a routine method (e.g. detection of captan and/or THPI by a GC-method).

Next to THPI and PI, the parent compounds captan and folpet also need to be analyzed to cover the full residue definition. Analysis of captan and folpet via LC-MS/MS is possible, but the measurement conditions need further optimization to improve sensitivity and robustness (data not shown). Nevertheless, captan and folpet can still be determined by well-established GC-based methods, provided that matrix effects are accounted for. Given the typically good sensitivity achieved for captan and folpet in GC, and the good sensitivity achieved for THPI and PI by the present procedure, the lowest MRLs for captan (sum) and folpet (sum) in acidic and non-acidic commodities of high water content (at 0.02* and 0.03* mg/kg respectively), are well enforceable.

The goal is to develop more sensitive LC-MS/MS methods for folpet and captan, either by implementing them in a method that covers them together with their respective degradants or in another routinely applicable method, preferably covering multiple analytes.

It is important to highlight, that captan and THPI as well as folpet and PI should be measured from the very same extract and within a short time interval in-between, in order to prevent errors deriving from transformations happening during sample preparation or in the extracts. It is furthermore important to highlight, that at least in theory, THPI may also originate from captafol, although captafol is reportedly not produced any more at global level. PI can also have various sources including phthalates and phthalanhydride.

Taking into account the molecular weights of PI, THPI, folpet and captan a conversion factor of 1.989 is required to express THPI as captan and a conversion factor of 2.016 is needed to express PI as folpet.

Document History

Action	When	Doc. Version
Experiments	Q2 2020: Final development of the LC-MS/MS method for the determination of PI and THPI Q3 2020: Validation of PI and THPI on grapes Q1 2022: Validation of PI / THPI in cucumber, wheat flour, peanut butter Q4 2022: Stability of Captan, Captafol and Folpet during QuEChERS	
Version of document	January 2022 March 2023	V1 V2