Analysis of Captan/THPI and of Folpet/PI via GC-MS/MS and LC-MS/MS

F. Hägele, D. Mack, E. Scherbaum and M. Anastassiades
E-Mail: Florian.Haegele@cvuas.bwl.de

Chemisches und Veterinäruntersuchungsamt Stuttgart

Introduction

Since 2016, the legal residue definitions for folpet and captan have been entailing their respective degradants phthalimide (PI) and tetrahydrophthalimide (THPI). Captan and folpet exhibit poor detection sensitivity using standard LC-MS/MS measurement conditions, and are thus typically analyzed by GC-techniques. Accurate GC-quantification of the parents is challenging, but manageable when matrix effects are taken care of. The quantification of THPI/PI is even more challenging as the GC-signals obtained do not originate from the THPI/PI fractions originally present in the samples/extracts (which need to be quantified) but also from the THPI/PI fractions that are formed during GC injection, through thermal degradation of captan and folpet, which are mostly also present. A simple addition of the GC-result of the parent with the result of the degradation product (expressed as parent) will typically lead to overestimated results as the parent losses are compensated through calibration. Several analytical observation reports can be found within the EURL-SRM website, dealing with the analysis of captan and folpet via GC, in which analyte protectants (APs) and ILISs are used to reduce degradation and compensate for matrix effects.

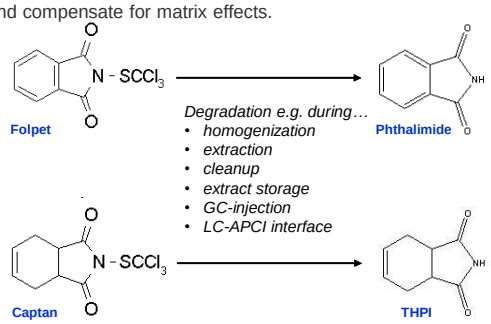

Fig. 1: *Degradation of captan and folpet to PI and THPI*

Methods

In the method presented here, the combination of GC-MS/MS and LC-MS/MS is employed to circumvent the problems associated with direct GC analysis of captan (sum) and folpet (sum). The parent compounds are analyzed via GC using APs for compensating matrix effects, and the degradation products, THPI and PI, via LC-ESI(neg)-MS/MS. At higher levels analysis of captan and folpet via LC-ESI(pos.)-MS/MS is also possible (see analytical observation document in EURL-SRM website), but the focus of this poster is on the LC-MS/MS measurement of THPI and PI. Measurement settings are shown in Tab. 1.

Tab. 1: *LC and ion-source instrumentation settings*

LC		Waters Acquity UPLC I-Class	
MS/MS		SCIEX API 5500 Q-Trap	
Column		Waters BEH C18 2.1x 100 mm 1.7 µm	
Pre-column		Waters BEH C18 2.1x 5 mm 1.7 µm	
ESI neg	**Source Temp.**	550 °C	
	Mobile Phase	A: 0.01% acetic acid in water (with 5% ACN) B: 0.01% acetic acid in acetonitrile	
Gradient		Time (min)	Mobile Phase A (%)
		0	95
		3	10
		6	10
		6.1	95
		11	95
Flow Rate		0.4 mL/min	
Injection Volume		2 µL	
Column Temp.		40 °C	

Following successful validation of the above LC-MS/MS analysis of THPI and PI in various commodities, market samples, previously analyzed by GC-MS/MS and found to contain captan- or folpet-related residues, were re-analyzed by LC-ESI(neg)-MS/MS to demonstrate the potential of overestimating the summed residue when parents and degradants are directly analyzed by GC-MS/MS. In addition, the parents were measured by LC-MS/MS in the ESI-pos. mode (more info see EURL-SRM analytical observation document).

Results

Tab. 2, shows a comparison of THPI and PI results obtained from analyzing QuEChERS-extracts of routine samples by …

a) GC-MS/MS (interfered by thermal degradation of parents) &

b) LC-ESI (neg)-MS/MS.

The parent compounds captan and folpet were analyzed by GC-MS/MS in parallel. Due to the breakdown of the parents during GC-injection, THPI and PI results were, as expected, significantly overestimated when using GC-MS/MS. Where the parent compounds previously degraded, due to food processing, the GC and LC results were very similar, essentially confirming the theory (see Tab. 3, processed food).

The parents were additionally measured by LC-MS/MS in the ESI-pos-mode (as NH_4^+-adducts) achieving results comparable to those obtained by GC-MS/MS, but measurement settings are not shown here. This option for the analysis of the parents is compromised at low levels (<0.05 mg/kg).

Tab. 2: *Comparison of GC and LC-MS/MS approach*

	GC-MS-MS (matrix-based calib - cucumber- + AP)				LC-ESI(neg)-MS/MS (solvent-based calib, generic IS - No ILIS)		Factor between GC and LC-result of THPI or PI
Commodity	**Captan* (mg/kg)**	**Folpet* (mg/kg)**	**THPI (mg/kg)**	**PI (mg/kg)**	**THPI (mg/kg)**	**PI (mg/kg)**	
Primary products							
Apple	0,25	-	0,16	-	0,046	-	**8,5**
Apple	0,026	-	0,055	-	0,032	-	**1,7**
Apricot	0,049	-	0,017	-	0,002*	-	**8,5**
Blueberry **(see table)**	0,180	-	0,092	-	0,014	-	**6,6**
Cherry **(see table)**	0,021	-	0,019	-	0,003*	-	**6,3**
Pear	0,36	-	0,13	-	0,070	-	**1,9**
Strawberry	0,019	-	0,030	-	0,013	-	**2,3**
Wine Grapes	-	0,68	-	0,27	-	0,055	**4,9**
Wine Grapes **(see table)**	-	0,070	-	0,043	-	0,013	**3,3**
Processed food							
Apple juice	-	-	0,070	0,003	0,073	-	**1,0**
Apricot, dried	-	-	0,33	0,005	0,29	-	**1,1**
Mango smoothie	-		0,042	-	0,049	-	**0,9**

* Captan and folpet were additionally analyzed by LC-ESI(pos)-MS/MS achieving comparable results to GC-MS/MS
** Traces; only target MRM was visible

Exemplary chromatograms for THPI and PI are shown in Fig. 2.

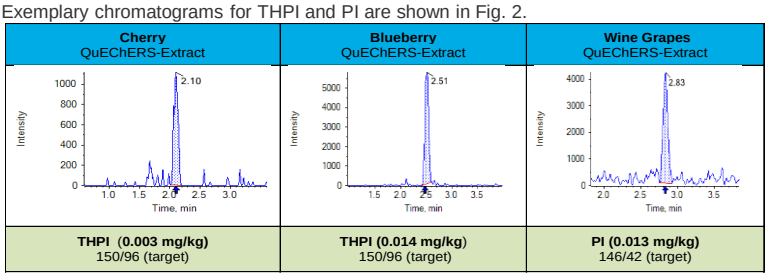

Fig. 2: *Exemplary LC-MS-MS chromatograms of PI and THPI with ESI negative mode using Sciex 5500 QTrap (injection volume 2 µL)*

Further interesting aspects

In the ESI-neg-mode PI-D4 interferes with one of the most prominent MRMs of THPI (m/z 150/42). Chromatographic separation between PI and THPI is thus indicated. This also applies to the pair folpet-D4 and captan, which share the MRMs 317/264; 319/266; 300/264 and 302/266 in the ESI-pos-mode.

In the APCI (neg) captan, folpet and their products can be analyzed in one go. The parents decompose to THPI and PI while heating the eluent prior to entering the APCI source, so only the degradants are measured. Apart of the mentioned mutual interference of PI-D_4 and THPI, an additional unusual MS interference was observed, which was caused by the oxidative dehydrogenation of THPI (and captan) to PI and of THPI-D_6 (and captan-D_6) to PI-D_4 (-$2H_2$ and -2HD respectively). The latter is isobaric to THPI, which limits the usefulness of THPI-D_6 and captan-D_6 as ILISs. A separation of all 4 compounds is thus paramount. Also here the sensitivity of the parents is lower than that achieved by GC-MS/MS.

Conclusions

The combination of GC-MS/MS for the analysis of captan and folpet with LC-ESI(neg)-MS/MS for the analysis of THPI and PI, offers a viable approach for the analysis of the full legal residue definition of captan and folpet. This approach circumvents the error-prone GC-analysis of THPI and PI. Still, measures have to be taken to ensure accurate GC-quantification of captan and folpet (e.g. use of ILIS and AP). Chromatographic separation of THPI and PI is needed.

EPRW 2020

Baden-Württemberg