CAPTAN AND FOLPET BY LC-MS/MS: IMPROVING IONISATION EFFICIENCIES OF MULTIRESIDUE PESTICIDES IN FOOD

Kari Organtini[1], Susan Leonard[1], Renata Jandova[2], Eimear McCall[2], and Gareth Cleland[1]

[1] Waters Corporation, Milford, MA 01757, USA
[2] Waters Corporation, Wilmslow, SK9 4AX, UK

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INTRODUCTION

Screening food samples for contaminants such as pesticides requires the use of GC-MS and LC-MS techniques. In order to cover a full suite of regulated compounds, several LC and GC methods are usually required that separately incorporate large suites of compounds, single residue methods, and "troublesome" compounds.

Of the troublesome compounds in the GC-MS suite of pesticide residues, the thiophthalimide fungicides, including captan and folpet, are amongst the most difficult to analyze. These compounds rapidly degrade in the GC inlet under normal splitless injection conditions used for multiresidue pesticide analysis methods. Captan and folpet lose the –$SCCl_3$ group to produce Tetrahydrophthalimide (THPI) and Phthalimide (PI), respectively. This degradation happens within as little as two injections making reproducible analysis nearly impossible. It is generally accepted that captan and folpet are GC compounds, although methods for their degradation products using APCI ionization using an LC-MS method have been reported.[1]

We assessed the possibility of developing an LC-MS/MS method to make the analysis of these compounds more robust and reliable. Electrospray ionization (ESI) and a novel LC-MS ionization technique (UniSpray or USI) were investigated to determine whether captan and folpet could be successfully analyzed with an LC-MS approach without the problems observed using GC analysis. The method evaluation was performed in challenging food matrices.

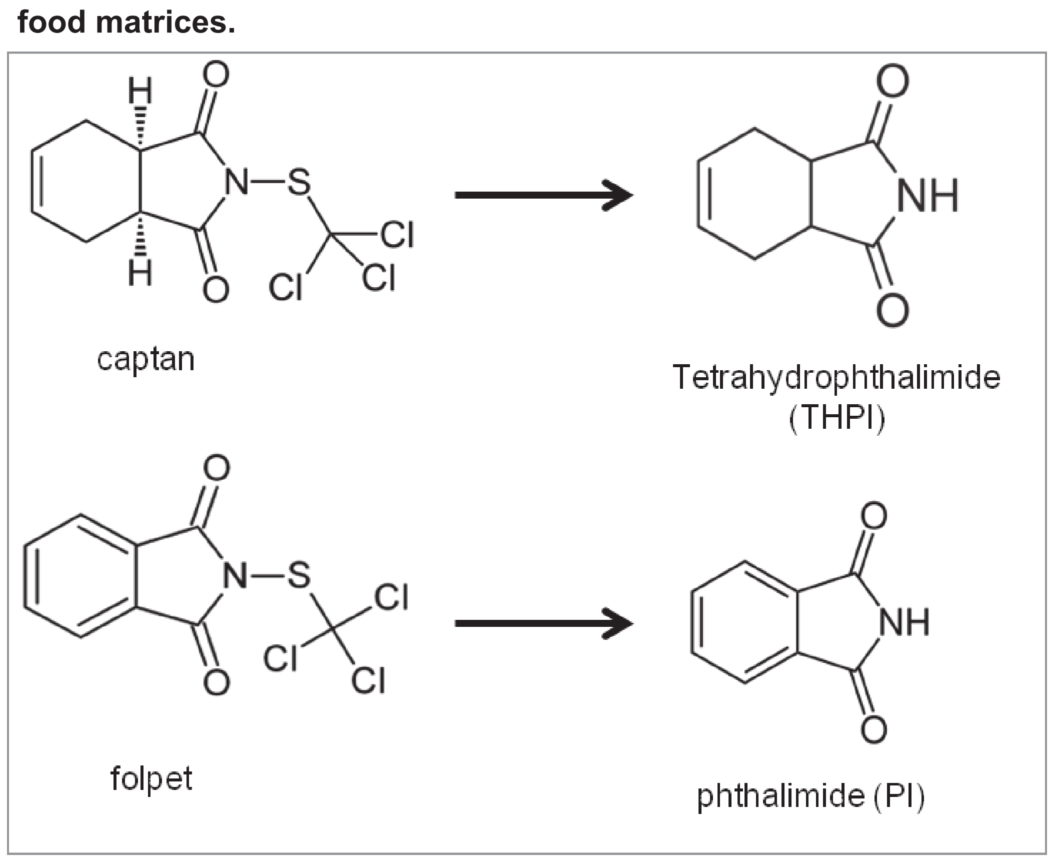

Figure 1. Structures of captan and folpet and their degradation products, THPI and PI.

RESULTS AND DISCUSSION

GC analysis of pesticides like captan and folpet is often not repeatable as the pesticides degrade in GC splitless injections, as demonstrated in Figure 4 showing three consecutive GC injections of captan in matrix. Analysis using the LC-MS/MS methods developed using Electrospray and UniSpray (Figure 4) was shown to be repeatable (n=25 injections). Linearity in matrix was excellent with R^2 values > 0.995 for all pesticides in each matrix in the range of 0.005 - 0.100 mg/kg. Limits of detection were well within the required EU maximum residue level (MRL) of 0.030 mg/kg in kale and 0.060 mg/kg in celery (Table 1).[4,5] The methods proved to be robust as RSDs for 25 injections in matrix were < 10%. Figure 5 shows the trend of peak area and associated %RSD for folpet in 25 injections of kale. Although both LC-MS ionization techniques were robust, UniSpray ionization produced greater ionization, resulting in an increase of peak areas for all compounds. Figure 6 illustrates the peak areas for each compound normalized to UniSpray peak area in kale and celery matrix.

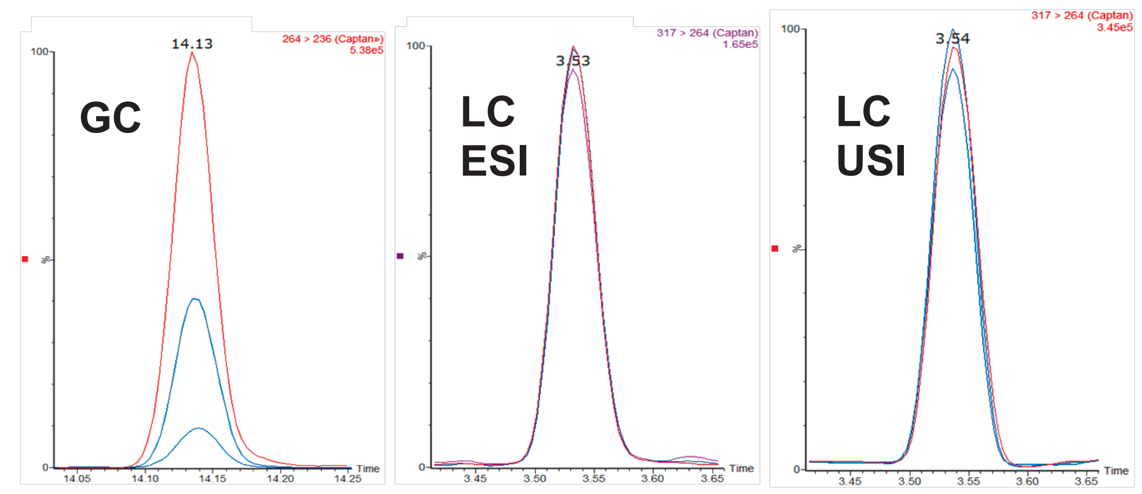

Figure 4. Three consecutive injections of captan in matrix on GC, Electrospray (LC ESI), and UniSpray (LC USI).

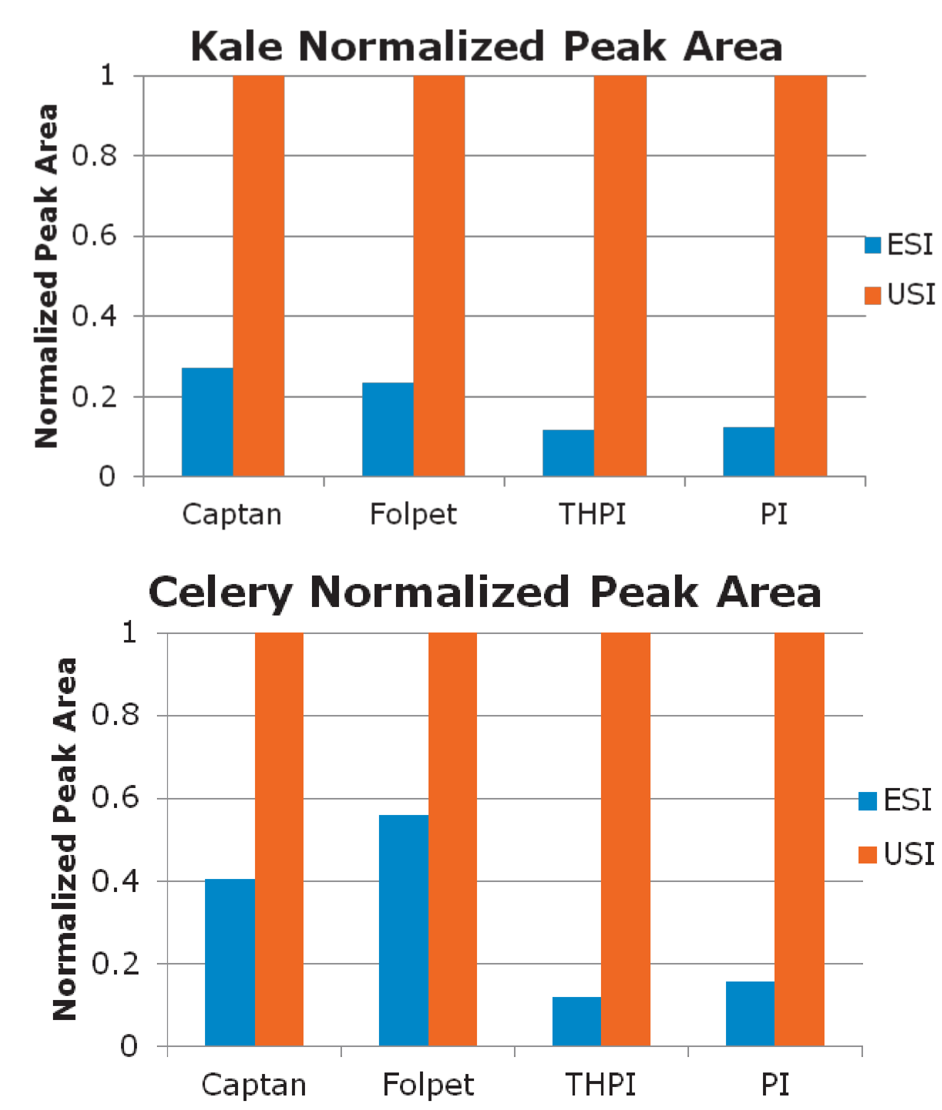

Figure 6. Comparison of the normalized peak areas of the pesticides in kale matrices using Electrospray and UniSpray ionization.

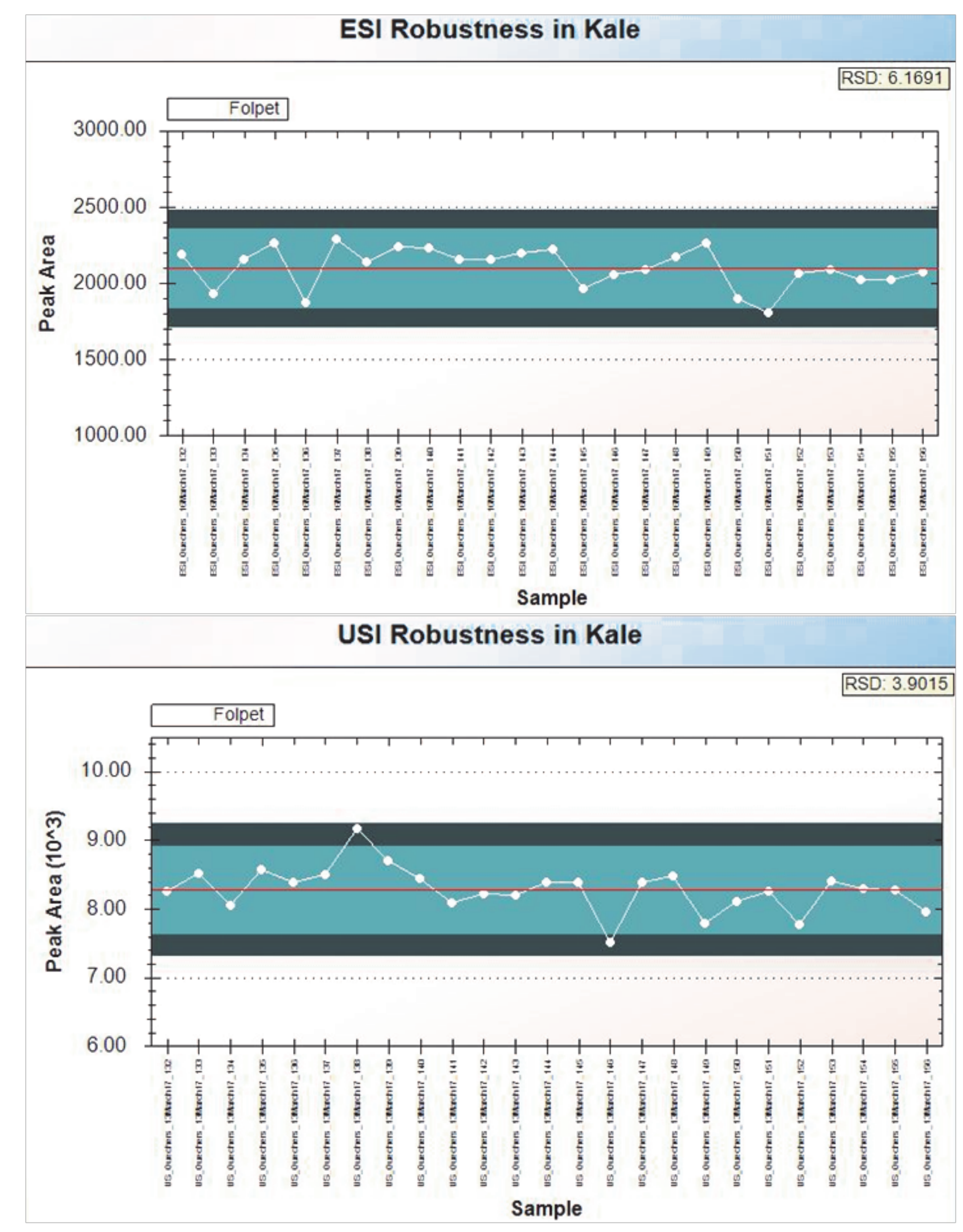

Figure 5. 25 injections of kale spiked with 0.010 mg/kg of folpet on (a) Electrospray and (b) UniSpray ionization techniques with associated RSD values. Red line indicates the mean, light blue shaded area represents +/- 2 standard deviations, dark blue shaded area represents +/- 3 standard deviations.

	USI LOD (ppb)			ESI LOD (ppb)		
	Solvent	Celery	Kale	Solvent	Celery	Kale
Captan	0.5	0.5	5.0	0.5	0.5	1.0
Folpet	0.5	5.0	0.5	0.5	0.5	5.0
THPI	0.1	0.5	0.5	0.5	0.5	0.5
PI	1.0	1.0	5.0	5.0	5.0	5.0

Table 1. Limit of Detections (LODs) of each pesticide in each matrix using both Electrospray and UniSpray ionization.

METHODS

How Does UniSpray Work?

UniSpray is an novel atmospheric ionization technique that allows for multimode ionization of both polar and non-polar analytes in a single injection.

A simplified diagram of how UniSpray ionization works is shown in Figure 2. The column effluent is nebulized in a grounded, heated probe and directed onto a stainless steel pin which is held under high voltage, creating smaller droplet sizes, which are ionized at impact. The nebulized flow bends around the surface of the impactor pin into the sample cone due to the Coanda Effect. The mechanism allows for increased ionization and sampling efficiency.[2,3]

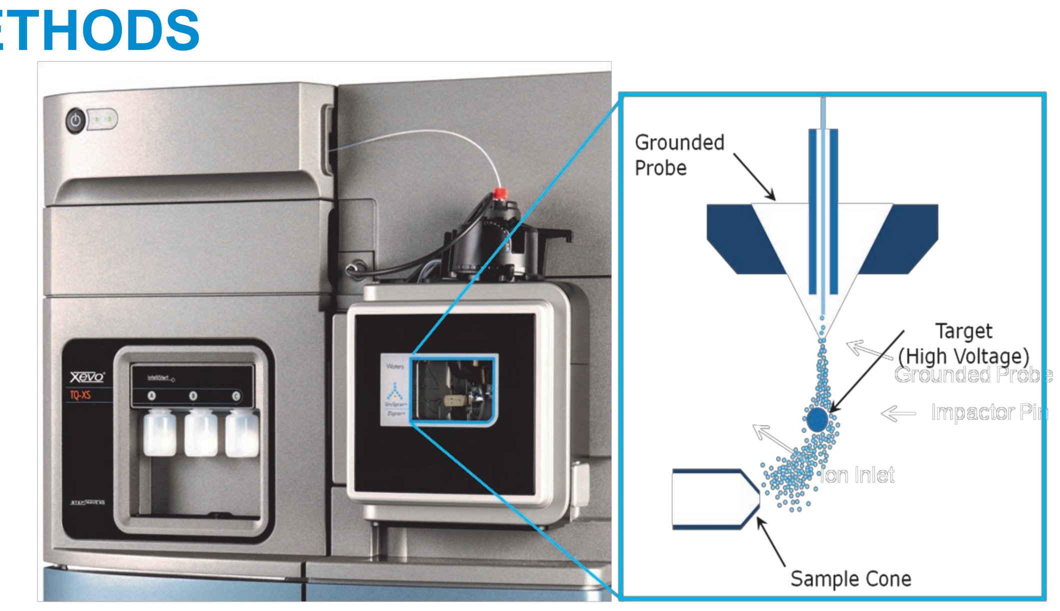

Figure 2. UniSpray source shown on the Xevo TQ-XS. Inset shows the heated, grounded probe directing the nebulized spray onto the impactor pin, on which a voltage is applied. The generated ions then enter the mass spectrometer through the ion inlet, or sample cone.

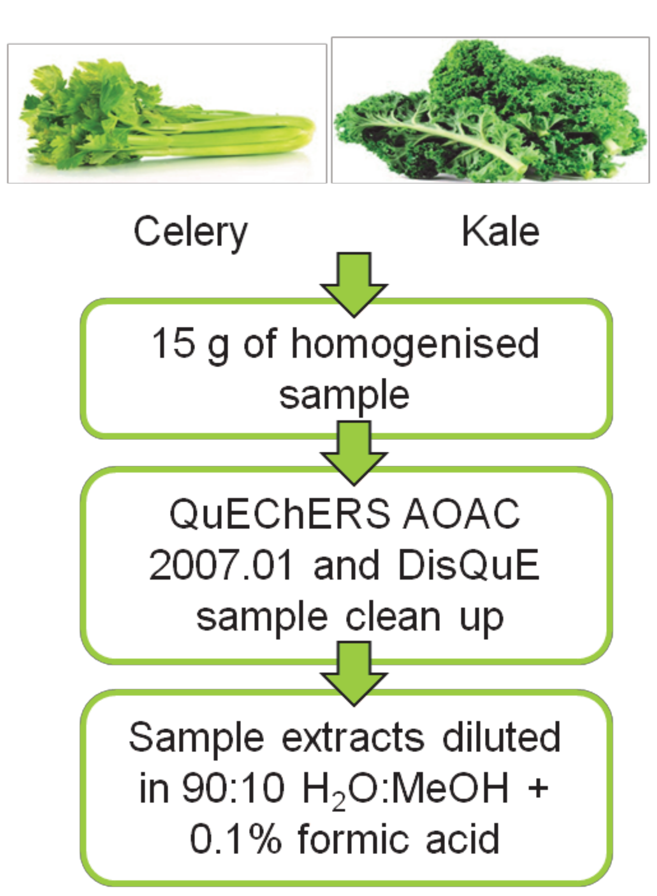

Figure 3. Overview of the sample extraction and clean up procedure used to prepare the sample matrices for analysis.

LC Conditions:

LC System: ACQUITY UPLC I Class
MS System: Xevo TQ-XS
Column: ACQUITY BEH C18 2.1 x 50 mm, 1.7um
Column Temperature: 45° C
Sample Temperature: 4° C
Flow Rate: 0.45 mL/min
Injection Volume: 10 µL
Mobile Phase A: Water + 0.1% formic acid + 0.05% ammonia
Mobile Phase B: Methanol + 0.1% formic acid + 0.05% ammonia
Gradient:

Time (min)	Flow (mL/min)	% A	% B
-	0.45	90	10
5	0.45	0	100
6	0.45	0	100
8	0.45	90	10

MS Conditions:

UniSpray
Impactor Voltage: 3 kV
Desolvation Temp: 300° C
Desolvation Flow: 1000 L/hr
Cone Flow: 600 L/hr

Electrospray
Capillary Voltage: 3kV
Desolvation Temp: 200° C
Desolvation Flow: 1000 L/hr
Cone Flow: 600 L/hr

CONCLUSIONS

- **An LC-MS/MS method was developed for the analysis of thiophthalimide fungicides captan and folpet as well as their degradation products THPI and PI.**
- **The LC-MS/MS analysis of captan and folpet was repeatable using both Electrospray and UniSpray ionization techniques. Compound degradation did not occur during sample analysis as compared to GC-MS analysis of the same compounds.**
- **Electrospray and UniSpray ionization produced very robust options for analysis methods of captan and folpet with RSDs < 10% in all matrices analyzed.**
- **The novel UniSpray ionization source provided enhanced ionization of all compounds studied when compared to Electrospray ionization.**
- **Limit of detection for captan and folpet in matrix were well below the regulated limits.**
- **The methods developed provide a viable alternative for analysis of thiophthalimide fungicides using LC-MS that is much more robust than GC-MS analysis.**

References

1. A. Berthet, M. Bouchard, P. Schupfer, D. Vernez, B. Danuser, C.K. Huynh, 2011. Liquid chromatography-tandem mass spectrometry (LC/APCI-MS/MS) method for the quantification of captan and folpet phthalimide metabolites in human plasma and urine. Anal Bioanal Chem, 399.
2. A. Lubin, S. Bajic, D. Cabooter, P. Augustijns, F. Cuyckens, 2016. Atmospheric pressure ionization using a high voltage target compared to electrospray ionization, J. Am. Soc. Mass Spectrom, 28.
3. A. Lubin, R. De Vries, D. Cabooter, P. Augustijns, F. Cuyckens, 2017. An atmospheric pressure ionization source using a high voltage target compared to Electrospray ionization for the LC/MS analysis of pharmaceutical compounds. J. Pharm. Biomed. Anal, 142.
4. European Union. Commission Regulation (EU) 2016/156. Amending Annexes II and III to Regulation (EC) No 396/2005 of the European Parliament and of the Council as regards maximum residue levels for boscalid, clothianidin, thiamethoxam, folpet and tolclofos-methyl in or on certain products. 2016.

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