

# PaperSpray methods for agrochemical analysis: Swab and homogenate testing for screening and targeted quantitation

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## ABSTRACT

**Purpose:** Two simplified and robust workflows for PaperSpray™ ionization-mass spectrometry of food samples – one for qualitative screening of agrochemicals on food surfaces, and a second for targeted pesticide quantitation in food homogenates.

**Methods:** The Swab/Spot/Spray method utilizes a fixed volume of solvent into which a single-use swab is immersed for 30 seconds. The wetted swab is used to collect material from a surface and transfer that material to a PaperSpray cartridge, followed by analysis by mass spectrometry. The homogenate method employs acetonitrile extraction followed by filtration and spotting onto the PaperSpray cartridge, which is then dried and used for mass spectrometry analysis by SRM.

**Results:** Diphenylamine was detected on unwashed apples using the Swab/Spot/Spray method and confirmed by MS/MS with comparison to a diphenylamine reference standard. Pyrimethanil was also tentatively identified. Using the homogenate-based method calibration curves were prepared for several agrochemicals in apple and orange matrices. Excellent linearity and reproducibility were observed across the calibration curve (0.05 mg/kg to 50 mg/kg).

## INTRODUCTION

PaperSpray ionization is a powerful tool for rapid analysis of samples requiring relatively little preconcentration or clean-up, rendering it an excellent fit for food safety applications such as targeted pesticide screening in food homogenates or from the surfaces of food products. In this poster we present two methods for PaperSpray-mass spectrometry analysis of foodstuffs: Swab/Spot/Spray for analysis of compounds present on the surface of food samples, and a more quantitative method for targeted pesticide measurement in food homogenates. The Swab/Spot/Spray method is a fast, readily standardized approach to surface sampling that is fully compatible with currently available commercial PaperSpray cartridges. The homogenate analysis method is analogous to a simplified QuEChERS sample preparation method, employing extraction and filtration followed by drying on the PaperSpray substrate prior to analysis.

## MATERIALS AND METHODS

### Sample Preparation

Orange and apple samples were obtained from a local vendor. Samples tested by the Swab/Spot/Spray method were not washed prior to sampling. Samples tested by the homogenate-based approach were washed with warm tap water and dried with paper towels before homogenization. Organic fruit was used for the homogenate-based method; analytes were spiked into the samples during the acetonitrile extraction process as described below. Diphenylamine and thiabendazole were purchased from Thermo Fisher Scientific. Boscalid was purchased from Sigma Aldrich. Boscalid and isotopically labeled internal standards were purchased from Toronto Research Chemicals.

### Test Methods

Surface testing employed a modified Thermo Scientific™ Velos Pro™ ion trap mass spectrometer and a ProSolia™ Velox 360™ PaperSpray ion source. A swab was immersed in a pre-measured volume of solvent (200 µL isopropanol) for 30 seconds, then used to wipe the surface of the fruit sample for 30 seconds. The wet swab was then touched to the substrate of the PaperSpray cartridge and held in place with light pressure for 30 seconds. The PaperSpray cartridge was then immediately loaded into the Velox 360 ion source for analysis.

Homogenate analysis employed a Thermo Scientific™ TSQ Altis™ triple stage quadrupole mass spectrometer and a ProSolia Velox 360 PaperSpray ion source unless otherwise stated. The homogenate was weighed into a Thomson™ eXtractor 3D|FV® filter vial (0.45 µm PVDF membrane) and acetonitrile was added (1 µL acetonitrile per mg homogenate). Internal standards and analytes (for spiked samples) were added in the acetonitrile. The filter vial was capped and vortexed for 1 minute. It was then filtered by depressing the plunger. Filtrate was spotted on PaperSpray cartridges in 5 µL aliquots and dried for at least 1 hour at room temperature. The cartridges were analyzed by PaperSpray mass spectrometry using 90/10/0.1 acetonitrile/water/formic acid (methanol with 0.1% formic acid was used with diphenylamine samples). All internal standards were used at a concentration of 1 µg/g (1 ppm).

### Data Analysis

Data analysis was performed using Thermo Scientific™ Freestyle™ and QuanBrowser™ software. The quantifying transition chromatogram was integrated for each analyte and internal standard. Calibration curves were generated by plotting the analyte/internal standard signal ratio vs. analyte concentration.

## SWAB/SPOT/SPRAY

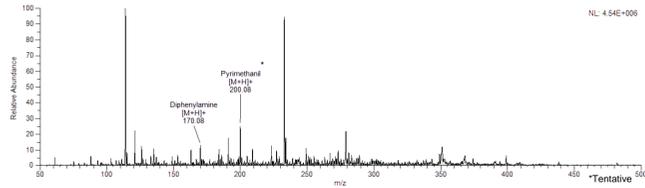
### Workflow

1. A disposable swab is wetted with a predefined volume of a suitable solvent (in this case, 200 µL of isopropanol).
2. The surface to be sampled from is wiped with the wetted swab. The area to be swabbed may be defined using a template, or the surface may simply be swabbed for a defined period of time. In the experiments described here, the surface was wiped for 30 seconds, covering approximately 1/3<sup>rd</sup> of the surface of a large apple in that time.
3. The tip of the swab is touched to the substrate in the PaperSpray cartridge for a defined period of time (30 seconds). As solvent wicks from the wet swab onto the dry paper, species collected from the surface are transferred as well.
4. The PaperSpray cartridge is immediately loaded into the PaperSpray ion source for analysis.

### Results

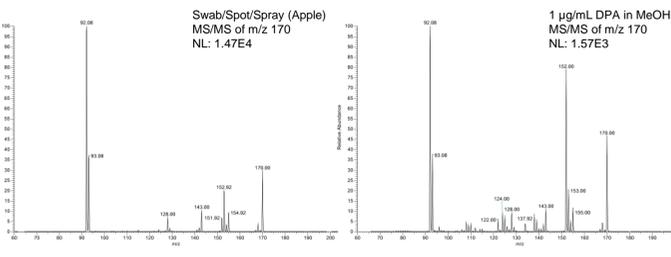
Two agrochemicals, diphenylamine and pyrimethanil, were identified from unwashed apples obtained from a local vendor.

**Figure 2. Swab/Spot/Spray PaperSpray-mass spectrum from surface sampling of an apple, analyzed using a modified Velos Pro. Two notable peaks corresponding to agrochemicals known to be used on apples were identified – 200.08 (pyrimethanil) and 170.08 (diphenylamine).**



The identities of the two species were verified by MS/MS. The presence of diphenylamine was confirmed by comparison to a sample of pure diphenylamine in methanol (see Figure 3, below), while the identity of pyrimethanil was determined by comparison to literature data.

**Figure 3. MS/MS spectra for m/z 170 from Swab/Spot/Spray of apple (left) and from a neat solution of 1 µg/mL diphenylamine in methanol (right, 5 µL on cartridge, dried 1 hr), analyzed using a modified Velos Pro instrument.**

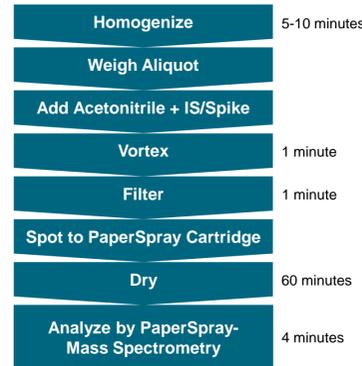


**Figure 1. An apple tested by the Swab/Spot/Spray method, with a swab and vial of pre-measured solvent.**



## HOMOGENATE

**Figure 4. Workflow for quantitative analysis of food samples by homogenization, acetonitrile extraction, and filtration followed by PaperSpray-mass spectrometry.**

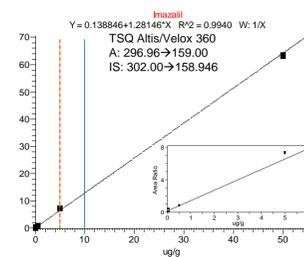


This workflow is analogous to a simplified QuEChERS method, optimized for speed, simplicity, and low cost rather than for maximum performance for multiple analytes. A simple acetonitrile extraction replaces the full extraction with salting used in QuEChERS, and filtration with Thomson filter vials is used as a faster alternative to centrifugation and decanting. The sample is spotted onto the PaperSpray cartridge and allowed to dry, and is then re-extracted during PaperSpray-mass spectrometry analysis, which provides a degree of sample clean-up as many matrix species are left bound to the cellulose substrate.

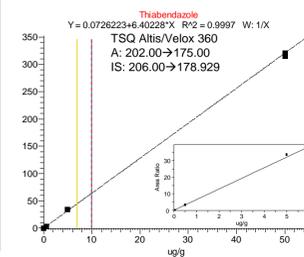
### Orange Homogenate

Calibration curves (Figures 5 and 6) were prepared for imazalil and thiabendazole in a matrix of navel orange homogenate. Imazalil and thiabendazole are both fungicides commonly used to protect citrus fruit. Both species yielded a highly linear fit from 0.05 ppm to 50 ppm. Relative standard deviations for analytical replicates of thiabendazole were consistently below 1%. Greater variability was observed for imazalil, with RSDs ranging from 0.3% in the 50 ppm sample to 16.6% in the 0.05 ppm sample.

**Figure 5. Calibration curve for imazalil in orange homogenate (0.05-50 ppm). The maximum residue limits for imazalil in orange for the United States,<sup>1</sup> the European Union,<sup>2</sup> and Japan<sup>3</sup> are marked in blue, gold, and red respectively. Inset: 0-5 ppm region.**



**Figure 6. Calibration curve for thiabendazole in orange homogenate (0.05 – 50 ppm). The maximum residue limits for thiabendazole in orange for the United States,<sup>1</sup> the European Union,<sup>2</sup> and Japan<sup>3</sup> are marked in blue, gold, and red respectively. Inset: 0-5 ppm region.**

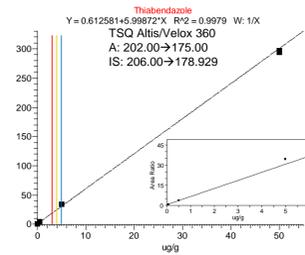


## HOMOGENATE

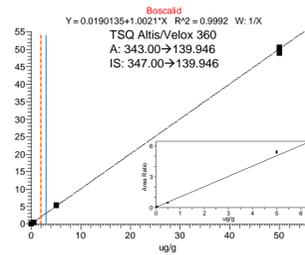
### Apple Homogenate

Calibration curves (Figures 7-10) were prepared in apple homogenate matrix using the same procedures as for orange. Four pesticides were tested in apple matrix: thiabendazole, imazalil, boscalid, and diphenylamine. Boscalid, like thiabendazole and imazalil, is a fungicide used on a variety of crops. Diphenylamine is an anti-scald agent used particularly on apples. It is commonly used in the United States but its use is much more restricted in the European Union.

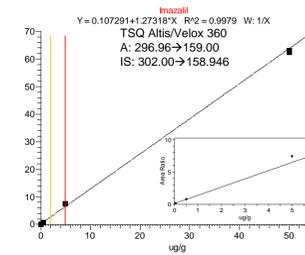
**Figure 7. Calibration curve for thiabendazole in apple homogenate (0.05-50 ppm). The maximum residue limits for thiabendazole in apple for the United States,<sup>1</sup> the European Union,<sup>2</sup> and Japan<sup>3</sup> are marked in blue, gold, and red respectively. Inset: 0-5 ppm region.**



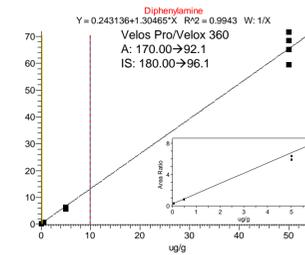
**Figure 9. Calibration curve for boscalid in apple homogenate (0.05-50 ppm). Maximum residue limits for boscalid in apple for the United States,<sup>1</sup> the European Union,<sup>2</sup> and Japan<sup>3</sup> are marked in blue, gold, and red respectively. Inset: 0-5 ppm region.**



**Figure 8. Calibration curve for imazalil in apple homogenate (0.05 – 50 ppm). The maximum residue limits for imazalil in apple for the European Union<sup>2</sup> and Japan<sup>3</sup> are marked in red and gold respectively. Inset: 0-5 ppm region.**



**Figure 10. Calibration curve for diphenylamine in apple homogenate (0.05 – 50 ppm). The maximum residue limits for diphenylamine in apple for the United States,<sup>1</sup> the European Union,<sup>2</sup> and Japan<sup>3</sup> are marked in red and gold respectively. Inset: 0-5 ppm region.**



The diphenylamine calibration curve was generated on a modified Velos Pro ion trap mass spectrometer. Spray voltages, transitions monitored, and CID parameters are listed in Table 1.

**Table 1. Spray voltage and MS/MS parameters for analytes and internal standards.**

	Spray Voltage	Transition	Collision Energy
Boscalid	4500 V	343.00→139.946	19.36 V
Boscalid-d4	4500 V	347.00→139.946	20.65 V
Diphenylamine	5000 V	170.00→92.1	40% (q=0.35)
Diphenylamine-d10	5000 V	180.00→96.1	40% (q=0.35)
Imazalil	5000 V	296.96→159.00	24.22 V
Imazalil-d5	5000 V	302.00→158.946	24.75 V
Thiabendazole	5000 V	202.00→175.00	26.26 V
Thiabendazole-d4	5000 V	206.00→178.929	26.34 V

## PRECISION & LINEARITY

**Table 2. Relative standard deviations and R<sup>2</sup> values for calibration curves. All curves were linear and fitted using 1/X weighting.**

	Relative Standard Deviation				Linearity R <sup>2</sup>
	0.05 ppm	0.5 ppm	5ppm	50 ppm	
Diphenylamine	16.57%	1.37%	4.39%	7.99%	0.9943
Boscalid	12.9%	0.7%	1.2%	1.7%	0.9992
Imazalil (apple)	15.7%	0.4%	0.4%	0.5%	0.9979
Imazalil (orange)	16.6%	3.2%	0.9%	0.3%	0.9977
Thiabendazole (apple)	5.4%	0.3%	0.1%	0.6%	0.9979
Thiabendazole (orange)	0.2%	0.1%	0.5%	0.8%	0.9997

## CONCLUSIONS

Two methods were developed for analysis of pesticides in food samples by PaperSpray-mass spectrometry.

- Swab/Spot/Spray used to detect and confirm pesticides on the surface of unwashed apples.

- Diphenylamine confirmed by MS/MS
- Tentative identification of pyrimethanil

- Calibration curves generated in apple and orange homogenate matrices using Extraction/Filtration method.

- Imazalil
- Thiabendazole
- Boscalid
- Diphenylamine

- Performance did not vary significantly with matrix.

- Further experiments required to determine limits of detection, but all LODs are well below the lowest relevant maximum residue limit for the USA, European Union, and Japan.

## REFERENCES

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3. Table of MRLs for Agricultural Chemicals. The Japan Food Chemical Research Foundation. Online. db.fccr.or.jp/front/ Accessed 7 May 2018.

## TRADEMARKS/LICENSING

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