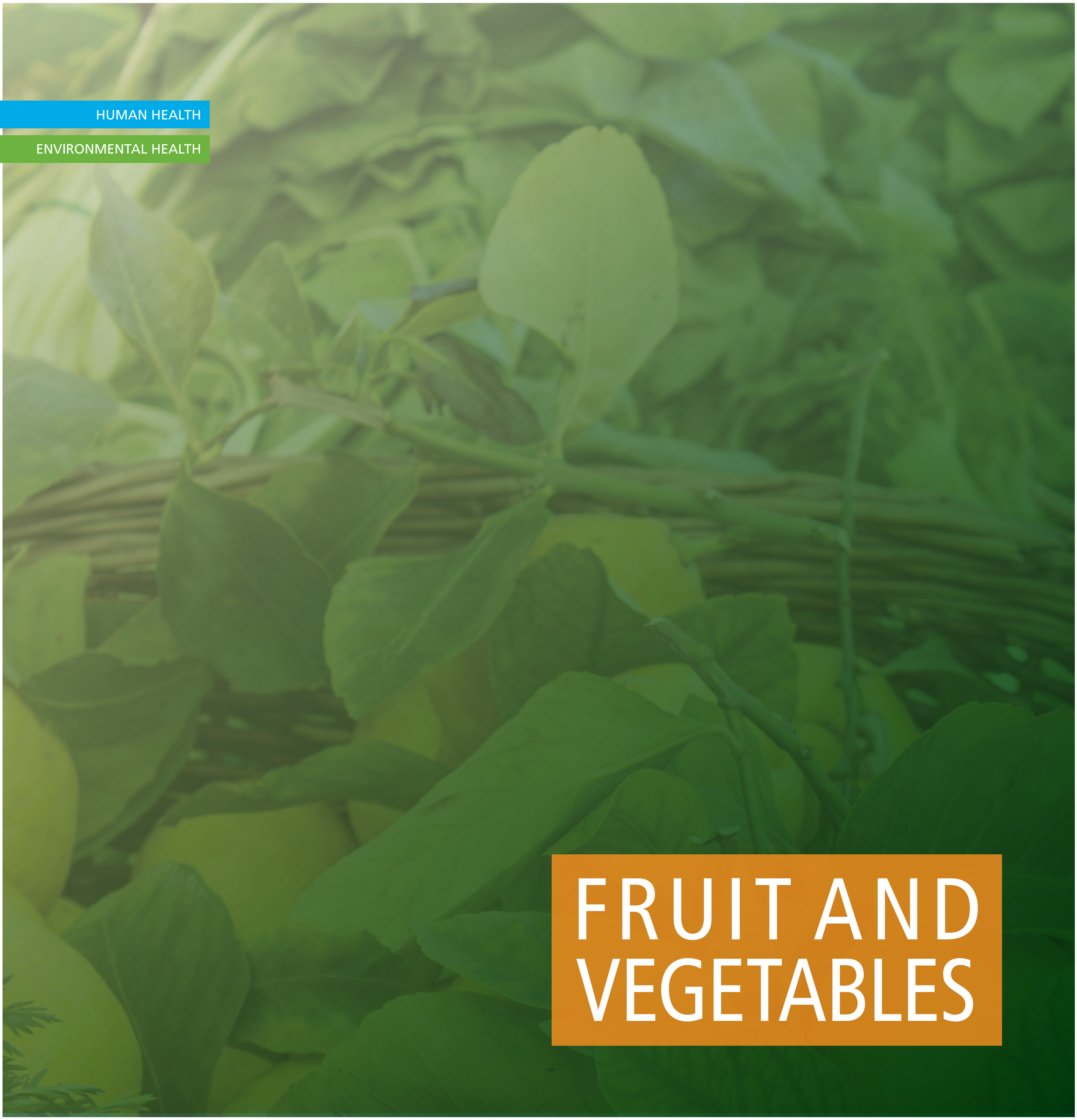
HUMAN HEALTH

ENVIRONMENTAL HEALTH



FRUIT AND VEGETABLES

Fruit and Vegetables Compendium

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Fruit and Vegetables

Liquid Chromatography/
Mass Spectrometry

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PerkinElmer, Inc.

Targeted Screening of 130 Pesticides in QuEChERS Extracts of Lettuce Leaves Using UHPLC-TOF and High Throughput Screening Software

Introduction:

The Food Quality protection Act (FQPA) in the United States (US) and the European Union (EU) directive 91/414/EEC require that if pesticides are present in food they are below agreed levels due to the health risk posed by pesticides [1, 2]. With the advent of large scale agricultural production, hundreds of pesticides have been synthesized in the last century and used widely to protect crops. Newer pesticides continue to be synthesized for crop usage which makes it important to analyze both targeted (or expected analytes) and non-targeted pesticides in food and in the environment. Unlike a triple quadrupole instrument that only measures targeted analytes (defined by selected multiple reaction monitoring of analyte ions or MRMs), the time-of-flight (TOF) mass spectrometer can measure both targeted and non-targeted analytes[3]. TOF mass spectrometers collect full spectrum information and hence the data can be re-examined for the presence of these "non-targeted" analytes. We present a study of pesticide analysis in a lettuce leaves extract that was obtained by the QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) method of food extraction. The lettuce extract was spiked with varying concentrations of a mix of 130 pesticides and analyzed by Ultra-High Pressure Liquid Chromatography-Mass Spectrometry (UHPLC-MS) with a PerkinElmer AxION® 2 TOF MS as the detector. We could detect the majority of the pesticides well within the EU limit of detection (LOD) requirement range of 10 ppb. The data was further analyzed using AxION Solo™ high throughput software. The presence of each of the analytes when detected above the 10 ppb threshold was given a specific color code which helped to rapidly screen for presence/absence of all 130 analytes in each sample. A combination of short run times and powerful screening software helped simplify analysis and also reduce the time of analysis.

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Experimental conditions

Sample Preparation:

Organically grown lettuce samples were prepared using the QuEChERS method. Homogenized lettuce leaves (10 g) were spiked with pesticide standards (at different concentrations) and 10 mL of acetonitrile was added. Salts (1 g sodium chloride, 4 g magnesium sulfate, 1 g sodium citrate and 0.5 g disodium hydrogen citrate) were added to the sample, shaken and centrifuged (3700 rpm) [EN 15662 QuEChERS extraction kit: N9306901]. The supernate (1 mL) was transferred to a dispersive SPE micro-centrifuge tube containing primary and secondary amine (PSA, 50 mg) and magnesium sulphate (150 mg) [EN 15662 QuEChERS clean-up kit: N9306920]. The mixture was shaken (30 s), centrifuged (3700 rpm for 1 min) and supernatant (1mL) was acidified by adding acetonitrile containing 5% formic acid (10µL) and filtered (0.22 um x 25 mm PTFE syringe filter: 02542926) prior to analysis.

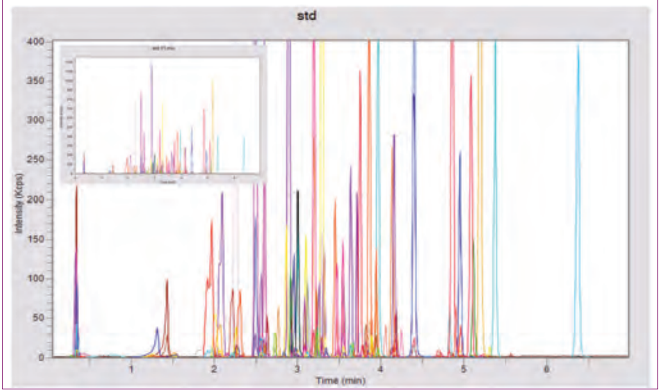


Figure 1. Extracted ion chromatograms (EICs) showing 130 pesticide standards analyzed by UHPLC-TOF MS.

LC conditions:

Pump: PerkinElmer Flexar™ FX-15 pump

Mobile phase A: Water containing 0.1% formic acid

Mobile phase B: Acetonitrile containing 0.1% formic acid

Gradient conditions: Starting at 5% B, linear gradient to 90% B in 5 mins, maintained at 90%B for another 2 mins.

Column used: PerkinElmer Brownlee™ SPP C-18, 2.1 x 50 mm, 2.7 µm (N9308402)

Guard Cartridge: N9308513

Guard Cartridge Holder: N9308534

Flow: 0.4 mL/min

Injection volume: 4 µL in partial fill mode

MS conditions:

Mass spectrometer: PerkinElmer AxION2 TOF

Ionization source: PerkinElmer Ultraspray™ 2 (Dual ESI source)

Ionization mode: positive

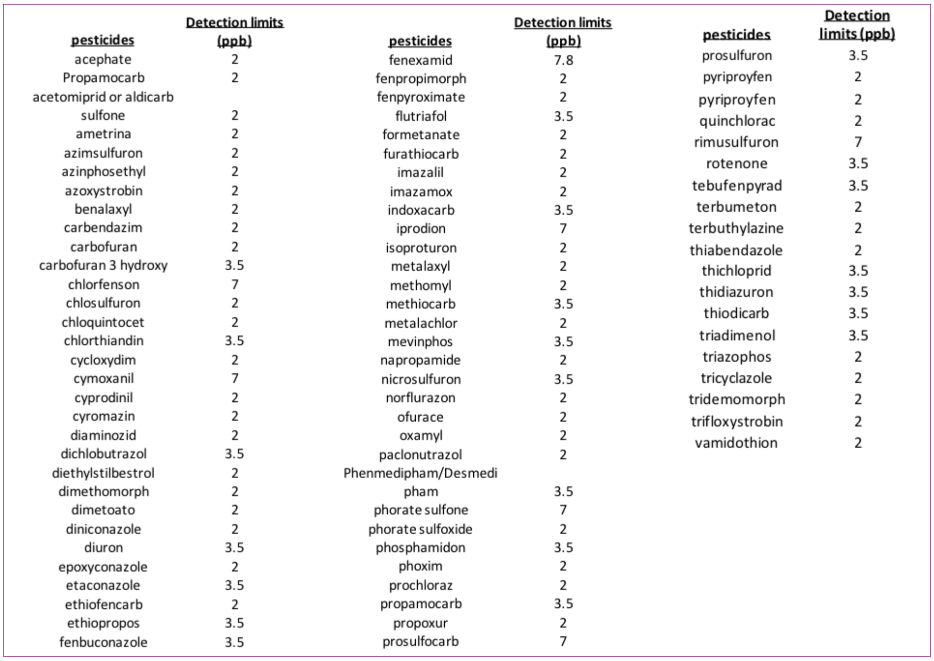
Capillary exit voltage: 100 V

TrapPulse™ mode: 100-1000 m/z (D7:65, D8:92)

Internal calibration was performed using m/z 118.08625 and 922.00979 as lock mass ions

Results and Discussion

The separation and analysis of 130 pesticide standards was achieved within 7 min. (Figure 1). The AxION 2 TOF was operated in the proprietary TrapPulse™ mode which increases the duty cycle of the TOF resulting in significant improvement in signal. Using this mode, we were able to achieve excellent detection limits of < 10 ppb for majority of the pesticides as shown in Table 1. These detection limits meet the requirements as specified by the EU directive.



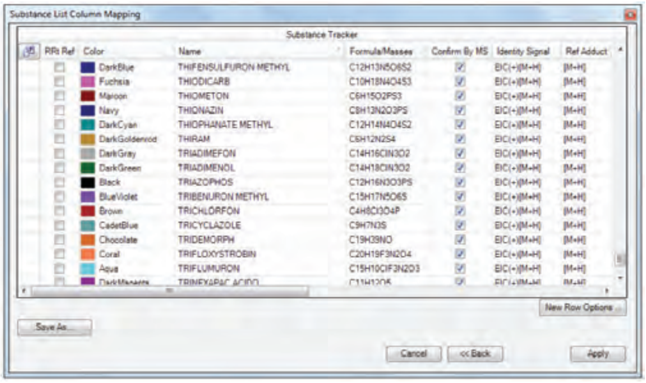
pesticides	Detection limits (ppb)	pesticides	Detection limits (ppb)	pesticides	Detection limits (ppb)
acephate	2	fenexamid	7.8	prosulfuron	3.5
Propamocarb	2	fenpropimorph	2	pyriproyfen	2
acetomiprid or aldicarb sulfone	2	fenpyroximate	2	pyriproyfen	2
ametrina	2	flutriafol	3.5	quinchlorac	2
azimsulfuron	2	formetanate	2	rimusulfuron	7
azinphosethyl	2	furathiocarb	2	rotenone	3.5
azoxystrobin	2	imazalil	2	tebufenpyrad	3.5
benalaxyl	2	imazamox	2	terbumeton	2
carbendazim	2	indoxacarb	3.5	terbuthylazine	2
carbofuran	2	iprodion	7	thiabendazole	2
carbofuran 3 hydroxy	3.5	isoproturon	2	thichloprid	3.5
chlorfenson	7	metalaxyl	2	thidiazuron	3.5
chlosulfuron	2	methomyl	2	thiodicarb	3.5
chloquintocet	2	methiocarb	3.5	triadimenol	3.5
chlorthiandin	3.5	metalachlor	2	triazophos	2
cycloxydim	2	mevinphos	3.5	tricyclazole	2
cymoxanil	7	napropamide	2	tridemomorph	2
cyprodinil	2	nicrosulfuron	3.5	trifloxystrobin	2
cyromazin	2	norflurazon	2	vamidothion	2
diaminozid	2	ofurace	2		
dichlobutrazol	3.5	oxamyl	2		
diethylstilbestrol	2	paclonutrazol	2		
dimethomorph	2	Phenmedipham/Desmedipham	3.5		
dimetoato	2	phorate sulfone	7		
diniconazole	2	phorate sulfoxide	2		
diuron	3.5	phosphamidon	3.5		
epoxyconazole	2	phoxim	2		
etaconazole	3.5	prochloraz	2		
ethiofencarb	2	propamocarb	3.5		
ethiopropos	3.5	propoxur	2		
fenbuconazole	3.5	prosulfocarb	7		

Table 1. Instrument detection limits (IDLs) for representative 80 pesticides at less than 10 ppb (signal/noise greater than or equal to 10).

AxION Solo software could rapidly identify the presence or absence of 130 pesticides in the lettuce extracts obtained by QuEChERS extraction process. AxION Solo automatically extracts ion chromatograms of the target analytes based on the accurate mass of the analytes. This software allows for easy import of the target analyte information including name and elemental composition from an excel spreadsheet (Figure 2). Several hundred analytes can be searched against this target list for presence of [M+H]+ ion or any adducts ions (Na+, K+ etc). In addition to searching against spectral information, the software can also search for target analytes based on user defined retention time windows which further improves specificity of detection. Even at the low 10 ppb concentration, the observed accurate masses of the target analytes in lettuce extracts was less than 5ppm (Table 2) meeting regulatory requirements. The presence of a target analyte is confirmed by AxION Solo based on the accurate mass of all detectable isotopes and also on the accuracy of the isotope profile ratio (Figure 3).

AxION Solo permitted quick visualization of the presence or absence of analytes in the samples (Figure 4). The presence of individual pesticides can be coded with a specific color for ease of identification. In addition to a "target view" which allows for quick visualization of individual target pesticides in a given set of samples, simultaneously information can be obtained on the presence or absence of all target pesticides for a selected sample as shown in Figure 4.

Since the EU regulation limits the presence of pesticides in food matrices to no greater than 10 ppb, scanning for several hundred pesticides in hundreds of batches of samples can be time consuming. AxION Solo allows for rapid visualization of the presence of pesticides above a 10 ppb concentration.Using the rich list of math expressions provided in the software, a custom expression for the area threshold for peak integration of a given pesticide can be set for a 10 ppb standard or alternatively, the peak integration threshold could also be set based on area ratio relative to an internal standard.



RRt Ref	Color	Name	Formula/Masses	Confirm By MS	Identity Signal	Ref Adduct
☐	DarkBlue	THIFENSULFURON METHYL	C12H13N5O6S2	☑	EIC(+)[M+H]	[M+H]
☐	Fuchsia	THIODICARB	C10H18N4O4S3	☑	EIC(+)[M+H]	[M+H]
☐	Maroon	THIOMETON	C6H15O2PS3	☑	EIC(+)[M+H]	[M+H]
☐	Navy	THIONAZIN	C8H13N2O3PS	☑	EIC(+)[M+H]	[M+H]
☐	DarkCyan	THIOPHANATE METHYL	C12H14N4O4S2	☑	EIC(+)[M+H]	[M+H]
☐	DarkGoldenrod	THIRAM	C6H12N2S4	☑	EIC(+)[M+H]	[M+H]
☐	DarkGray	TRIADIMEFON	C14H16ClN3O2	☑	EIC(+)[M+H]	[M+H]
☐	DarkGreen	TRIADIMENOL	C14H18ClN3O2	☑	EIC(+)[M+H]	[M+H]
☐	Black	TRIAZOPHOS	C12H16N3O3PS	☑	EIC(+)[M+H]	[M+H]
☐	BlueViolet	TRIBENURON METHYL	C15H17N5O6S	☑	EIC(+)[M+H]	[M+H]
☐	Brown	TRICHLORFON	C4H8Cl3O4P	☑	EIC(+)[M+H]	[M+H]
☐	CadetBlue	TRICYCLAZOLE	C9H7N3S	☑	EIC(+)[M+H]	[M+H]
☐	Chocolate	TRIDEMORPH	C19H39NO	☑	EIC(+)[M+H]	[M+H]
☐	Coral	TRIFLOXYSTROBIN	C20H19F3N2O4	☑	EIC(+)[M+H]	[M+H]
☐	Aqua	TRIFLUMURON	C15H10ClF3N2O3	☑	EIC(+)[M+H]	[M+H]

Figure 2. Substance or target list containing compound names and elemental composition can be set up in AxION Solo or easily imported from an excel spreadsheet. .

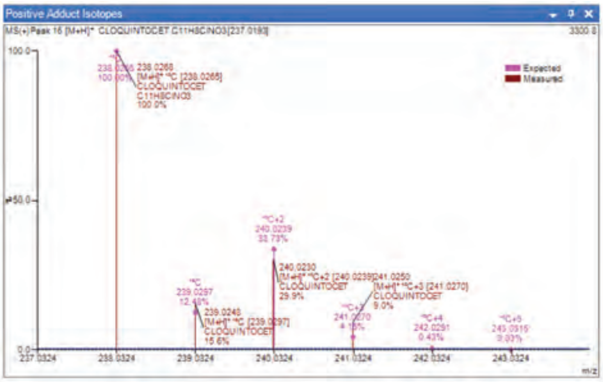
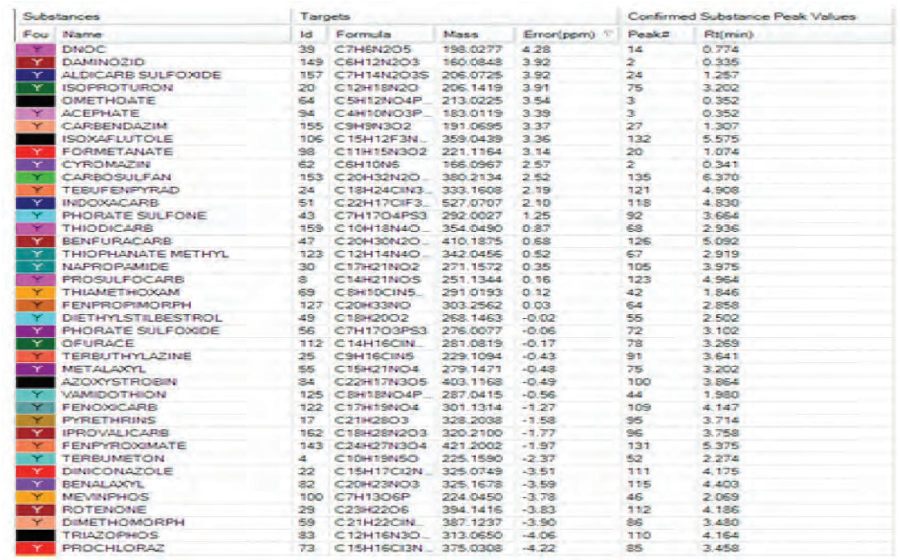


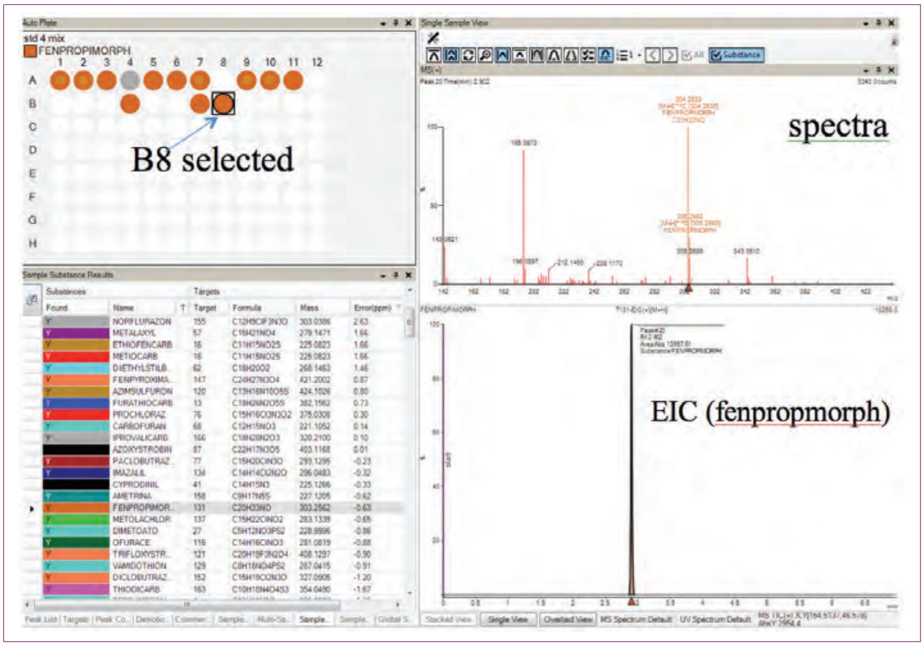
Figure 3. AxION Solo software allows for the confirmation of the presence of cloquintocet by matching accurate mass information of all detected isotopes to theoretical values and also matching theoretical and observed isotope profiles. Cloquintocet has a mass error of less than 2 ppm and the observed isotope ratio for A+1 is within 3% of the expected ratio.



Substances		Targets				Confirmed Substance Peak Values	
Fou	Name	Id	Formula	Mass	Error(ppm)	Peak#	Rt(min)
Y	DNOC	39	C7H6N2O5	198.0277	4.28	14	0.774
Y	DAMINOZID	149	C6H12N2O3	160.0848	3.92	2	0.335
Y	ALDICARB SULFOXIDE	157	C7H14N2O3S	206.0725	3.92	24	1.257
Y	ISOPROTURON	20	C12H18N2O	206.1419	3.91	75	3.202
	OMETHOATE	64	C5H12NO4P...	213.0225	3.54	3	0.352
Y	ACEPHATE	94	C4H10NO3P...	183.0119	3.39	3	0.352
Y	CARBENDAZIM	155	C9H9N3O2	191.0695	3.37	27	1.307
	ISOXAFLUTOLE	106	C15H12F3N...	359.0439	3.36	132	5.575
Y	FORMETANATE	98	C11H15N3O2	221.1164	3.14	20	1.074
Y	CYROMAZIN	62	C6H10N6	166.0967	2.57	2	0.341
Y	CARBOSULFAN	153	C20H32N2O...	380.2134	2.52	135	6.370
Y	TEBUFENPYRAD	24	C18H24ClN3...	333.1608	2.19	121	4.908
Y	INDOXACARB	51	C22H17ClF3...	527.0707	2.10	118	4.830
Y	PHORATE SULFONE	43	C7H17O4PS3	292.0027	1.25	92	3.664
Y	THIODICARB	159	C10H18N4O...	354.0490	0.87	68	2.936
Y	BENFURACARB	47	C20H30N2O...	410.1875	0.68	126	5.092
Y	THIOPHANATE METHYL	123	C12H14N4O...	342.0456	0.52	67	2.919
Y	NAPROPAMIDE	30	C17H21NO2	271.1572	0.35	105	3.975
Y	PROSULFOCARB	8	C14H21NOS	251.1344	0.16	123	4.964
Y	THIAMETHOXAM	69	C8H10ClN5...	291.0193	0.12	42	1.846
Y	FENPROPIMORPH	127	C20H33NO	303.2562	0.03	64	2.858
Y	DIETHYLSTILBESTROL	49	C18H20O2	268.1463	-0.02	55	2.502
Y	PHORATE SULFOXIDE	56	C7H17O3PS3	276.0077	-0.06	72	3.102
Y	OFURACE	112	C14H16ClN...	281.0819	-0.17	78	3.269
Y	TERBUTHYLAZINE	25	C9H16ClN5	229.1094	-0.43	91	3.641
Y	METALAXYL	55	C15H21NO4	279.1471	-0.48	75	3.202
	AZOXYSTROBIN	84	C22H17N3O5	403.1168	-0.49	100	3.864
Y	VAMIDOTHION	125	C8H18NO4P...	287.0415	-0.56	44	1.980
Y	FENOXICARB	122	C17H19NO4	301.1314	-1.27	109	4.147
Y	PYRETHRINS	17	C21H28O3	328.2038	-1.58	95	3.714
Y	IPROVALICARB	162	C18H28N2O3	320.2100	-1.77	96	3.758
Y	FENPYROXIMATE	143	C24H27N3O4	421.2002	-1.97	131	5.375
Y	TERBUMETON	4	C10H19N5O	225.1590	-2.37	52	2.274
Y	DINICONAZOLE	22	C15H17Cl2N...	325.0749	-3.51	111	4.175
Y	BENALAXYL	82	C20H23NO3	325.1678	-3.59	115	4.403
Y	MEVINPHOS	100	C7H13O6P	224.0450	-3.78	46	2.069
Y	ROTENONE	29	C23H22O6	394.1416	-3.83	112	4.186
Y	DIMETHOMORPH	59	C21H22ClN...	387.1237	-3.90	86	3.480
	TRIAZOPHOS	83	C12H16N3O...	313.0650	-4.06	110	4.164
Y	PROCHLORAZ	73	C15H16Cl3N...	375.0308	-4.22	85	3.458

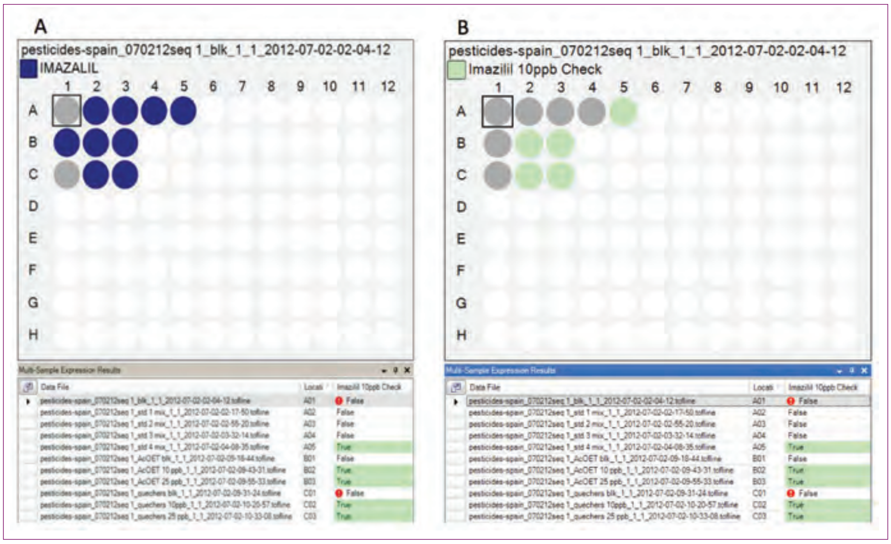
Table 2. Accurate mass of representative pesticides at 10 ppb concentration spiked in QuEChERS extracts of lettuce leaves.

The samples with area thresholds above the set value get flagged with an alternative color which can be visualized in the expression view of the software (Figures 5A and 5B). Using this easy to read color-coded view, we can very quickly identify the samples that contain pesticides over the regulated limit. Both single sample and batch reports can rapidly be generated by the software.(Figure 6). Batch reports can be exported into excel spreadsheets for further analysis.



Found	Name	T	Target	Formula	Mass	Error(ppm)
Y	NORFLURAZON		155	C12H9ClF3N3O	303.0386	2.63
Y	METALAXYL		57	C15H21NO4	279.1471	1.66
Y	ETHIOFENCARB		16	C11H15NO2S	225.0823	1.66
Y	METIOCARB		16	C11H15NO2S	225.0823	1.66
Y	DIETHYLSTILB...		62	C18H20O2	268.1463	1.46
Y	FENPYROXIMA...		147	C24H27N3O4	421.2002	0.87
Y	AZIMSULFURON		120	C13H16N10O5S	424.1026	0.80
Y	FURATHIOCARB		13	C18H26N2O5S	382.1562	0.73
Y	PROCHLORAZ		76	C15H16Cl3N3O2	375.0308	0.30
Y	CARBOFURAN		68	C12H15NO3	221.1052	0.14
Y	IPROVALICARB		166	C18H28N2O3	320.2100	0.10
	AZOXYSTROBIN		87	C22H17N3O5	403.1168	0.01
Y	PACLOBUTRAZ...		77	C15H20ClN3O	293.1295	-0.23
Y	IMAZALIL		134	C14H14Cl2N2O	296.0483	-0.32
	CYPRODINIL		41	C14H15N3	225.1266	-0.33
Y	AMETRINA		158	C9H17N5S	227.1205	-0.62
Y	FENPROPIMOR...		131	C20H33NO	303.2562	-0.63
Y	METOLACHLOR		137	C15H22ClNO2	283.1339	-0.65
Y	DIMETOATO		27	C5H12NO3PS2	228.9996	-0.86
Y	OFURACE		116	C14H16ClNO3	281.0819	-0.88
Y	TRIFLOXYSTR...		121	C20H19F3N2O4	408.1297	-0.90
Y	VAMIDOTHION		129	C8H18NO4PS2	287.0415	-0.91
Y	DICLOBUTRAZ...		152	C15H19Cl2N3O	327.0905	-1.20
Y	THIODICARB		163	C10H18N4O4S3	354.0490	-1.67

Figure 4. The top left hand corner shows the presence (orange color) and the absence (grey color) of fenpropmorph in different vials. The remaining pesticides detected in the selected vial B8 are shown in the bottom left hand corner.



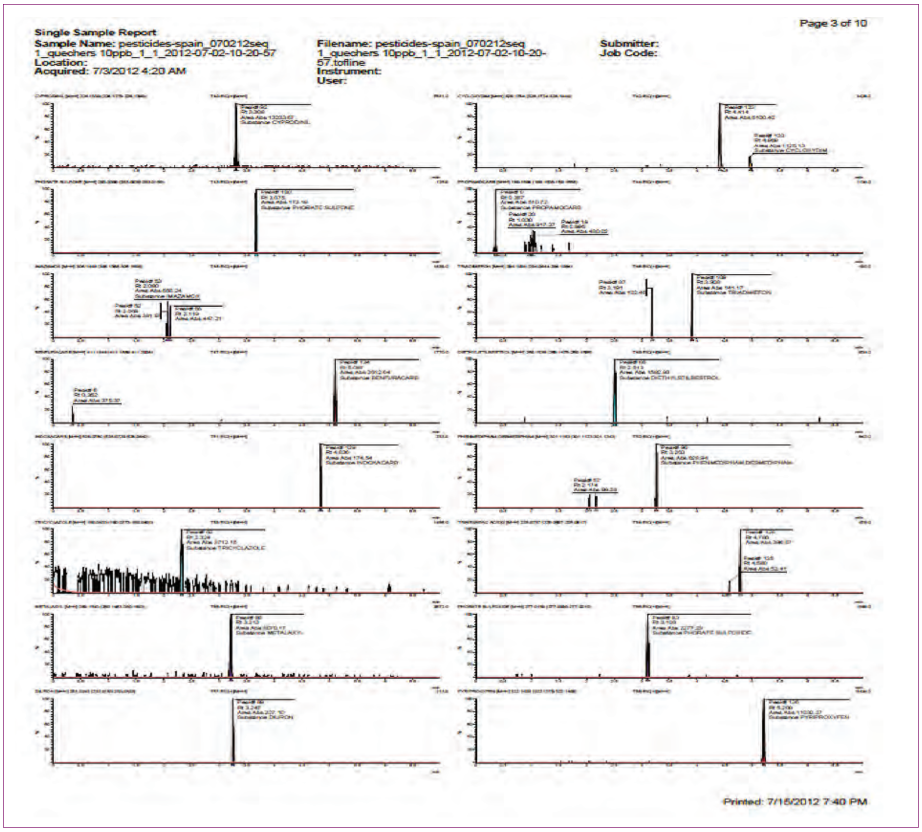
A

Data File	Locati	Imazilil 10ppb Check
pesticides-spain_070212seq 1_blk_1_1_2012-07-02-02-04-12.tofline	A01	❗ False
pesticides-spain_070212seq 1_std 1 mix_1_1_2012-07-02-02-17-50.tofline	A02	False
pesticides-spain_070212seq 1_std 2 mix_1_1_2012-07-02-02-55-20.tofline	A03	False
pesticides-spain_070212seq 1_std 3 mix_1_1_2012-07-02-03-32-14.tofline	A04	False
pesticides-spain_070212seq 1_std 4 mix_1_1_2012-07-02-04-08-35.tofline	A05	True
pesticides-spain_070212seq 1_AcOET blk_1_1_2012-07-02-09-18-44.tofline	B01	False
pesticides-spain_070212seq 1_AcOET 10 ppb_1_1_2012-07-02-09-43-31.tofline	B02	True
pesticides-spain_070212seq 1_AcOET 25 ppb_1_1_2012-07-02-09-55-33.tofline	B03	True
pesticides-spain_070212seq 1_quechers blk_1_1_2012-07-02-09-31-24.tofline	C01	❗ False
pesticides-spain_070212seq 1_quechers 10ppb_1_1_2012-07-02-10-20-57.tofline	C02	True
pesticides-spain_070212seq 1_quechers 25 ppb_1_1_2012-07-02-10-33-08.tofline	C03	True

B

Data File	Locati	Imazilil 10ppb Check
pesticides-spain_070212seq 1_blk_1_1_2012-07-02-02-04-12.tofline	A01	❗ False
pesticides-spain_070212seq 1_std 1 mix_1_1_2012-07-02-02-17-50.tofline	A02	False
pesticides-spain_070212seq 1_std 2 mix_1_1_2012-07-02-02-55-20.tofline	A03	False
pesticides-spain_070212seq 1_std 3 mix_1_1_2012-07-02-03-32-14.tofline	A04	False
pesticides-spain_070212seq 1_std 4 mix_1_1_2012-07-02-04-08-35.tofline	A05	True
pesticides-spain_070212seq 1_AcOET blk_1_1_2012-07-02-09-18-44.tofline	B01	False
pesticides-spain_070212seq 1_AcOET 10 ppb_1_1_2012-07-02-09-43-31.tofline	B02	True
pesticides-spain_070212seq 1_AcOET 25 ppb_1_1_2012-07-02-09-55-33.tofline	B03	True
pesticides-spain_070212seq 1_quechers blk_1_1_2012-07-02-09-31-24.tofline	C01	❗ False
pesticides-spain_070212seq 1_quechers 10ppb_1_1_2012-07-02-10-20-57.tofline	C02	True
pesticides-spain_070212seq 1_quechers 25 ppb_1_1_2012-07-02-10-33-08.tofline	C03	True

Figure 5A , B. Panel A shows the presence (blue color) and complete absence (grey color) of imazalil in samples. Panel B shows the presence of imazalil in samples at greater than or equal to 10 ppb concentration in green color while the grey colored vials show imazalil in samples at less than 10 ppb concentration.



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Single Sample Report
Sample Name: pesticides-spain_070212seq 1_quechers 10ppb_1_1_2012-07-02-10-20-57
Location:
Acquired: 7/3/2012 4:20 AM

Filename: pesticides-spain_070212seq 1_quechers 10ppb_1_1_2012-07-02-10-20-57.tofline
Instrument:
User:

Submitter:
Job Code:

Printed: 7/16/2012 7:40 PM

Figure 6. Single sample report.

Conclusions

The AxION 2 TOF MS can very easily detect less than or equal to 10 ppb concentration of an extended panel of commonly regulated pesticides. Both accurate mass and retention time can be used to confirm the presence of the target pesticides in samples. Using the powerful high throughput software AxION Solo, the user can quickly identify the presence or absence of hundreds of pesticides in large batches of samples using an easy to read color coded scheme. In addition, the software allows for user defined area thresholds for peak integration of each analyte for quick visualization of the presence or absence of analytes at or above a regulatory limit of 10 ppb concentration.

Acknowledgments

The authors would like to acknowledge Begona Arribas (PerkinElmer, Spain) for providing the pesticide standards and samples.

References

1.EU Food Directives, 2002, 91/414/EEC

2.Food Quality Protection Act, 1998 (FQPA)

3.Picó Y, Blasco C, Farré M, Barceló D *J AOAC Int*. 92 (2009) 734.

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010959A_01

APPLICATION BRIEF

ICP - Mass Spectrometry

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The Elemental Analysis of Spinach with the NexION 300/350 ICP-MS

Introduction

Trace metals in food can be highly toxic or nutritionally beneficial, depending on the type of metal present and its concentration. Naturally present in many foods, some elements are added to boost the nutritional value and enhance consumers' well-being, while others may be toxic at higher concentrations. Therefore, the elemental analysis of food requires the ability to measure both trace and high levels.

The elemental capabilities and dynamic range of inductively coupled plasma mass spectrometry (ICP-MS) make it ideally suited for the analysis of food materials. The ultratrace detection limits of ICP-MS permit the determination of low-level contaminants, such as Pb, As, Se, and Hg, while the macro-level nutritional elements, such as Ca, Mg, K, and Na, can be quantified using the extended dynamic range capability of ICP-MS which provides the ability to measure concentrations over nine orders of magnitude. However, there are still a number of challenges to overcome, including complex sample matrices, high levels of dissolved solids, and interferences. With the proper ICP-MS instrumental conditions and design, all of these issues can be overcome, allowing for the successful analysis of food samples, as described elsewhere[1]. This work will focus on the analysis of spinach, an important food staple, especially in the developing world.

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Experimental

Sample Preparation

NIST® 1570a Spinach was used in this work. Approximately 0.5-0.6 g were digested in duplicate with 5 mL of nitric acid (Fisher Scientific™, Optima grade) and 2 mL of hydrogen peroxide (Fisher Scientific™, Optima grade) in pre-cleaned PTFE microwave sample vessels. The digestion program consisted of 30 min of heating and 15 min of cooling, as shown in Table 1. All samples were completely dissolved, resulting in clear solutions that were diluted to a final volume of 50 mL with deionized water. No further sample dilutions were necessary. Gold was added to all solutions at a final concentration of 200 µg/L to stabilize mercury. Preparation blanks, consisting of the acid mixture, were taken through the same microwave digestion program as the samples.

Table 1. Microwave Digestion Program.

Step	Power (W)	Ramp (min)	Hold (min)
1	500	1	4
2	1000	5	5
3	1400	5	10
4 (cooling)	0	—	15

Instrumental Conditions

All data in this study were generated under normal operating conditions on a PerkinElmer NexION® 300/350X ICP-MS using an autosampler. The instrumental operating conditions are shown in Table 2.

Table 2. ICP-MS Instrumental Operating Conditions for this Application.

Parameter	Value
Nebulizer	Glass concentric
Spray chamber	Glass cyclonic
Cones	Nickel
Plasma gas flow	18.0 L/min
Auxiliary gas flow	1.2 L/min
Nebulizer gas flow	0.98 L/min
Sample uptake rate	300 µL/min
RF power	1600 W
Total integration time	0.5 (1.5 seconds for As, Se, Hg)
Replicates per sample	3
Universal Cell Technology™ *	Collision mode

*PerkinElmer, Inc.

Calibration

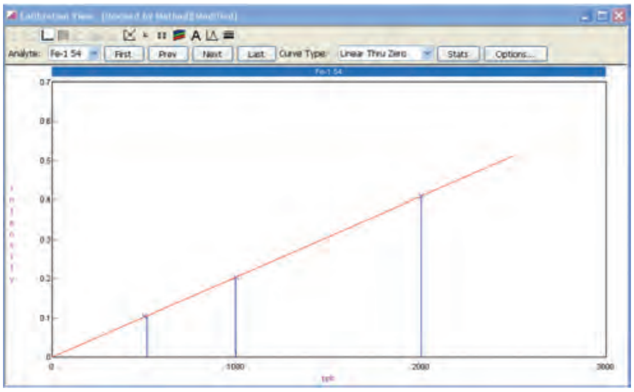
Multielement calibration standards, representing all the analytes in the SRMs, were made up from PerkinElmer Pure single and multielement standards and diluted into 10% HNO_3. Gold was added to all solutions at a final concentration of 200 µg/L to stabilize mercury. Calibration standard ranges were based on whether the analyte was expected to be a high-level nutritional element like potassium (K) or sodium (Na), low/medium-level essential element like manganese (Mn) or iron (Fe), or trace/ultratrace contaminant such as lead (Pb) or mercury (Hg).

Depending on the certified value of the analytes, five different calibration ranges were made up to cover the complete range of elements being determined:

- High-level nutritional analytes: 0-300 ppm
- Medium-level essential analytes: 0-20 ppm
- Low-level essential analytes: 0-2 ppm
- Trace-level contaminants: 0-200 ppb
- Ultratrace-level contaminants: 0-20 ppb

Figures 1 to 5 show representative calibration curves for each range.

In addition to the analyte elements used for the multielement calibration, the standards, blanks, and samples were also spiked on-line using a mixing tee with a solution of ^{6}Li, Sc, Ge, In, and Tb for internal standardization across the full mass range. Acetic acid was added to the internal standard solution to compensate for residual carbon left over from the sample digestion.

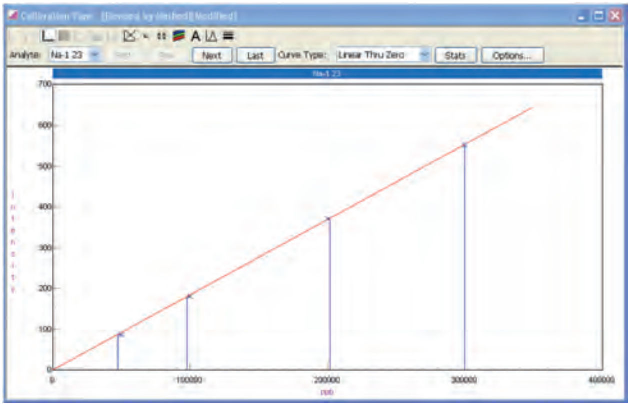


Analyte: Fe-1 54

Curve Type: Linear Thru Zero

^{54}Fe Correlation Coefficient = 0.99997.

Figure 1. Calibration curves for ^{54}Fe (0-2 ppm).

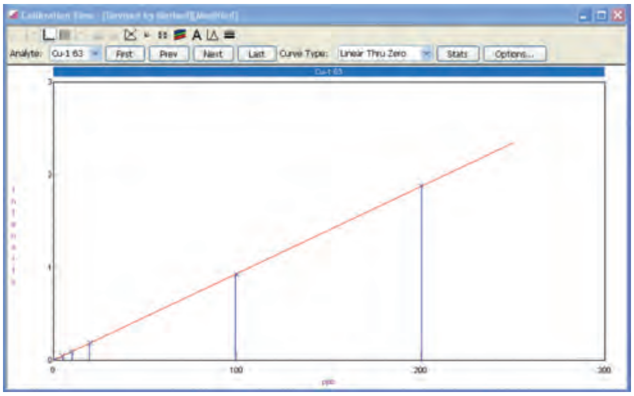


Analyte: Na-1 23

Curve Type: Linear Thru Zero

^{23}Na Correlation Coefficient = 0.99996.

Figure 2. Calibration curve for ^{23}Na (0-300 ppm).

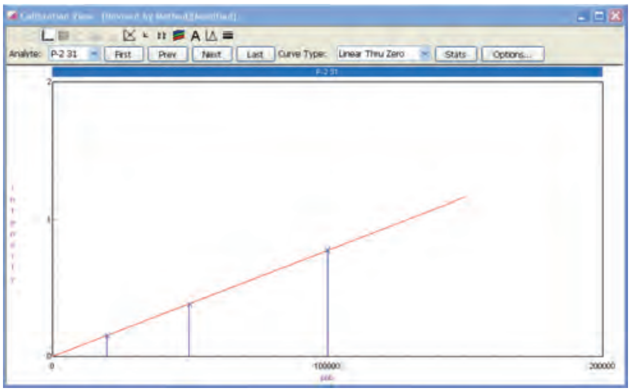


Analyte: Cu-1 63

Curve Type: Linear Thru Zero

^{63}Cu Correlation Coefficient = 0.99999.

Figure 3. Calibration curve for ^{63}Cu (0-200 ppb).

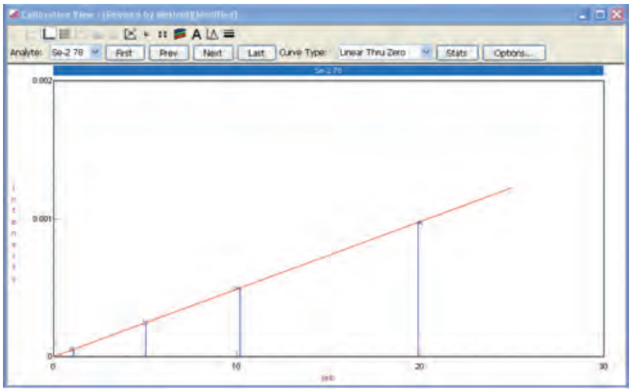


Analyte: P-2 31

Curve Type: Linear Thru Zero

^{31}P Correlation Coefficient = 0.99999.

Figure 4. Calibration curve for ^{31}P (0-100 ppm).



Analyte: Se-2 78

Curve Type: Linear Thru Zero

^{78}Se Correlation Coefficient = 0.99995.

Figure 5. Calibration curve for ^{78}Se (0-20 ppb).

Results

Quantitative results for two sample preparations of the NIST® 1570a Spinach reference materials are shown in Table 3. All elements in every sample were determined with Universal Cell operating in Collision mode using helium as the cell gas. Figures in parentheses ( ) in the Reference Value column are not certified values but are included for information purposes only. The data show very good agreement with the certified values, especially for the elements that suffer from known spectral interferences. The elements that are outside the specified limits are mostly the ones that are well recognized as environmental contaminants, which have most likely been impacted by the sample preparation procedure.

Table 3. Analysis of NIST® 1570a Spinach using the NexION 300/350 ICP-MS.

Element	Mass (amu)	Reference Value (mg/kg)	Experimental Value (mg/kg)
B	11	37.6 ±1.0	37.3
Na	23	18180 ±430	17350
Mg	26	(8900)	8600
Al	27	310 ±11	200
P	31	5180 ±110	4810
S	34	(4600)	4400
K	39	29030 ±520	26600
Ca	44	15270 ±410	15040
V	51	0.57 ±.003	0.58
Cr	52	–	1.63
Fe	54	–	265
Mn	55	75.9±1.9	77.9
Co	59	0.39±0.05	0.37
Ni	60	2.14 ±0.10	1.97
Cu	63	12.2 ±0.6	11.6
Zn	66	82 ±3	80
As	75	0.068 ±0.012	0.081
Se	78	0.117 ±0.009	0.21
Sr	88	55.6 ±0.8	58.1
Mo	98	–	0.39
Cd	111	2.89 ±0.07	2.83
Sn	118	–	0.027
Sb	121	–	0.007
Ba	137	–	5.8
Hg	202	0.030 ±0.003	0.028
Pb	208	(0.20)	0.16
Tl	205	–	0.018
Th	232	0.048 ±0.003	0.045
U	238	(0.155 ±0.023)	0.154

Conclusion

This work has demonstrated the ability of PerkinElmer's NexION 300/350X ICP-MS to effectively measure macro-level nutritional elements in the same analysis run as lower-level elements, without having to dilute the samples. The agreement between experimental and certified results for NIST® 1570a Spinach demonstrate the accuracy of the analysis. Instrument design characteristics eliminate deposition on the ion optics, leading to long-term stability in high-matrix samples, while permitting trace levels to be accurately measured.

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APPLICATION NOTE

Mass Spectrometry

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Rapid Screening and Quantitation of Postharvest Fungicides on Citrus Fruits Using AxION DSA/TOF and Flexar SQ MS

Introduction

Thiabendazole and Imazalil are the major post-harvest fungicides used on surface of citrus fruits such as oranges, grapefruits and lemons to prevent mold formation and control postharvest decay by various fungal pathogens in citrus fruits[1]. Figure 1 shows the structure of two fungicides. These substances have toxicity in higher doses, with effects such as liver and intestinal disorders and carcinogenic effects. The MRL (maximum residue limits) of imazalil and thiabendazole for conventional citrus fruits are 10 ppm and 5 ppm in the U.S. and Europe, respectively[2,3]. The use of these fungicides on organic citrus fruits is prohibited. The maximum residue limits for these compounds on conventional citrus fruits and possible toxic effects require that these compounds should be monitored before consumption of fruit to ensure food safety. Previously, these fungicides on citrus fruits have been measured by different LC/MS techniques with varying sample preparation techniques such as liquid-liquid extraction[4,5] and QUECHERS[6,7].

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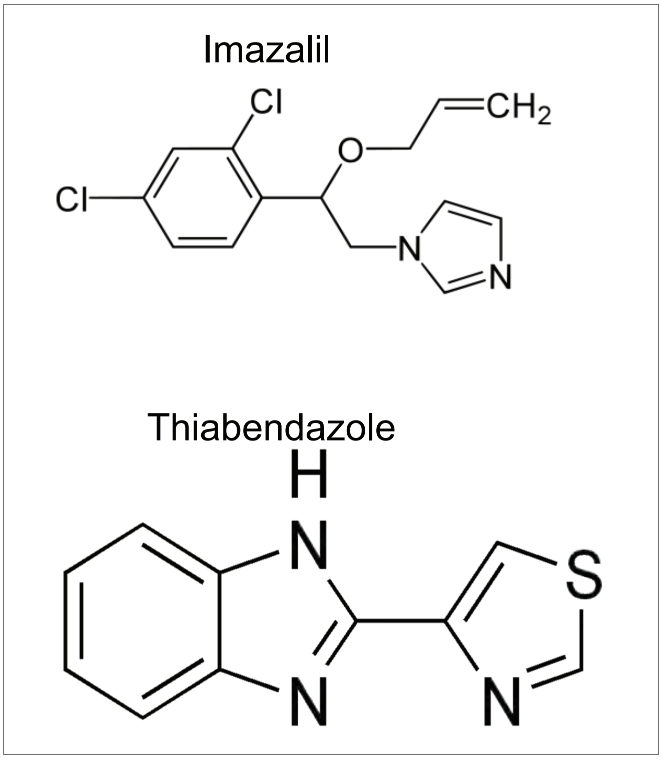

Figure 1. Structure of two fungicides.

The main drawback of these methods is that a great deal of time and money is consumed in the method development, sample preparation and analysis time. In this work, we demonstrated that the AxION® Direct Sample Analysis (DSA™) system integrated with the AxION 2 Time-of-Flight (TOF) mass spectrometer can be used for rapid screening of fungicides on conventional and organic citrus fruit samples with minimal sample preparation. After initial rapid screening, only the citrus fruits samples showing potential presence of fungicides were selected for quantitation using the Flexar™ FX-10 coupled with the Flexar SQ 300 MS (LC/SQ) with Ultraspray™ ESI Source. This work shows an improved and productive work flow by screening samples using DSA/TOF in 10 seconds per sample followed by LC/SQ for only those samples that tested positive. This reduction in number of total samples analyzed using chromatography decreases the amount of total time spent for sample analysis as well as decreases lab overhead costs with reduction in solvent usage and disposal.

Method

Sample Preparation

Five conventional citrus fruits and two organic citrus fruits were obtained from local supermarket. These citrus fruits were peeled and 10 gm of the peel was extracted in 30 ml of acetonitrile. After extraction, 5 µL of extracted sample was pipetted onto stainless mesh for rapid screening measurement with DSA/TOF. After that, acetonitrile extracted samples were cleaned with modified one step QUECHERS method before running them with LC/MS. 1.5 ml of ACN extract was added to tube with 25 mg of PSA adsorbent and 150 mg $MgSO_4$ and centrifuged at 7800 rpm for 10 minute. After centrifuging, 0.6 ml of supernatant was dried under nitrogen for 30 min and reconstituted in 0.6 ml of 35/65 methanol/water and was used for analysis with LC/MS.

DSA/TOF Parameters

Five µl of each acetonitrile extracted sample was pipetted directly onto the stainless mesh of the AxION DSA system for ionization and analysis. The DSA/TOF experimental parameters were as follows: corona current of 5 µA, heater temperature of 300 °C. The AxION 2 TOF MS was run in positive ionization mode with flight voltage of -8000 V. The capillary exit voltage was set to 100 V for the analysis. Mass spectra were acquired in a range of m/z 100-700 at an acquisition rate of 5 spectra/s. All samples were analyzed within 10 s. To obtain high mass accuracy, the AxION 2 TOF instrument was calibrated before each analysis by infusing a calibrant solution into the DSA source at 10 µl/min.

LC/SQ MS Parameters

After rapid screening of samples with DSA/TOF, the samples showing potential presence of fungicides were further analyzed for quantitation using the LC/SQ MS. The separation of fungicides was carried out using a PerkinElmer Brownlee™ Supra C18 column (2.1x100 mm, 3 µm) at flow rate of 0.5 ml/min and using a linear gradient of 35% methanol in water to 95 % methanol in water with 0.05% acetic acid in 5 minutes. The column temperature was maintained at 30 °C and injection volume was 3 µL. The ESI source temperature and drying gas flow rate were 350 °C and 15 L/min. The SIM dwell time was 100 msec. Both molecular and fragment ions of fungicides, imazalil and thiabendazole, were monitored to provide further confirmation of their presence in citrus fruits by LC/MS. The optimum capillary exit voltages for measurement of thiabendazole molecular and fragment ion were 90 and 160 V, respectively. The optimum capillary exit voltages for measurement of imazalil molecular and fragment ion were 80 and 140 V, respectively.

Results

Fungicide Screening by DSA/TOF

Figure 2 and Figure 3 show extracted ion chromatogram (EIC) for thiabendazole and imazalil for two organic citrus fruits and five conventional citrus fruits. The data shows that thiabendazole was present in all five conventional citrus fruits, whereas imazalil was present in 3 out of 5 conventional citrus fruits. Organic citrus fruits should be absent of fungicides as they are prohibited from use. The potential presence of fungicides shown using DSA/TOF suggests that these samples should be further investigated using LC/SQ MS. The data in Figures 2-4 suggests that these fungicides were present at lower level in organic orange as compared to other conventional citrus fruits. The zoomed in EIC data for organic grapefruit showed that these fungicides were completely absent. Figure 5 and Figure 6 showed the mass spectra of conventional and organic orange, respectively. The presence of fungicides in these citrus fruit samples was further confirmed by below 5 ppm mass accuracy of measurements with DSA/TOF. The data with DSA/TOF showed that one or both fungicides was present in all conventional citrus fruits, whereas both fungicides show potential presence in 1 out of 2 organic citrus fruits. This work showed that DSA/TOF can also be used to perform initial screening for the presence of fungicides on fruits that can then be further analyzed using LC/SQ for traditional quantitation.

Fungicide Quantitation by LC/SQ MS

Figure 7 shows SIM for molecular ion and fragment ion for 1 ppm standard of 2 fungicides using the LC/SQ. The on column detection limit for thiabendazole and imazalil was 0.3 ppb and 0.45 ppb, respectively. Figures 8 and 9 demonstrated that the response for 2 fungicides was linear from 1 ppb to 1000 ppb with correlation coefficient R^2 better than 0.9933. Figure 10 displayed SIM for molecular ion and fragment ion for 2 fungicides in conventional orange brand #1. Further confirmation of the presence of fungicides in citrus fruits was obtained using in source CID (collision induced decomposition) and monitoring their fragment ion at higher capillary exit voltage and retention time matching with the standard. Table 1 showed the level of fungicides measured in five conventional citrus fruit samples and one contaminated organic citrus fruit sample. The data showed the amount of fungicides on conventional citrus fruit samples was below MRL value of 5ppm. Also, it confirmed that one of organic citrus fruit sample was contaminated with low levels of fungicide as suggested by the DSA/TOF screening method.

Conclusions

This method shows the first work for rapid screening of fungicides in conventional and organic citrus fruits using DSA/TOF in 10 seconds. The data showed that fungicides were present in all five conventional citrus fruits and one out of two organic citrus fruits. The mass accuracy of all measurements with DSA/TOF was less than 5 ppm with external calibration. All samples were screened, with minimal sample preparation, in 10 sec. per sample. After rapid screening of citrus fruit samples for fungicides, the samples with fungicides were selected for quantitation with LC/SQ MS. A five minute LC/MS method was developed for quantitation of fungicides in citrus fruits. Both molecular and fragments ions were monitored in SIM mode for both imazalil and thiabendazole to confirm their presence in citrus fruit samples. All tested conventional citrus fruit samples had total fungicide amount (0.5 to 1.5 ppm) which was less than MRL of 5 ppm. The organic grapefruit sample showed absence of fungicides, where as organic orange showed presence of total fungicide amount of about 0.02 ppm.

Table 1. Fungicide levels measured on one organic and five conventional citrus fruits bought at a local grocery store using LC/SQ MS.

Sample	Thiabendazole (ppm)	Imazalil (ppm)
Organic orange	0.013	0.009
Orange Brand # 1	0.490	0.726
Orange Brand # 2	0.290	Not Detected
Orange Brand # 3	0.649	0.578
Grapefruit	0.509	Not Detected
Lemon	0.250	1.390

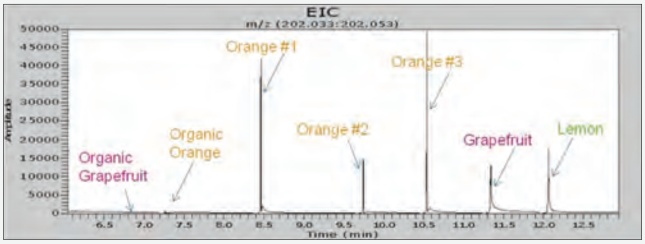

Figure 2. Extracted ion chromatogram for thiabendazole in two organic and five conventional citrus fruits using DSA/TOF.

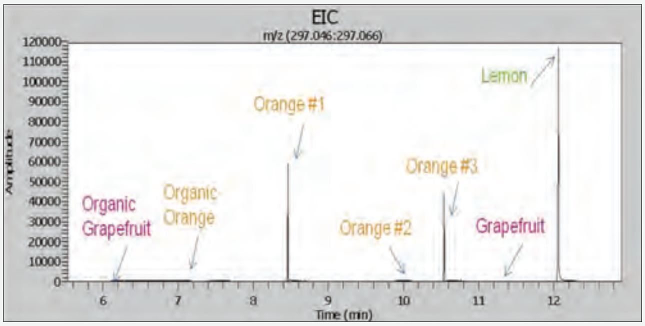

Figure 3. Extracted ion chromatogram for imazalil in two organic and five conventional citrus fruits using DSA/TOF.

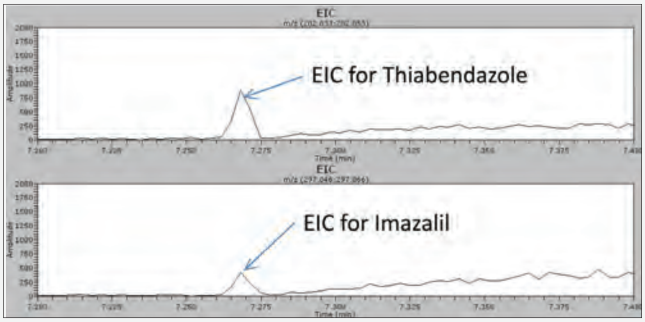

Figure 4. Zoomed in extracted ion chromatogram for imazalil and thiabendazole in organic orange using DSA/TOF.

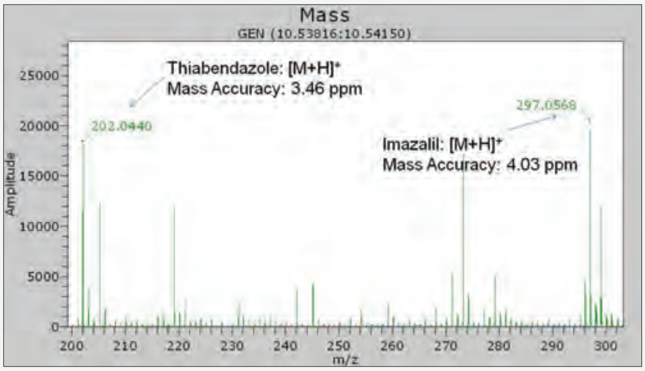

Figure 5. Mass spectra of conventional orange #3 obtained with DSA/TOF.

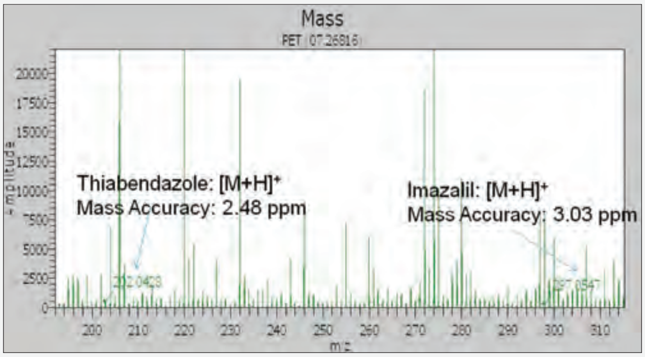

Figure 6. Mass spectraspectra of organic orange obtained with DSA/TOF.

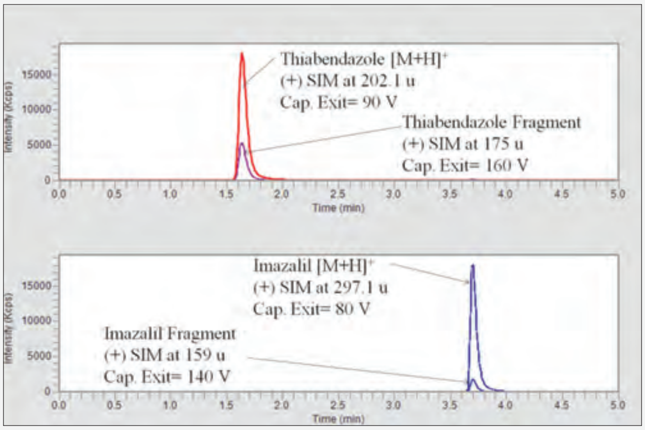

Figure 7. SIM of 1 ppm standard of two fungicides using LC/SQ MS.

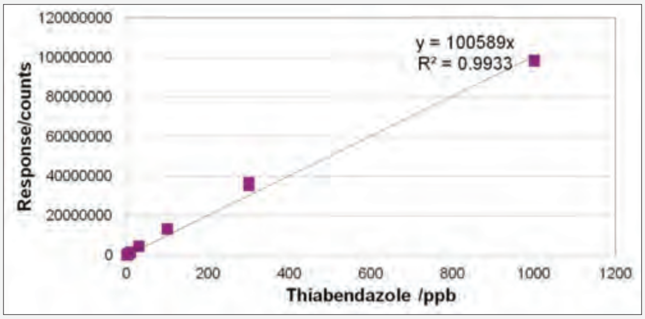

Figure 8. Calibration curve for thiabendazole over concentration range from 1 to 1000 ppb obtained with LC/SQ MS.

With this workflow, the samples can be screened rapidly with DSA/TOF and can be divided into two categories of positive and negative samples. After initial screening, only positive samples need to be run for quantitation with LC/MS. In comparison to other established workflows such as LC/MS and GC/MS, the workflow, which couples DSA/TOF with LC/MS, will reduce total analysis time and reduce solvent usage which decreases laboratory overhead costs and improves overall productivity.

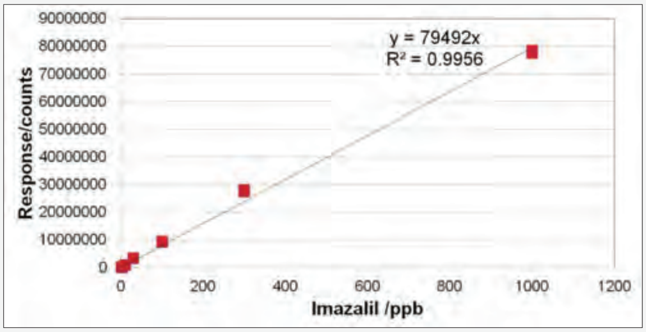

Figure 9. Calibration curve for imazalil over concentration range from 1 to 1000 ppb obtained with LC/SQ MS.

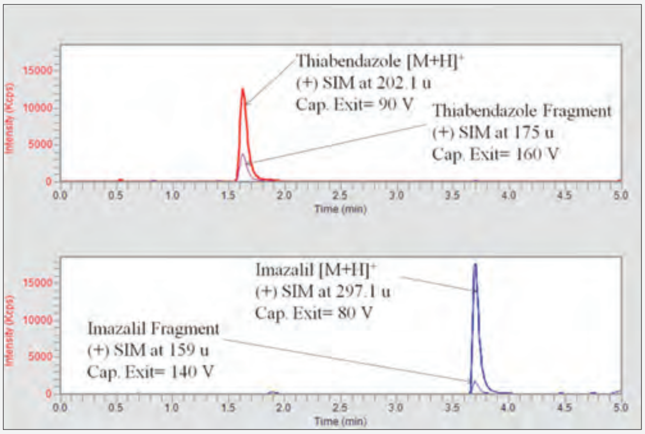

Figure 10. SIM of two fungicides in peel extract of conventional orange #1 using LC/SQ MS

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APPLICATION NOTE

Gas Chromatography/
Mass Spectrometry

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The Extraction and Quantification of Limonene from Citrus Rinds Using GC/MS

Introduction

D-Limonene, shown in Figure 1, is a common naturally occurring compound with a citrus scent. It is often used as an additive in food products and fragrances, and is classified by the U.S. Food and Drug Administration (FDA) as Generally Recognized as Safe (GRAS)[1]. It has also been approved by the U.S. Environmental Protection Agency (EPA) for usage as a natural pesticide and insect repellent[1]. Limonene has also been studied for its anti-carcinogenic properties[2]. Orange oil, which contains a considerable amount of limonene, has numerous applications including a combustant in engines[3], a powerful degreaser in cleaning applications, and a natural pesticide[4]. These uses may require a known concentration of limonene with a limited amount of impurities. This exemplifies the need for a reliable method of extraction of limonene from its natural source, citrus rinds, followed by a quantitative analysis of the extract for limonene and possible impurities.

A method for the extraction and quantification of limonene from citrus fruit peels is discussed in this applications note. Beyond demonstrating the use of GC/MS in the analysis of citrus fruit for limonene content, this application demonstrates a simple, inexpensive technique to introduce students to method development, calibration and quantification using a chromatographic technique. The analysis of citrus fruit for limonene may be an ideal laboratory assignment at the undergraduate level. The techniques used are safe, simple and easy.

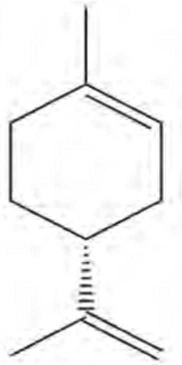

Figure 1. Molecular structure of limonene.

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Experimental

External Calibration Curve

A limonene standard (SPEX CertiPrep®, Metuchen, NJ) with a concentration of 1000 µg/mL was diluted to 100 µg/mL by a 10:1 dilution with methanol. The remaining solutions were prepared by serial 2:1 dilutions resulting in final limonene concentrations of 50, 25, 12.5, and 6.25 µg/mL.

Extraction of limonene

Samples of lemon, orange, and grapefruit rinds were carefully collected using a razor blade. The samples were checked to ensure that none of the white flesh under the rind was included in the sample, as shown in Figures 2 and 3. The white flesh contributes to the mass of the sample but contains little limonene; this makes the rinds appear to have a lower limonene concentration. Then each sample was cut down to a mass of approximately 0.1 g. The rind samples were each placed in 7 mL vials with 5 mL of methanol. The vials were shaken vigorously for 5 minutes and then allowed to stand for an additional 5 minutes. After the 10-minute extraction was complete, 0.5-mL aliquots of methanol from each vial were diluted volumetrically (20:1 for lemon and grapefruit rinds, and 10:1 for orange rind). These dilutions were necessary in order to prepare solutions with concentrations of analyte within the range of the previously prepared calibration curve.

Analysis and quantification of limonene

The analysis of the standards and samples was performed with a PerkinElmer® Clarus® 560 D GC/MS, using the parameters shown in Table 1. The GC was fitted with a capillary injector port using a 4-mm standard glass liner packed with quartz wool configured for split operation (PerkinElmer Part No. N6121010). A PerkinElmer Elite™-5ms (30 m x 0.25 mm x 0.25 µm) column (PerkinElmer Part No. N9316282) was used throughout; the details of the method are shown in Tables 1 and 2.

Figure 2. Example of an orange rind being cut with a razor blade.

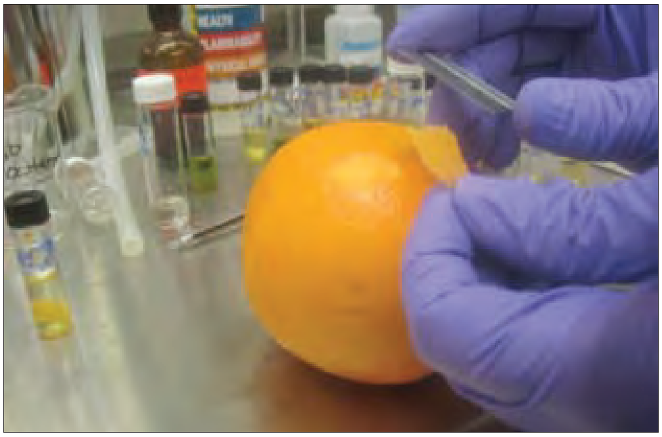

Figure 3. Example of a good sample of orange rind. (None of the white flesh is on the sample).

***Table 1.* Operation Specifications for GC.**

Gas Chromatograph:	**PerkinElmer Clarus 500 GC**		
Analytical Column:	Elite-5ms (30 m x 0.25 mm x 0.25 µm)		
Injector-Port Type:	Capillary		
Injector-Port Temp:	250 ˚C		
Injection Type:	Split (20 mL/min)		
Syringe Volume:	5 µL		
Injection Volume:	0.5 µL		
Injection Speed:	Fast		
Rinse Solvent:	Methanol		
Carrier-Gas Program:	1 mL/min		
Oven Program:	**Temperature**	**Hold Time**	**Rate**
	80 ˚C	3 min	5 ˚C/min
	140 ˚C	0 min	45 ˚C/min
	275 ˚C	Hold	

***Table 2.* Operation Specifications for MS.**

Mass Spectrometer:	**PerkinElmer Clarus 560 D MS**
GC Inlet Temp:	250 ˚C
Ion-Source Temp:	250 ˚C
Function Type:	Full Scan
Full-Scan Range:	*m/z* 40-300
Full-Scan Time:	0.15 sec
Interscan Delay:	0.05 sec
Solvent Delay:	2.5 min

Discussion

Mass spectra for the limonene standard and limonene in the extract are shown in Figure 4. The limonene spectrum and retention time in the standard matched those of the fruit extract, and a NIST library search also supported the identification as limonene. The chromatogram for *m/z* 136 was chosen for quantification because it is a unique, high *m/z* peak that is relatively abundant; higher *m/z* peaks generally experience a better signal-to-noise ratio.

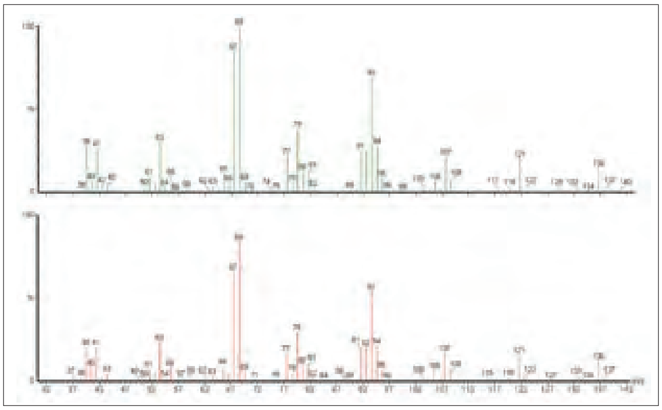

Figure 4. Mass spectrum for limonene in the rind extract (top) and in the standard (bottom).

The extracted *m/z* 136 ion chromatogram for the orange sample is shown in Figure 5. The amount of limonene in each sample was quantified by plotting a calibration curve using the instrument response at *m/z* 136, shown in Figure 6. The linear regression analysis of the calibration curve in Figure 6 yielded Equation 1, which was used to calculate the concentration of limonene in the sample. These concentrations were then used to calculate the concentrations of the undiluted solutions, which were then used to determine the wt/wt % of limonene in each fruit's rind; these results are shown in Table 3.

Equation 1: $y = 941.4172x + 2317.1604$

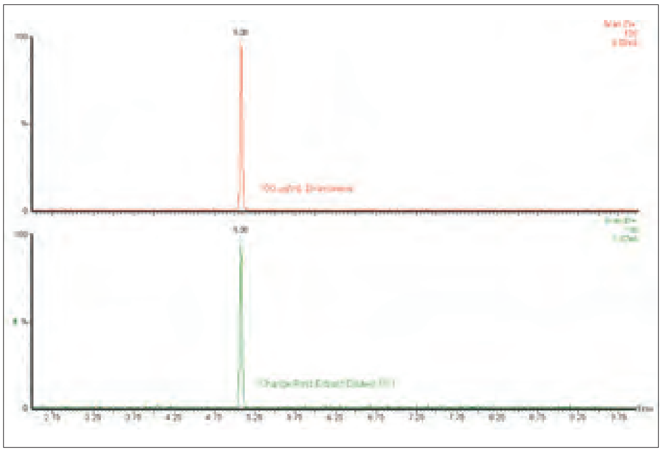

Figure 5. The extracted ion chromatogram for the 100 µg/mL limonene standard (top) and the diluted orange extract (bottom) at *m/z* 136.

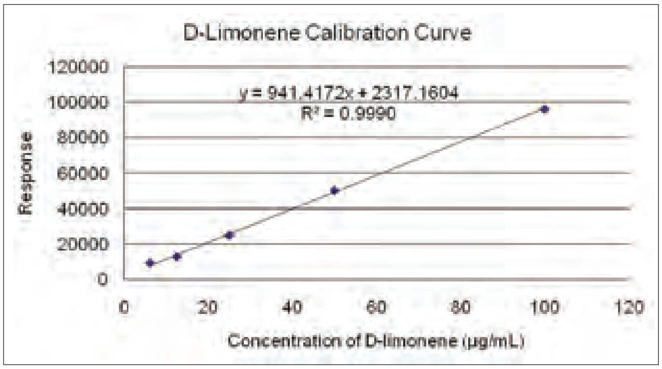

Figure 6. Calibration curve of limonene used to quantify the samples.

***Table 3.* Intermediate and Final Results for the Analysis of Lemon, Grapefruit and Orange Rinds.**

	Diluted Conc. (µg/mL)	Undiluted Conc. (µg/mL)	Mass Extracted (µg)	Mass of Sample (g)	% wt/wt
Lemon	35.66	713.2	3566	0.1199	2.97
Grapefruit	44.55	891.0	4455	0.1557	2.86
Orange	35.62	356.2	1781	0.1096	1.63

Conclusion

This application note demonstrates a simple extraction and quantification method for limonene using GC/MS. The limonene extraction and calibration curve preparation were discussed, as well as the method for analysis. The results obtained by following this method were presented along with the final %wt/wt of oil in the rinds. It was discovered that while all of these fruits had limonene in their rinds, lemon contained the highest concentration. Students conducting this analysis will gain valuable experience in sample preparation, solid-liquid extractions, and one of the most sensitive analytical techniques for the analysis of volatile compounds.

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