APPLICATION NOTE

Liquid Chromatography/ Mass Spectrometry

Authors:

Sharanya Reddy

Ariovaldo Bisi

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.

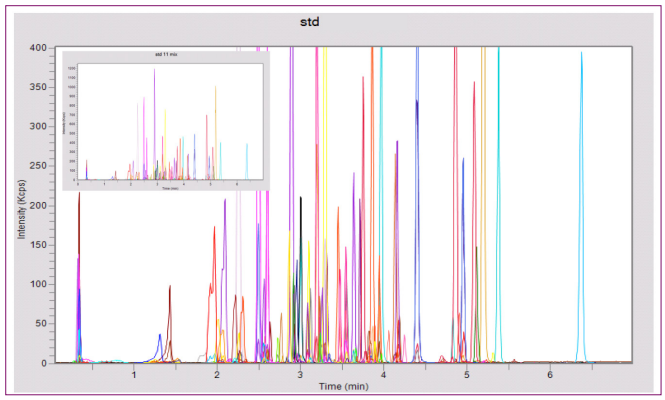

Figure 1. Extracted ion chromatograms (EICs) showing 130 pesticide standards analyzed by UHPLC-TOF MS.

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		fenpyroximate	2	pyriproyfen	2
sulfone	2	flutriafol	3.5	quinchlorac	2
ametrina	2	formetanate	2	rimusulfuron	7
azimsulfuron	2	furathiocarb	2	rotenone	3.5
azinphosethyl	2	imazalil	2	tebufenpyrad	3.5
azoxystrobin	2	imazamox	2	terbumeton	2
benalaxyl	2	indoxacarb	3.5	terbuthylazine	2
carbendazim	2	iprodion	7	thiabendazole	2
carbofuran	2	isoproturon	2	thichloprid	3.5
carbofuran 3 hydroxy	3.5	metalaxyl	2	thidiazuron	3.5
chlorfenson	7	methomyl	2	thiodicarb	3.5
chlosulfuron	2	methiocarb	3.5	triadimenol	3.5
chloquintocet	2	metalachlor	2	triazophos	2
chlorthiandin	3.5	mevinphos	3.5	tricyclazole	2
cycloxydim	2	napropamide	2	tridemomorph	2
cymoxanil	7	nicrosulfuron	3.5	trifloxystrobin	2
cyprodinil	2	norflurazon	2	vamidothion	2
cyromazin	2	ofurace	2		
diaminozid	2	oxamyl	2		
dichlobutrazol	3.5	paclonutrazol	2		
diethylstilbestrol	2	Phenmedipham/Desmedi			
dimethomorph	2	pham	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.

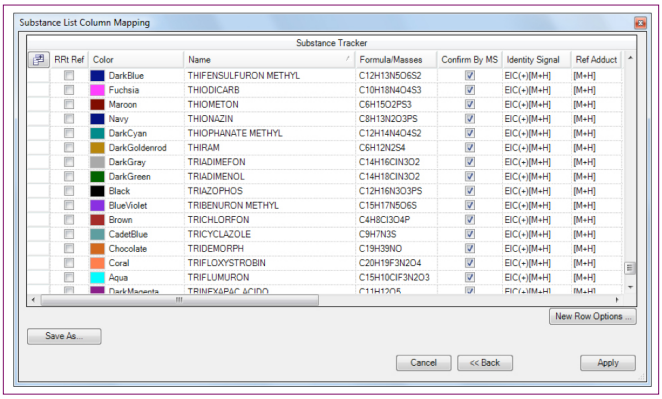

Figure 2. Substance or target list containing compound names and elemental composition can be set up in AxION Solo or easily imported from an excel spreadsheet. .

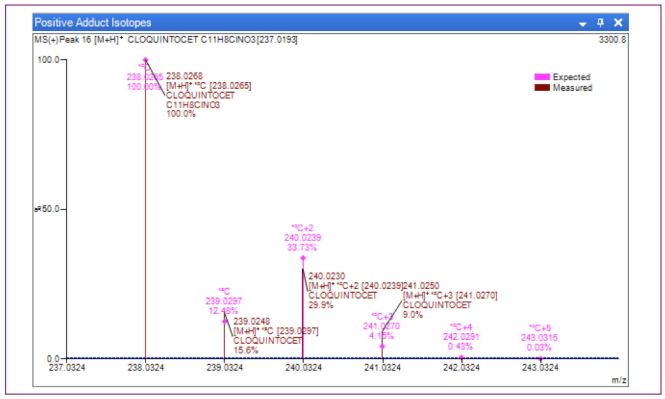

Figure 3. AxION Solo software allows for the confirmation of the presence of cloquintocet by matching accurate mass information of all detected isotopes to theoretical values and also matching theoretical and observed isotope profiles. Cloquintocet has a mass error of less than 2 ppm and the observed 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
Y	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
Y	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
Y	AZOXYSTROBIN	84	C22H17N3O5	403.1168	-0.49	100	3.864
Y	VAMIDOTHION	125	C8H18NO4P...	287.0415	-0.56	44	1.980
Y	FENOXYCARB	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	-3.73	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.

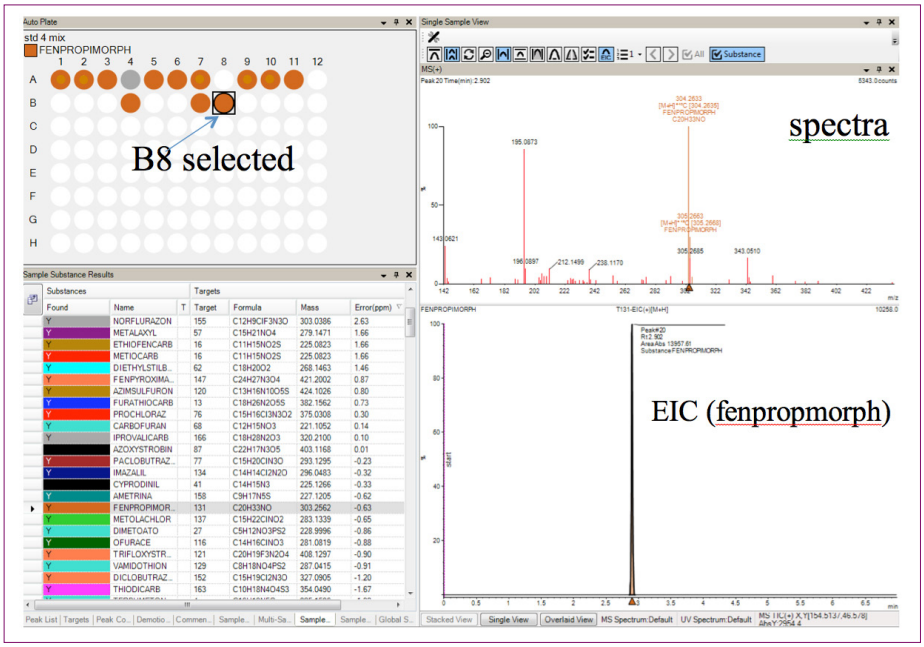

Figure 4. The top left hand corner shows the presence (orange color) and the absence (grey color) of fenpropmorph in different vials. The remaining pesticides detected in the selected vial B8 are shown in the bottom left hand corner.

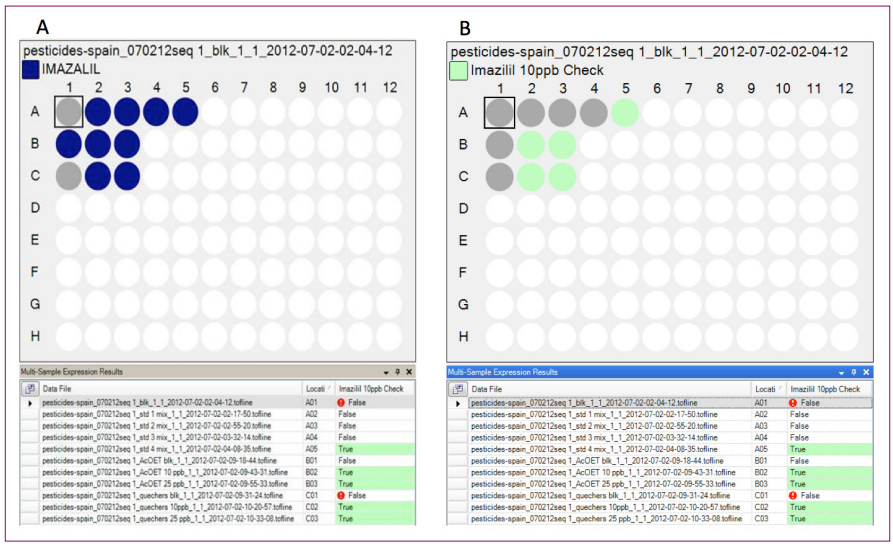

Figure 5A , B. Panel A shows the presence (blue color) and complete absence (grey color) of imazalil in samples. Panel B shows the presence of imazalil in samples at greater than or equal to 10 ppb concentration in green color while the grey colored vials show imazalil in samples at less than 10 ppb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

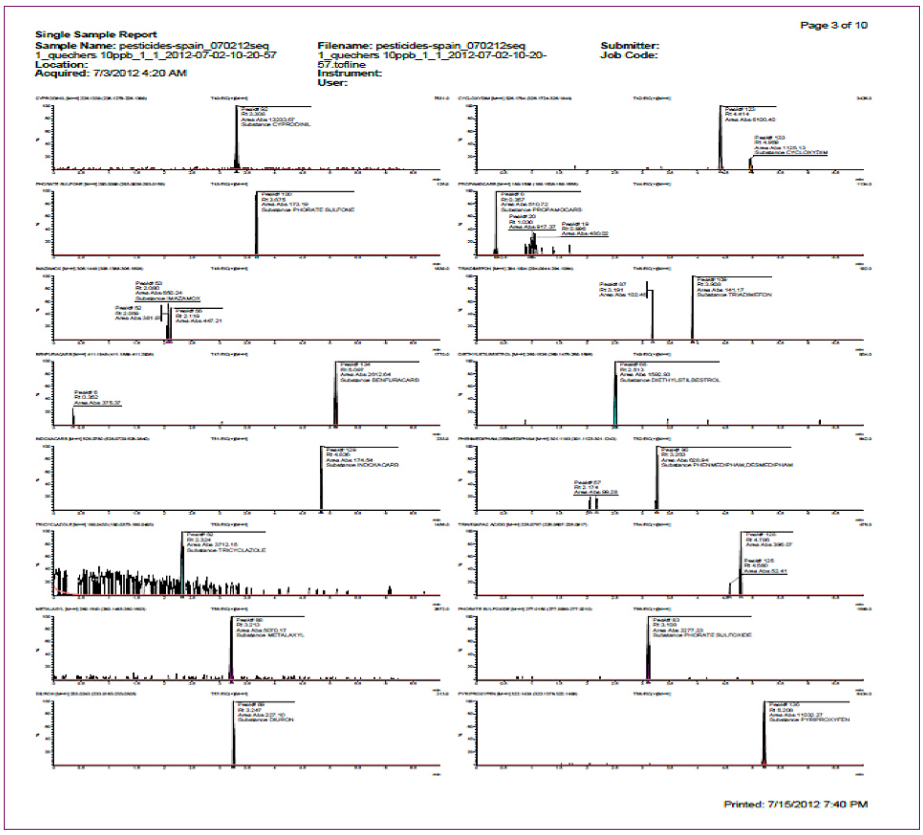

Figure 6. Single sample report.

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.

PerkinElmer, Inc.
940 Winter Street
Waltham, MA 02451 USA
P: (800) 762-4000 or
(+1) 203-925-4602
www.perkinelmer.com

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