Application Note LCMS-97

Quantitation of 250 Pesticides in Fruit Juices with Positive/Negative Switching LC-MS/MS

Abstract

A study was carried out using the Bruker EVOQ Elite liquid chromatography-triple quadrupole mass spectrometer (LC-MS/MS) in multiple reaction monitoring (MRM) mode to quantify 250 pesticide compounds in apple, cranberry, whit grape, orange and vegetable juice. A single method performed automatic positive and negative ion switching for over 500 MRM transitions in 18 minutes including re-equilibration, considerably faster than separate positive and negative quantification methods. With fast and straightforward 'dilute-and-shoot' sample preparation, good linearity, sensitivity and response factor relative standard deviation (RSD) were achieved throughout.

Introduction

A number of factors are increasing the strain on modern food testers. The increased variety of pesticides employed in global crop production, along with the expanding international export market, has seen the quantity of samples and monitored analytes rise substantially. To continue to meet productivity targets, contract and QC labs around the world are in need of detection methods that reduce the analytical cycle time while delivering enhanced sensitivity, reliability and robustness.

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Pesticides	EVOQ LC-TQ
LC-MS/MS	Compound Based Scanning
Positive/negative switching	

Fig. 1: EVOQ Elite triple quadrupole mass spectrometer coupled to a Bruker Advance UHPLC.

Liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) in multiple reaction monitoring mode (MRM) is widely considered the gold standard for pesticide quantification. However, conventional LC-MS/MS systems are unable to scan for both positive and negative ions simultaneously. This means that for complete analytical confidence, conventional LC-MS/MS techniques must run duplicate analyses in both positive and negative modes to account for co-eluting compounds. This calls for twice as many samples and double the analysis time.

The Bruker EVOQ Elite triple quadrupole LC-MS system has several advanced developments that improve the efficiency and sensitivity of pesticide detection. Chief amongst these is smart Compound Based Scanning (CBS) software which enables the simultaneous detection of both cationic (positive) or anionic (negative) species. CBS computes and assigns a scan window and dwell times for each pesticide, automatically switching between positive and negative modes to accelerate analysis times with simplified method development.

In addition, a vacuum insulated probed heated electrospray ionization (VIP-HESI) source efficiently ionizes polar, semi-volatile and thermally labile pesticides without any loss in sensitivity. The EVOQ'sIQ Dual Ion Funnel and Active Exhaust ensures that high signal to noise ratios and superior robustness are maintained even for trace pesticide compounds. Straightforward 'dilute-and-shoot' sample preparation requires no sample enrichment, further reducing the time and effort expended on analysis.

To illustrate the speed and sensitivity of this method for food testing, the EVOQ Elite triple quadrupole mass spectrometer was used to quantify 250 pesticides in apple, cranberry, white grape, orange, and vegetable juice. Only one method with positive negative switching was used for over 500 MRM transitions.

Experimental

Sample Preparation

50 µL fruit juice was mixed with 450 µL of solvent (MeOH/water, 10/90, v/v) in the filter vial (Part number 85531-5, Thomson Instrument Company) using a press filter plunger (0.2 µm PVDF) to filter. A single injection of standard calibration solutions were injected before the juice samples and duplicate injections of standard calibration solutions were performed after the sample.

Instruments

The EVOQ Elite triple quadrupole mass spectrometer was coupled to a Bruker Advance Ultra High Performance Liquid Chromatography (UHPLC) system (Figure 1).

UHPLC Parameters:
Column: YMC-Pack ODS-AQ 3 µm, 150 mm x 3 mm (I.D.)
Column Temperature: 40 °C

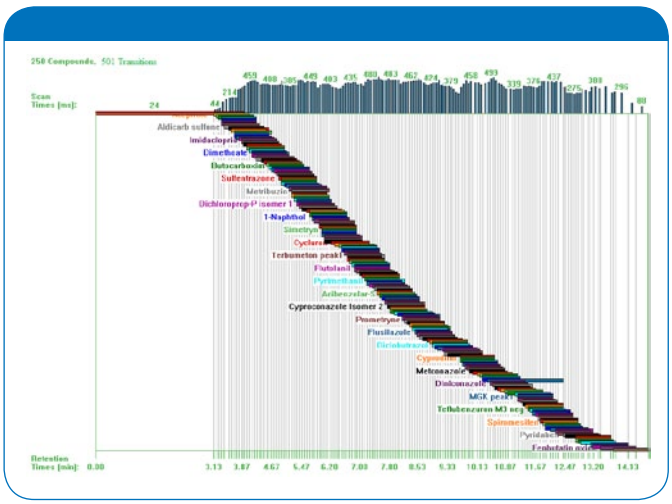

Fig. 2: Compound Based Scanning (CBS) timed MRM windows for 250 pesticides with rapid polarity switching to detect positive and negative compounds.

Injection Volume: 30 µL
Mobile Phase A: 5 mM ammonium fluoride in water
Mobile Phase B: Methanol
Gradient:

Time (s)	%A	%B	Flow(µL/min)
0.00	90	10	400
0.20	90	10	400
0.20	30	70	400
10.0	0	100	400
15.0	0	100	400
15.1	90	10	500
17.5	90	10	500
18.0	90	10	400

MS Parameters:
Source: HESI
Spray Voltage (Positive): 4000V
Spray Voltage (Negative) 4000V
Cone Gas Flow: 20-unit
Cone Temperature: 250 °C
Heated Probe Gas Flow: 40 –unit
Heated Probe Temperature: 400 °C
Nebulizer Gas Flow: 60 – unit
Exhaust Gas: On

	Name	Retention Time	RT Window	CAS Number	Retention Index	Scan Type	Scan Time (ms)	Polarity
139	Mefenacet	8.12	0.90		0	MRM	16	Positive
140	Paclitaxel	8.14	0.90		0	MRM	16	Positive
141	Tetraconazole	8.15	0.90		0	MRM	16	Positive
142	Iprovalicarb Isomer 1 and 2	8.18	0.90		0	MRM	16	Positive
143	Cyproconazole Isomer 2	8.19	0.90		0	MRM	16	Positive
144	Flufenacet	8.24	0.90		0	MRM	16	Positive
145	Fenhexamid	8.26	0.90		0	MRM	17	Positive
146	Spirotetramat	8.29	0.90		0	MRM	17	Positive
147	Bromucanozole Isomer 1	8.29	0.90		0	MRM	17	Positive
148	Fluquinconazole	8.31	0.90		0	MRM	17	Positive
149	Triticonazole	8.43	0.90		0	MRM	17	Positive
150	Fenarimol	8.51	0.90		0	MRM	17	Positive
151	Cyazofamid	8.63	0.90		0	MRM	19	Positive
152	Fipronil Neg	8.63	0.90		0	MRM	19	Negative
153	Mepanipyrim	8.64	0.90		0	MRM	19	Positive
154	Prometryne	8.65	0.90		0	MRM	19	Positive
155	Fenbuconazole	8.67	0.90		0	MRM	19	Positive
156	Epoxiconazole	8.77	0.90		0	MRM	19	Positive
157	Etaconazole Isomer 1 and 2	8.79	0.90		0	MRM	19	Positive
158	Clethodim Isomer 2	8.81	0.90		0	MRM	19	Positive
159	Flubendiamide	8.86	0.90		0	MRM	19	Positive
160	Spiroxamine Isomer 1 and 2	8.87	0.90		0	MRM	19	Positive
161	Terbutryn	8.91	0.90		0	MRM	19	Positive
162	Flusilazole	8.91	0.90		0	MRM	19	Positive
163	Diflubenzuron	8.96	0.90		0	MRM	19	Positive

	Precursor	Product	Collision Energy	Q1 Resolution	Q3 Resolution	Custom Res	Scan Time (%)	Qualifier Ion	Qualifier Ratio	Quantifier Ion
1	435.00	330.00	20.00	Unit (0.7)	Custom	1.00	50.00%	☑	123.10%	☐
2	435.00	250.00	20.00	Unit (0.7)	Custom	1.00	50.00%	☐		☑
3								☐		☐
4								☐		☐
5								☐		☐
6								☐		☐
7								☐		☐

Fig. 3: MRM method for screened pesticides. Positive Cyazofamid and negative Fipronil have the same retention time and assigned scan time.

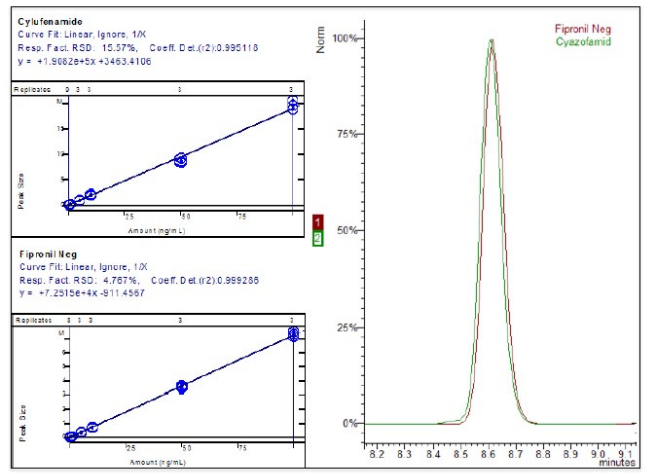

Fig. 4: Calibration curves for negative pesticide Fipronil (left top) and positive pesticide Cyazofamid (left bottom).

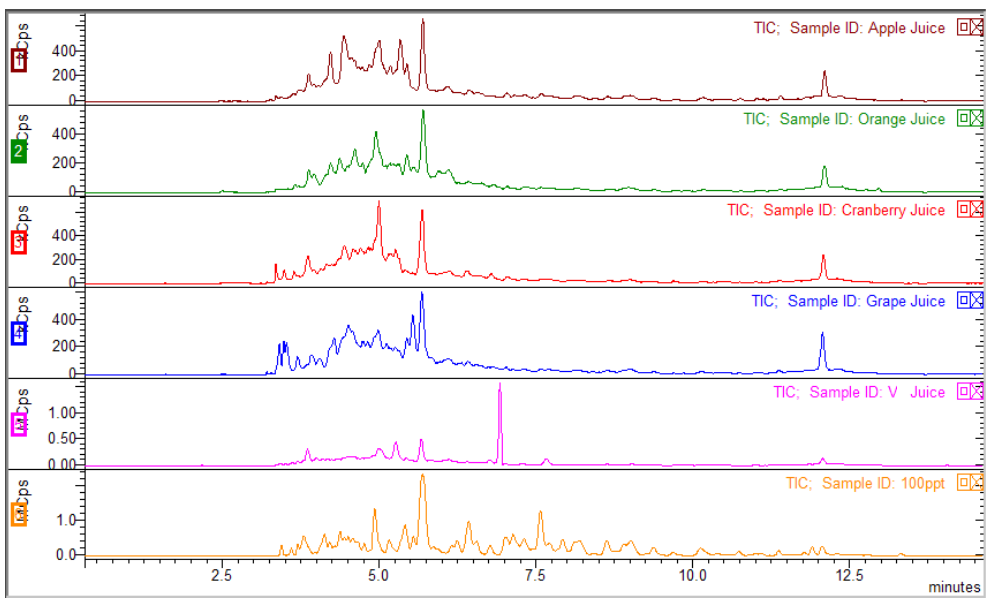

Fig. 5: Total Ion Chromatograms of (top to bottom): Apple juice, orange juice, cranberry juice, white grape juice, vegetable juice and a 100 ppt standard.

Table 1: A total of 12 pesticides were detected in orange, cranberry, apple or vegetable juice. No pesticides were detected in white grape juice.

Fruit Juice=>	Apple Juice	Orange Juice	Cranberry Juice	White Grape Juice	Vegetable Juice
Pesticide	µg/L (ppb)				
Azoxystrobin	ND	ND	0.32	ND	0.48
Boscalid	ND	ND	0.16	ND	ND
Carbaryl	ND	0.39	1.47	ND	ND
Carbofuran	ND	0.14	ND	ND	ND
Dimethoate	ND	0.30	ND	ND	ND
Imidacloprid	ND	ND	0.60	ND	0.20
Mandipropamid	ND	ND	0.59	ND	ND
Metalaxyl	ND	ND	0.21	ND	ND
Methoxyfenozide	ND	ND	ND	ND	0.84
Tebuconazole	ND	ND	0.32	ND	ND
Thiabendazole	1.8	ND	ND	ND	ND

Results and Discussion

The built in CBS software automatically assigned dwell times for timed MRM detection, based on peak width and data points required. This enabled the polarity switching of 250 positive and negative compounds for over 500 MRM transitions with minimal manual effort required for method development (Figure 2).

The detection of Cyazofamid and Fipronil provides an excellent example of the need for rapid polarity switching in a large and complex analysis method. Positive charged pesticide Cyazofamid and negatively charged Fipronil have the same retention time and are assigned the same scan time (Figure 3).

Figure 4 shows the calibration curve for negative pesticide Fipronil (left top) and positive pesticide Cyazofamid (left bottom) and their co-eluting chromatographic plots. The calibration of triplicate injections showed excellent linearity, between 0.01 ppb and 10 ppb, and R2 of 0.999 for both pesticides. The response factor % RSD over three orders of range was <5% indicating good reproducibility.

A total of twelve pesticides were detected in the apple, orange, cranberry or vegetable juice samples (Figure 5 and Table 1). There were no pesticides detected in the white grape juice. The multiple pesticides detected in orange, cranberry and vegetable juice suggested that the juice may come from multiple sources of raw materials or from pooled juices.

Conclusion

The Bruker EVOQ Elite triple quadrupole mass spectrometry system enabled the quantification of 250 positive and negatively charged pesticides with a single method in 18 minutes. Good linearity, sensitivity and RSD's were achieved for over 500 MRM transitions, proving the superior robustness and sensitivity of the EVOQ Elite for rapid multi-residue pesticide quantification.

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