



Application Note CA-1816264

Multi-residue Analysis of Pesticides in Zucchini Using EVOQ™ Triple Quadrupole LC-MS System

Abstract

An MRM method is developed to analyze 87 pesticides in zucchini matrix using the Bruker EVOQ triple quadrupole LC-MS system. The innovative designs of the ion source and ion optics improve the sensitivity, reproducibility and robustness significantly. The software feature of compound based scanning (CBS) with the built-in MRM library and the auto-calculation of scan time without the use of segmentation, is implemented to facilitate fast MRM method development. In the current work, analysis of 87 pesticides in zucchini matrix shows good sensitivity, linearity, and robustness.

Introduction

Pesticide residue is a global food safety concern. There are about 880 active substances formulated in the available pesticide products, which belong to more than 100 substance categories.¹ Monitoring and regulating pesticide levels in food becomes an essential task for governments and related agencies. Triple quadrupole mass spectrometry operated in multiple reaction monitoring (MRM) mode is one of the gold standard methods for target analysis, due to its high sensitivity, selectivity, and rapid scan speed.²

Coupled to liquid chromatography (LC), triple quadrupole tandem mass spectrometry (LC-MS/MS) has become the most widely used tool for trace level pesticide analysis. In the current study, a multi-residue analysis method has been developed for pesticide screening in zucchini matrix using Bruker EVOQ LC-MS/MS system. EVOQ mass spectrometer highlights the new design features of vacuum insulated probe for heated ESI (VIP HESI), active exhaust ion source, orifice vacuum interface and interlaced quadrupole dual ion funnel, which help increase the efficiency of ionization and ion transmission, improving sensitivity and robustness significantly. Bruker MS Workstation (MSWS) software system is implemented with a unique feature - compound based scanning (CBS). CBS links MRM transitions to compound names in the method and MRM methods can be easily set up by importing compounds from the built-in factory library. CBS also features timed MRM that the scan time of each compound is automatically calculated based on the defined peak width and expected scan points. With CBS, an MRM-based method for analyzing 87 pesticides in zucchini QuEChERS (Quick, Easy, Cheap, Effective, Rugged, Safe) extracts was easily developed. The results demonstrate great linearity and system robustness.

Experimental

Sample preparation

Pesticide-free zucchini was purchased from a local organic grocery store. A modified AOAC QuEChERS extraction protocol³ was used in sample preparation described as follow: homogenize 15 g pesticide-free zucchini; spike in 50 ppb ISTD d5-Atrazine and 87 pesticide mix standard. Three levels of pesticide spiked-in zucchini samples at 10, 30, 100 ppb were prepared respectively. To each sample, add 15 mL 1% acetic acid in ACN, 1.5 g NaOAc, 6 g anhydrous MgSO₄, shake and centrifuge, and transfer 7 mL supernatant to a clean-up vessel with 50 mg primary secondary amine (PSA) and 150 mg MgSO₄. Shake and centrifuge the vessel. Dry 5 mL cleaned-up sample and reconstitute it in 1 mL ACN. Final extracts were diluted with 3 times of water for EVOQ analysis

Instrument setup

Liquid chromatography

Instrument: Bruker Advance™ UHPLC system
Column: Restek Ultra Aqueous C18,
100 mm × 2.1 mm, 3 μm
Injection volume: 10 μL
Flow rate: 300 μL/min
Mobile phase A: H₂O + 10 mM NH₄Ac
Mobile phase B: MeOH + 10 mM NH₄Ac

Gradient:

Time (min)	Solvent B (%)
0	5
2	25
10	90
12	95
15	95
15.1	5
20	5

Mass spectrometry

Instrument EVOQ Qube™ triple quadrupole mass spectrometer
Ion Source ESI+, 4500 V
Cone gas 10 units
Probe Gas 50 units
Nebulizing Gas 50 units
Heated Probe Temperature 400 °C
Heated Cone Temperature 350 °C
Active Exhaust on
Collision gas Argon, 1.5 mTorr
MRM Transitions: See Table 1

Compound name	RT (min)	MRM 1 (CE)	MRM 2 (CE)
Methamidophos	2.64	142 > 94 (20)	142 > 125 (20)
Acephate	3.20	184 > 143 (15)	184 > 49 (35)
Omethoate	3.95	214 > 125 (20)	214 > 183 (13)
Aldicarb sulfone	4.44	223 > 86 (20)	223 > 148 (15)
Dinotefuran	4.55	203 > 129 (20)	203 > 157 (15)
Aldicarb sulfoxide	4.65	207 > 132 (10)	207 > 89 (20)
Oxamyl	4.79	237 > 72 (25)	237 > 90 (10)
Thiomethoxam	5.76	292 > 211 (20)	292 > 181 (35)
Carbofuran-3OH	5.93	238 > 163 (20)	238 > 181 (15)
Mevinphos	6.22	225 > 127 (15)	225 > 193 (10)
Dimethoate	6.23	230 > 125 (19)	230 > 171 (14)
Clothianidin	6.30	250 > 169 (15)	250 > 132 (20)
Imidacloprid	6.57	256 > 209 (25)	256 > 175 (30)
Aldicarb	7.14	208 > 116 (10)	208 > 89 (25)
Acetamiprid	7.26	223 > 126 (30)	223 > 99 (55)
Carbendazim	7.30	192 > 160 (25)	192 > 132 (40)
EPTC	7.38	190 > 128 (15)	190 > 86 (25)
Cyanazine	7.57	241 > 214 (14)	241 > 104 (28)
Propoxur	7.62	210 > 111 (15)	210 > 168 (15)
Bromacil	7.79	261 > 205 (10)	261 > 113 (6)
Carbofuran	7.79	222 > 123 (25)	222 > 165 (15)
Dichlorovos	7.83	238 > 221 (18)	238 > 109 (20)
Metribuzin	7.84	215 > 187 (17)	215 > 131 (17)
Quinlorac	8.11	242 > 224 (12)	242 > 196 (26)
Simazine	8.17	202 > 124 (13.5)	202 > 132 (15.5)
Carbaryl	8.34	202 > 145 (12)	202 > 127 (30)
Tebuthiuron	8.50	229 > 172 (18)	229 > 116 (28)
Thiabendazole	8.55	202 > 175 (35)	202 > 131 (45)
Propachlor	8.78	212 > 170 (15)	212 > 94 (25)

Compound name	RT (min)	MRM 1 (CE)	MRM 2 (CE)
Atrazine	8.90	216 > 174 (22)	216 > 104 (21)
Primicarb	9.01	239 > 72 (35)	239 > 182 (25)
Methidathion	9.04	303 > 85 (23)	303 > 145 (5)
Hexazinone	9.08	253 > 253 (4.5)	253 > 171 (17)
Simetryn	9.10	214 > 144 (30)	214 > 124 (30)
Diphenamid	9.16	240 > 134 (20)	240 > 167 (24)
Phosmet	9.42	318 > 133 (25)	318 > 160 (20)
Norfluzone	9.44	304 > 284 (25)	304 > 88 (39)
Iprodione	9.48	330 > 143 (25)	330 > 245 (15)
Azinphos-methyl	9.49	318 > 132 (11)	318 > 170 (13)
Propazine	9.50	230 > 146 (20)	230 > 188 (15)
Azoxystrobin	9.50	404 > 372 (15)	404 > 329 (32)
Malathion	9.51	331 > 99 (25)	331 > 127 (13)
Disulfoton sulfone	9.54	307 > 171 (20)	307 > 261 (15)
Methoxyfenozide	9.60	369 > 149 (25)	369 > 313 (15)
Prometon	9.61	226 > 142 (35)	226 > 86 (40)
Propyzamide	9.61	256 > 173	256 > 190 (15)
Methiocarb	9.63	226 > 169 (15)	226 > 121 (30)
Terbacil	9.66	217 > 144 (30)	217 > 161 (30)
Ametryn	9.70	228 > 186 (19)	228 > 96 (22)
Chlorobenzilate	9.71	342 > 307 (15)	342 > 139 (25)
Boscalid	9.72	343 > 307 (30)	343 > 140 (25)
Fludioxonil	9.73	266 > 229 (25)	266 > 227 (15)
Triadimenol	9.84	296 > 70 (30)	296 > 227 (20)
Molinate	9.98	188 > 126 (16)	188 > 83 (20)
Cyproconazole	9.99	292 > 70 (20)	292 > 125 (32)
Difenoconazole	10.00	406 > 251 (25)	406 > 188 (42)
Phorate sulfone	10.00	293 > 171 (20)	293 > 247 (15)
Myclobutanil	10.03	289 > 70 (19)	289 > 125 (31)

Compound name	RT (min)	MRM 1 (CE)	MRM 2 (CE)
Pyrimethanil	10.06	200 > 107 (26)	200 > 82 (30)
Tebuconazole	10.10	353 > 133 (25)	353 > 297 (15)
Fenarimol	10.13	331 > 268 (18)	331 > 189 (38)
Metolachlor	10.16	284 > 176 (23.5)	284 > 252 (13)
Prometryn	10.18	242 > 158 (24)	242 > 200 (20)
Azinphos-ethyl	10.24	346 > 132 (11)	346 > 160 (13)
Parathion	10.28	292 > 236 (10)	292 > 97 (20)
Terbutryn	10.37	242 > 186 (30)	242 > 68 (65)
Tetrachlorovinphos	10.38	367 > 127 (25)	367 > 241 (20)
Fenbuconazole	10.46	337 > 125 (35)	337 > 70 (27)
Diazinon	10.60	305 > 169 (25)	305 > 153 (23)
Phorate	10.62	261 > 75 (14)	261 > 143 (19)
Tebuconazole	10.66	308 > 70 (21)	308 > 125 (32)
Primiphos Methyl	10.72	306 > 164 (10)	306 > 108 (34)
Vernolate	10.84	204 > 128 (11)	204 > 44 (19)
Trifloxystrobin	10.85	409 > 186 (25)	409 > 206 (20)
Propiconazole	10.89	342 > 69 (21)	342 > 159 (29)
Pyraclostrobin	10.93	288 > 194 (20)	288 > 163 (30)
Cycloate	11.01	216 > 154 (20)	216 > 134 (20)
Cyprodinil	11.02	226 > 93 (28)	226 > 108 (22)
Butylate	11.10	218 > 156 (15)	218 > 57 (25)
Terbufos	11.17	289 > 103 (12)	289 > 58 (21)
Ethion	11.30	385 > 199 (15)	385 > 143 (29)
Fenvelerate	11.40	437 > 167 (17)	437 > 125 (35)
Trialete	11.56	306 > 145 (20)	306 > 86 (10)
Cypermethrin	11.85	433 > 191 (14)	433 > 193 (14)
Tribufos	12.18	315 > 169 (25)	315 > 202 (20)
Permethrin	12.42	408 > 183 (22)	408 > 355 (10)
Spinosad	15.48	733 > 142 (35)	733 > 98 (50)

Table 1. The optimized MRM transitions of 87 pesticides in the method

Results and discussions

Easy MRM method development using MSWS 8.1 with CBS

Figure 1 summarizes the step-wise MRM method development workflow using the EVOQ data system MSWS 8.1.

1) Search for the targeted compounds in the built-in factory library (Figure 1a) and export them into the acquisition method. MSWS 8.1 comes with a compound library containing thousands of compound MRM transitions and optimized collision energy (CE) values. Compounds with MRM transitions in the library can be directly added into the method builder.

2) Input the retention time of each compound in the method builder. Note that CBS links MRM transitions to each compound automatically in the method table (Figure 1b) to avoid setup of a different data processing method by tediously sorting retention time and compound names after data acquisition.

3) Calculate the scan time for each compound by a mouse click (Figure 1c) and start data acquisition. By specifying the average peak width with the desired scan points, scan time (dwell time) of each compound will be automatically computed and updated in the acquisition method, making a timed MRM method.

4) After data acquisition, update retention time, calibration level, and ion ratios in the acquisition method by a few mouse clicks and finalize the MRM method (Figure 1d-f). The Compound Table as a post-data handling tool contains all the MRM method parameters (Figure 1d). MSWS 8.1 can also synchronize between the acquisition and data handling methods by updating parameters such as retention time, calibration levels, and the ion ratios (Figure 1e-f).

With the ease CBS feature in MSWS 8.1, the effort required for a large MRM method development is dramatically minimized, therefore significantly speeds up setting up a multi-residue analysis method using EVOQ system.

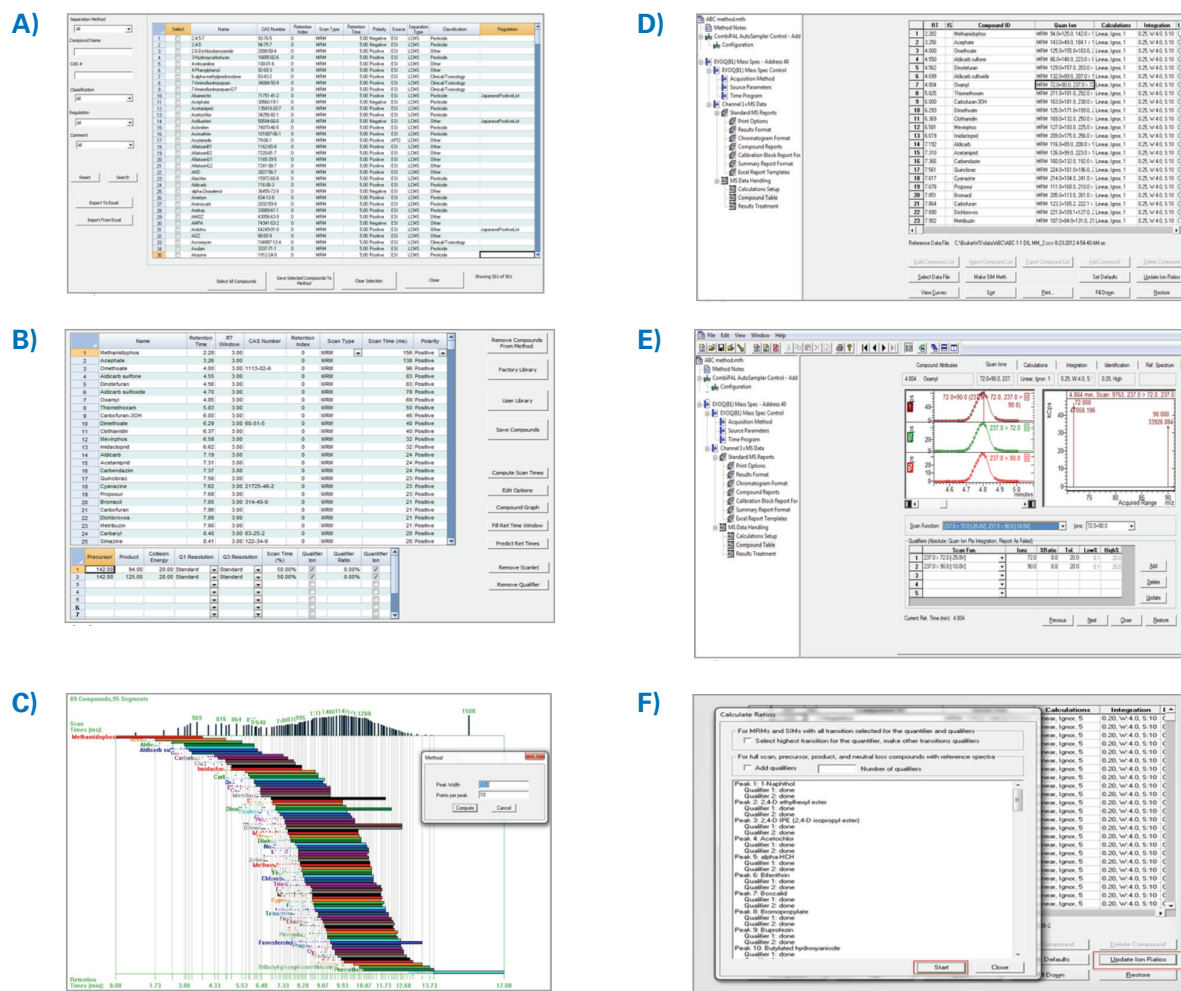


Figure 1. The MRM method development workflow using Bruker MSWS 8.1

Linearity and robustness performance

The 10 ppb pesticides spiked in zucchini are easily detected, representative ion chromatograms as shown in Figure 2. Three calibration levels were used for quantitation of 87 pesticides in zucchini. More than 75% pesticides have a calibration curve that could be fitted with $y=ax+b$ model with $R^2 > 0.995$, indicating good linear instrument response for pesticide analysis in QuEChERS matrix. For six consecutive injections of 30 ppb spiked-in zucchini samples, 80% pesticides have a relative standard deviation (%RSD) less than 8% which proves the great robustness of the method against QuEChERS extracts. Representative R^2 and %RSD values ($n=6$) for selected pesticides are listed in Table 2.

Conclusions

An MRM method was developed for analysis of 87 pesticides in zucchini using the Bruker EVOQ LC-MS/MS system. The new software features of Bruker MSWS 8.1, such as the built-in factory library, CBS, significantly simplify and speed up MRM method development when compared to currently available routine workflows. The results of pesticide analysis in zucchini QuEChERS extracts show good sensitivity, linearity, and robustness of the instrument, demonstrating EVOQ LC-MS/MS a well suitable system for analyzing pesticides in complex matrices.

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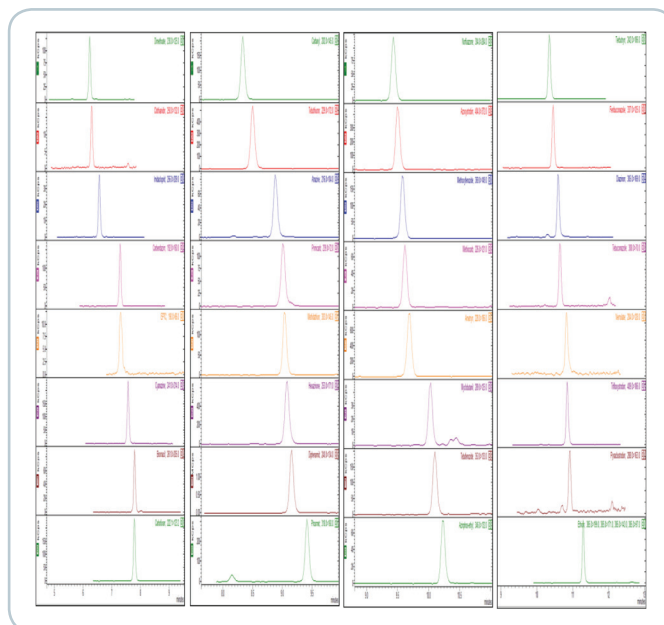


Figure 2. Representative ion chromatograms of 10 ppb pesticides in zucchini matrix

Compound name	R ²	%RSD (n=6)
Atrazine	0.998	3.07
Azinphos-ethyl	0.991	2.86
Azinphos-methyl	0.992	3.25
Bromacil	.0999	2.53
Carbofuran	0.995	3.69
Clothianidin	0.996	1.50
Imidacloprid	0.998	3.19
Malathion	0.997	3.12
Methiocarb	0.996	3.17
Methoxyfenozide	0.994	2.85
Mevinphos	0.997	1.69
Myclobutanil	0.999	3.09
Phosmet	0.997	2.08
Propazine	0.999	2.45
Propiconazole	0.998	2.27
Tebuconazole	0.998	3.16
Trifloxystrobin	0.998	2.00

Table 2. A few examples of pesticides with R^2 values and %RSDs (30 ppb, $n=6$)

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