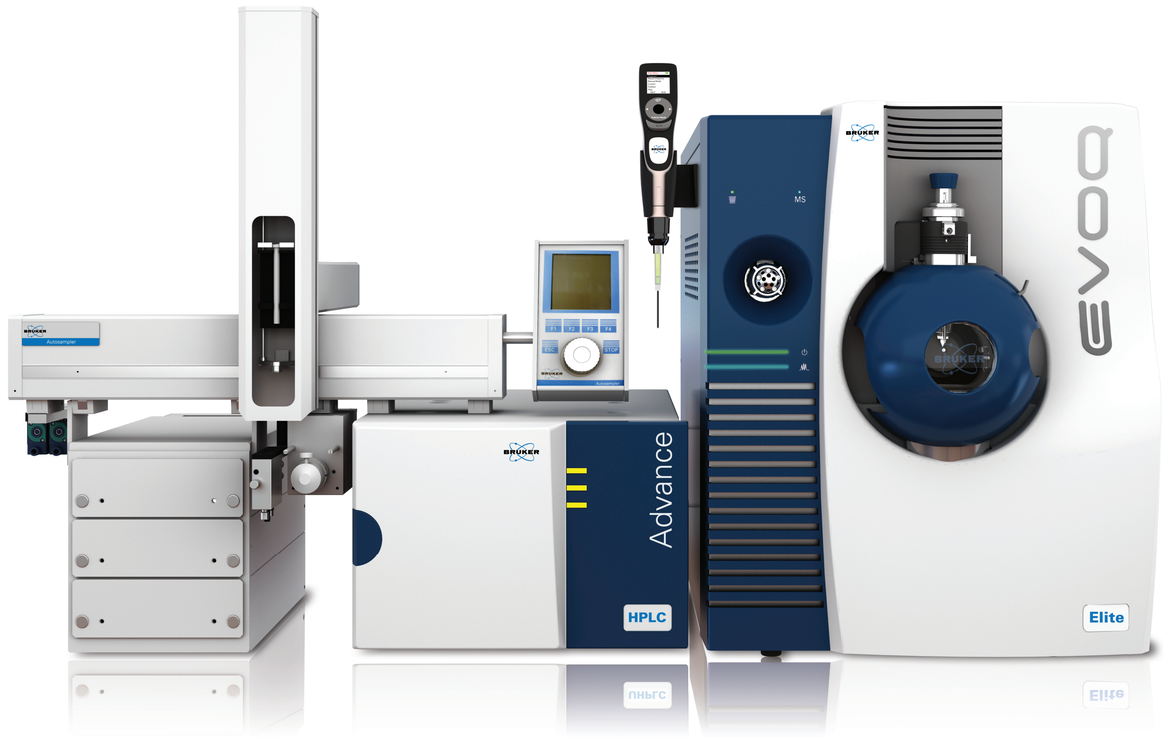

Application Note #1824184

Rapid and Simple Approaches to Multi-residue Pesticide Analysis in Fruits and Vegetables on both GC-MS/MS and LC-MS/MS

Abstract

This study demonstrates a multi-residue pesticide analysis method for complex food matrices prepared by a modified QuEChERS protocol using a Bruker SCION GC-MS/MS and EVOQ LC-MS/MS system. The sample preparation method is well suited for direct injection on both GC-MS/MS and LC-MS/MS systems. The Compound Based Scanning (CBS) feature enables fast multiple reaction monitoring (MRM) method development on both the EVOQ and SCION. Excellent linearity was achieved on both instruments and good sensitivity of low ppb (1 ppb) for GC-MS/MS analysis and sub-ppb (0.1 ppb) for LC-MS/MS analysis is demonstrated.

Introduction

Tandem mass spectrometry coupled to chromatography systems such as GC-MS/MS and LC-MS/MS, operating in MRM mode, has emerged as the industry standard method for monitoring residues in fruits and vegetables. However, a continuing challenge in multi-residue MS analysis is finding a sample preparation method that is as easy, fast and cost-efficient as possible.

A modified QuEChERS preparation protocol has been developed at the US FDA laboratory in Irvine.[1] QuEChERS, an acronym for quick, easy, cheap, effective, rugged and safe, is an encompassing method that can extract multiple classes of pesticides from a wide variety of samples. The methodology developed by the US FDA lab in Irvine presents an alternative to the conventional QuEChERS technique, allowing the extracted matrix to be diluted and injected directly into the GC/LC-MS to save time. In the following study, the modified QuEChERS sample preparation protocol is shown to be a simple, less expensive and unified alternative to the conventional QuEChERS techniques.

Samples prepared by this method can be analyzed by both GC-MS and LC-MS, and so in this study analysis by both the Bruker SCION GC-MS/MS and EVOQ LC-MS/MS is demonstrated. The novel features offered by the EVOQ and SCION instruments make them eminently suited to the detection of trace pesticide components within complex food samples. The Vacuum Insulated Probe Heated Electrospray (VIP-HESI), IQ Dual Ion Funnel and Active Exhaust on the EVOQ ensure high signal-to-noise ratios and superior robustness while SCION's Axial Ion Source and novel Lens-Free design delivers ultra-high sensitivity and reproducibility for routine analyses. Both systems come with CBS, an easy to use software feature which enables rapid method development for multi-residue assays.

Method

Three vegetable matrix samples of rice, avocado and spinach representing low moisture content, fatty content and high moisture content vegetable groups, respectively, were extracted using the following modified QuEChERS protocol developed at US FDA Lab at Irvine (Figure 1).

Thirty pesticides amenable for both GC-MS and LC-MS were spiked into the three extracted vegetable matrices. Calibration solutions were diluted using extracted blank matrices and prepared for analysis using the EVOQ LC-MS/MS and the SCION GC-MS/MS.

The MRM method development workflow was set up using CBS as shown in Figure 2. The target pesticides (Table 1) were selected from the software's MRM library before being exported to the CBS method editor. The dwell time for each MRM is automatically calculated based on its retention time window (timed MRM). A "built-in" processing method allows for easy updates of the retention times and method parameters and automatically updates qualitative and qualitative ion ratios based on the standards.

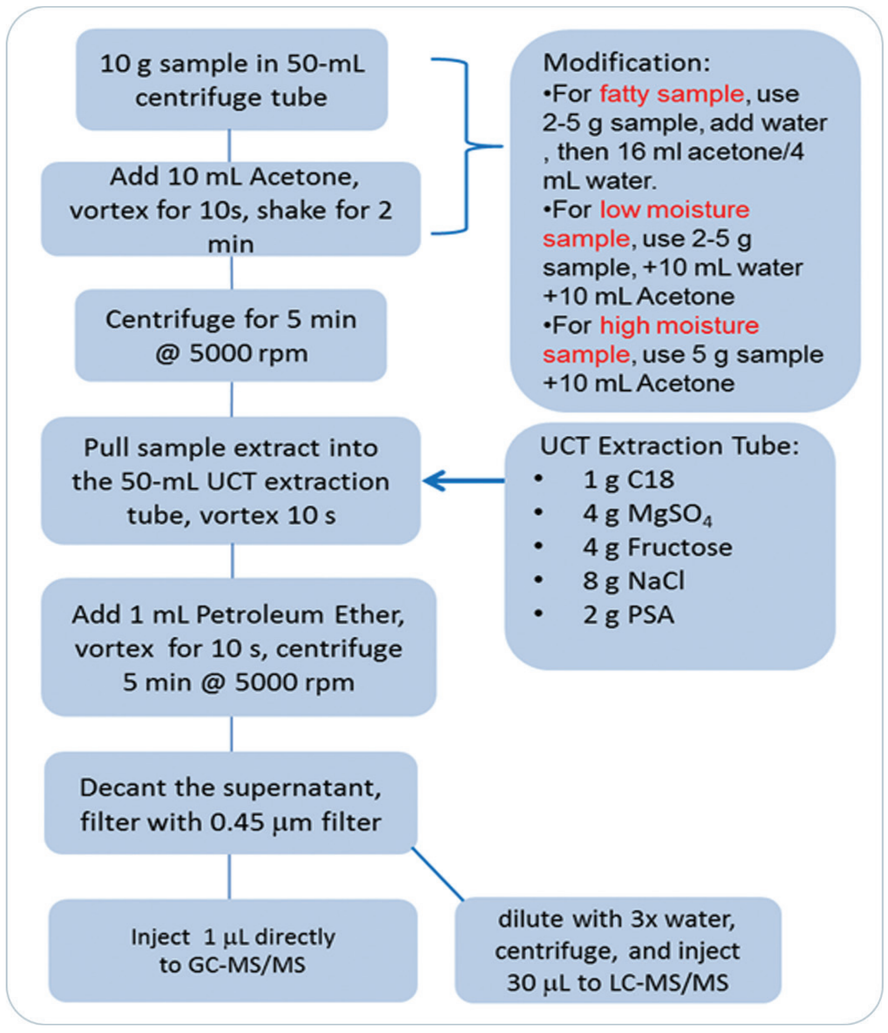

Figure 1: A modified QuEChERS sample preparation protocol developed at the US FDA lab at Irvine, USA.

Instrumentation set up

SCION GC-MS/MS

Calibration range	1, 2, 5, 10, 50, 100 ppb
MS condition	Instrument: Bruker SCION TQ Ionization: EI mode Electron energy: 70eV Emission current: 80 µA Source temperature: 260 °C Transfer line temperature: 300 °C Q2 gas: Ar, 1.5 mTorr
GC conditions	Instrument: Bruker 436 GC Injector: 280 °C Injection mode: Splitless, 1 µL injection Flow rate: He, 1 mL/min
GC Column	Bruker BR-5MS 30m x 0.25mm, 0.25 µm
Oven Program	80 °C, hold 2 min; 160 °C (20 °C/min); 280 °C (5 °C/min); 300 °C (20 °C/min), hold 5 min; Total run time: 36 min

EVOQ LC-MS/MS analysis

Calibration range	0.1, 0.5, 1, 5, 10, 20, 50, 100 ppb
MS condition	Instrument: Bruker EVOQ Elite TQ Ionization: ESI positive ESI voltage: 4000 V Cone Gas Flow: 20 units Cone Gas Temperature: 300 °C Heated Probe Gas Flow: 40 units Heated Probe Temperature: 450 °C Nebulizer Gas Flow: 50 units Exhaust Gas: on Q2 Pressure: Ar, 1.5 mTorr
LC condition	Instrument: Bruker Advance UHPLC Mobile phase: A: 0.1% formic acid in water B: Acetonitrile Column: YMC-Pack Pro C18 RS, 3 µm, 50mm × 2.0 mm I.D. Column Temperature: 40 °C Flow rate: 0.4 mL/min Injection volume: 30 µL
LC gradient	Time (min) %A %B 0 70 30 0.2 70 30 4.0 5 95 5.0 5 95 5.1 70 30 8.0 70 30
Injection	30 µL

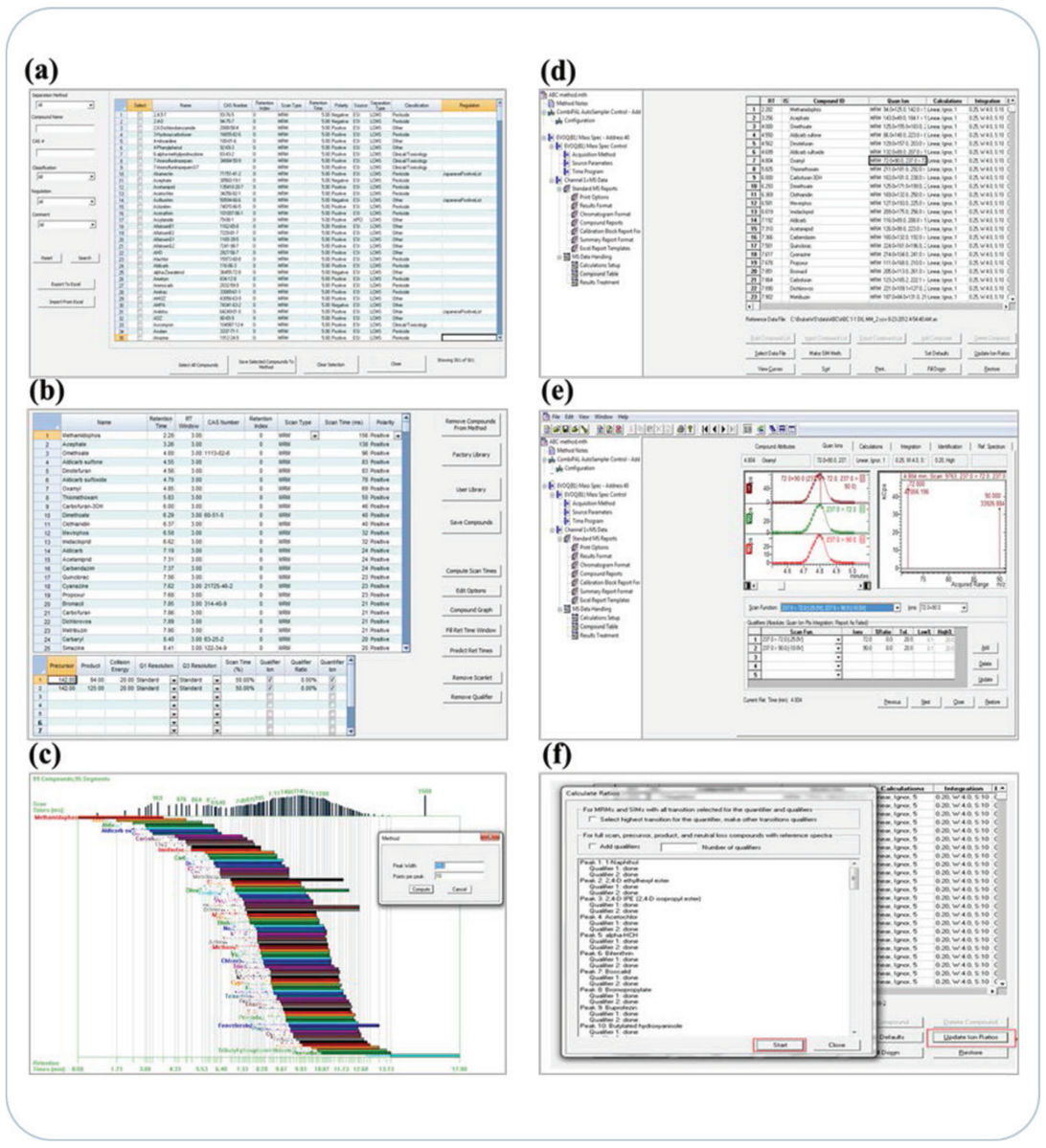

Figure 2: MRM method development workflow using Bruker Compound Based Scanning (CBS) software: a) select target pesticides from MRM library; b) export to CBS compound method editor; c) auto-calculate scan time for timed-MRM; d) "built-in" processing method; e) easy update of RT and method parameters; f) auto update Quan/Qual ion ratios from the result of a standard.

Compound name	GC-MS /MS		LC-MS /MS	
	MRM 1 (eV)	MRM 2 (eV)	MRM 1 (eV)	MRM 2 (eV)
Ametryn	212 > 94 (20)	212 > 122 (10)	228 > 186 (17)	228 > 68 (30)
Azaconazole	217 > 173 (15)	217 > 145 (25)	300 > 159 (15)	300 > 231 (15)
Azoxystrobin	344 > 156 (35)	344 > 329 (10)	404 > 372 (14)	404 > 344 (23)
Benalaxyl	206 > 162 (10)	206 > 132 (18)	326 > 148 (24)	326 > 208 (15)
Bromacil	205 > 162 (15)	205 > 188 (15)	261 > 205 (12)	261 > 188 (25)
Butralin	244 > 132 (20)	266 > 190 (10)	296 > 222 (15)	296 > 240 (12)
Carboxine	235 > 87 (20)	235 > 143 (20)	236 > 143 (16)	236 > 87 (27)
Clomazone	204 > 78 (30)	204 > 107 (20)	240 > 125 (23)	240 > 89 (30)
Coumaphos	362 > 109 (15)	362 > 226 (15)	363 > 227 (20)	363 > 211 (28)
Diethofencarb	267 > 197 (15)	267 > 225 (10)	268 > 180 (15)	268 > 226 (5)
Diniconazole	268 > 171 (20)	268 > 232 (10)	326 > 148 (23)	326 > 208 (14)
Fenamidone	238 > 103 (20)	268 > 180 (20)	312 > 236 (15)	312 > 92 (23)
Fenamiphos	303 > 154 (15)	303 > 228 (10)	304 > 217 (21)	304 > 202 (34)
Fenbuconazol	198 > 102 (25)	198 > 129 (15)	337 > 125 (29)	337 > 70 (25)
Fenothiocarb	160 > 72 (10)	160 > 106 (10)	254 > 160 (8)	254 > 72 (10)
Fenpropimorph	128 > 70 (10)	303 > 128 (10)	304 > 147 (29)	304 > 119 (30)
Flusilazole	233 > 152 (15)	315 > 233 (10)	316 > 247 (16)	316 > 165 (28)
Hexaconazole	214 > 152 (20)	214 > 159 (20)	314 > 70 (10)	314 > 159 (25)
Hexazinone	171 > 71 (15)	171 > 85 (15)	253 > 171 (16)	253 > 71 (25)
Imazalil	215 > 41 (20)	215 > 173 (10)	297 > 159 (23)	297 > 255 (12)
Isoprocarb	136 > 103 (25)	136 > 121 (10)	194 > 95 (10)	194 > 77 (25)
Myclobutanil	179 > 125 (15)	179 > 152 (10)	289 > 70 (10)	289 > 125 (25)
Napropamide	128 > 72 (5)	271 > 128 (10)	272 > 171 (19)	272 > 129 (18)
Pendimethalin	252 > 160 (10)	252 > 191 (10)	282 > 212 (5)	282 > 194 (15)
Pyriproxifen	136 > 41 (10)	136 > 96 (12)	322 > 185 (25)	322 > 134 (25)
Tebuconazole	250 > 125 (10)	250 > 163 (10)	308 > 125 (33)	308 > 70 (38)
Thiabendazole	201 > 130 (25)	201 > 174 (15)	202 > 175 (23)	202 > 131 (32)
Thiamethoxam	247 > 139 (15)	247 > 182 (10)	292 > 181 (15)	292 > 211 (15)
Tricyclazole	189 > 135 (20)	189 > 162 (10)	190 > 163 (20)	190 > 136 (27)
Triflumizole	206 > 179 (15)	278 > 73 (10)	346 > 43 (15)	346 > 73 (10)

Table 1: MRM transitions of 30 pesticides by GC-MS/MS and LC-MS/MS systems.

Results and Discussion

Excellent sensitivity was achieved for multi-residue pesticide in various vegetable matrices using both the Bruker SCION GC-MS/MS and EVOQ LC-MS/MS systems. Examples of 1 and 5 ppb spiked samples in a spinach QuEChERS matrix analyzed by LC-MS/MS and GC-MS/MS is shown in Figure 3. Table 2 illustrates the calibration results for the pesticides in different matrices for the two systems. R^2 values show that excellent linearity was achieved within each matrix.

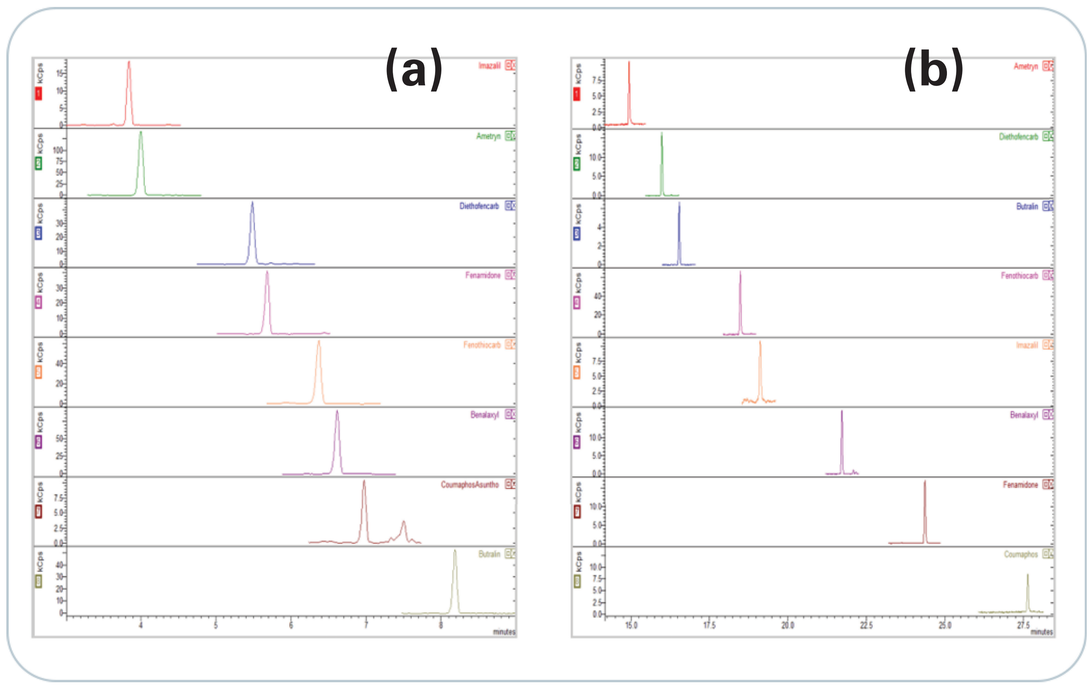

Figure 3: (a) MRM chromatograms for selected pesticides at 1 ppb in a spinach extract by LC-MS/MS. (b) MRM chromatograms for selected pesticides at 5 ppb in a spinach extract by GC-MS/MS.

Compound name	Control		Rice		Avocado		Spinach	
	GC-MS/MS 1 - 100 ppb	LC-MS/MS 0.1 - 100 ppb	GC-MS/MS 1 - 100 ppb	LC-MS/MS 0.1 - 100 ppb	GC-MS/MS 1 - 100 ppb	LC-MS/MS 0.1 - 100 ppb	GC-MS/MS 1 - 100 ppb	LC-MS/MS 0.1 - 100 ppb
Ametryn	0.998	0.999	1.000	0.994	0.999	0.993	1.000	0.998
Azaconazole	0.997	0.999	0.999	0.995	0.999	0.994	1.000	0.999
Azoxystrobin	0.995	0.987	0.999	0.999	0.999	0.997	0.999	0.997
Benalaxyl	0.998	0.997	1.000	0.999	0.999	0.988	1.000	0.991
Bromacil	0.997	0.997	0.999	0.997	0.999	0.992	1.000	0.999
Butralin	0.995	0.999	0.999	0.998	0.997	0.998	1.000	0.993
Carboxine	0.997	0.992	1.000	0.998	0.996	0.999	0.998	0.995
Clomazone	0.998	0.991	1.000	1.000	0.999	0.998	1.000	0.990
Coumaphos	0.994	0.997	1.000	0.987	0.998	0.998	1.000	0.993
Diethofencarb	0.997	0.997	0.999	0.999	0.999	0.995	1.000	0.989
Diniconazole	0.996	0.998	0.999	0.999	0.999	0.986	1.000	0.998
Fenamidone	0.996	0.996	1.000	1.000	0.999	0.999	1.000	0.997
Fenamiphos	0.995	0.993	1.000	1.000	0.999	0.998	1.000	0.993
Fenbuconazol	0.993	0.997	0.999	0.999	0.999	0.995	0.999	1.000
Fenothiocarb	0.997	0.998	0.999	0.995	0.999	0.992	1.000	0.997
Fenpropimorph	0.997	0.995	0.999	0.997	0.999	1.000	1.000	0.992
Flusilazole	0.997	0.998	0.999	1.000	0.999	1.000	1.000	0.998
Hexaconazole	0.995	0.996	0.999	0.999	0.999	0.996	1.000	0.999
Hexazinone	0.997	1.000	1.000	0.989	0.999	0.992	1.000	0.996
Imazalil	0.993	0.989	0.999	0.992	0.999	0.997	0.987	0.992
Isoprocarb	0.998	0.994	1.000	0.998	0.999	0.997	1.000	0.991
Myclobutanil	0.997	0.996	0.999	0.998	0.999	0.998	1.000	0.990
Napropamide	0.999	0.995	1.000	0.995	0.999	0.994	0.999	0.998
Pendimethalin	0.993	0.998	0.998	0.999	0.997	0.998	0.999	0.994
Pyriproxifen	0.997	0.999	0.999	1.000	0.999	1.000	1.000	0.997
Tebuconazole	0.995	0.994	1.000	0.999	0.999	0.993	1.000	0.997
Thiabendazole	0.983	1.000	0.999	0.997	0.995	0.996	0.999	1.000
Thiamethoxam	0.998	0.997	0.999	1.000	0.989	1.000	0.998	0.997
Tricyclazole	0.994	0.991	0.999	0.995	N/A	0.999	0.992	0.996
Triflumizole	0.996	0.996	0.999	0.998	0.999	0.993	1.000	0.997

Table 2: R^2 values for the calibration of pesticides in different matrices using GC-MS/MS and LC-MS/MS.

Conclusion

The multi-residue pesticide methods developed on the Bruker SCION GC-MS/MS and EVOQ LC-MS/MS systems have shown superior sensitivity and reproducibility for the analysis of complex food matrices prepared by an FDA modified QuEChERS protocol. The Compound Based Scanning feature and Bruker's pesticide libraries enabled rapid method development and ensured optimal dwell times for each MRM transition. Unique hardware innovations such as the EVOQ's Active Exhaust and IQ Dual Ion Funnel and the SCION's Axial Ion Source and Lens-Free design ensure sustained high performance is maintained during routine, high volume, high throughput quantitative analyses.

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Reference:

1. FDA/ORA/DFS, LIB# 4495, Irvine Rapid Analytical Method: A rapid SPE Multiresidue Method for the Analysis of Polar and Non-polar Pesticides in High Moisture Food products by Olusegun Ajayi, et. al

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