Multiclass Determination of Organic Contaminants in Red Chili and Turmeric Powders

Using the Agilent EMR—Lipid Sample Cleanup and Agilent 6495 Triple Quadrupole LC/MS System in a Single Method of Analysis

Application Note

Food safety

Authors

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Abstract

This application note describes a UHPLC/MS/MS based multiresidue analysis method for the determination of multiple organic contaminants in a single method of analysis for spice samples. These contaminants include more than 235 pesticides, 20 pyrrolizidine alkaloids, nine mycotoxins, and six illegal dyes. The method benefits from the increased chromatographic resolution of the Agilent 1290 Infinity II UHPLC system, as well as the versatile ionization capabilities of the Agilent Jet Stream ionization source and the innate sensitivity of the Agilent 6495 triple quadrupole LC/MS system. The method has been applied for the analysis of pesticide, mycotoxins, pyrrolizidine alkaloids (PAs), and illegal dye residues in complex matrixes such as red chili powder and turmeric powder. Matrix effects associated with electrospray ionization were controlled by effective sample cleanup before injection.

The results demonstrate that the increased sensitivity of the 6495 triple quadrupole LC/MS system enables the accurate quantitation of targeted pesticides in the sample extracts with high precision and excellent robustness. Most of these pesticides were detected below the maximum residue limits (MRLs) specified by the European Commission.

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Introduction

Analysis of agrochemical residues in red chili powder and turmeric samples is always considered difficult because of its complex nature. During sample preparation, matrix components may also get co-extracted along with the target compounds, leading to poor sensitivity and robustness, and loss in precision. In addition, reduced column performance and more periodic source cleaning would also be required, leading to loss in productivity. In this study, attempts were made to improve sample preparation method to improve the recovery and increase column life with minimal source maintenance. Choosing matrix-free transitions from Agilent MRM Databases offered accurate determination of the residues. The Quick, Easy, Cheap, Effective, Rugged and Safe (QuEChERS) Enhanced Matrix Removal—Lipid (EMR—Lipid) technique was used for extraction of analytes of interest. The red chili and turmeric extracts were analyzed using an Agilent 1290 Infinity II LC with an Agilent 6495 triple quadrupole LC/MS system in dynamic multiple reaction monitoring (dMRM) modes. Red chili and turmeric are popular spices in India and across the world. Every year, millions of tons of red chili and turmeric are exported from India. Red chili is principally used in food preparation in Indian and Asian food cuisines, whereas turmeric is used in medicinal formulations in addition to its use in food preparations. India is one of the world's largest producer, consumer, and exporter of these two commodities. The intensive use of different agrochemicals during different stages of production, from cultivation to harvest, storage, and transportation stages has resulted in concerns over consumer exposure to pesticides and various other toxins, creating potential health risks. Therefore, it is necessary to provide effective residue analysis methods. Table 1 shows the major organic and inorganic constituents of red chili and turmeric samples.

Matrix effects in electrospray ionization (ESI), which change considerably between different food samples, present a significant challenge to the accurate quantitation of pesticides and other organic contaminants in food. There are different strategies to compensate for matrix effects such as matrix-matched calibrations, standard addition, or the use of internal standards and dilution experiments. Dilution experiments are performed by diluting the final extracts with neat solvents or diluents. Dilutions are performed at 2, 5, 10, 25, 50, or 100 fold depending upon the sensitivity of the equipment.

Table 1. Organic and Inorganic Constituents of Red Chili and Turmeric

Constituents	Quantity per 100 g wet basis
Red chili	
Energy	40 Kcal
Carbohydrates (g)	8.8
Sugars (g)	5.3
Dietary fiber (g)	1.5
Fat (g)	0.4
Protein (g)	1.9
Water (g)	75–85
Vitamin A (µg)	48
Beta-carotene (µg)	534
Vitamin B6 (mg)	0.51
Vitamin C (mg)	144
Iron (mg)	1
Magnesium (mg)	23
Potassium (mg)	322
Capsaicin (g)	0.01–6.0
Turmeric	
Ascorbic acid (mg)	50
Ash (g)	6.8
Calcium (g)	0.2
Carbohydrate (g)	69.9
Fat (g)	8.9
Food energy (Kcal)	390.0
Iron (mg)	47.5
Niacin (mg)	4.8
Potassium (mg)	200.0
Phosphorus (mg)	260.0
Protein (g)	8.5
Riboflavin (mg)	0.19
Sodium (mg)	30.0
Thiamine (mg)	0.09
Water (g)	6.0

In this study, matrix-matched calibration standards were used for accurate quantitation of analytes as the use of internal standards is expensive. Dilution experiments were also performed to check and evaluate the matrix effect.

This application note describes the performance of a single method of analysis for various types of chemical contaminants in red chili and turmeric powders (dry). It also describes the advantages of using EMR—Lipid QuEChERS sample cleanup and ease of instrument method set up. This study demonstrates a reliable UHPLC/MS/MS method for the screening and quantitation of hundreds of pesticides in food samples. The method was developed using the LC/MS Pesticide tMRM database (p/n G1733CA).

Transitions for all compounds in the comprehensive pesticide standard mix (p/n 5190–0551) and few other contaminants such as aflatoxins, pyrrolizidine alkaloids (PAs), illegal dyes and few mycotoxins of interest were included in the method. A 1290 Infinity II UHPLC system was coupled to the highly sensitive 6495 triple quadrupole LC/MS system, operated in dMRM mode with fast polarity switching.

Experimental

Table 2. List of Pesticides, Mycotoxins, PAs, and Dyes (continued next page)

Pesticides

2,4-D	Chlorpyriphos	Ethirimol	Furathiocarb	Methamidophos	Promecarb
Acephate	Chlorsulfuron	Ethoprophos	Halofenozide	Methidathion	Prometon
Acetamiprid	Clethodim	Ethoxyquin	Halosulfuron-methyl	Methomyl	Propamocarb
Aldicarb	Clofentezin	Etofenprox	Hexaconazole	Methoprotryne	Propaquizafop
Aldicarb fragment	Clomazone	Famoxadon	Hexythiazox	Metobromuron	Propetamophos
Amidosulfuron	Coumaphos	Fenamidon	Hydramethylnon	Metolachlor	Propiconazole
Aminocarb	Cyazofamid	Fenamiphos	Imazalil	Metrafenone	Propoxur
Avermectin B1a	Cycloate	Fenazaquin	Imidacloprid	Metribuzin	Propyzamid
Azaconazole	Cycluron	Fenbuconazole	Indoxacarb	Metsulfuron-methyl	Proquinazid
Azamethiphos	Cymiazol	Fenhexamid	Ioxynil	Mevinphos	Prosulfocarb
Azinphos-methyl	Cymoxanil (Curzate)	Fenobucarb	Ipconazole	Mexacarbate	Pymetrozin
Azoxystrobin	Cyproconazole	Fenoxycarb	Iprovalicarb	Molinate	Pyracarbolid
Beflubutamid	Cyprodinil	Fenpropidin	Isocarbophos	Monocrotophos (Azodrin)	Pyraclostrobin
Benalaxyl	Diethyltoluamide (DEET)	Fenpyroximat	Isoprothiolane	Myclobutanil	Pyridaben
Bentazone	Desmedipham	Fenuron	Isoxaben	Nicosulfuron	Pyrimethanil
Benzoximate	Dichlorvos	Fipronil	Isoxaflutole	Nitenpyram	Pyriproxyfen
Bifenazate	Diethofencarb	Flazasulfuron	Ivermectin B1a	Novaluron	Quinmerac
Bifenthrin	Difenoconazole	Flonicamid	Kresoxim methyl	Omethoat	Quinoxyfen
Bispyribac	Diflubenzuron	Flubendiamide	Lenacil	Oxadiazon	Rimsulfuron
Bitertanol	Diflufenican	Fludioxonil	Linuron	Oxamyl	Rotenone
Boscalid (Nicobifen)	Dimethachlor	Flufenacet	Lufenuron	Oxasulfuron	Secbumeton
Bromoxynil	Dimethoate	Flufenoxuron	Malaoxon	Penconazole	Spinosyn A
Bromuconazole	Dimethomorph	Flumetsulam	Malathion	Pencycuron	Spinosyn D
Bupirimate	Dimethomorph_1	Fluometuron	Mandipropamid	Pendimethalin	Spirodiclofen
Buprofezin	Dimoxystrobin	Fluopicolide	MCPB (4-(MCB))	Phenmedipham	Spiromesifen
Butocarboxim	Diniconazole	Fluopyram	MCPP	Phosalone	Spirotetramat
Carbaryl	Dinotefuran	Fluoxastrobin	Mecarbam	Phosmet	Spiroxamine
Carbendazim	Dinoterb	Fluquinconazole	Mepanipyrim	Phosphamidon	Sulfentrazone
Carbofuran	Dioxacarb	Flusilazole	Mesosulfuron-methyl	Phoxim	Tebufenozid
Carboxin	Dithianon	Flutriafol	Metaflumizone	Picolinafen	Tebufenpyrad
Carfentrazone-ethyl	Diuron	Foramsulfuron	Metalaxyl	Picoxystrobin	Tebuthiuron
Chlorantraniliprole	DNOC	Forchlorfenuron	Metamitron	Pirimicarb	Teflubenzuron
Chlorfenvinphos	Epoxyconazol	Fosthiazate	Metazachlor	Pirimiphos-methyl	Temephos
Chlorotoluron	Ethidimuron	Fuberidazole	Metconazole	Prochloraz	Tetraconazole
Chloroxuron	Ethion	Furalaxyl	Methabenzthiazuron	Profenofos	Thiabendazol

Pesticides (continued)	Mycotoxins	Pyrrolizidine alkaloids (PAs)	Dyes
Thiacloprid	Alfatoxin B1	Echimidin	Sudan I
Thiamethoxam	Alfatoxin B2	Echimidin-N-oxide	Sudan II
Thidiazuron	Alfatoxin G1	Erucifolin	Sudan III
Thifensulfuron-methyl	Alfatoxin G2	Erucifolin-N-oxide	Sudan IV
Thiodicarb	Deoxynivalenol	Europin	Sudan Orange G
Triadimefon	Fumonisin B1	Europin-N-oxide	Quinoline yellow
Triadimenol	Fumonisin B2	Heliotrin	
Triasulfuron	HT-2 toxin	Heliotrin-N-oxide	
Triazophos	Ochratoxin	Intermedin	
Tribenuron-methyl	T-2 toxin	Jacobin	
Trichodesmin	Zeralenone	Jacobin-N-oxide	
Tricyclazol		Lasiocarprin	
Trietazin		Lasiocarprin-N-oxide	
Trifloxystrobin		Monocrotalin	
Triflumizol		Monocrotalin-N-oxide	
Triflumuron		Retrorsin	
Trimethacarb		Senecionin	
Triticonazole		Senecionin-N-oxide	
Uniconazole-P		Seneciphyllin-N-oxide	
Vamidothion		Senecivernin	
Zoxamide		Senecivernin-N-oxide	
		Senkirkin	

Reagents and chemicals

All reagents and solvents purchased were LC/MS grade. Formic acid, acetic acid, acetonitrile (ACN), and methanol were purchased from Fluka (Sigma-Aldrich Bangalore, India). Ultrapure water was produced using a Milli-Q integral system equipped with a 0.22-µm point-of-use membrane filter cartridge. Ammonium formate buffer (5 M) (p/n G1946 85021) and the pesticide standards comprehensive pesticide test mixture (p/n 5190-0551) were from Agilent.

Immediately before use, the eight sub mixes of the comprehensive pesticide mixture and the stock solutions of mycotoxins, illegal dyes, and mixture of pyrrolizidine alkaloids (PAs) were combined and further diluted with acetonitrile. This process produced a final working solution containing more than 235 pesticides, mycotoxins, and illegal dyes at a concentration of 500 ng/mL, and aflatoxins and PAs at a concentration of 100 ng/mL. This solution was used for spiking studies and to spike the QuEChERS extracts for the preparation of the calibration samples. Acetic acid 1 % in ACN was prepared by adding 10 mL of acetic acid to 990 mL of ACN.

Equipment and materials

- Agilent 1290 Infinity II binary pump LC (G7120A)
- Agilent 1290 Infinity II multisampler (G7167B)
- Agilent 1290 Infinity multicolumn thermostat (G7116B)
- Agilent 6495 triple quadrupole LC/MS with electrospray ionization
- Agilent Bond Elut QuEChERS extraction tubes (p/n 5982-5755)
- Agilent Bond Elut QuEChERS dispersive SPE Enhanced Matrix Removal-Lipid (p/n 5982-1010)
- Agilent Bond Elut QuEChERS Enhanced Matrix Removal—Lipid, final polish tube (p/n 5982-0101)
- Agilent ZORBAX RRHD Eclipse plus C18, 2.1 × 150 mm, 1.8 µm (p/n 959759-902)
- Eppendorf pipettes, 200 µL and 5 mL
- Beckman Coulter Allegra X-22-R centrifuge
- Digital vortex mixer (Vortex Genie-2)

Separation was carried out using a 1290 Infinity II binary UHPLC system, coupled to a 6495 triple quadrupole LC/MS System equipped with an Agilent Jet Stream Electrospray ionization (ESI) source. Agilent MassHunter Workstation Software (v. B.07.01) was used for data acquisition and v. B.07.00 for data analysis.

Analytical method

Table 3 summarizes the 1290 Infinity II UHPLC system conditions, and Table 4 shows the summary of the 6495 triple quadrupole parameters. Identification of polarity, precursor and product ions, as well as optimization of collision energies, was taken from the Agilent pesticide tMRM LC/MS application kit and was further optimized using Agilent MassHunter optimizer software. Analysis was carried out in both positive and negative ESI in dMRM mode in a single analytical run. A 1-µL sample of the final extract was injected into the UHPLC/MS/MS. Data were evaluated using Agilent MassHunter quantitative analysis software. Calibration was done using matrix-matched standard solutions, 1/x weighted, linear, and quadratic calibration curves.

Sample preparation

Red chili powder and turmeric powder samples were bought from a local market. A modified EMR—Lipid QuEChERS technique was used for sample preparation.

The procedure involved a QuEChERS AOAC extraction followed by EMR—Lipid dSPE and polish salts. EMR—Lipid provides far superior matrix removal. First, 2 g of dried spice powder sample (red chili/turmeric powder) was weighed into a 50-mL centrifuge tube. Then, 10 mL of distilled water was added to the centrifuge tube and allowed to hydrate for 30 minutes. Next, 10 mL of ACN, acidified with 1 % acetic acid, was added and the tube and agitated vigorously for 1 minute. The contents of Agilent Bond Elut QuEChERS extraction kit (p/n 5982-5755), magnesium sulfate (6 g), and sodium acetate (1.5 g), were added to the tube. After vigorous agitation for 1 minute, the tube was centrifuged at 6,000 rpm for 5 minutes. Then, 5 mL of the ACN layer was transferred to an Agilent 15-mL centrifuge tube (p/n 5982-1010) containing Agilent Bond Elut EMR—Lipid dSPE material, which was hydrated before use through the addition of 5 mL of water. Whole contents were mixed and agitated vigorously for 1 minute, then centrifuged at 13,000 rpm for 5 minutes. Next, 5 mL of the content was transferred to a 15-mL Agilent Bond Elut EMR—Lipid Polish tube (p/n 5982-0101) containing anhydrous magnesium sulfate and sodium chloride. The tube was shaken vigorously for 1 minute, then was centrifuged at 13,000 rpm for 5 minutes.

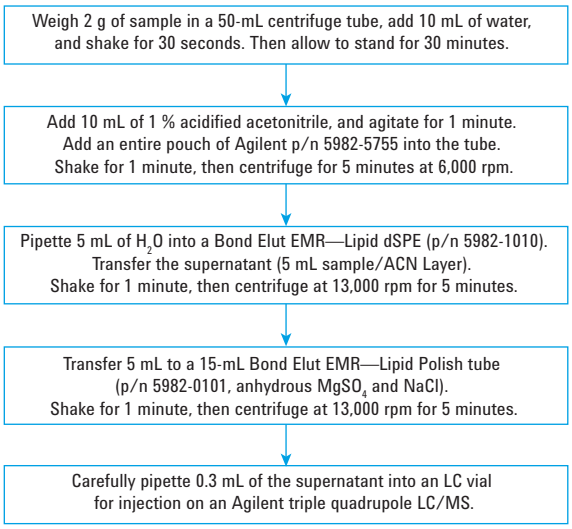


Figure 1. Flow chart of sample preparation.

Sample analysis

A binary gradient method with 5 mM ammonium formate and 0.1 % formic acid in water and in methanol were used as mobile phase. Chromatographic separations were achieved using a 1290 Infinity II (U)HPLC system. LC parameters such as flow rate, gradient composition, and gradient program were optimized and are presented in Table 3. MS source parameters were optimized to achieve higher responses for low sensitive analytes (Table 4). The multiple reaction monitoring (MRM) transitions were taken from Agilent MRM databases including Agilent pesticides and mycotoxins databases. Other MRM transitions were added manually using Agilent optimizer tool.

All the available transitions from the database were analyzed, and two transitions chosen by considering peak shape, abundance, ion ratio, and interferences from the matrix to set up of a method: one for quantifier and another for qualifier. Table 1 shows a list of pesticides and other analytes. The 6495 triple quadrupole LC/MS was operated as MS/MS, and was used for quantitation in MRM mode.

Instrument calibration

Red chili and turmeric matrix-matched calibration standards of pesticides, mycotoxins, and dyes were prepared at concentrations of 0.02, 0.05, 0.1, 0.2, 0.5, 1.0, 2.0, 5.0, 10.0, 20, 50, and 100 ng/mL. Aflatoxins and pyrrolizidine alkaloids were prepared at concentrations of 0.004, 0.01, 0.02, 0.04, 0.1, 0.2, 0.4, 1.0, 2.0, 4.0, 10.0, and 20 ng/mL.

Instrumental parameters

Table 3. LC Parameters

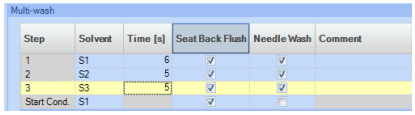
Parameter	Value
Column	Agilent ZORBAX RRHD Eclipse plus C-18, 2.1 × 150 mm, 1.8 µm (p/n 959759–902)
Column oven temperature	40 °C
Injection volume	1 µL
Autosampler temperature	6 °C
Needle wash	6 seconds with IPA, 5 seconds with MeOH, and 5 seconds with ACN
	
Mobile phase	A) 5 mM ammonium formate + 0.1 % formic acid in water B) 5 mM ammonium formate + 0.1 % formic acid in methanol
Flow rate	0.5 mL/min
Gradient program	Time (min) %B 0 5 1.5 5 4 50 17 98 23 98 24 5
Stop time	24 minutes
Post run time	2 minutes

Table 4. MS Parameters

Parameter	Value
Ionization mode	Electrospray ionization in simultaneous positive and negative ionization
Scan mode	Dynamic MRM
Drying gas temperature	200 °C
Drying gas flow	17 L/min
Sheath gas temperature	400 °C
Sheath gas flow	11 L/min
Nebulizer pressure	35 psi
Nozzle voltage	300/500 (positive/negative)
Capillary voltage	3,000 (positive/negative)
EMV gain	200
iFunnel parameters	
High-pressure RF	150
Low-pressure RF	60

Method performance

The sample preparation method described in this application note was validated by performing recovery experiments at three different concentrations of 5, 10, and 50 ng/g for pesticides, mycotoxins, and dyes, and 1, 2, and 10 ng/g for aflatoxins and PAs. The recovery was between 70–130 % for 73 % of the tested compounds in red chili powder, and 79 % of the tested compounds in turmeric powder.

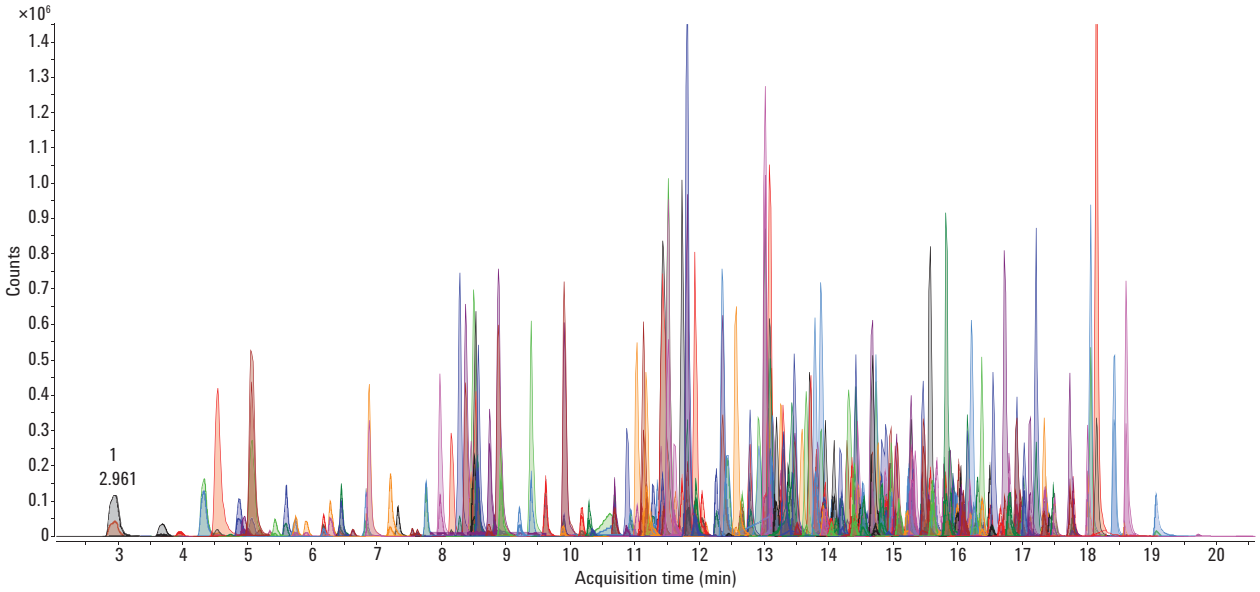


Figure 2. Overlay of MRM chromatograms of standards at 20 ng/mL.

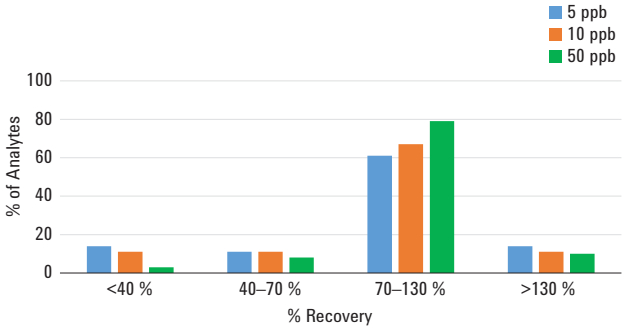


Figure 3. Recovery distribution across concentrations of 5, 10, and 50 ng/g for chili.

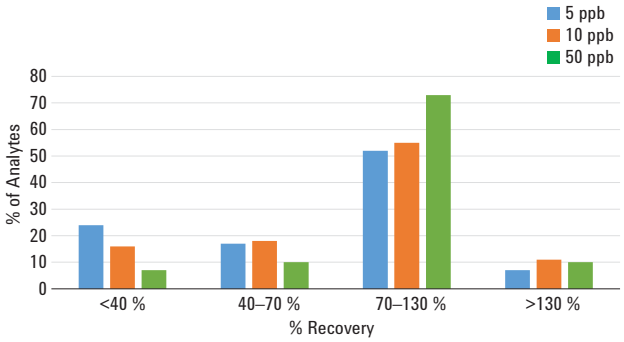


Figure 5. Recovery distribution across concentrations of 5, 10, and 50 ng/g for turmeric.

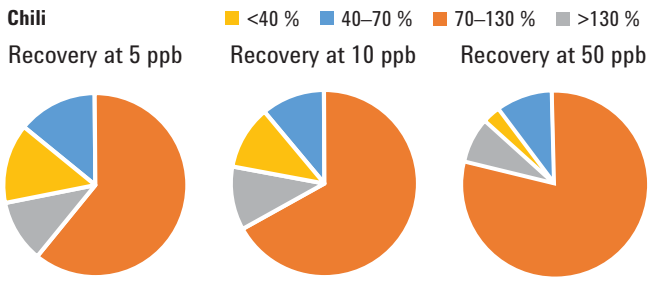


Figure 4. Recovery distribution across concentrations of 5, 10, and 50 ng/g for chili.

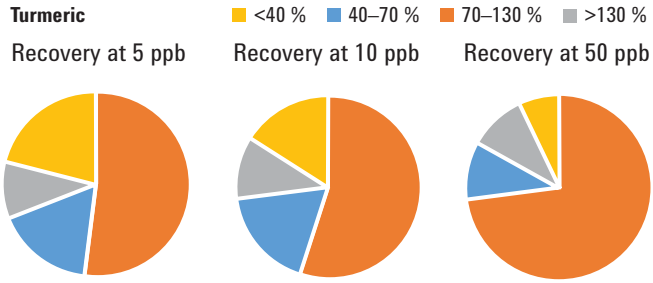


Figure 6. Recovery distribution across concentrations of 5, 10, and 50 ng/g for turmeric.

Conclusion

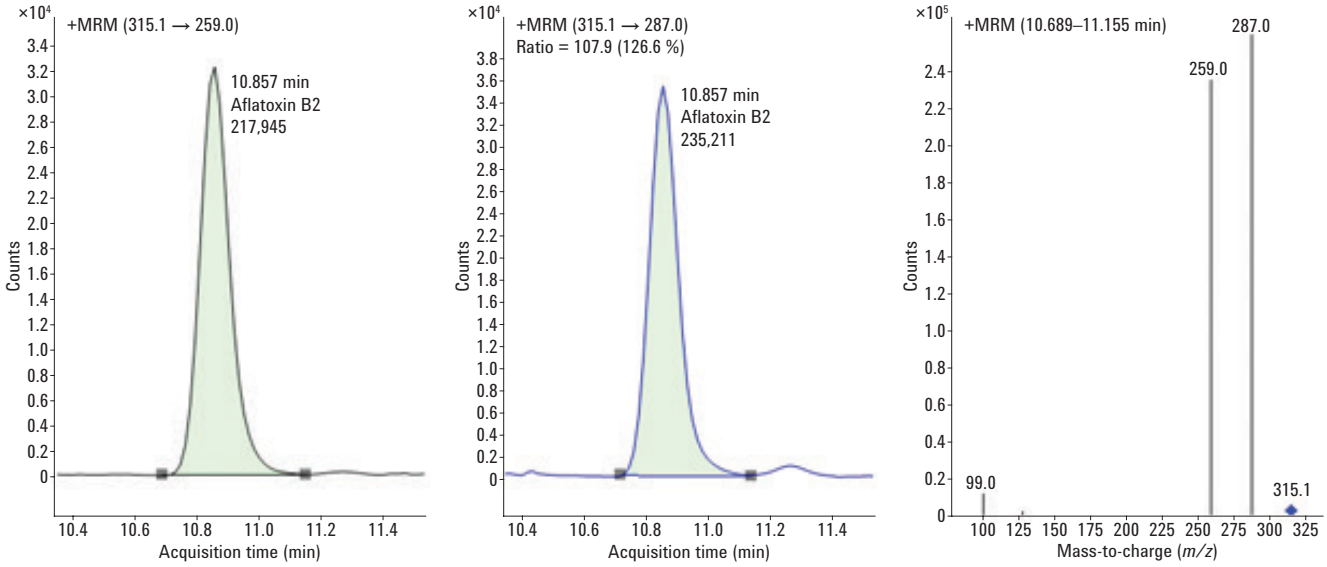
A single method of analysis for the estimation of multicomponent residues of pesticides, mycotoxins, illegal dyes, and PAs in spice samples was developed using Agilent 1290 Infinity II LC and Agilent 6495 triple quadrupole MS systems. The new multicomponent LC/MS/MS method was successfully applied to two different types of spice samples: red chili powder and turmeric powder. Analysis of dried red chili powder and dried turmeric powder is challenging in sample preparation and analysis due to their complex matrices.

Effort was taken to remove co-extracts and other interfering carotenoids, curcumins, and other pigments to improve column life and to achieve required recovery. A modified EMR—Lipid QuEChERS sample preparation technique was used, which involved extraction with acetonitrile followed by an EMR—dispersive cleanup step and EMR—Polish.

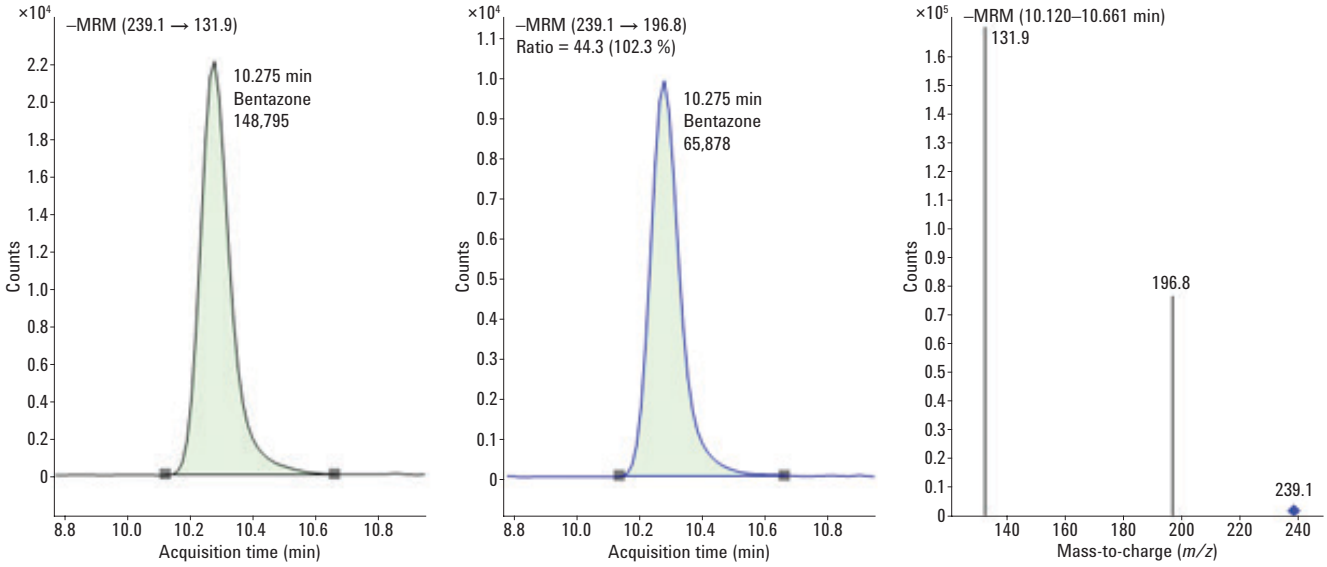
The recoveries were found to be within 70–130 %, with good repeatability for most of the compounds. In terms of sensitivity, the developed method showed LOQs mostly lower than the currently available MRLs for most of the compounds in the list. The consistency of results was found to be stable for both turmeric and red chili extracts with RSDs for triplicate injections found to be <20 % for the recovery experiments. Overall, the optimized sample preparation and LC/MS/MS method gave reliable results and performance characteristics, showing that this method can be used as a quantitative assay down to regulated levels for all the compounds in the target list. The method has the advantages of high sensitivity, selectivity, accuracy, and throughput. It has been successfully applied for red chili and turmeric powder samples, and can be extended to other spices and similar food matricies.

Appendix

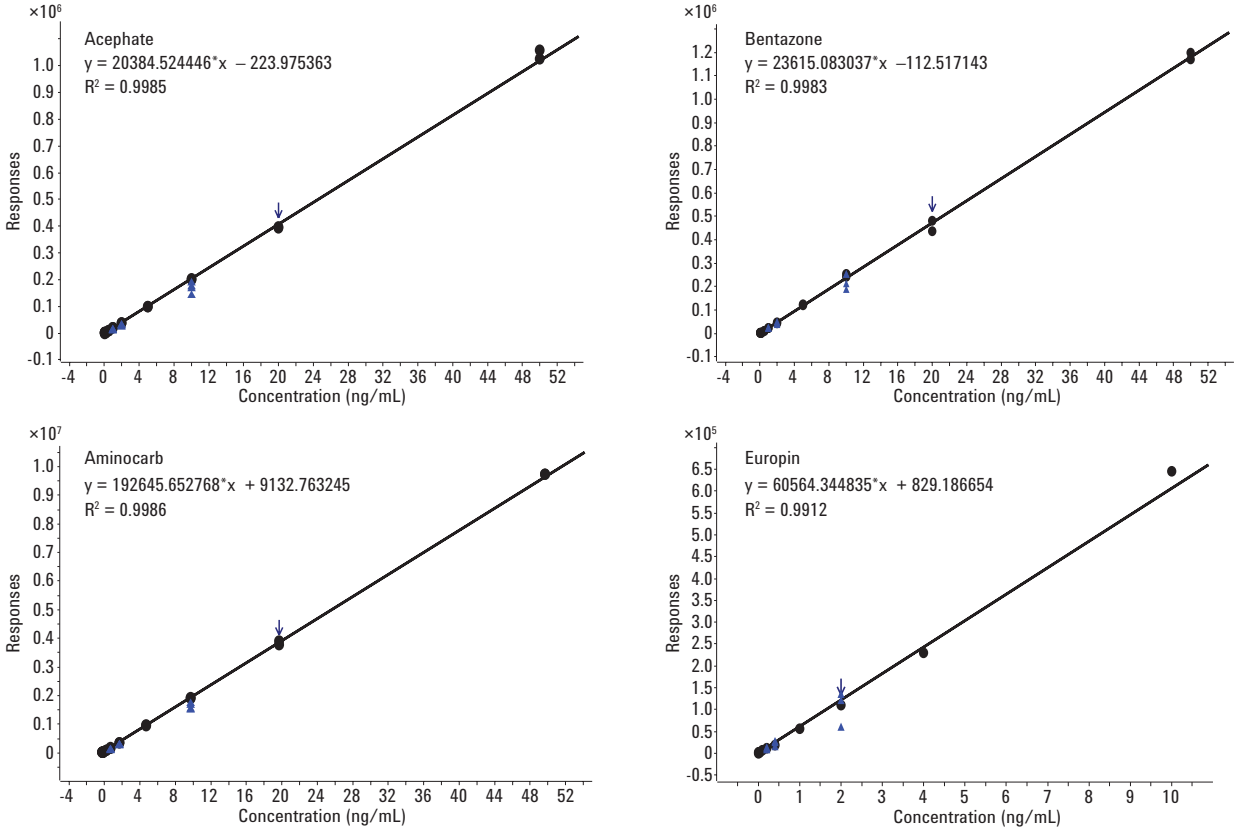
Aflatoxin B2 at a concentration of 0.02 ng/g concentration



Bentazone at a concentration of 1.0 ng/g concentration



Calibration curves for Acephate, Aminocarb, Bentazone, and Europin



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