Optimizing Sample Preparation for LC/MS/MS of Pesticide Residues in Herbal Teas

Application Note

Food Testing & Agriculture

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Introduction

Herbal teas are deeply woven into a variety of cultures and generally associated with the promotion of good health by lowering the risk of diseases such as cancer, stroke, and osteoporosis [1,2]. The high demand for tea also requires implementation of modern agricultural practices, which include the application of pesticides to maintain the integrity of crops. Measuring the large number of permitted and banned pesticides at increasingly low maximum residue limits requires fast, robust, and efficient methods for herbal commodities [3]. Sample preparation is an essential step in this process, especially for botanical matrices, which are often complex and contain interfering matrix compounds that result in ion suppression, coelution, and instrument contamination.

This study implements the Quick, Easy, Cheap, Effective, Rugged, and Safe (QuEChERS) technique for the preparation of herbal black and green tea samples. QuEChERS follows three easy steps; 1) extraction using organic solvent and partitioning salts, 2) sample cleanup with adsorbent materials (dispersive sorbents), and 3) LC or GC analysis, or both [4,5]. Dispersive cleanup sorbents can include C18, primary secondary amine (PSA), and graphitized carbon black (GCB). The use of GCB is increasingly important for tea samples that contain a large amount of pigment; however, it must be used with discretion as it can also remove analytes of interest, particularly compounds with planar geometry.

A QuEChERS protocol was optimized using Agilent Bond Elut QuEChERS premeasured kits to provide fast and easy method development. The method gives adequate cleanup for the highly pigmented samples and delivers high recovery and reproducibility for the majority of pesticides, without significant retention of planar pesticides.

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Materials and Methods

HPLC-grade acetonitrile was from Honeywell International, Inc. Reverse osmosis water was prepared using a Millipore water purification system. Formic acid (98%) was ACS grade and purchased from Sigma-Aldrich Corp. Tea samples were from multiple suppliers and were generously provided by a collaborator. Standards were acquired from Accustandards as 100 µg/mL solutions in acetonitrile. They were mixed and diluted in acetonitrile to the appropriate concentrations and stored in a freezer at –3 °C.

Sample preparation

Extraction: Agilent Bond Elut QuEChERS Original Extraction Tubes for 10 g samples (p/n 5982-5550)

Dispersive SPE cleanup: Agilent Bond Elut QuEChERS Dispersive Universal Kit, 15 mL dispersive SPE tubes (400 mg PSA, 400 mg C18, 45 mg GCB, 1,200 mg $MgSO_4$) (p/n 5982-0029)

Filtration: Captiva Premium Syringe Filter, nylon membrane, 15 mm, 0.2 µm (p/n 5190-5088)

QuEChERS extraction

Dried tea (1 ± 0.01 g) was weighed in 50 mL centrifuge tubes and the samples were spiked as required.

Water (10 mL) was added to the centrifuge tubes followed by capping and vortexing for 1 minute. After the samples were thoroughly wetted, acetonitrile (10 mL) containing internal standard (dimethoate-d6, diuron-d6, and diazinon-d10 at 50 ng/mL) was added to the centrifuge tubes, which were then capped, hand shaken for 1 minute, and placed in an ultrasonic bath for 15 minutes. The tubes were then removed, Bond Elut QuEChERS original extraction salts (4 g $MgSO_4$, 1 g NaCl) were added, and the tubes were shaken vigorously by hand for 1 minute. The samples were subsequently centrifuged for 5 minutes at 4,000 rpm, resulting in phase separation between aqueous and organic solvents (Figure 1, Step 1).

Dispersive solid phase extraction

Following centrifugation, the upper acetonitrile layer (6 mL) was transferred to 15 mL tubes from the Bond Elut QuEChERS Dispersive Universal Kit. The tubes were vortexed for 1 minute followed by centrifugation at 4,000 rpm for 3 minutes. After centrifugation, 2 mL of the cleaned extracts were added to 10 mL test tubes and evaporated to near dryness under a stream of nitrogen. The samples were reconstituted with 0.7 mL H_2O + 0.1% formic acid (FA), 0.2 mL acetonitrile, and 0.1 mL acetonitrile or calibration standards. Next, the samples were syringe filtered into autosampler vials for LC/MS/MS analysis (Figure 1, Step 2).

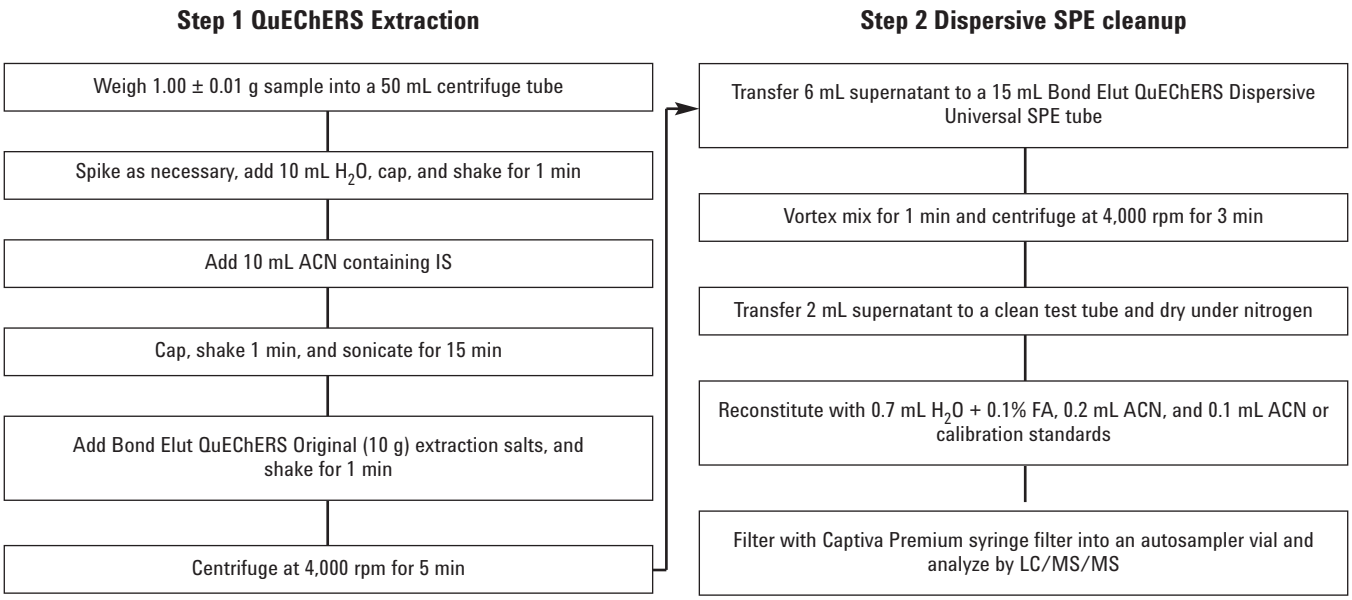

Figure 1. QuEChERS workflow for the extraction and cleanup of green and black teas.

LC conditions

Column:	Agilent ZORBAX RRHD Eclipse Plus C18, 2.1 × 150 mm, 1.8 µm (p/n 959759-902)	
Eluent:	A, Water + 0.1% formic acid B, acetonitrile + 0.1% formic acid	
Injection volume:	10 µL	
Flow rate:	0.2 mL/min	
Gradient:	Time (min)	% B
	0.0	5
	0.5	5
	5.0	60
	7.0	80
	12	95
	15	95
Temperature:	22 °C	
Instruments:	Agilent 1290 Infinity LC, Agilent 6410 Triple Quadrupole LC/MS System	

MS source parameters

Mode:	ESI+
Gas temperature:	300 °C
Gas flow:	7 L/min
Nebulizer:	35 psi
Capillary:	3,500 V

Table 1 shows a list of MS/MS parameters by compound, and Figure 2 is an MRM overlay of a 100 ppb spike for all the pesticides.

Calibration curves and linearity

Stock standard solutions were prepared by mixing the certified standard ampules. Working standards were created by making the appropriate dilutions with acetonitrile. Matrix-matched standards were prepared at 0.5, 1, 5, 10, 20, 50, 100, and 200 ng/mL levels by taking blank green and black tea samples through the QuEChERS protocol and adding the appropriate working standard during solvent reconstitution. Three internal standards were spiked into all samples at 50 ng/mL and taken through the entire QuEChERS workflow. The standards generated linear calibration curves with an R^2 of 0.992 or greater (data not shown).

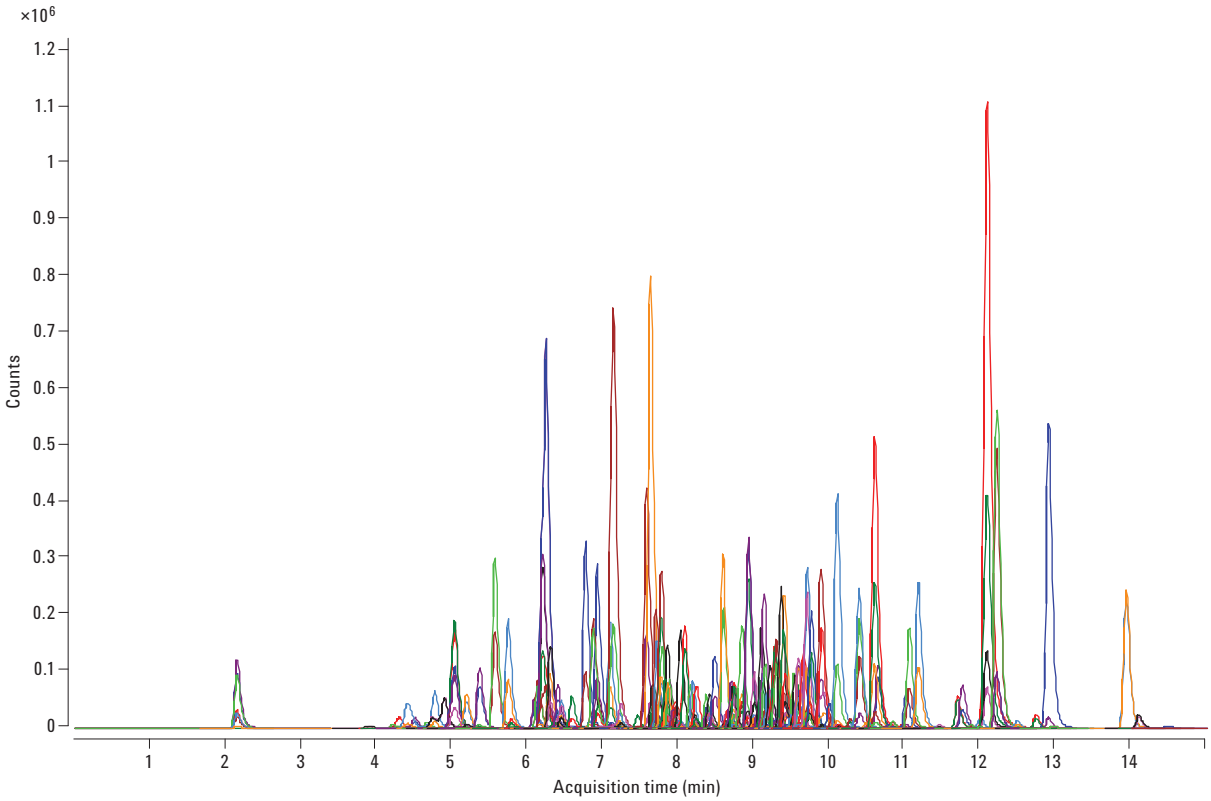

Figure 2. An MRM chromatogram overlay of a 100 ppb spike in black tea for the 176 pesticides analyzed in this study.

Results and Discussion

Selection of QuEChERS salts and cleanup kit

Several extraction salt kits were evaluated in this study to achieve optimal recovery and reproducibility. After testing the Bond Elut QuEChERS Original, AOAC 2007.01, and EN 15662 extraction salts, it was determined that the original extraction kit containing 1 g NaCl and 4 g $MgSO_4$ gave the overall highest recoveries. Due to the large amount of pigment coextracted with the analytes of interest, it was necessary to select a dispersive cleanup kit containing GCB. It was found that the Bond Elut QuEChERS Universal kit, containing PSA, C18, GCB, and $MgSO_4$, delivered significantly cleaner samples, greater recoveries, and only minimal to moderate retention of planar pesticides (carbendazim, thiabendazole, tricyclazole, and pyrimethanil).

Recovery and reproducibility

This method delivered excellent recovery and reproducibility for the vast majority of the 176 pesticides in green and black tea matrices. For green tea samples, 82% of the pesticide recoveries at 10 ppb and 92% at 100 ppb were between 70 and 120%. For black tea samples, 76% of the pesticide recoveries at 10 ppb and 88% at 100 ppb were between 70 and 120%. As shown in Figure 3, this was accompanied with a relatively low number of pesticide recoveries below 50% and some nondetected pesticides. A full list of pesticides and the respective recoveries and relative standard deviation is in Table 2.

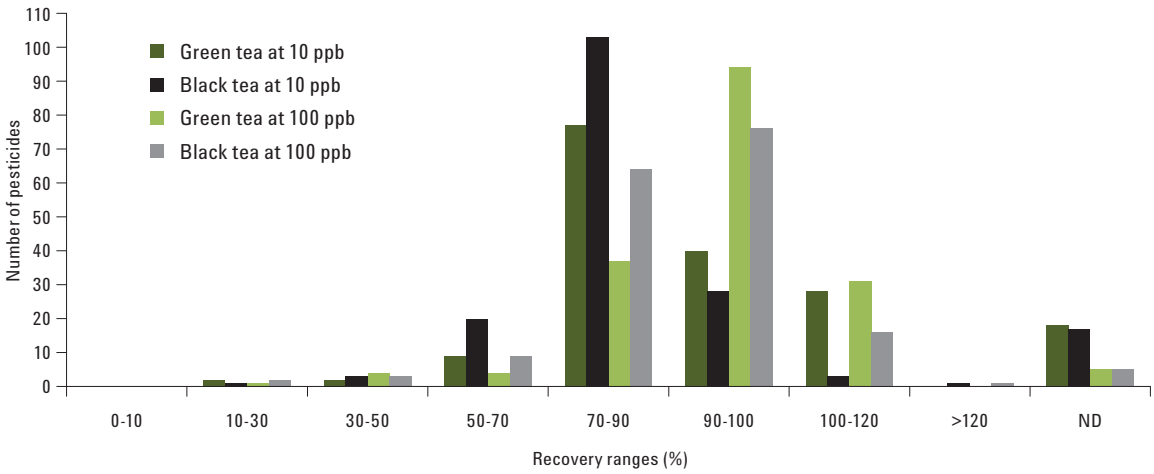

Figure 3. Plot of recovery ranges for 176 pesticides.

Table 1. List of compounds used in this study and their respective MS/MS parameters.

Compound	Precursor ion	MRM 1	Collision energy 1 (V)	MRM 2	Collision energy 2 (V)	Fragmentor (V)	Retention time (min)
Propamocarb	189.2	144.1	10	102.1	15	120	2.128
Aminocarb	209.1	152.1	10	137.1	20	120	2.145
Pymetrozine	218.1	105.0	20	79.0	20	110	2.148
Acephate	184.0	143.0	5	95.0	20	90	2.154
Methamidophos	142.0	125.0	10	94.0	15	80	3.902
Omethoate	214.0	183.0	5	125.0	20	80	4.495
Aldicarb sulfoxide	207.1	132.1	5	89.0	5	80	4.662
Carbendazim	192.1	160.1	20	132.1	25	90	4.753
Thiabendazole	202.0	175.0	30	131.1	40	120	4.889
Fuberidazole	185.1	157.1	25	156.0	30	120	5.02
Mexacarbate	223.0	166.0	10	151.0	20	110	5.023
Nitenpyram	271.0	237.0	15	224.0	15	100	5.126
Monocrotophos	224.0	193.0	0	127.0	10	100	5.18
Butoxycarboxim	223.0	166.0	0	106.0	5	90	5.185
Aldicarb sulfone	223.1	148.0	5	76.0	5	80	5.321
Dicrotophos	238.0	127.0	5	112.1	15	90	5.357
Pirimicarb	239.2	182.1	15	72.0	20	120	5.562
Vamidothion	288.1	146.1	10	118.0	20	80	5.734
Thiamethoxam	292.0	211.0	10	181.0	20	80	5.779
Amitraz	294.1	163.0	10	121.9	35	110	5.791
Mevinphos	225.0	193.0	1	127.0	15	80	6.084
Carbofuran-3-hydroxy	255.1	220.1	5	163.1	15	70	6.13
Clothianidin	250.0	169.0	5	132.0	15	90	6.16
Secbumeton	226.2	170.0	15	142.0	25	100	6.203
Prometon	226.2	184.1	20	142.1	20	120	6.244
Terbumeton	226.2	170.1	15	114.1	20	120	6.244
Fenuron	165.1	120.0	15	72.0	15	120	6.29
Simetryn	214.1	144.1	20	124.1	20	120	6.301
Imidacloprid	256.1	209.1	10	175.0	10	80	6.342
Tricyclazole	190.0	163.0	25	136.0	30	120	6.391
Dimethoate-d6	236.0	205.0	5	171.0	10	80	6.437
Dimethoate	230.0	199.0	5	171.0	10	80	6.437
Acetamiprid	223.1	126.0	15	56.0	15	80	6.443
Imazalil	297.1	255.0	20	159.0	20	160	6.6
Tebuthiuron	229.1	172.1	15	116.0	20	120	6.768
Metoxuron	229.1	156.0	20	72.0	20	120	6.77
Cymoxanil	199.1	128.1	5	111.0	15	80	6.813
Ametryn	228.1	186.1	20	96.1	25	120	6.919
Carbetamide	237.1	192.1	5	118.0	10	80	6.932
Thiacloprid	253.0	186.0	10	126.0	15	120	6.937
Methoprotryne	272.2	198.1	20	170.1	30	140	6.97
Methabenzthiazuron	222.1	165.1	15	150.0	20	120	7.097
Thidiazuron	221.0	128.0	12	102.0	8	100	7.097
Aldicarb	116.1	89.0	5	70.0	5	80	7.107
Fenpropimorph	304.3	147.1	30	130.0	30	120	7.114
Spiroxamine	298.3	144.1	20	100.1	20	120	7.168
Etoxazole	360.2	141.0	28	113.0	50	155	7.213

Compound	Precursor ion	MRM 1	Collision energy 1 (V)	MRM 2	Collision energy 2 (V)	Fragmentor (V)	Retention time (min)
Oxadixyl	279.1	219.1	10	102.0	10	80	7.236
Thiophanate-methyl	343.0	192.0	21	151.0	20	100	7.463
Prometryn	242.1	200.1	20	158.1	20	120	7.578
Mesotrione	340.1	227.8	49	104.1	15	80	7.61
Terbutryn	242.1	186.1	15	71.0	20	120	7.626
Metribuzin	215.1	187.1	15	131.0	20	120	7.672
Spinosad A	732.5	142.1	10	98.0	10	80	7.688
Flutriafol	302.1	123.0	20	70.0	15	120	7.7
Propoxur	210.1	168.1	5	111.0	10	80	7.72
Dioxacarb	224.0	167.1	0	123.1	15	80	7.743
Bendiocarb	224.1	167.1	5	109.0	10	80	7.748
Formetanate	222.1	165.1	15	120.0	20	120	7.763
Cycluron	199.4	89.0	15	72.0	25	120	7.771
Carbofuran	222.1	165.1	10	123.0	15	120	7.783
Pyrimethanil	200.1	183.0	25	107.1	25	120	7.82
Forchlorfenuron	248.1	155.1	12	129.1	12	95	7.848
Chlorotoluron	213.1	140.0	20	72.0	20	120	7.854
Fluometuron	233.1	160.0	20	72.0	20	120	7.877
Diuron	233.0	160.0	20	72.0	20	120	7.877
Diuron-d6	239.0	78.0	20			120	7.877
Sulfentrazone	404.5	307.1	20	307.1	20	110	7.906
Pyracarbolid	218.1	125.1	12	55.1	36	115	7.955
Spinosad D	746.5	558.4	5	142.1	15	120	7.971
Carbaryl	202.1	145.1	5	117.1	10	80	7.977
Thiofanox	241.1	184.0	5	57.1	15	120	8.019
Isoproturon	207.2	165.1	15	72.0	15	120	8.025
Metalaxyl	280.2	220.1	10	192.0	15	120	8.085
Atrazine	216.1	174.1	15	132.0	20	120	8.102
Ethiofencarb	226.1	164.1	5	107.1	5	80	8.155
Carboxin	236.1	143.0	15	87.0	20	120	8.188
Monolinuron	215.1	148.1	10	126.0	15	120	8.217
Emamectin B1a	886.7	158.0	40	126.0	40	150	8.271
Isoprocarb	194.0	137.0	20	95.0	5	80	8.361
Nuarimol	315.1	252.1	25	81.0	30	120	8.371
Prochloraz	376.0	308.0	10	266.0	10	80	8.407
Bupirimate	317.2	272.0	20	166.1	25	120	8.419
Metobromuron	259.0	170.0	15	148.1	15	120	8.423
Hydramethylnon	495.2	323.1	28	151.0	50	220	8.525
Paclobutrazol	294.1	165.0	20	70.0	20	120	8.589
Triadimenol	296.1	227.1	5	70.0	10	80	8.594
Propham	180.1	138.1	5	120.0	15	80	8.629
Ethiprole	397.1	351.3	19	255.4	37	110	8.654
Cyprodinil	226.1	108.0	30	93.0	40	120	8.693
Desmedipham	301.1	182.1	5	136.0	20	80	8.723

Compound	Precursor ion	MRM 1	Collision energy 1 (V)	MRM 2	Collision energy 2 (V)	Fragmentor (V)	Retention time (min)
Phenmedipham	301.1	168.1	5	136.1	20	80	8.727
Siduron	233.0	137.0	15	94.0	15	100	8.742
Triticonazole	318.1	125.2	41	70.1	15	110	8.749
Fipronil	437.0	368.0	30	263.0	40	120	8.76
Cyproconazole	292.1	125.0	15	70.0	15	120	8.841
Fludioxonil	229.0	185.0	15	158.0	20	120	8.896
Methiocarb	226.1	169.1	5	121.1	10	80	8.916
Iprovalicarb	321.2	203.1	5	119.1	20	80	8.921
Furalaxyl	302.1	242.1	10	95.0	27	110	8.926
Fenarimol	331.0	268.0	25	81.0	30	120	9.002
Diethofencarb	268.2	226.1	5	152.0	20	80	9.002
Isoxaflutole	360.1	251.0	10	69.0	10	120	9.006
Bromuconazole	376.0	159.0	20	70.0	20	80	9.04
Azoxystrobin	404.1	372.1	10	344.1	15	120	9.062
Linuron	249.0	182.0	15	160.0	20	120	9.083
Myclobutanil	289.1	125.0	20	70.0	15	120	9.096
Promecarb	208.1	151.1	5	109.1	10	80	9.098
Tetraconazole	372.0	159.1	36	70.1	16	150	9.116
Epoxiconazole	330.1	141.0	20	121.0	20	120	9.136
Fenamidone	312.1	236.1	4	92.1	20	120	9.154
Diclobutrazol	328.1	159.0	20	70.0	20	120	9.185
Triadimefon	294.1	197.0	15	69.0	20	120	9.216
Flusilazole	316.1	247.1	15	165.0	20	120	9.279
Fenhexamid	302.1	97.1	25	55.1	30	80	9.294
Mefenacet	299.1	148.1	8	120.1	24	110	9.297
Fluquinconazole	376.0	349.1	23	307.1	23	80	9.357
Tebuconazole	308.2	151.0	20	70.0	20	120	9.364
Acibenzolar-S-methyl	211.0	136.0	20	91.0	25	120	9.39
Methoxifenozide	369.0	313.0	0	149.0	15	90	9.395
Bifenazate	301.0	198.0	5	170.0	20	80	9.396
Fenbuconazol	337.1	125.0	20	70.0	20	120	9.42
Ethofumesate	304.1	287.1	4	241.1	4	90	9.43
Triflumizole	346.1	278.1	5	73.1	10	80	9.438
Diflubenzuron	311.0	158.0	10	141.0	15	80	9.503
Bitertanol	338.2	269.2	5	99.0	10	80	9.529
Flutolanil	324.0	282.0	10	262.0	20	120	9.55
Mepanipyrim	224.1	106.0	25	77.0	30	120	9.578
Mepronil	270.0	228.0	10	119.0	25	120	9.597
Fenoxycarb	302.1	116.1	10	88.0	20	120	9.602
Fluoxastrobin	459.0	427.1	15	188.0	40	130	9.603
Hexaconazole	314.1	159.0	10	70.0	20	120	9.658
Molinate	188.1	126.0	10	83.0	15	120	9.663
Penconazol	284.1	159.0	20	70.0	15	120	9.7
Metconazole	320.2	125.0	30	70.0	25	120	9.706

Compound	Precursor ion	MRM 1	Collision energy 1 (V)	MRM 2	Collision energy 2 (V)	Fragmentor (V)	Retention time (min)
Butafenacil	492.0	348.8	10	330.8	23	90	9.729
Flufenacet	364.1	194.1	5	152.1	10	80	9.741
Dimoxystrobin	327.1	205.0	5	116.0	20	110	9.771
Propiconazole	342.1	159.0	20	69.0	20	120	9.891
Tebufenozide	353.2	297.2	5	133.1	20	80	9.895
Prothioconazole	344.1	189.0	20	125.0	40	90	9.895
Diniconazole	326.1	159.0	30	70.0	25	120	9.901
Neburon	275.1	88.1	15	57.0	20	120	10.004
Carfentrazone-ethyl	412.0	366.0	15	346.0	20	120	10.043
Picoxystrobin	368.1	205.1	5	145.1	20	80	10.115
Triflumuron	359.0	156.0	20	139.0	20	120	10.124
Kresoxim-methyl	314.1	267.0	5	206.1	5	80	10.141
Famoxadone	392.2	331.2	4	238.1	12	90	10.284
Benalaxyl	326.2	294.0	5	148.1	10	120	10.403
Ipconazole	334.1	125.0	45	70.1	22	110	10.406
Hexaflumuron	461.0	158.0	10	141.0	20	120	10.499
Zoxamide	336.0	187.0	16	159.0	36	125	10.628
Fenazaquin	307.2	161.1	15	57.0	20	120	10.636
Pyraclostrobin	388.1	194.1	10	163.0	20	120	10.645
Dimethomorph	388.1	301.1	20	165.0	25	120	10.654
Buprofezin	306.2	201.1	10	116.1	15	120	10.655
Clofentezine	303.0	138.1	8	102.1	40	99	10.79
Diazinon	305.1	169.0	20	153.1	20	160	10.856
Diazinon-d10	315.1	163.1	20			160	10.856
Indoxacarb	528.0	203.0	36	150.0	16	120	10.858
Malathion	331.0	126.9	5	99.0	10	80	10.886
Teflubenzuron	381.0	158.0	10	141.0	15	80	10.966
Thiobencarb	258.3	125.1	25	100.1	5	100	11.033
Trifloxystrobin	409.1	206.1	10	186.1	15	120	11.072
Benzoximate	364.1	199.0	5	105.0	20	80	11.197
Lufenuron	511.0	158.0	10	141.0	20	80	11.475
Tebufenpyrad	334.0	145.0	24	117.0	32	175	11.709
Quinoxyfen	308.0	272.0	24	197.0	32	135	11.789
Clethodim	360.1	240.2	12	136.1	28	131	11.891
Piperonyl butoxide	356.1	177.0	8	119.0	37	110	12.108
Furathiocarb	383.2	252.1	10	195.1	15	120	12.113
Pyriproxifen	322.1	185.0	16	96.1	8	100	12.234
Chlorfluazuron	540.0	382.9	15	158.0	15	120	12.508
Eprinomectin B1a	914.6	468.3	5	330.3	10	150	12.71
Hexythiazox	353.1	228.0	10	168.1	20	120	12.769
Fenpyroximate	422.2	366.2	15	135.0	40	130	12.914
Propargite	373.1	81.0	25	57.0	25	160	13.004
Pyridaben	365.1	309.1	10	147.0	20	80	13.952
Spirodiclofen	411.0	313.0	5	71.2	15	110	14.144
Avermectin B1a	890.6	305.0	20	145.2	43	130	14.503

Table 2. List of compounds used in this study and their recoveries and relative standard deviations (RSDs) in herbal green and black tea (n = 4).

	Green tea				Black tea			
Compound	**10 ppb % recovery**	**10 ppb % rsd**	**100 ppb % recovery**	**100 ppb % rsd**	**10 ppb % recovery**	**10 ppb % rsd**	**100 ppb % recovery**	**100 ppb % rsd**
Propamocarb	46.6	6.0	52.0	8.7	63.1	17.6	56.0	5.7
Aminocarb	85.7	7.3	95.0	4.7	92.3	8.5	92.4	3.1
Pymetrozine	30.3	7.3	32.8	5.4	ND		10.0	9.8
Acephate	80.5	5.2	85.7	4.2	99.7	3.2	91.5	2.7
Methamidophos	85.1	4.8	78.4	2.3	66.5	14.8	71.5	7.1
Omethoate	81.7	11.4	91.6	7.4	83.4	4.4	89.5	4.7
Aldicarb sulfoxide	73.7	14.2	85.0	7.3	92.8	13.8	82.5	3.6
Carbendazim	95.9	12.6	85.1	8.9	67.2	8.4	73.9	6.1
Thiabendazole	59.1	7.4	66.6	6.4	55.2	8.0	65.3	6.9
Fuberidazole	71.9	2.0	84.5	2.0	67.8	6.0	77.6	4.1
Mexacarbate	81.6	4.2	95.5	3.5	124.7	17.5	94.9	8.8
Nitenpyram	67.2	8.5	73.6	4.5	57.6	10.2	61.0	2.9
Monocrotophos	100.1	6.2	82.9	4.4	103.2	6.4	81.5	9.5
Butoxycarboxim	91.7	7.6	99.2	5.4	89.9	11.5	110.3	6.0
Aldicarb sulfone	88.9	2.3	105.7	6.9	95.4	5.9	98.1	3.1
Dicrotophos	86.1	5.3	91.0	8.9	78.2	4.0	79.7	5.7
Pirimicarb	ND	ND	104.6	5.9	82.8	24.0	80.8	8.1
Vamidothion	95.6	15.1	91.2	7.5	72.2	5.1	90.2	4.5
Thiamethoxam	87.7	5.0	88.9	7.7	79.0	5.5	85.6	7.6
Amitraz	21.8	21.5	31.4	8.2	ND		18.9	15.9
Mevinphos	73.9	11.8	93.7	7.2	66.3	14.0	86.7	4.5
Carbofuran-3-hydroxy	ND		80.3	8.7	42.9	33.5	75.2	15.0
Clothianidin	104.4	16.1	99.8	12.5	71.6	11.0	104.3	3.0
Secbumeton	90.5	1.6	95.4	9.3	87.2	7.4	89.8	7.7
Prometon	86.9	6.5	96.5	8.7	89.6	5.0	89.0	4.2
Terbumeton	110.3	7.5	101.2	3.2	85.6	6.1	98.2	1.2
Fenuron	82.8	9.4	93.1	7.1	83.6	6.5	91.5	4.1
Simetryn	93.7	5.2	100.1	2.1	90.2	7.3	91.1	4.1
Imidacloprid	110.1	4.1	90.7	3.5	82.4	7.1	91.2	3.6
Tricyclazole	60.1	4.4	67.1	4.1	59.9	3.8	65.4	2.6
Dimethoate	102.9	10.0	103.3	9.3	98.8	4.4	93.0	4.9
Acetamiprid	93.3	9.0	96.6	4.7	82.8	8.5	85.8	5.0
Imazalil	90.1	3.2	91.0	7.5	84.7	5.5	89.8	2.8
Tebuthiuron	83.1	7.8	91.3	5.2	81.6	4.4	87.6	2.8
Metoxuron	99.2	7.1	96.0	9.3	90.4	8.1	89.3	2.0
Cymoxanil	86.9	6.8	87.2	11.6	87.6	5.5	84.3	7.6
Ametryn	97.6	11.4	97.2	3.1	90.1	14.2	97.2	5.1
Carbetamide	100.3	5.9	98.1	8.2	88.7	4.9	96.4	3.9
Thiacloprid	85.4	4.7	91.6	9.6	80.6	8.6	87.3	7.2
Methoprotryne	94.4	3.2	99.3	2.1	94.6	10.0	102.8	5.6
Methabenzthiazuron	90.4	4.3	94.9	9.6	86.2	4.4	90.5	4.3

	Green tea				Black tea			
Compound	10 ppb % recovery	10 ppb % rsd	100 ppb % recovery	100 ppb % rsd	10 ppb % recovery	10 ppb % rsd	100 ppb % recovery	100 ppb % rsd
Thidiazuron	69.2	13.4	72.6	9.3	68.2	5.0	77.3	3.7
Aldicarb	90.0	4.1	96.3	7.1	80.6	7.0	94.0	6.1
Fenpropimorph	80.1	4.8	88.8	10.2	89.5	7.3	93.2	5.3
Spiroxamine	79.4	6.5	84.1	5.6	74.0	3.3	84.5	2.8
Etoxazole	84.3	3.3	95.9	10.9	98.8	7.7	122.3	10.1
Thiophanate-methyl	ND		80.2	7.5	ND		77.2	7.5
Prometryn	81.9	5.6	84.0	4.4	78.9	6.3	84.0	3.4
Mesotrione	ND		ND		ND		ND	
Terbutryn	96.3	9.3	90.2	3.9	79.7	6.8	80.9	4.0
Metribuzin	89.1	6.2	98.0	7.7	83.8	5.9	94.9	5.2
Spinosad A	73.5	3.5	85.1	6.1	72.5	6.4	85.4	4.9
Flutriafol	79.8	5.4	93.5	4.7	82.3	7.8	94.7	6.5
Propoxur	119.7	10.1	111.8	7.7	91.6	8.0	102.0	6.9
Dioxacarb	98.9	3.3	106.7	3.5	89.9	4.1	99.2	5.3
Bendiocarb	112.6	13.7	105.1	7.5	74.2	12.1	90.1	5.5
Formetanate	74.9	10.8	76.0	5.7	60.8	4.3	68.7	3.0
Cycluron	95.5	6.3	104.6	5.6	85.9	5.1	92.2	3.8
Carbofuran	90.4	4.3	96.8	6.5	85.4	5.5	90.4	5.3
Pyrimethanil	72.6	6.8	71.8	9.5	78.2	8.5	82.4	8.4
Forchlorfenuron	81.5	7.3	76.9	4.2	33.6	15.5	79.9	4.2
Chlorotoluron	95.1	9.3	105.6	3.9	87.6	8.5	91.9	6.2
Fluometuron	91.3	5.4	94.6	2.4	90.1	6.9	87.3	5.3
Diuron	90.7	4.5	97.2	7.5	86.1	6.4	88.9	5.0
Sulfentrazone	66.2	9.4	88.0	2.3	11.8	36.7	37.0	14.7
Pyracarbolid	96.7	9.1	95.5	3.6	83.2	5.7	91.5	4.6
Spinosad D	ND		55.7	14.9	ND		57.7	18.9
Carbaryl	100.9	5.3	100.5	5.8	93.1	4.2	96.9	2.9
Thiofanox	ND		109.0	13.2	ND		105.0	10.2
Isoproturon	110.0	7.0	108.3	8.8	87.9	11.5	94.5	3.1
Metalaxyl	88.0	4.6	93.8	3.0	87.5	6.9	86.2	4.8
Atrazine	89.0	6.7	91.5	5.7	88.5	4.7	93.4	5.8
Ethiofencarb	101.1	7.1	110.4	4.6	95.5	8.7	97.2	7.1
Carboxin	83.5	6.6	85.9	7.5	67.1	14.1	84.9	2.5
Monolinuron	84.9	4.8	95.7	8.0	89.8	7.0	95.1	6.2
Emamectin B1a	ND		40.4	29.5	ND		38.5	12.8
Isoprocarb	ND		97.9	5.4	ND		85.9	4.4
Nuarimol	80.7	7.2	86.9	11.3	77.8	6.4	89.6	5.0
Prochloraz	77.7	9.6	89.4	9.6	87.6	7.5	94.7	7.4
Bupirimate	90.8	8.8	95.4	2.1	84.0	9.4	85.3	4.3
Metobromuron	89.1	8.3	90.7	5.3	88.1	16.5	90.7	4.3
Hydramethylnon	20.9	4.6	22.8	8.3	48.7	8.6	53.3	6.3
Paclobutrazol	81.6	7.0	94.3	4.0	81.5	11.8	85.5	3.1
Triadimenol	81.7	3.1	93.1	3.5	85.0	4.2	90.6	6.5
Propham	ND		90.8	4.5	ND		89.8	7.5
Ethiprole	92.0	6.2	93.3	5.2	85.4	12.8	96.3	4.2
Cyprodinil	89.6	9.0	91.1	3.7	67.8	7.6	93.1	1.7

	Green tea				Black tea			
Compound	10 ppb % recovery	10 ppb % rsd	100 ppb % recovery	100 ppb % rsd	10 ppb % recovery	10 ppb % rsd	100 ppb % recovery	100 ppb % rsd
Desmedipham	55.2	21.7	96.2	3.5	62.4	9.5	94.2	6.5
Phenmedipham	63.2	11.7	109.2	4.5	96.4	10.5	95.3	4.8
Siduron	96.8	4.7	94.1	2.0	79.3	6.7	83.6	3.3
Triticonazole	76.9	6.6	91.2	8.2	79.0	11.4	92.5	4.6
Fipronil	ND		101.0	11.6	ND		60.9	17.9
Cyproconazole	87.7	6.9	96.0	3.3	82.4	8.2	83.4	7.2
Fludioxonil	86.9	14.5	100.2	3.9	78.7	10.8	99.2	4.9
Methiocarb	83.8	12.1	90.3	6.4	85.0	8.2	95.1	4.4
Iprovalicarb	70.7	10.4	87.8	4.2	81.5	15.1	79.3	11.3
Furalaxyl	80.4	5.7	91.4	3.9	81.7	7.8	94.4	1.9
Fenarimol	82.6	7.8	91.5	7.1	81.9	5.4	85.4	5.1
Diethofencarb	ND		ND		ND		ND	
Isoxaflutole	102.8	8.9	97.3	6.2	117.7	10.7	95.9	7.0
Bromuconazole	85.6	9.0	90.8	5.2	62.9	17.4	71.8	11.0
Azoxystrobin	104.4	8.2	106.2	6.7	92.4	5.0	101.7	4.7
Linuron	95.4	7.4	92.7	3.7	82.7	5.0	82.1	2.8
Myclobutanil	84.0	4.3	96.5	7.9	87.5	7.5	92.7	6.9
Promecarb	ND		88.7	3.5	ND		95.7	3.5
Tetraconazole	72.5	6.0	89.6	7.4	79.5	6.2	89.8	6.0
Epoxiconazole	86.0	5.1	98.6	3.9	90.9	3.9	90.0	5.5
Fenamidone	94.1	6.8	103.0	2.7	91.2	8.8	101.0	3.7
Diclobutrazol	91.4	16.8	92.2	5.1	78.5	10.4	87.2	5.1
Triadimefon	100.3	8.9	97.1	3.7	83.4	11.5	95.1	7.7
Flusilazole	95.4	6.9	91.8	4.4	90.4	9.5	85.5	5.3
Fenhexamid	84.8	5.7	96.3	4.9	78.6	4.1	90.1	2.5
Mefenacet	101.4	6.3	99.8	3.2	84.5	8.5	103.8	6.2
Fluquinconazole	95.1	9.9	99.9	3.5	84.4	4.4	95.2	3.6
Tebuconazole	97.5	12.8	89.9	5.6	89.7	11.0	86.1	14.5
Acibenzolar-S-methyl	85.0	7.1	90.4	3.7	52.0	22.9	94.4	4.7
Methoxifenozide	112.0	2.2	107.8	9.1	102.7	7.8	101.8	6.6
Bifenazate	95.8	9.1	91.1	8.2	63.5	13.3	56.9	23.0
Fenbuconazol	95.8	14.8	94.0	7.3	81.6	17.9	93.0	7.3
Ethofumesate	86.7	10.9	99.6	5.8	90.6	9.4	100.6	4.8
Triflumizole	54.8	19.6	97.8	14.6	75.3	17.2	94.8	10.6
Diflubenzuron	103.0	12.9	91.8	10.6	86.2	13.2	96.2	9.5
Bitertanol	85.6	7.1	91.8	6.4	80.3	5.2	92.3	4.4
Flutolanil	85.6	5.0	90.9	4.0	79.3	8.0	86.3	3.7
Mepanipyrim	92.9	6.7	99.2	6.8	90.7	6.2	96.2	1.8
Mepronil	92.8	6.7	96.9	3.7	83.1	9.7	79.6	3.5
Fenoxycarb	97.1	7.4	98.5	5.4	90.3	12.5	92.8	8.5
Fluoxastrobin	94.5	6.1	103.9	8.6	89.5	8.2	101.3	3.2
Hexaconazole	76.0	5.9	90.0	5.4	77.8	8.1	88.7	5.8
Molinate	90.9	12.6	99.7	2.6	92.3	15.1	95.7	7.6
Penconazol	81.4	5.8	95.3	3.5	83.0	7.5	89.4	7.2
Metconazole	96.3	10.7	97.3	5.1	75.6	8.7	77.9	7.3
Butafenacil	109.1	9.4	103.0	2.5	80.6	16.0	93.5	6.9

	Green tea				Black tea			
Compound	10 ppb % recovery	10 ppb % rsd	100 ppb % recovery	100 ppb % rsd	10 ppb % recovery	10 ppb % rsd	100 ppb % recovery	100 ppb % rsd
Flufenacet	109.8	7.8	102.6	5.6	82.3	9.4	85.6	5.6
Dimoxystrobin	104.9	3.7	108.5	9.0	93.1	5.7	102.1	5.1
Propiconazole	80.3	5.7	94.1	6.7	87.7	7.8	96.4	4.3
Tebufenozide	111.5	6.0	112.6	11.6	91.3	5.1	97.8	7.5
Prothioconazole	ND		ND		ND		ND	
Diniconazole	86.0	8.2	93.4	4.5	75.0	6.1	91.0	5.9
Neburon	88.5	8.2	94.8	5.1	73.5	4.9	92.3	5.1
Carfentrazone-ethyl	87.0	4.0	98.9	6.6	87.9	8.9	95.7	5.2
Picoxystrobin	106.5	8.1	100.1	4.2	81.6	8.4	93.1	3.2
Triflumuron	104.8	20.3	103.8	9.6	72.3	12.2	84.8	8.6
Kresoxim-methyl	104.5	9.4	94.9	7.6	87.6	8.8	100.1	5.5
Famoxadone	ND		ND		ND		ND	
Benalaxyl	105.2	7.5	113.2	9.0	85.7	4.2	96.6	5.6
Ipconazole	80.3	2.4	95.5	3.9	76.2	6.1	92.2	4.5
Hexaflumuron	ND		87.0	10.9	ND		77.1	21.0
Zoxamide	87.0	3.0	100.6	5.3	85.9	7.1	91.2	6.6
Fenazaquin	85.0	6.6	88.4	2.8	85.3	4.8	98.4	2.5
Pyraclostrobin	104.5	10.5	98.2	3.4	93.2	9.9	85.0	13.0
Dimethomorph	73.6	6.7	92.3	2.5	78.6	12.4	96.3	2.5
Buprofezin	ND		98.9	7.6	ND		97.9	9.6
Clofentezine	98.7	14.1	93.3	9.4	77.9	5.7	90.1	4.3
Diazinon	90.0	8.0	93.2	5.5	92.9	6.2	84.6	7.5
Indoxacarb	90.3	10.3	92.3	9.3	83.2	6.7	100.6	4.4
Malathion	96.6	7.2	92.6	3.8	95.2	11.8	97.6	1.8
Teflubenzuron	82.6	5.6	90.5	9.3	78.0	10.4	88.8	7.4
Thiobencarb	83.1	3.3	92.2	6.0	79.1	6.3	89.2	6.5
Trifloxystrobin	107.8	15.4	105.1	5.1	83.0	10.9	104.1	5.1
Benzoximate	84.5	3.1	99.9	4.9	75.1	11.0	91.9	4.8
Lufenuron	84.0	3.9	92.8	7.3	77.9	12.5	93.0	5.5
Tebufenpyrad	75.0	5.7	86.8	8.8	73.5	6.7	88.3	5.6
Quinoxyfen	74.2	5.4	87.4	7.8	65.4	4.9	74.4	8.9
Clethodim	55.3	18.2	47.4	8.4	50.5	22.8	48.5	14.0
Piperonyl butoxide	86.9	7.1	89.2	8.0	89.3	6.7	93.5	6.6
Furathiocarb	70.4	16.5	93.2	7.0	71.3	23.1	92.4	4.8
Pyriproxifen	86.1	8.3	89.3	8.2	73.5	4.9	87.3	3.9
Chlorfluazuron	ND		88.4	4.7	54.2	16.7	84.1	8.4
Eprinomectin B1a	ND		ND		ND		ND	
Hexythiazox	72.4	12.3	89.2	2.7	63.3	21.6	91.2	5.7
Fenpyroximate	100.5	9.5	103.5	5.3	86.3	11.5	97.5	3.3
Propargite	75.4	16.8	93.7	4.0	77.6	16.1	91.8	5.8
Pyridaben	83.7	4.1	85.3	9.6	74.8	9.1	87.9	5.9
Spirodiclofen	81.9	4.8	87.1	6.0	80.5	4.7	85.2	4.1
Avermectin B1a	85.1	7.8	91.9	14.3	73.1	8.0	102.5	8.1
Abamectin	77.7	9.2	94.1	16.8	70.0	6.9	102.3	6.3

ND = not detected

Conclusions

QuEChERS sample preparation was optimized for the LC/MS/MS analysis of 176 pesticides in herbal green and black teas. The method provided fast separation and sensitive detection on the UPLC/MS/MS platform. Sample preparation gave adequate cleanup for the highly pigmented tea samples and delivered high recoveries for most of the pesticides.

While LC/MS/MS is a powerful tool for pesticide residue analysis, it cannot accommodate some groups of pesticides that are GC-amenable. Future studies will investigate the optimized analysis of pesticides in dried botanical matrices by GC/MS/MS. Several of these GC-amenable pesticides can be retained by GCB sorbents and so pigment removal must carefully balance sample cleanup with analyte recovery. The combination of LC and GC workflows will provide a complete solution for the analysis of pesticides in green and black teas and provide methodologies that can be further transferred to other dried botanical matrices of high complexity.

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