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Analysis of Agricultural Residues on Tea Using d-SPE Sample Preparation with GC-NCI-MS and UHPLC-MS/MS

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This study presents new sample preparation and analytical procedures for the quantification of pesticides on processed tea leaves. The new method includes tea extraction and dispersive solid phase extraction (d-SPE) to prepare gas chromatography (GC) and ultrahigh-performance liquid chromatography (UHPLC)-ready samples, providing a fast and cost-effective solution for time-sensitive industrial analysis to fulfill regulatory requirements. Both GC−negative chemical ionization mass spectrometry (GC-NCI-MS) and UHPLC−tandem mass spectrometry (UHPLC-MS/MS) were employed to produce highly sensitive and reproducible data. Excellent limits of detection (typically below 1 μg/kg for GC and 10 μg/kg for UHPLC), wide linearity ranges, and good recoveries (mostly >70%) were achieved on the selected pesticides. Twenty-seven tea samples purchased from local grocery stores were analyzed using the newly developed methods. Among the pesticides analyzed, endosulfan sulfate and kelthane were the most frequently detected by GC-NCI-MS and imidacloprid and acetamiprid by UHPLC-MS/MS in these teas. The samples were found to be relatively clean, with <1 mg/kg of total pesticide residues. The organic-labeled teas were significantly cleaner than nonorganic ones. The cost per gram of tea did not correlate with pesticide residue levels detected.

KEYWORDS: Pesticides; GC-NCI-MS; UHPLC-MS/MS; d-SPE; tea

INTRODUCTION

As a product consumed in all parts of the world, tea from the plant of *Camellia sinensis* is easy to find on grocery store shelves. There are many ways to classify teas: how they are processed (resulting in varieties of green tea, oolong tea, black tea, pu'er tea, etc.); by the region in which the tea was grown (Darjeeling tea, Ceylon tea, etc.); by its organic certification; and by additives (Earl Gray tea, jasmine tea, tea with chamomile flowers, etc.). The availability and cost of organically grown tea are often the limiting factor that contributes to the purchase decision when consumers are in their local market. Most tea farmers and producers may apply pesticides for crop protection and value protection. Local, regional, national, and global regulatory guidelines may proscribe certain pesticides for use on tea which, in theory, protects consumers from these compounds. Relying on these regulations for protection, however, requires implicit trust that the rules will indeed be followed. To monitor the tea-growing practices and to help enable public trust of organically certified tea products, analytical laboratories need reliable and practical pesticide residue analysis methods that are easily adapted for modern day analytical equipment. The primary goals of the work presented herein were to create an improved method based upon commercially available products, to use common analytical instrumentation that is found in most commercial laboratories, and to have acceptable validation parameters that would be useful for compliance to the myriad of regulatory agencies throughout the world.

Great strides have been made in method development of tea leaf analysis, and just in the past decade there have been many agricultural pesticide residue analysis methods that were developed for use on tea leaf, especially using GC instruments. In 2003, Seiji and Kazuhiro published a method for the determination of agricultural residues (ARs) using AR-class dependent SPE cleanup and then analysis by either GC-FPD, NPD, or ECD (flame photometric detector, nitrogen−phosphorus detector, and electron capture detector, respectively) (*1*). The instrument manufacturer Gerstel (*2*) reported analysis of ARs using their stir bar sorptive extraction and thermal desorption GC-MS technique. A similar study using the Pegasus GC time-of-flight MS (GC-TOFMS) was presented by LECO in cooperation with Gerstel in another application note in 2008 (*3*). Another instrument manufacturer, Thermo Scientific (*4*), was one of the first to report the application of the QuEChERS (quick, easy, cheap, effective, rugged, and safe) sample preparation approach to the analysis of ARs in green tea by ion trap GC-MS^n. The original unbuffered QuEChERS sample preparation method was reported by Anastassiades et al. in 2003 (*5*).

As one of the world's largest producers of tea, China has been active in the development of methods for the analysis of ARs in tea leaf. As a result, several bodies of work have been published in China with growing emphasis on lowering detection limits and increasing the number of analytes detected. Recent work in 2005

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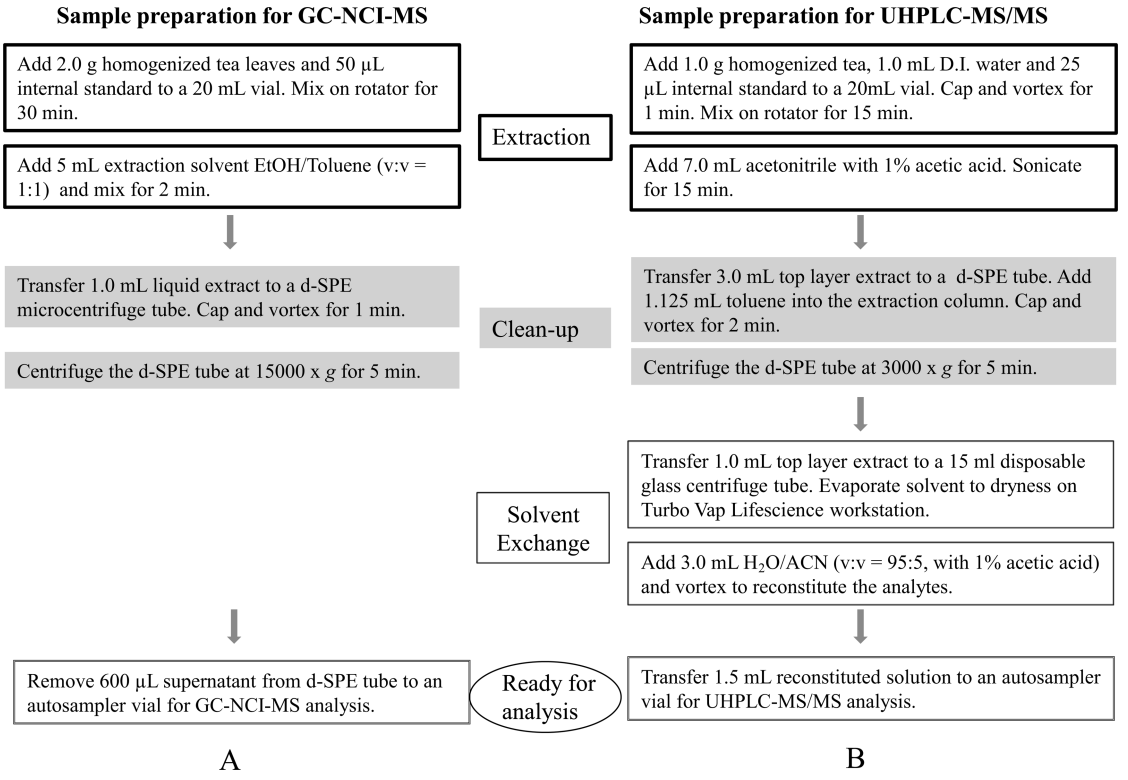

Figure 1. Flowchart of sample preparation for AR analysis by GC-NCI-MS (**A**) and UHPLC-MS/MS (**B**).

included response surface optimization for AR determination by matrix solid-phase dispersion and GC by Hu et al. (*6*). A 2006 paper from the Chinese Entry−Exit Inspection and Quarantine Bureau detailed the analysis of organochlorine residues using normal phase Florisil SPE for sample preparation and GC-MS for analyte detection (*7*). Concurrent to the previous work, Peng et al. from the Southern Yangtze University published their study of an interlaboratory validation using SPE and GC-MS for nine organic heterocyclic ARs (*8*). A third Chinese Entry−Exit Inspection and Quarantine Bureau in Shaoxing published a 2008 paper on the determination of ARs using sample preparation with accelerated solvent extraction (ASE), then gel permeation chromatography (GPC), and then Florisil SPE (*9*). The analytes included organophosphorous, organochlorine, and pyrethroid ARs that were quantified using GC-MS. An ambitious team collaborating with the Hunan Entry−Exit Inspection and Quarantine Bureau first published a paper in 2007 on the determination of 102 ARs in tea by GC-MS (*10*) and then followed that with a 2009 article on the analysis of 103 ARs in tea by LC-MS/MS (*11*). These papers indicate the importance of not only increasing the number of analytes in the panel of targets but also including ARs that are more suited to the use of LC-MS/MS as a detection methodology.

Important research has also come out of India as well. The work in India focused on the dissipation rates of pesticides on the tea leaf and the transfer of residues into tea brew (*12*−*16*). A recent LC-MS/MS method to monitor ARs in tea, tea infusion, and spent tea leaves was reported by Kanrar et al. (*12*). By focusing on LC-MS/MS methodology, the organochlorine ARs were not included in this study. Niessen has summarized AR analytes and their potential modes of detection with various LC-MS/MS ion sources (*17*). It is important to note that multiclass AR analyses will need both LC-MS/MS and some type of GC-MS instrumentation to fulfill the requirement set by regulatory agencies. Consequently, research in both areas is critical to the continuing evolution of AR detection on tea leaf.

Despite all of these advances, however, there are still considerable challenges for AR analysis on tea in daily industrial operations, which demand fast turnaround and cost-effectiveness in addition to greater sensitivity and reliability. Interferences from the complex matrix make sample extraction and cleanup not only expensive but also unacceptably lengthy. On the sensitivity and reliability side, the complicated background prevents quantification of some very important target analytes at low levels. The ARs we listed in this research have been carefully selected on the basis of tea-related pesticide regulations by the Euroepan Union (EU), Japan, and Codex. This method uses UHPLC-MS/MS in the positive electrospray mode, which is sensitive for all of the ARs except the organochlorine residues. We used GC-NCI-MS as a more selective, but still easy to implement, detection method for the organochlorine and halogen-containing pyrethroid ARs (*18*). The results presented below will cover the flexible analysis of multiclass ARs using GC-NCI-MS and UHPLC-MS/MS. Typical LODs were in the low parts per billion (μg/kg) range with good analyte recoveries and linearity. The method was then applied to a selection of commercial teas purchased at local markets in the Atlanta, GA, area. The presented method is easily expandable to additional ARs, can be adapted to newer MS models of instrumentation, and is well-suited to meet the regulations for multiclass ARs.

MATERIALS AND METHODS

Materials and Standards Preparation. HPLC grade acetonitrile (ACN) was purchased from Riedel-de Haën (Muskegon, MI); ethanol (EtOH) and methanol (MeOH) were from Sigma-Aldrich (St. Louis, MO); ACS grade toluene and reagent grade acetic acid were from J. T. Baker (Phillipsburg, NJ); and ammonium formate was from Fluka/Sigma-Aldrich (St. Louis, MO). Four deuterium isotope labeled internal standards for UHPLC-MS/MS analysis were purchased from CDN isotopes (Pinte-Claire, Quebec, Canada). DI water was provided on-site. Sorbents for the dispersive solid-phase extraction were obtained from United Chemical Technologies (UCT) (Bristol, PA).

Pesticide standards were purchased from Sigma-Aldrich (St. Louis, MO) and Chem Service (West Chester, PA). Pesticide stock solutions and internal standards were prepared in ACN with 0.1% acetic acid for GC-NCI/MS and with 1.0% acetic acid for UHPLC-MS/MS.

Commercial tea samples were purchased from local grocery stores (Atlanta, GA). Whole tea leaves were ground into small pieces (< 2 mm). For the quality control (QC) and calibration matrix, we selected a prescreened AR-free black tea.

Sample Preparation for GC-NCI-MS. Figure 1A contains the sample preparation procedure for GC-NCI-MS: weigh 2.00 ± 0.05 g of homogenized tea sample into a 20 mL Wheaton vial with screw cap; add 50 μL of internal standard (2,4,6-trichloroanisole and flucythrinate, each 5 μg/mL); cap and mix on a rotator for 30 min; to extract, add 5 mL of EtOH/toluene (v/v = 1:1) and vortex for 2 min; transfer 1.0 mL of liquid extract to a d-SPE tube (UCT ENVIRO-CLEAN extraction column, CUMPSCB2CT, 150 mg of $MgSO_4$, 50 mg of primary secondary amine (PSA), and 50 mg of graphitized carbon black GCB); cap and vortex the d-SPE tube for 1 min; centrifuge for 5 min at 15000*g*; transfer 600 μL of supernatant to an autosampler vial for GC-NCI-MS analysis. For QC test samples, 200 μL of QC standard (0.5 μg/mL endosulfan I, τ-fluvalinate, and tetradifon) was added to AR-free tea matrix in addition to the internal standards. For the calibration curve, 200 μL of calibration solution of various concentrations (0.1, 0.5, 1.0, 3.0, 5.0, and 10 μg/mL of ARs) was added to the AR-free tea matrix in addition to the internal standards. The corresponding amounts of the calibration points are 0.01, 0.05, 0.10, 0.30, 0.50, and 1.00 mg/kg.

GC-NCI-MS Analysis. Tea extracts were analyzed on an Agilent Technologies (Palo Alto, CA) 6890 GC equipped with an HP-5 MS column (Agilent Technologies, 0.25 μm film thickness, 30 m × 0.25 mm), a 7683B autoinjector, and a 5973N mass selective detector (MSD). Selective ion monitoring (SIM) was performed in the NCI mode with methane as the reagent gas at 40% flow (2 mL/min). Injector and interface temperatures were set at 250 and 280 °C, respectively. Helium was used as the carrier gas at 1.7 mL/min constant flow. Injection volume was 2 μL in the splitless mode. The column temperature was programmed as follows: starting at 70 °C and holding for 0.8 min; increasing at 15 °C/min to 150 °C and holding for 12 min; increasing at 5 °C/min to 200 °C and holding for 2.3 min; increasing at 20 °C/min to 260 °C and holding for 14.0 min; and finally ramping at 15 °C/min to 290 °C and holding for 9.0 min. The total run time was 58 min. The MSD operating parameters were set by the tune file. EM volts were set at tune +200 V, emission current at 160 μA, quadrupole temperature at 150 °C, ion source temperature at 200 °C, and transfer line temperature at 280 °C.

For quantification, the peak areas of the target ions (see **Table 1**) were first normalized with those of the internal standards, and the quantity was determined using the standard curve from matrix-matched calibration standards. Identification was established by retention time and one to three qualifier to target ion ratios, which were determined by injection of individual pesticide standards using full scan with *m*/*z* from 15 to 700 Da.

Sample Preparation for UHPLC-MS/MS. Figure 1B illustrates the sample preparation procedure for UHPLC-MS/MS: weigh 1.00 ± 0.05 g of homogenized tea sample into a 20 mL Wheaton vial with screw cap; add 1.0 mL of water and 25 μL of internal standard (methamidophos-d_6 and carbaryl-d_7, 4.0 μg/mL; fenthion-d_6, 40.0 μg/mL); mix on a rotator for 15 min; add 7 mL of ACN (with 1% acetic acid) and sonicate the mixture (Branson Ultrasonic Cleaner B52, 50/60 Hz, 240 W) for 15 min; transfer 3.0 mL of liquid extract to a d-SPE tube (UCT ENVIRO-CLEAN extraction column, ECMPSCB15CT, 900 mg of $MgSO_4$, 300 mg of PSA, and 150 mg of carbon black) and add 1.125 mL of toluene; cap and vortex the d-SPE tube for 2 min; centrifuge (RESEK Q-Sep 3000) for 5 min at 3000*g* and transfer 1.0 mL of supernatant to a 15 mL disposable glass centrifuge tube. The solution was evaporated to dryness with a nitrogen stream in a Caliper LifeSciences Turbo Vap LV with a 40 °C water bath for 20 min. The residue was reconstituted in 3.0 mL of water/ACN (v/v = 95:5 with 1% acetic acid), and 1.5 mL of reconstituted solution was transferred to an autosampler vial for UHPLC-MS/MS analysis. For QC samples, add 50 μL of QC standard (5.0 μg/mL acephate, acetamiprid, azoxystrobin, hexaconazole) to AR-free tea matrix in addition to the internal standard and water. For the calibration curve, 50 μL of calibration solution at different concentrations (1.0, 2.0, 4.0, 6.0, and 10 μg/mL of ARs analyzed) was added to the AR-free tea matrix in addition to the internal standard and water. The corresponding amounts of the calibration points are 0.05, 0.10, 0.20, 0.30, and 0.50 mg/kg, respectively.

Table 1. Results for Pesticides Quantified by GC-NCI-MS

pesticide	ions[a] (*m*/*z*)	recovery[b] (%)	LOD[c] (μg/kg)	RSD[d] (%)
4,4-DDE	35, 37	101	0.11	2.06
bifenthrin	205, 386, 241	111	0.20	4.61
chlorfenapyr	349, 347, 351, 350	104	0.03	2.25
chlorpyrifos	313, 315, 212, 214	103	0.02	1.65
cyfluthrin, total	207, 209, 171	99	0.32	1.80
cyhalothrin, λ	241, 205, 243	102	0.08	4.22
cypermethrin, total	207, 209, 171	100	0.59	2.03
deltamethrin	79, 81, 297, 217	105	0.14	5.16
dichlorvos	125, 35, 37	109	4.35	2.49
endosulfan I	35, 37, 242, 406	101	0.30	1.84
endosulfan II	35, 406, 408, 404	103	0.23	3.31
endosulfan sulfate	386, 388, 384, 97	100	0.07	2.54
EPN	138, 154, 323	101	0.09	2.79
fenpropathrin	141, 142	106	0.09	1.73
fenvalerate	211, 213, 167, 212	102	0.24	1.62
flufenoxuron	341, 321, 295, 249	88	2.33	8.21
hexachlorobenzene	284, 286, 282, 288	71	0.05	3.11
kelthane	250, 252, 251, 254	107	0.09	1.94
lindane	35, 71, 70, 37	88	0.27	2.08
lufenuron	147, 205, 313, 335	94	0.44	2.88
o,p-DDD	35, 37, 246, 248	104	0.25	2.23
p,p-DDD	35, 37, 71, 73	103	0.14	2.05
p,p-DDT	35, 37, 71, 73	101	0.23	2.44
paclobutrazol	293, 295, 166, 207	111	3.29	2.99
pentachlorophenol	35, 230, 230, 196	91	1.24	1.45
permethrin, total	207, 35	104	2.71	1.67
prochloraz	35, 161	88	0.38	5.70
quintozene	249, 251, 247, 265	92	0.18	2.13
S-421	35, 37, 70, 71	104	0.12	1.34
teflubenzuron	223, 224, 225, 227	77	0.29	6.42
tetradifon	320, 318, 322, 245	98	0.02	2.73
τ-fluvalinate	294, 296, 295	97	0.13	2.67

[a] SIM ions. The first one listed is used for quantification. [b] Recovery rate with ARs fortified at 100 μg/kg, *n* = 3. [c] LOD, limit of detection, *n* = 7, calculated as 3 times the standard deviation of the quantification results at low concentration levels. [d] RSD, relative standard deviation, *n* = 7.

UHPLC-MS/MS Analysis. UHPLC-MS/MS analysis was performed on a Waters ACQUITY ultraperformance liquid chromatographic system (Milford, MA) interfaced to a Quattro Premier XE tandem quadrupole mass spectrometer. Chromatographic separation was carried out using a Waters ACQUITY UPLC BEH phenyl analytical column (1.7 μm particle size, 2.1 × 50 mm). The mobile phase was 5 mM ammonium formate in deionized water (mobile phase A) and 5 mM ammonium formate in methanol (mobile phase B). Flow rate was 0.70 mL/min. The elution started at 5% B for 0.10 min, followed by B increasing linearly to 25% at 0.50 min, 30% at 1.00 min, 40% at 1.5 min, 60% at 2.0 min, 65% at 2.5 min, 75% at 3.0 min, 90% at 3.5 min, 99% at 4.0 min, 99% at 4.5 min, and 5% at 4.51 min. The total run time, including the conditioning of the column to the initial conditions, was 5.25 min. Injection volume was 10 μL. The autosampler component temperature was set at 5 °C and the column temperature at 40 °C.

MS was acquired in electrospray (ESI) positive ion mode using multiple reaction monitoring (MRM). The most abundant MS/MS transitions were monitored (**Table 2**). The MS source conditions were as follows: capillary voltage, 0.5 kV; extractor voltage, 4 V; RF lens, 1.0 V; source temperature, 150 °C; desolvation temperature, 350 °C; desolvation gas (N_2) flow, 1105 L/H; and cone gas (N_2) flow, 2 L/H. MS/MS conditions were optimized for each pesticide (**Table 2**).

Quantification was based on the peak areas of the quantitative MS/MS transition of the analyte normalized to those of the internal standards and the standard curve from matrix-matched calibration standards using Waters Quanlynx v4.1 software. Identification was established by the retention time and two pairs of MRM transitions (**Table 2**). Ion ratios of the primary (quantitative) transition and secondary (confirmative) transition were compared to the matrix-matched standards (20% limits on ion ratio).

Table 2. Results for Pesticides Quantified by UHPLC-MS/MS

pesticide	RT[a] (min)	MS/MS[b] ion transition (*m*/*z*)	CV[c] (V)	CE[d] (eV)	int std[e]	recovery[f] (%)	LOD[g] (μg/kg)	RSD[h] (%)
acephate	0.55	183.8 > 142.7 (48.9)	17	7 (19)	me	92	0.59	4.17
acetamiprid	1.61	222.9 > 125.7 (55.9)	28	21 (16)	ca	98	1.49	3.43
azoxystrobin	2.74	404.1 > 372.0 (343.9)	20	15 (25)	ca	79	2.97	8.76
boscalid	2.63	342.8 > 306.9 (139.8)	31	18 (17)	ca	74	5.40	12.5
buprofezin	3.35	306.1 > 201.0 (115.8)	20	11 (17)	ca	87	1.16	4.05
carbendazim	1.39	192.0 > 160.1 (132.0)	25	15 (30)	fe	43	3.75	14.0
carbofuran	2.07	222.1 > 164.9 (123.0)	20	10 (20)	ca	104	0.96	5.40
diethofencarb	2.43	268.0 > 225.9 (123.8)	14	9 (31)	ca	95	2.09	5.10
difenoconazole	3.29	405.8 > 250.8 (110.7)	33	25 (57)	fe	80	14.7	19.2
dimethoate	1.39	229.8 > 198.8 (124.7)	18	8 (21)	ca	112	1.51	5.67
ethiofencarb	2.20	225.9 > 106.8 (169.1)	15	15 (5)	ca	96	3.48	3.83
ethion	3.48	384.9 > 198.8 (96.7)	24	11 (43)	fe	104	8.01	19.9
fenazaquin	3.56	307.1 > 161.0 (146.9)	30	15 (20)	fe	81	18.7	18.4
fenhexamid	2.64	301.8 > 96.7 (54.9)	37	24 (39)	ca	78	3.93	7.23
fenthion	3.00	278.9 > 169.0 (105.1)	30	20 (25)	fe	71	17.0	27.0
fipronil	2.87	436.7 > 367.8 (254.8)	33	18 (32)	ca	62	9.40	15.6
flusilazole	2.94	316.0 > 247.0 (164.9)	35	18 (25)	fe	69	4.32	16.0
hexaconazole	2.99	314.1 > 70.0 (158.9)	30	20 (25)	ca	80	5.43	14.0
imidacloprid	1.43	255.9 > 208.9 (174.9)	22	17 (18)	ca	94	3.37	5.27
isocarbophos	2.35	230.8 > 120.7 (64.9)	36	19 (37)	ca	104	1.41	4.31
methamidophos	0.42	142.0 > 93.9 (124.8)	20	15 (15)	me	92	3.41	4.92
methomyl	0.87	162.9 > 88.1 (106.1)	10	10 (10)	me	102	1.42	5.44
monocrotophos	0.94	224.0 > 126.8 (97.7)	20	15 (13)	ca	101	1.23	6.01
omethoate	0.66	213.9 > 182.7 (124.7)	21	10 (22)	me	92	0.51	3.15
pirimicarb	2.25	239.0 > 181.9 (71.9)	27	16 (22)	ca	104	1.31	5.27
profenofos	3.28	372.8 > 302.9 (345.0)	30	20 (15)	fe	86	1.62	20.1
propamocarb	0.90	189.0 > 102.0 (74.1)	25	15 (25)	me	13	1.78	9.31
propargite	3.52	368.1 > 231.1 (174.9)	18	11 (11)	fe	97	4.07	19.6
pyridaben	3.66	365.0 > 308.9 (146.9)	22	12 (26)	fe	107	20.4	20.6
pyrimethanil	2.39	199.8 > 106.7 (81.6)	43	22 (27)	fe	72	5.43	13.4
quinalphos	2.90	298.9 > 162.8 (146.8)	28	23 (25)	fe	81	8.46	15.6
tebuconazole	2.92	308.1 > 70.1 (125.0)	30	20 (20)	ca	77	2.23	8.90
tebufenozide	2.83	353.0 > 132.7 (296.8)	11	20 (7)	ca	78	3.12	5.38
triadimenol	2.63	296.0 > 70.1 (99.1)	15	10 (15)	ca	91	12.9	12.3
triazophos	2.78	314.0 > 161.8 (118.9)	30	21 (35)	ca	76	4.02	10.9
trichlorfon	1.19	256.6 > 108.7 (220.6)	24	18 (11)	ca	100	2.45	5.57
methamidophos-d_6	0.44	148.0 > 97.0	20	15		internal standard		
carbaryl-d_7	2.11	209.0 > 152.1	15	10		internal standard		
fenthion-d_6	2.94	284.9 > 169.0	30	20		internal standard		

[a] RT, retention time. [b] The first pair of transition ions is primary and used for quantification. The number in the parentheses is the second product ion monitored from the same precursor ion. [c] CV, cone voltage. [d] CE, collision energy. The first number is collision energy of the primary transition. The number in parentheses is that of the secondary transition. [e] Int std, internal standard: "me" stands for methamidophos-d_6; "ca" for carbaryl-d_7; and "fe" for fenthion-d_6. [f] Recovery rate with ARs fortified at 200 μg/kg, $n = 3$. [g] LOD, limit of detection, $n = 7$, calculated as 3 times the standard deviation of the quantification results at low concentration levels. [h] RSD, relative standard deviation, $n = 7$.

RESULTS AND DISCUSSION

Sample Preparation Development. To analyze ARs with different properties, we utilized both GC-NCI-MS and UHPLC-MS/MS as certain compounds can be quantified at reasonable levels only by GC-NCI-MS, whereas others only by UHPLC-MS/MS. **Figure 1** contains sample preparation procedures for both GC-NCI-MS and UHPLC-MS/MS.

For GC-NCI-MS (**Figure 1A**), we tested various solvents and solvent combinations for extraction with different chemical properties. Among those tested, which included acetonitrile, ethanol, hexanes, isopropanol, methanol, and toluene, the mixture of ethanol and toluene (50:50, v/v) provided the best overall extraction efficiency. Other than ARs, the extracts contain many other components, such as pigments, lipids, and alkaloids, which can severely interfere with AR analysis. To remove undesirable components and reduce sample complexity, we employed d-SPE. A UCT ENVIRO-CLEAN extraction column (CUMPSCB2CT, 150 mg of $MgSO_4$, 50 mg of PSA, and 50 mg of GCB) proved to be efficient and cost-effective.

For UHPLC-MS/MS (**Figure 1B**), we first homogenized the tea products, which enabled a smaller sample size (1.0 g) and less solvents and reagents and improved extraction efficiency. The pre-extraction wetting with water was found to be critical for effective extraction of certain polar ARs. Several reported buffered and unbuffered QuEChERs methods were tested for sample preparation. We studied a variety of solvents for extraction, such as acetonitrile, methanol, and isopropanol, and multiple d-SPE cleanup methods, including C18, PSA, GCB, $MgSO_4$, and their combinations. These conditions yielded extracts from clear solutions to dark-colored and cloudy suspensions. They, however, suffered one or more of the following drawbacks, which rendered them ineffective as routine methods: low recovery rate, complex and high background, and lengthy process time. After extensive optimization, we employed acetonitrile (with 1% acetic acid to stabilize basic ARs) as the extraction solvent and the UCT ENVIRO-CLEAN extraction column (ECMPSCB15CT, 900 mg of $MgSO_4$, 300 mg of PSA, and 150 mg of GCB) for cleanup. This procedure removed most pigments and other undesirable matrix while producing generally high recovery rate. GCB, however, negatively affected the recovery of planar pesticides, such as carbendazim. To solve this problem, toluene was added at an 8:3 (ACN/toluene, v/v) ratio during cleanup as noted by Zhao and Stevens (*19*). Compared to cartridge-based cleanup methods

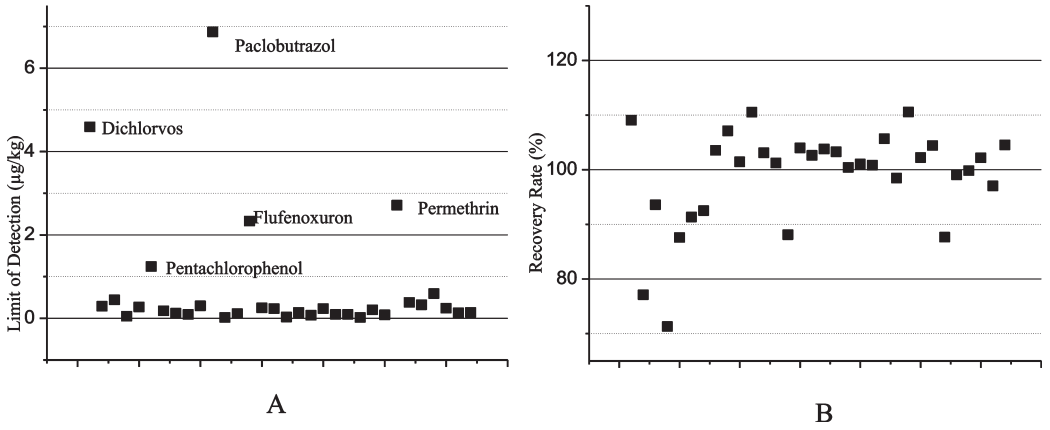

Figure 2. Results of AR analysis in tea using GC-NCI-MS. The LODs of most analytes were below 1 μg/kg (**A**). The recovery rate was between 70 and 120% (**B**). The five analytes with higher LODs (between 1 and 7 μg/kg) are labeled with their names.

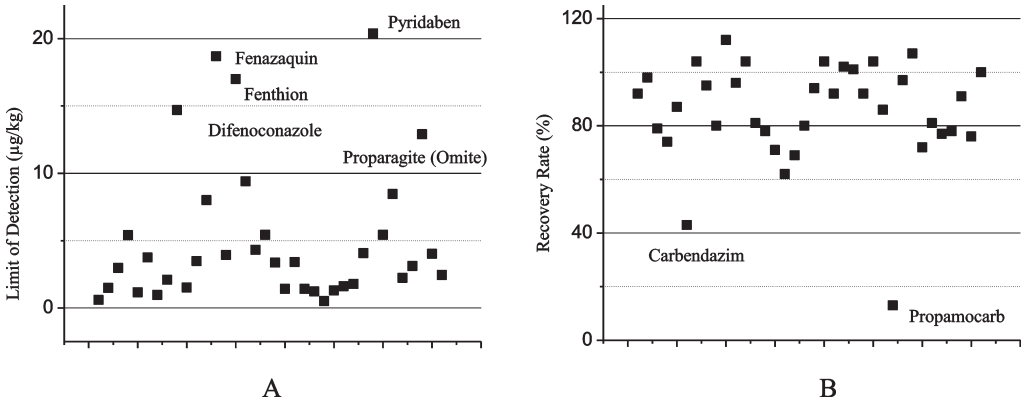

Figure 3. Results of AR analysis in tea using UHPLC-MS/MS. LODs of most analytes were below 10 μg/kg (**A**). The five analytes with higher LODs (between 10 and 22 μg/kg) are labeled with their names. The recovery rate for most analytes was between 70 and 120%, with the exceptions of carbendazim and propamocarb (**B**).

our d-SPE-based procedure significantly decreased the reagents consumed, saved processing time, and minimized the cost, thereby making it possible to be utilized in industrial settings, which demand large numbers of samples to be analyzed in short turnaround times. The cleaned extract was reconstituted in 3.0 mL of water/acetonitrile (95:5 (v/v) with 1% acetic acid) for UHPLC-MS/MS analysis. The 95:5 ratio was decided to be optimal because it efficiently dissolved the ARs and gave the best peak shape for all pesticides monitored. A 10 μL sample was injected for each analytical run. Normally, smaller injection volumes were used to avoid overloading the column. Because the concentrations of ARs present in the samples were low and the extracts were clean, column overloading was not a major concern.

Method Evaluation. GC-NCI-MS has been reported for the identification and quantification of low levels of pollutants with high confidence (*20*). It has been applied for pesticide analysis in complex matrices such as plasma, serum, and human fluids (*21*−*23*). Initially, we used GC-MS with electron impact (EI) ionization to analyze the ARs in tea samples. The resulting mass spectra provided useful fragmentation ions for identification. However, the limit of detection (LOD) suffered from matrix interference, and the EI SIM did not meet the requirements of regulatory parameters. NCI-MS was tested and selected for its higher sensitivity and better accuracy. The experimental results are listed in **Table 1**. The LODs were calculated as 3 times the standard deviation (n = 7) of the pesticide quantitative results at low concentration levels. The LODs of most pesticides were below 1 μg/kg. Those with high LODs are labeled with their names in **Figure 2A**. The recovery rates of 32 ARs fortified at 100 μg/kg were between 70 and 120%, as listed in **Table 1**. The relative standard deviations (RSDs) were < 10% (n = 7). The calibration curve ranged from 10 to 1000 μg/kg with $R^2 \geq 0.996$ for all compounds.

The cone voltage for each pesticide was optimized to achieve the precursor ion with the highest sensitivity using direct infusion of individual pesticide for UHPLC-MS/MS. Several ionization methods that include ESI, APCI, and APPI were evaluated. Although ESI was found to be the most suitable, other ionization techniques had capabilities that are worth noting. In some cases APCI provided better ionization compared to ESI. APPI was found to have less noise and lower baselines for a limited number of compounds, although it did not perform as well for the majority of targets. ESI was finally selected for its overall better sensitivity and lower LODs. The experimental results are listed in **Table 2**. The LODs of most pesticides analyzed were below 10 μg/kg. Five pesticides with LODs between 10 and 20 μg/kg are labeled with their names (**Figure 3A**). The recovery rates of 36 ARs fortified at 200 μg/kg are listed in **Table 2**. For most compounds the recovery was in the range of 70−120% (**Figure 3B**). For all but one compound, fenthion, the RSDs were ≤20% (n = 7). The linearity range of most calibration curves was from 50 to 500 μg/kg with $R^2 \geq 0.990\%$.

On the basis of the LOD data, the limits of quantification (LOQs, calculated as 3 times the LODs) of the method developed

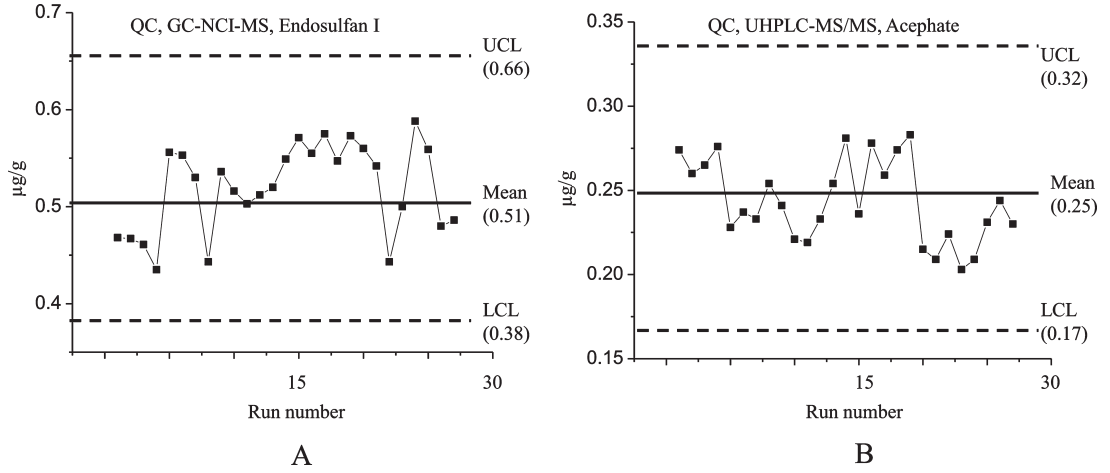

Figure 4. Control chart of endosulfan I (**A**) and acephate (**B**). Data shown were acquired on different dates over 2 months. Upper control limit (UCL) and lower control limit (LCL) were calculated as mean plus and minus three standard deviations. Target values were 0.5 mg/kg endosulfan I and 0.25 mg/kg acephate. All of the measured values were within the acceptable range.

Table 3. Analysis Results of Commercial Tea Samples Purchased from Grocery Stores

tea ID	geographic origin listed on package	tea type	organic tea	tea bag	price (U.S. cents/g)	total ARs (μg/kg)
1	China	pu'er		yes	2	12
2	India	black		yes	11.7	493
3	Pakistan	black			1.1	51
4	Argentina	green		yes	9.3	10
5	Sri Lanka	black	yes	yes	10	nd[a]
6	Japan	green		yes	10	245
7	Indonesia	black		yes	4	163
8	India	black			13	183
9	Taiwan	green			4.3	1684
10	China	green (with bergamot oil)	yes	yes	18.4	1159
11	Taiwan	oolong		yes	11.3	1358
12	China	pu'er		yes	2	383
13	India	black	yes	yes	9.5	9
14	China	white		yes	20	15
15	China	oolong		yes	7.1	265
16	China	white			10	350
17	India	black	yes	yes	25.6	35
18	Japan	green	yes	yes	11.4	48
19	Bangladesh	black	yes	yes	27.1	4
20	Bangladesh	white	yes	yes	30.2	2
21	China	green	yes		3	nd
22	USA	black		yes	3.1	89
23	Japan	green	yes	yes	16.6	16
24	China	oolong		yes	9.2	632
25	China	green (with jasmine)		yes	25.6	1
26	unknown	black	yes	yes	2.5	nd
27	China	pu'er (with herbal)			2.6	17

[a] nd, not detected.

were much lower than the maximum residue limits (MRLs) established by Japanese, EU, and Codex regulations. **Figure 4A** contains the plot of endosulfan I, which was one of the three QC compounds of GC-NCI-MS and is being targeted for a global ban. **Figure 4B** contains the similar plot for acephate, a representative of four QC compounds in UHPLC-MS/MS analysis. The data were collected over a period of 2 months, which reflects both intraday and interday variations. The QC results proved that our method was reliable and precise. It is worth noting that both our GC-MS (Agilent 6890N-5973N) and UHPLC-MS/MS (Waters Micromass Quattro Premier XE tandem quadrupole mass spectrometer) are not the most current models, and significant improvement of LODs should be expected on state-of-the-art instruments.

Commercial Tea Sample Analysis. We tested 27 commercial tea samples from local grocery stores for ARs. There are six kinds of tea based on the respective processing of the tea leaves: black, green, white, yellow, oolong, and pu'er. We tested five types except the yellow tea, which we could not find in the local stores we visited. Most of the teas came in premeasured bagged units of 1−2 g. The tea samples included nine black teas, nine green teas, three oolong teas, three white teas, and three pu'er teas. **Table 3** summarizes the information and the total amount of ARs detected. The amount of total ARs for each tea sample is shown in **Figure 5A**. Oolong tea, a type of partially oxidized tea, happened to have relatively higher AR level among the samples we analyzed. All three oolong tea samples had significant amounts of ARs. Green tea samples had a wide range of ARs. We detected high

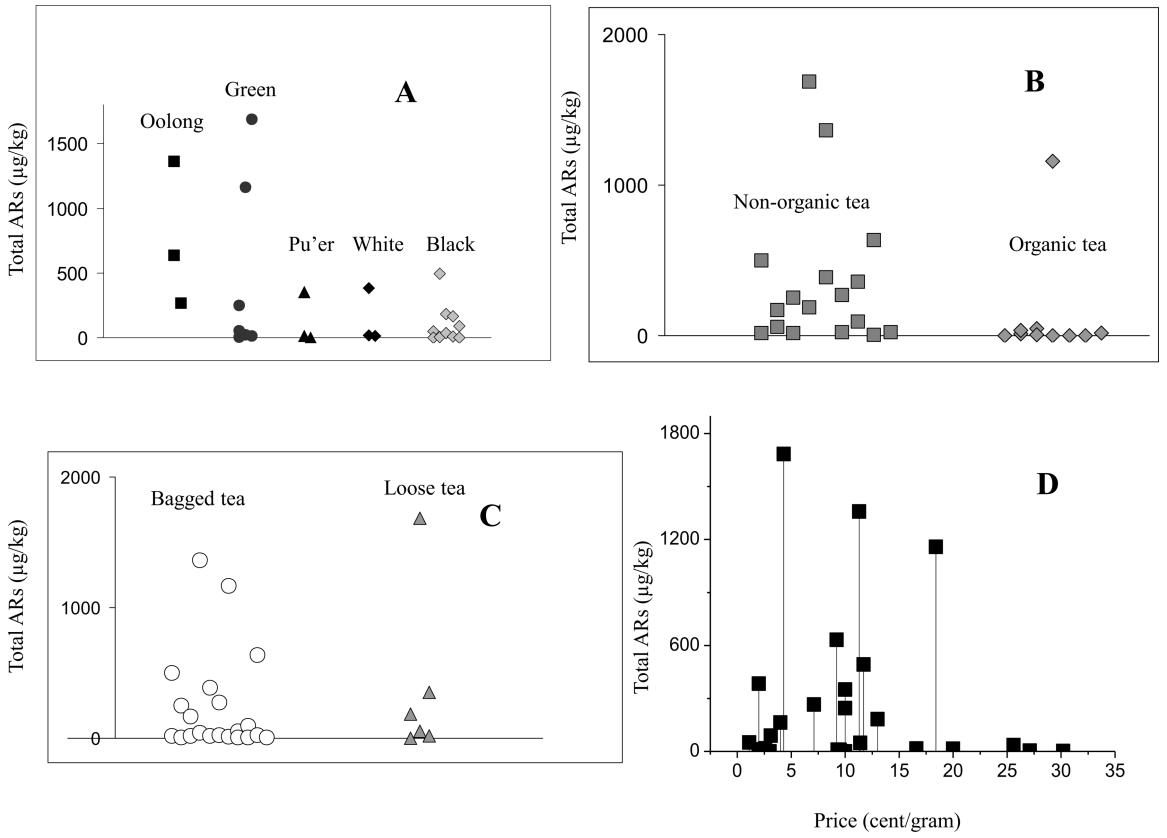

Figure 5. Analysis results of commercial teas. The total ARs detected in different types of tea (**A**) are illustrated. The tested organic teas were generally cleaner (**B**). AR amounts in bagged tea are similar to those in loose teas (**C**). There was no clear relation between AR levels and prices among the samples we tested (**D**).

levels of ARs from two of the nine samples studied. Pu'er, white, and black teas had similar amounts of ARs at the lower end. Of the 27 teas we analyzed, 10 were labeled as "organic" (8 as "USDA organic"). The ARs found in organic tea, with one exception, were considerably less than in its counterpart (average amount of total ARs was 350 μg/kg) (**Figure 5B**). Three samples without detectable ARs were all organic labeled. The total ARs of most organic tea were < 50 μg/kg. However, sample 10, labeled as USDA organic, had total ARs of 1159 μg/kg, which is even higher than most nonorganic. We did notice some nonorganic labeled tea was also quite clean with low total ARs. Commercial tea comes as either individual small tea bags or loose tea. As illustrated in **Figure 5C**, there was no obvious difference between bagged or loose tea among the samples we purchased. This suggests the total AR amount difference can be caused by other factors or their combinations such as sources (company/brands) and tea categories rather than the package. Finally, we compared the price of tea samples and their AR levels. There was no direct relationship as shown in **Figure 5D**. This indicates that tea pricing is based on many other factors. These factors could be brand, packaging, origin, and processing methods.

For the 68 ARs analyzed, some pesticides had been detected more frequently than others (**Table 4**). Endosulfan sulfate and kelthane, the most frequently found, were detected in 15 of 27 tea samples. Both of them are organochlorine pesticides, have moderate solubility in water, and have been reported to remain on tea plants (*12*, *13*). Among pesticides analyzed by UHPLC-MS/MS, imidacloprid and acetamiprid were the most commonly detected, from 12 and 11 of the 27 samples, respectively. They are commonly used neonicotinoid insecticides and have been frequently found in tea products.

Table 4. Commonly Detected ARs from 27 Tea Samples from Grocery Stores

compound name	no. of samples with positive result, of 27 total
endosulfan sulfate	15
PP-kelthane	15
cypermethrin	12
endosulfan I	12
imidacloprid	12
acetamiprid	11
endosulfan II	11
cyhalothrin, λ	10
fenpropathrin	9
chlorpyrifos	8
p,p-DDT	8

Regulatory Perspective. Tea beverage is an increasingly integral part of popular culture around the world, and as such, the regulations that govern its production and use are becoming increasingly important. Globally, pesticide regulations on tea are not as comprehensive as they are with other commodities. In fact, there are currently only two major regions that have established firm pesticide guidelines for tea imports, the European Union and Japan. Codex lists tea tolerances on only 11 of its total 227 listed pesticides, roughly 5%. The United States has fewer maximum residue limits set on ARs for tea leaf, where no tolerance is regulated as zero tolerance. It is the EU's guidelines that are the most stringent, and with a significant share of China's tea imports going to the EU, this fact has serious implications not only for trade but also for compliance concerns. In 2001 the EU reduced tea tolerances by 100-fold, which significantly affected trade routes into the affected countries (*24*). Most EU tea tolerances are reduced to the limit of detection (LOD), which is typically

≤0.1 ppm. In 2009 the EU once again tightened restrictions by proposing a controversial ban of 22 pesticides. These types of restrictions along with the general lack of global guidelines for pesticide regulations on tea suggest a need for analytical methodology that will allow accurate reporting at very sensitive detection levels.

We have developed a quick, robust, sensitive, and cost-effective method for quantification of 68 ARs in tea samples, which can be easily expanded. This method is based on commercially available reagents and can be readily adapted by analytical laboratories with common instrumentation platforms. We for the first time reported that GC-NCI-MS can be effectively employed for the analysis of ARs on tea samples with simple d-SPE cleanup, and we optimized sample preparation and analytical conditions for UHPLC-MS/MS. Our new method yields low LODs and LOQs, good recovery rates, and wide linear ranges. The low cost, high reliability, and fast turnaround meet the requirements of daily operations in industrial settings. Starting from commonly available d-SPE tubes based on the very popular QuEChERS methodology, we have successfully adapted this new method to analyze ARs in various commercial tea samples available on the market.

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LITERATURE CITED

(1) Seiji, N.; Kazuhiro, H. Simultaneous determination method of pesticides in leaf of tea. *Annu. Rep. Fukuoka City Inst. Hyg. Environ.* **2003**, *28*, 160−164.

(2) Ochiai, N.; Sasamoto, K.; Kanda, H.; Yamagami, T.; Frank, D.; Pat, S. Multi-residue method for determination of 85 pesticides in vegetables, fruits and green tea by stir bar sorptive extraction and thermal desorption GC-MS. GERSTEL Global Analytical Solutions AppNote, 2004; 4.

(3) Libardoni, M.; Heim, J.; Ochiai, N.; Sasamato, K. Trace level organochlorine and organophosphorous pesticides analysis in green tea by SBSE-GC-TOFMS. LECO Life Science and Chemical Analysis Solutions, 2008; 3, form 203-821-337.

(4) Steiniger, D.; Lu, G.; Bulter, J.; Phillips, E.; Fintschenko, Y. Multiresidue pesticide analysis in green tea by a modified QuEChERS extraction and ion trap GC/MSn analysis, Thermo Scientific Technical Note 10295, 2009.

(5) Anastassiades, M.; Lehotay, S. J.; Stajnbaher, D.; Schenck, F. J. Fast and easy multiresidue method employing acetonitrile extraction/partitioning and "dispersive solid-phase extraction" for the determination of pesticide residues in produce. *J. AOAC Int.* **2003**, *86*, 412−431.

(6) Hu, Y.; Zheng, P.; He, Y.; Sheng, G. Response surface optimization for determination of pesticide multiresidues by matrix solid-phase dispersion and gas chromatography. *J. Chromatogr., A* **2005**, *1098*, 188−193.

(7) Zhao, Q.; Jin, B.; Xie, L.; Wu, W.; Lan, F.; Lin, L.; Han, R. Determination of 25 organochlorine pesticides in tea by gas chromatography−mass spectrometry. *Chin. J. Chromatogr.* **2006**, *24* (6), 629−632.

(8) Peng, C.; Kuang, H.; Li, X.; Xu, C. Evaluation and interlaboratory validation of a GC-MS method for analysis of pesticide residues in tea. *Chem. Pap.* **2007**, *61*, 1−5.

(9) Hu, B.; Song, W.; Xie, L.; Shao, T. Determination of 33 pesticides in tea by accelerated solvent extraction-gel permeation and solid-phase extraction purification−gas chromatography−mass spectrometry. *Chin. J. Chromatogr.* **2008**, *26* (1), 22−28.

(10) Huang, Z.; Li, Y.; Chen, B.; Yao, S. Simultaneous determination of 102 pesticide residues in Chinese teas by gas chromatography−mass spectrometry. *J. Chromatogr., B* **2007**, *853*, 154−162.

(11) Huang, Z.; Zhang, Y.; Wang, L.; Ding, L.; Wang, M.; Yan, H.; Li, Y.; Zhu, S. Simultaneous determination of 103 pesticide residues in tea samples by LC-MS/MS. *J. Sep. Sci.* **2009**, *32*, 1294−1301.

(12) Kanrar, B.; Mandal, S.; Bhattacharyya, A. Validation and uncertainty analysis of a multiresidue method for 42 pesticides in made tea, tea infusion and spent leaves using ethyl acetate extraction and liquid chromatography−tandem mass spectrometry. *J. Chromatogr., A* **2010**, *1217* (12), 1926−1933.

(13) Manikandan, N.; Seenivasan, S.; Ganapathy, M. N. K.; Muraleedharan, N. N.; Selvasundaram, R. Leaching of residues of certain pesticides from black tea to brew. *Food Chem.* **2009**, *113*, 522−525.

(14) Tewary, D. K.; Kumar, V.; Ravindranath, S. D.; Shanker, A. Dissipation behavior of bifenthrin residues in tea and its brew. *Food Control* **2005**, *16*, 231−237.

(15) Seenivasan, S.; Muraleedharan, N. N. Residues of λ-cyhalothrin in tea. *Food Chem. Toxicol.* **2009**, *47*, 502−505.

(16) Sood, C.; Jaggi, S.; Kumar, V.; Ravindranath, S. D.; Shanker, A. How manufacturing processes affect the level of pesticide residues in tea. *J. Sci. Food Agric.* **2004**, *84*, 2123−2127.

(17) Niessen, W. M. A. Group-specific fragmentation of pesticides and related compounds in liquid chromatography−tandem mass spectrometry. *J. Chromatogr., A* **2010**, *1217* (25), 4061−4070.

(18) Tagami, T.; Kajimura, K.; Satsuki, Y.; Nakamura, A.; Okihashi, M.; Kitagawa, Y.; Takatori, S.; Kitagawa, M. Simultaneous analysis of 10 pyrethroid pesticides in natural medicines by GC/MS with negative chemical ionization. *Yakugaku Zasshi* (*Pharm. Soc. Jpn.*) **2006**, *126* (10), 991−995.

(19) Zhao, L.; Stevens, J. Optimizing recoveries of planar pesticides in spinach using toluene and the AOAC QuEChERS kits with graphitized carbon. *123rd AOAC International Annual Meeting*; 2008; p-T-141.

(20) Rivera-Rodrígues, L.; Rodrígues-Estrella, R.; Ellington, J. J.; Evans, J. J. Quantification of low levels of organochlorine pesticides using small volumes (≤100 μL) of plasma of wild birds through gas chromatography negative chemical ionization mass spectrometry. *Environ. Pollut.* **2007**, *148*, 654−662.

(21) Aprea, C.; Colosio, C.; Mammone, T.; Minoia, C.; Maroni, M. Biological monitoring of pesticide exposure: a review of analytical methods. *J. Chromatogr., B* **2002**, *769*, 191−219.

(22) Conka, K.; Drobná, B.; Kocan, A.; Petrík, J. Simple solid-phase extraction method for determination of polychlorinated biphenyls and selected organochlorine pesticides in human serum. *J. Chromatogr., A* **2005**, *1084*, 33−38.

(23) Covaci, A.; Schepens, P. Simplified method for determination of organochlorine pollutants in human serum by solid-phase disk extraction and gas chromatography. *Chemosphere* **2001**, *43*, 439−447.

(24) Heim, J.; Libardoni, M.; Ochiai, N.; Sasamoto, K. Trace-level organochlorine and organophosphorus pesticides analysis by SBSE-GC-TOFMS and SBSE-GCxGC-TofMS. *LC−GC Chromatogr. Online* **2008**, March.

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