JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

pubs.acs.org/JAFC

Development and Validation of a Method for the Determination of 159 Pesticide Residues in Tobacco by Gas Chromatography−Tandem Mass Spectrometry

Xiaoshui Chen, Zhaoyang Bian, Hongwei Hou, Fei Yang, Shanshan Liu, Gangling Tang,*,‖ and Qingyuan Hu*,‖

China National Tobacco Quality Supervision and Test Center, No. 2 Fengyang Street, Zhengzhou High and New Technology Industries Development Zone, Zhengzhou 450001, China

Supporting Information

ABSTRACT: A multiresidue gas chromatography−tandem mass spectrometry (GC-MS/MS) method was developed for the analysis of 159 multiclass pesticides in tobacco. A modified QuEChERS sample preparation technique, based on acetonitrile extraction and toluene dilution, followed by dispersive solid-phase extraction (d-SPE) cleanup using primary−secondary amine (PSA) and octadecyl (C18) sorbents, was used for sample treatment. Key performance parameters investigated were linearity, recovery, relative standard deviation (RSD), limit of detection, and limit of quantitation. With the exception of chinomethionate and folpet, recoveries for pesticides ranged from 69 to 141%, and the RSDs ranged from 2 to 27%. The validated method was applied to the analysis of 118 real samples, and positive results were obtained for 116 samples, with 25 different pesticides being detected.

KEYWORDS: *tobacco, pesticide residues, QuEChERS, gas chromatography−tandem mass spectrometry (GC-MS/MS)*

INTRODUCTION

Nowadays, pesticides are widely applied in the cultivation and storage of vegetables, fruits, and other related commodities such as tea and tobacco. However, pesticide residues in agricultural products are frequently detected, and they may have adverse effects on human health.[1,2] To protect the consumer and control pesticide residue levels, some government agencies and international organizations have set maximum residue limits (MRLs) for many pesticide compounds and commodities. Guidance residue levels (GRLs)[3] of 118 pesticides in tobacco have been issued by the Agro-Chemical Advisory Committee (ACAC) of Cooperation Center for Scientific Research Relative to Tobacco (CORESTA). The 2008 GRL list contains different classes of pesticides, such as organochlorine, organophosphorus, and pyrethroids. At the same time, sensitive and selective multiresidue analytical methods are needed to satisfy the demand for monitoring pesticide residues at low concentration levels in agricultural produce.

Multiresidue methods have been applied widely for analysis of pesticides in diverse matrices such as vegetables[4−6] and fruits.[7,8] The simple, high-throughput and low-cost QuEChERS (quick, easy, cheap, effective, rugged, and safe) sample preparation method is now widely accepted since it was first published[9] 10 years ago. Moreover, the QuEChERS method, when coupled to gas chromatography−tandem mass spectrometry (GC-MS/MS) or liquid chromatography−tandem mass spectrometry (LC-MS/MS), has been successfully used for the determination of more than 100 pesticides in many different matrices.[10−12]

Tobacco is considered a challenge for multiresidue analysis. On the one hand, recoveries for most pesticides need to be in the range of 70−120%. On the other hand, the amount of coextractives needs to be as little as possible to minimize matrix effects and related instrumental problems. Until recently, only a few papers related to pesticide detection in tobacco have been published. They involve mainly gas chromatography (GC),[13] high-performance liquid chromatography (HPLC), GC-MS/MS,[14] and LC-MS/MS.[15,16] However, most of these methods require extensive sample treatment procedures such as pressurized liquid extraction and solid-phase extraction. Lee et al. compared three sample preparation methods, that is, liquid−liquid extraction, pressurized liquid extraction, and QuEChERS, for the determination of 49 pesticide residues in tobacco by GC-MS/MS.[17] In their work, the QuEChERS method performed best in terms of recovery and precision data, but like other existing methods, the number of pesticides tested for was quite limited.

In this paper, a simple and sensitive multiresidue method, using a modified QuEChERS method in combination with GC-MS/MS, was developed and validated to identify and quantify 159 multiclass pesticides in tobacco. The 159 GC-amenable pesticides were selected because of their presence in the CORESTA GRLs list and in the lists of pesticides banned or recommended for tobacco cultivation in China. The two main objectives of the present work were as follows: (1) to provide a critical comparison of methods that feature different buffer systems and different GC sample introduction steps and (2) to validate the multiresidue method and document performance

Received: February 25, 2013
Revised: May 21, 2013
Accepted: May 23, 2013
Published: May 23, 2013

ACS Publications © 2013 American Chemical Society

Table 1. Multireaction Monitoring (MRM) Parameters of the 159 Pesticides and Internal Standard (TPP) in GC-MS/MS Method

pesticide	t_R (min)	MRM transitions, m/z (collision energy, eV)	
		quantification	identification
acephate	9.53	136.0/93.9 (12)	136.0/42.1 (8)
acetamiprid	35.75	152.1/116.0 (15)	166.1/139.0 (9)
acibenzolar-*S*-methyl	17.93	182.0/181.0 (8)	182.0/153.0 (15)
alachlor	17.66	188.1/160.1(10)	160.1/132.1(12)
aldrin	20.81	262.9/192.9 (32)	262.9/227.9 (20)
azinphos-ethyl	39.28	160.0/132.0(5)	160.0/104.0(10)
azinphos-methyl	37.83	160.0/132.0(5)	160.0/104.0(10)
azoxystrobin	46.57	344.1/156.1(25)	344.1/329.1(15)
benalaxyl	33.42	234.1/174.1(10)	266.1/148.1(15)
benfluralin	12.17	292.1/264.1(10)	292.1/160.1(21)
bifenthrin	36.71	181.1/166.1(10)	181.1/153.1(9)
bitertanol	40.37,40.60	170.1/141.1(20)	170.1/115.1(25)
bromacil	19.72	205.0/188.0(15)	207.0/190.0(15)
bromophos	27.61	331.1/315.9(15)	331.1/285.8(25)
butralin	22.91	266.1/220.1(10)	266.1/190.1(15)
cadusafos	12.50	159.1/130.8(8)	159.1/96.9(15)
captafol	10.08	151.0/79.0(18)	151.0/122.0(10)
captan	25.54	149.0/70.0(12)	149.0/79.0(10)
carbaryl	17.89	144.1/115.1(25)	144.1/116.1(15)
carbofuran	13.40	221.1/164.1(5)	164.1/149.1(10)
3-hydroxycarbofuran	17.46	179.9/137.0(12)	136.9/106.9(10)
chinomethionate	26.72	234.0/206.0(10)	206.0/148.0(15)
chlorantraniliprole	36.47	278.0/249.0(20)	278.0/215.0(20)
chlordane (*cis*-)	27.81	372.8/265.9(15)	409.8/374.8(5)
chlordane (*trans*-)	26.79	372.8/265.9(15)	409.8/374.8(5)
chlordimeform	12.08	196.0/181.0(10)	181.0/140.0(15)
chlorfenvinphos (*E*)	24.59	267.0/159.0(15)	323.0/267.0(15)
chlorfenvinphos (*Z*)	25.51	267.0/159.0(15)	323.0/267.0(15)
chlornitrofen	33.38	317.0/287.0(10)	319.0/289.0(10)
chlorpyrifos (-ethyl)	20.93	313.9/258.0(15)	313.9/285.9(7)
chlorpyrifos-methyl	17.14	285.9/093.0(20)	285.9/270.9(15)
chlorthal-dimethyl	21.34	331.9/300.9(10)	300.9/222.9(20)
chorobenzilate	31.74	251.0/139.0(15)	253.0/141.0(15)
clomazone	13.82	125.0/89.0(18)	204.0/107.0(15)
cyfluthrin	41.81, 42.04, 42.15, 42.25	163.0/127.0(5)	206.0/151.0(20)
cyhalothrin	39.00	208.1/181.0(10)	197.0/141.0(13)
cypermethrin	42.46, 42.69, 42.79, 42.89	181.0/152.0(25)	163.0/127.0(10)
dazomet	13.19	162.0/89.0(8)	162.0/129.0(5)
DBCP	6.60	157.0/75.0(8)	157.0/77.0(8)
o,p′-DDT	32.19	234.9/165.0(20)	236.9/165.0(25)
p,p′-DDT	34.06	234.9/165.0(25)	236.9/165.0(20)
p,p'-DDD	32.10	237.0/165.0(25)	235.0/165.0(20)
o,p'-DDD	29.98	235.0/165.0(20)	235.0/199.0(13)
o,p′-DDE	27.29	246.0/176.0(30)	317.9/246.0(20)
p,p′-DDE	29.64	246.0/176.0(25)	248.0/176.0(25)
deltamethrin	46.12	253.0/93.0(13)	253.0/174.0(13)
demeton-*S*-methyl	11.53	87.9/60.0(5)	141.8/78.9(12)
demeton-*S*-methyl sulfone	18.71	168.8/125.0(8)	168.8/109.0(12)
demeto-O	11.34	143.0/115.0(10)	171.0/115.0(15)
demeto-S	13.17	143.0/115.0(10)	170.0/114.0(10)
diazinon	14.56	304.1/179.1(15)	179.1/137.1(15)
dichlorvos	8.08	185.0/93.0(12)	185.0/109.0(15)
dicloran	13.27	206.0/176.0(10)	208.0/178.0(10)
dieldrin	29.50	276.9/240.9(10)	276.9/206.9(20)
difenoconazole	45.27,45.43	325.1/267.0(15)	323.1/265.0(15)
diflubenzuron	7.13	153.0/091.0(20)	153.0/125.0(20)
dimefox	5.54	154.1/58.0(10)	154.1/111.1(15)
dimetachlone	22.04	243.0/187.0(10)	187.0/152.0(10)

dx.doi.org/10.1021/jf400887x | J. Agric. Food Chem. 2013, 61, 5746−5757

Table 1. continued

pesticide	t_R (min)	MRM transitions, m/z (collision energy, eV)	
		quantification	identification
dimethoate	13.22	125.0/79.0(10)	229.0/87.0(8)
dimethomorph (*E*)	47.79	301.1/165.1(15)	301.1/273.1(10)
dimethomorph (*Z*)	46.68	301.1/165.1(15)	301.1/273.1(10)
diphenamid	23.07	239.1/167.1(10)	167.1/165.1(20)
disulfoton	15.05	274.0/88.0(5)	274.0/245.0(5)
disulfoton sulfoxide	8.73	124.8/96.9(5)	152.9/96.9(10)
disulfoton sulfone	27.75	213.0/153.0(8)	213.0/125.0(10)
α-endosulfan	27.72	240.9/205.9(15)	264.9/192.9(17)
β-endosulfan	31.46	240.9/205.9(20)	195.0/159.9(10)
endosulfan-sulfate	33.60	273.9/238.9(15)	271.9/236.9(20)
endrin	30.76	262.9/192.9(26)	262.9/190.9(25)
EPN	36.41	169.0/141.0(10)	169.0/77.0(20)
ethion	32.32	231.0/129.0(20)	231.0/175.0(10)
ethoprophos	11.72	158.0/114.0(10)	158.0/130.0(5)
famoxadone	47.01	329.9/224.1(10)	329.9/237.1(10)
fenamiphos	28.87	303.1/288.1(10)	303.1/260.1(15)
fenamiphos sulfoxide	35.83	303.9/196.1(10)	303.9/234.1(10)
fenamiphos sulfone	36.06	319.9/292.0(10)	319.9/214.1(15)
fenchlorphos	18.33	284.9/269.9(13)	284.9/239.9(20)
fenitrothion	19.42	277.0/260.0(5)	277.0/109.0(20)
fensulfothion	31.77	293.0/97.0(20)	293.0/125.0(5)
fenthion	21.23	278.0/109.0(18)	278.0/169.0(15)
fenthion sulfoxide	31.68	278.8/108.9(15)	278.8/152.9(12)
fenthion sulfone	32.01	309.8/104.9(12)	309.8/135.9(15)
Σ fenvalerate	44.28,44.77	419.1/225.1(10)	419.1/167.1(10)
Σ flucythrinate	42.83,43.26	199.0/157.0(8)	199.0/107.0(20)
flumetralin	28.18	143.0/107.0(20)	143.0/108.0(20)
folpet	26.07	259.9/130.0(11)	259.9/95.0(15)
fonofos	14.45	246.0/137.0(10)	137.0/109.0(10)
formothion	16.09	126.0/93.0(8)	125.0/78.9(8)
HCH (α-)	12.87	218.9/182.9(10)	218.9/144.9(20)
HCH (β-)	13.73	218.9/182.9(10)	218.9/144.9(20)
HCH (δ-)	15.30	218.9/182.9(10)	180.9/144.9(15)
γ-HCH (lindane)	14.10	180.9/144.9(15)	180.9/109.0(25)
heptachlor	18.08	271.9/236.9(20)	269.9/234.9(12)
heptachlor epoxides (*cis*-)	24.73	354.8/264.9(15)	352.8/262.9(15)
heptachlor epoxides (*trans*-)	25.10	216.9/181.9(15)	288.9/218.9(15)
heptenophos	10.89	250.0/89.0(25)	250.0/124.0(5)
hexachlorobenzene	13.05	283.8/248.8(20)	283.8/213.9(25)
indoxacarb	45.92	203.0/133.9(12)	203.0/106.1(20)
iprobenfos	15.86	204.1/91.0(10)	204.1/121.9(15)
iprodione	36.17	314.0/245.0(10)	314.0/271.0(10)
isazophos	15.15	257.0/162.0(10)	257.0/119.0(20)
isopropalin	24.05	280.2/238.1(10)	280.2/180.1(15)
isoprothiolane	29.27	290.0/118.0(15)	290.0/204.0(5)
leptophos	37.90	377.0/362.0(20)	377.0/269.0(30)
malathion	20.42	173.0/99.0(15)	173.0/127.0(5)
metalaxyl	18.19	234.1/174.1(10)	206.1/132.1(25)
methamidophos	7.90	141.0/95.0(10)	141.0/80.0(15)
methidathion	26.84	145.0/85.0(10)	145.0/58.0(15)
methiocarb	19.56	168.1/153.1(10)	168.1/109.0(15)
methiocarb sulfone	18.88	199.9/121.0(15)	199.9/136.9(10)
methomyl	6.08	104.9/87.9(5)	104.9/58.0(12)
methoprene	27.39	152.9/110.9(10)	191.0/107.0(15)
methoxychlor	36.80	227.0/169.0(25)	227.0/212.0(15)
metolachlor	20.68	162.0/133.0(15)	238.0/162.0(12)
mevinphos	9.43	127.0/109.0(10)	192.0/127.0(12)
mirex	38.53	271.8/236.8(15)	269.8/234.8(15)
monocrotophos	12.28	192.1/127.0(10)	192.1/164.0(7)

dx.doi.org/10.1021/jf400887x | J. Agric. Food Chem. 2013, 61, 5746−5757

Table 1. continued

pesticide	t_R (min)	MRM transitions, m/z (collision energy, eV) quantification	identification
myclobutanil	29.98	179.0/125.0(15)	179.0/152.0(10)
napropamide	28.62	271.0/72.0(12)	271.0/128.0(8)
nitrofen	31.00	202.0/139.0(21)	283.0/253.0(10)
omethoate	11.13	156.0/109.9(8)	156.0/79.9(15)
oxadixyl	32.02	163.1/132.1(10)	163.1/105.1(20)
oxamyl	10.42	162.1/115.0(8)	162.1/145.0(8)
parathion (-ethyl)	21.60	291.0/109.0(15)	291.0/137.0(10)
parathion-methyl	17.49	263.0/109.0(15)	263.0/153.0(5)
penconazole	24.89	248.1/157.0(25)	248.1/192.0(15)
pendimethalin	24.41	252.1/162.1(12)	252.1/191.1(7)
permethrin (*cis*-)	40.60	183.0/153.0(15)	183.0/168.0(15)
permethrin (*trans*-)	40.90	183.0/153.0(15)	183.0/168.0(15)
phorate	12.65	121.0/65.0(10)	121.0/93.0(6)
phosalone	37.85	182.0/111.0(15)	182.0/138.0(10)
phosphamidon (*E*)	14.60	264.1/127.0(10)	264.1/193.0(10)
phosphamidon (*Z*)	16.61	264.1/127.0(10)	264.1/193.0(10)
piperonyl butoxide	35.45	176.0/131.0(12)	176.0/145.0(12)
pirimicarb	15.82	238.1/166.1(15)	166.1/96.1(10)
pirimiphos-methyl	19.42	290.1/233.1(10)	290.1/125.0(20)
profenofos	29.44	336.9/267.0(15)	336.9/309.0(8)
propoxur	11.34	152.1/110.1(10)	110.1/64.0(15)
prothiofos	29.15	308.8/238.9(15)	308.8/220.9(25)
pyrazophos	39.27	221.1/193.0(10)	221.1/177.0(15)
quinalphos	25.89	146.0/118.0(10)	146.0/91.0(20)
quizalofop-*P*-ethyl	42.76	299.0/192.0(26)	372.0/299.0(10)
schradan	13.42	243.0/153.0(10)	199.0/135.0(15)
teflubenzuron	9.06	197.0/135.0(25)	197.0/142.0(25)
tefluthrin	15.30	197.0/141.0(10)	197.0/161.0(10)
terbufos	14.27	231.0/175.0(15)	231.0/203.0(10)
terbufos sulfone	24.74	152.8/96.9(10)	198.8/142.9(10)
tetrachlorvinphos	27.65	330.9/109.0(17)	330.9/315.9(17)
tetradifon	37.57	356.0/229.0(12)	354.0/229.0(12)
thiamethoxam	23.45	212.0/139.0(10)	247.0/212.0(5)
thionazin	11.28	192.0/96.0(10)	248.1/140.0(10)
triadimefon	21.96	208.0/181.0(8)	208.0/127.0(12)
triadimenol	26.22,26.88	168.0/70.0(10)	128.0/100.0(10)
triazophos	33.09	161.0/134.0(10)	161.0/105.0(13)
trichlorfon	9.80	145.0/109.0(12)	109.0/79.0(10)
triflumuron	9.75	139.0/110.9(15)	139.0/75.0(25)
trifluralin	12.10	306.1/264.1(10)	306.1/160.1(20)
uniconazole	29.63	234.0/165.0(10)	234.0/137.0(15)
vamidothion	27.50	145.0/87.0(5)	145.0/112.0(5)
TPP	35.01	326.1/325.1(10)	326.1/233.1(10)

parameters such as linearity, recovery, matrix effects, limits of detection, and limits of quantitation. In addition, the developed method has been applied to the analysis of 118 tobacco samples.

MATERIALS AND METHODS

Reagents and Materials. Ultra-gradient HPLC-grade acetonitrile and ultra-resi-analyzed grade toluene were purchased from J. T. Baker (Phillipsburg, NJ, USA). Ultrapure water was obtained from a Milli-Q system from Millipore (Milford, MA, USA). The analytical reagent grade sodium chloride and magnesium sulfate anhydrous were ordered from Northern Tianyi Chemical Reagent Inc. (Tianjin, China). Primary−secondary amine (PSA) and octadecyl (C18) sorbents were from Supelco (Bellefonte, PA, USA). Certified pesticide analytical standards (see Table 1) and internal standard (triphenyl phosphate, TPP) with purity >92% were purchased from Dr. Ehrenstorfer (Augsburg, Germany).

Standard Solutions Preparation. Individual pesticide stock standard solutions (1000 mg/L) were prepared in toluene (when necessary, acetone or methanol was added as cosolvent). A multistandard mixture, containing 2 mg/L of each pesticide, was then prepared by transferring 0.2 mL of each stock solution to a 100 mL flask, also in toluene. The working calibration standard solutions were prepared by appropriate dilution of the 2 mg/L mixture with toluene to 10 mL flasks containing 100 μL of internal standard solution (TPP at 20 mg/L). The concentrations of the multistandard solutions were 5, 10, 20, 50, 100, 200, and 500 μg/L. After evaporation under a stream of nitrogen, the residue for the 1 mL blank tobacco (pesticide-free tobacco) extract was redissolved in 1 mL of the multistandard solution to realize matrix-matched working standard solutions of 5, 10, 20, 50, 100, 200, and 500 μg/L. The individual stock

dx.doi.org/10.1021/jf400887x | J. Agric. Food Chem. 2013, 61, 5746−5757

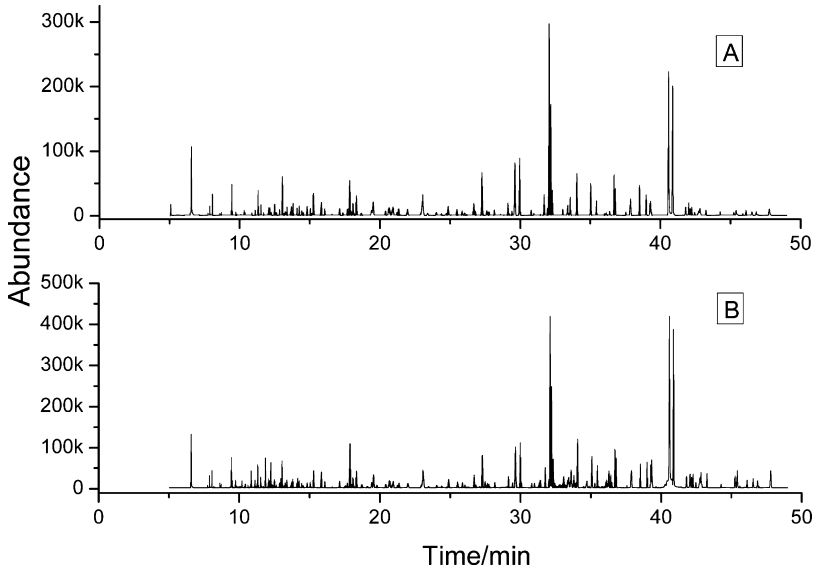

Figure 1. Total ion chromatograms (TICs) of (A) a standard solution of 159 pesticides and (B) the extract of a blank tobacco sample spiked with standard at 0.1 mg/L.

solutions (1000 mg/L) in toluene and the multistandard solution (2 mg/L) were stored at −20 °C.

Sample Treatment. Approximately 2 g of pulverized tobacco was weighed into a 50 mL centrifuge tube. Next, 10 mL of ultrapure water was added to hydrate the tobacco sample, and it was shaken in a vortex for 30 s and then left to stand for 10 min. After that, 10 mL of acetonitrile and 100 μL of internal standard solution (TPP at 20 mg/L) were added, and the tube was vortexed for 2 min. To avoid possible thermal decomposition of some pesticides in the salting-out procedure, the tube was frozen at −20 °C for at least 10 min. Then a salt mixture of 4 g of anhydrous magnesium sulfate ($MgSO_4$), 1 g of sodium chloride (NaCl), 1 g of trisodium citrate dihydrate ($Na_3Cit{\cdot}2H_2O$), and 0.5 g of disodium hydrogen citrate sesquihydrate ($Na_2HCit{\cdot}1.5H_2O$) were added and the contents immediately hand-shaken for 30 s. A further 5 mL of toluene was added, and the tube was vortexed for 2 min (here, the centrifugation step was omitted as there was enough supernatant after vortexing). One milliliter of the upper layer extract was then transferred to a 2 mL centrifuge tube containing 150 mg of $MgSO_4$, 50 mg of PSA, and 50 mg of C18. The mixture was vortexed for 2 min and centrifuged for 5 min. After centrifugation, the cleaned upper layer extract was transferred to an autosampler vial.

Blank tobacco samples were prepared in the same way as the sample treatment procedure mentioned above, but without the addition of the internal standard solution. The matrix-matched working standard solutions were prepared with the blank tobacco extracts as mentioned earlier.

GC-MS/MS Analysis. GC analysis was performed using a Trace GC Ultra GC, coupled with an AI/AS 3000 autosampler and a TSQ Quantum GC triple-quadrupole MS (Thermo Fisher, MA, USA). Analytes were separated with a TR-pesticide column from Thermo Fisher (30 m × 0.25 mm × 0.25 μm). A 5 m × 0.25 mm guard column of uncoated fused silica was connected to the separation column at the inlet end to prevent high-boiling nonvolatile compounds from entering the separation column. Helium (purity = 99.999%), at a constant flow rate of 1.2 mL/min, was used as the carrier gas. One microliter extracts were injected in the PTV in splitless mode and with a splitless time of 1 min. The injection inlet temperature was held at 70 °C for 0.02 min after injection, programmed at 14.5 °C/s to 200 °C, held for 1 min, programmed to 280 °C at 10 °C/s, and held for 5 min. The oven temperature programming was as follows: initial temperature, 90 °C (held for 5 min); increased by 25 °C/min to 180 °C (held for 15 min); increased by 5 °C/min to 280 °C; and held for 6.5 min. To prevent some pesticides (such as trichlorfon) from degradation in the gasification process in the liner, the injector and oven temperature in the first 15 min was set lower. For the post-run condition, the column was held at 300 °C for 5 min. Total run time was 55.1 min, and the equilibration time for the next run was 5 min.

The temperature of the transfer line was set at 280 °C and that of the ion source at 250 °C. The ionization mode was electron impact at 70 eV, and the filament current was 50 μA. Argon (purity = 99.999%) was used as the collision gas, and the collision cell pressure was 1.0 mTorr. The analysis was performed with a solvent delay of 5 min to prevent instrument damage. The instrument was run in multiple-reaction monitoring mode. A Thermo Fisher Xcalibur 2.1 workstation was used for instrument control, method development, and data acquisition.

RESULTS AND DISCUSSION

Target Pesticide Scope Selection. A comprehensive list of pesticides was selected on the basis of the following:

List 1 contained one hundred and twenty-five GC-amenable pesticides from the list of the CORESTA 2008 GRLs. The

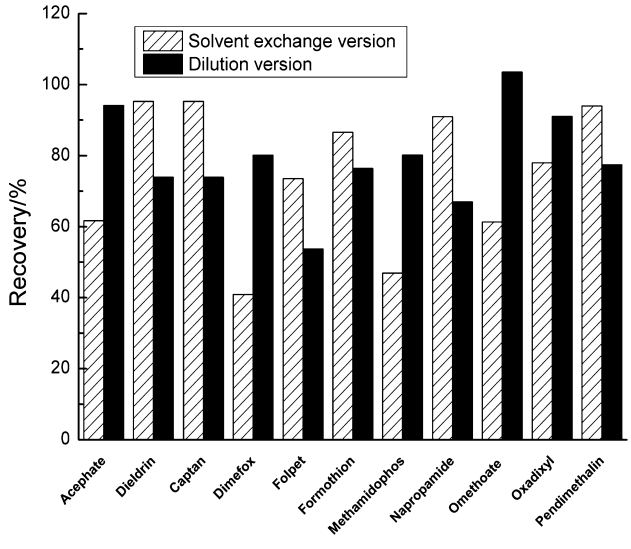

Figure 2. Recoveries of selected pesticides obtained from two different preparation methods (solvent exchange version and dilution version).

dx.doi.org/10.1021/jf400887x | J. Agric. Food Chem. 2013, 61, 5746−5757

Table 2. Result of the 2012 CORESTA Joint Experiment

no.	pesticide	dilution[a]	solvent exchange[b]	CORESTA result[c]
1	bifenthrin	1.37	1.58	1.55
2	bitertanol	0.24	0.26	0.19
3	carbofuran	0.42	0.66	0.64
4	chlorthal-dimethyl	0.31	0.31	0.30
5	clomazone	0.18	0.19	0.17
6	cyhalothrin	0.38	0.37	0.39
7	difenoconazole	0.82	0.96	0.91
8	dimethoate	–[d]	–[e]	0.07
9	fenamiphos sulfoxide	0.18	0.16	0.18
10	iprodione	0.23	–[e]	0.27
11	pendimethalin	2.91	3.22	2.99
12	profenofos	0.12	0.13	0.13
13	thiamethoxam	3.01	2.62	2.79
14	triadimefon	0.27	0.28	0.27

[a]Sample treatment in November 2012. [b]Sample treatment in May 2012. [c]The average score of the pesticides obtained from about 20 independent laboratories all over the world. [d]Not detected. [e]Not tested.

compounds selected represented 78.1% of the current GRL list. *List 2* comprised 17 GC-amenable pesticides from the list of the 2012 joint experiment of the Agrochemicals Analysis Subgroup of CORESTA. *List 3* included 17 GC-amenable pesticides from the list of banned or currently recommended pesticides for use in tobacco production in China. On the basis of selections from the above three lists, the total number of pesticides selected was 159.

The pesticides investigated in this study included most of those that were widely used in tobacco cultivation and amenable to GC. The optimized GC-MS/MS parameters, including retention times, precursor ions, product ions, and collision energies for the 159 pesticides, are shown in Table 1. Figure 1A shows the total ion chromatogram (TIC) of a standard solution of the 159 pesticides at 0.1 mg/L, and Figure 1B shows the TIC of the extract of a blank tobacco sample spiked with standards at 0.1 mg/L.

During method optimization and validation, key experimental parameters, that is, instrument robustness, stability of standard solutions, and matrix effects, were tested, and the results are presented in the Supporting Information (Figures S1, S2, and S3 and Table S1).

Optimization of Sample Treatment. In general, multiresidue methods consist of three basic steps, that is, extraction, cleanup, and measurement. The objective of the sample treatment is to increase the extraction efficiency of the analytes and to minimize the coextraction of matrix constituents. In general, a compromise needs to be found between sample extraction and cleanup. The sample treatment procedure employed here was based on the popular QuEChERS scheme, which involves a liquid partitioning between acetonitrile and water with anhydrous magnesium sulfate and sodium chloride followed by a dispersive-solid phase extraction (d-SPE) cleanup with PSA and C18. In principle, as described earlier, 10 mL of water was added to the tobacco sample to provide the optimal conditions for extraction by acetonitrile. We also did a minor modification to the method by freezing the tube to a temperature of −20 °C for at least 10 min before the salting-out procedure. This additional step was considered to be of benefit to some thermolabile pesticides by reducing exothermicity in the next step. In the optimization experiments, analyte recoveries and the amounts of coextracted components were used as indices of performance.

Comparison of Various QuEChERS Extraction Methods. Anastassiades first proposed the original QuEChERS scheme[9] and subsequently described a citrate-buffering version, which became accepted as European Committee for Standardization (CEN) Standard Method EN 15662.[21] Lehotay modified the original version to use acetate-buffering conditions, and it became AOAC Official Method 2007.01.[18,19] Buffer systems were introduced to achieve a constant pH of 5 during extraction to improve the stability of some pH-dependent pesticides in different matrix extracts. In this study, a recovery test was used to evaluate the performance of these three QuEChERS methods. The specific experimental procedures for all three methods were as described under Sample Treatment except for differences in salt compositions in the salting-out step as indicated below.

(1) For the original (unbuffered) method, salt composition was 4 g of $MgSO_4$ and 1 g of NaCl.
(2) For the CEN (citrate-buffered) method, salt composition was 4 g of $MgSO_4$, 1 g of NaCl, 1 g of $Na_3Cit{\cdot}2H_2O$, and 0.5 g of $Na_2HCit{\cdot}1.5H_2O$.
(3) For the AOAC (acetate-buffering) method, salt composition was 4 g of $MgSO_4$ and 1 g of NaOAc.

(To unbuffered and citrate-buffered samples was added 10 mL of acetonitrile as extraction solvent; to acetate-buffered sample was added 10 mL of acetonitrile with 1% HOAc as extraction solvent).

At a spike level of 0.1 mg/kg in tobacco, as shown in Figure S4 of the Supporting Information, most pesticides analyzed in this study provided acceptable recoveries of between 70 and 120% regardless of the QuEChERS method used. For the unbuffered system, some pesticides, such as folpet, acephate, and formothion, gave lower recoveries compared with the other two methods.

Cleanup Procedure. Tobacco is a complex material that contains thousands of components which might coextract in the sample cleanup stage. The coextracted matrix components could interfere with performance during GC-MS/MS analysis in different ways such as a direct matrix effect, the shifting of retention times, the skewing of peak shape, and interference with targeted transitions. Therefore, reducing the amount of coextracted matrix components is critical to achieving good GC-MS/MS performance.

PSA, C18, and graphitized carbon black (GCB) are commonly used adsorbents for d-SPE in the cleanup procedure of the QuEChERS method. PSA is the base sorbent used for cleanup as it can remove many matrices such as sugars, fatty acids, and organic acids. C18 can help to remove lipids and nonpolar interferences, and GCB can reduce pigments and sterols. Because of a high affinity for planar pesticides (e.g., hexachlorobenzene, chlorothalonil), GCB was not used in this study. The influences of different amounts of PSA (25, 50, and 75 mg/1 mL acetonitrile extract) with or without a constant amount of C18 (50 mg/1 mL acetonitrile extract) were investigated. The cleanup efficiency was determined by the amount of coextracted matrix calculated by gravimetric analysis.[4,20] The crude acetonitrile extract and the processed extracts were evaporated to dryness under nitrogen gas, and the remaining coextractives were weighed. As shown in Figure S5 of the Supporting Information, the coextractives decreased with

dx.doi.org/10.1021/jf400887x | J. Agric. Food Chem. 2013, 61, 5746−5757

Table 3. Method Validation Data: Linear Ranges, Correlation Coefficients (R^2), Recoveries (n = 6), Relative Standard Deviations (RSDs), Limits of Detection (LODs), and Quantification (LOQs) of 159 Pesticides Obtained by GC-MS/MS Analysis of Tobacco

			recovery, % (RSD, %)				
pesticide	linear range (μg/L)	R^2	50 μg/kg	250 μg/kg	500 μg/kg	LOD (μg/kg)	LOQ (μg/kg)
acephate	5−500	0.9998	112 (7)	88 (7)	83 (13)	10	50
acetamiprid	5−500	0.9877	78 (10)	108 (2)	117 (4)	10	50
acibenzolar-*S*-methyl	5−500	1.0000	92 (12)	88 (7)	90 (8)	10	50
alachlor	5−500	0.9998	116 (13)	101 (6)	107 (12)	10	50
aldrin	5−500	0.9999	79 (10)	90 (7)	94 (9)	5	50
azinphos-ethyl	5−500	0.9998	102 (7)	93 (4)	92 (4)	5	50
azinphos-methyl	10−500	0.9854	95 (7)	92 (3)	88 (5)	10	50
azoxystrobin	5−500	0.9997	89 (10)	87 (4)	87 (4)	5	50
benalaxyl	5−500	0.9999	103 (11)	106 (4)	106 (3)	10	50
benfluralin	5−500	0.9998	92 (13)	101 (14)	113 (11)	5	50
bifenthrin	5−500	0.9985	123 (6)	104 (3)	100 (3)	5	50
bitertanol	5−500	0.9952	81 (5)	80 (4)	84 (3)	5	50
bromacil	5−500	0.9999	90 (13)	88 (8)	86 (3)	5	50
bromophos	5−500	0.9988	85 (11)	96 (32)	103 (15)	5	50
butralin	5−500	0.9982	93 (10)	89 (6)	94 (9)	5	50
cadusafos	5−500	0.9996	89 (11)	94 (9)	98 (6)	5	50
captafol	5−500	0.9965	98 (7)	96 (6)	106 (10)	10	50
captan	10−500	0.9926	81 (13)	72 (13)	76 (7)	20	50
carbaryl	5−500	0.9999	108 (7)	82 (9)	88 (9)	5	50
carbofuran	5−500	0.9964	112 (8)	110 (12)	117 (12)	10	50
3-hydroxycarbofuran	5−500	0.9999	96 (10)	92 (14)	102 (5)	10	50
chinomethionate	5−500	0.9999	40 (21)	33 (19)	37 (12)	5	NG[a]
chlorantraniliprole	5−500	0.9987	106 (19)	82 (11)	82 (8)	10	50
chlordane (*cis*-)	5−500	0.9994	74 (10)	98 (12)	103 (11)	5	50
chlordane (*trans*-)	5−500	0.9989	73 (14)	98 (9)	97 (11)	5	50
chlordimeform	5−500	0.9982	93 (19)	96 (12)	97 (11)	10	50
chlorfenvinphos (*E*)	5−500	0.9982	103 (5)	84 (11)	82 (10)	10	50
chlorfenvinphos (*Z*)	5−500	0.9999	88 (6)	92 (7)	94 (10)	5	50
chlornitrofen	5−500	0.9977	95 (7)	88 (9)	90 (5)	5	50
chlorpyrifos (-ethyl)	5−500	0.9998	98 (10)	96 (5)	102 (10)	5	50
chlorpyrifos-methyl	5−500	0.9993	95 (5)	90 (9)	99 (10)	5	50
chlorthal-dimethyl	5−500	0.9998	74 (10)	89 (11)	91 (10)	5	50
chorobenzilate	5−500	0.9998	86 (9)	101 (5)	101 (3)	5	50
clomazone	5−500	0.9997	104 (4)	97 (5)	100 (10)	5	50
cyfluthrin	5−500	0.9992	85 (7)	94 (6)	93 (3)	5	50
cyhalothrin	5−500	0.9997	99 (10)	100 (5)	97 (3)	5	50
cypermethrin	5−500	0.9993	101 (14)	97 (7)	89 (6)	10	50
dazomet	5−500	0.9999	77 (14)	86 (10)	85 (5)	10	50
DBCP	5−500	0.9999	102 (9)	92 (5)	91 (9)	5	50
o,*p*′-DDT	5−500	0.9997	91 (4)	92 (3)	95 (2)	2	50
p,*p*′-DDT	5−500	0.9989	87 (7)	91 (4)	94 (3)	2	50
p,*p*′-DDD	5−500	0.9999	99 (4)	98 (5)	100 (2)	2	50
o,*p*′-DDD	5−500	1.0000	78 (9)	96 (3)	99 (3)	2	50
o,*p*′-DDE	5−500	1.0000	78 (10)	96 (5)	98 (3)	2	50
p,*p*′-DDE	5−500	1.0000	74 (10)	94 (5)	96 (3)	5	50
deltamethrin	5−500	1.0000	75 (10)	88 (8)	91 (4)	5	50
demeton-*S*-methyl	5−500	0.9999	104 (11)	82 (7)	87 (9)	5	50
demeton-*S*-methyl sulfone	5−500	0.9995	114 (8)	94 (12)	90 (7)	5	50
demeto-O	10−500	0.9930	98 (10)	74 (12)	83 (13)	20	50
demeto-S	10−500	0.9966	84 (12)	89 (16)	87 (10)	20	50
diazinon	5−500	0.9968	92 (8)	70 (9)	83 (13)	10	50
dichlorvos	5−500	0.9995	121 (7)	117 (5)	120 (11)	5	50
dicloran	5−500	0.9998	95 (12)	95 (4)	99 (9)	5	50
dieldrin	5−500	0.9996	73 (9)	96 (10)	92 (11)	5	50
difenoconazole	5−500	0.9997	91 (8)	89 (3)	89 (3)	5	50
diflubenzuron	5−500	0.9990	101 (16)	100 (4)	101 (11)	10	50
dimefox	5−500	1.0000	71 (7)	102 (10)	103 (5)	5	50

dx.doi.org/10.1021/jf400887x | J. Agric. Food Chem. 2013, 61, 5746−5757

Table 3. continued

pesticide	linear range (μg/L)	R^2	recovery, % (RSD, %) 50 μg/kg	250 μg/kg	500 μg/kg	LOD (μg/kg)	LOQ (μg/kg)
dimetachlone	5−500	0.9999	92 (11)	94 (9)	96 (4)	5	50
dimethoate	50−500	1.0000	ND[b]	94 (23)	108 (18)	50	250
dimethomorph (*E*)	5−500	0.9987	91 (7)	87 (2)	87 (3)	5	50
dimethomorph (*Z*)	5−500	0.9997	102 (5)	92 (3)	90 (2)	5	50
diphenamid	5−500	0.9999	92 (5)	95 (3)	91 (4)	5	50
disulfoton	5−500	0.9986	74 (21)	72 (22)	87 (10)	10	50
disulfoton sulfoxide	5−500	0.9933	91 (18)	93 (14)	103 (6)	10	50
disulfoton sulfone	5−500	0.9980	94 (11)	96 (9)	90 (4)	10	50
α-endosulfan	5−500	0.9998	76 (15)	84 (12)	89 (9)	5	50
β-endosulfan	5−500	0.9992	100 (12)	90 (15)	94 (13)	5	50
endosulfan-sulfate	5−500	0.9999	93 (7)	88 (6)	90 (3)	5	50
endrin	5−500	0.9998	91 (10)	92 (5)	95 (5)	5	50
EPN	5−500	0.9978	141 (11)	95 (6)	87 (5)	5	50
ethion	5−500	0.9999	99 (7)	107 (5)	104 (3)	5	50
ethoprophos	5−500	0.9991	70 (11)	88 (13)	89 (12)	10	50
famoxadone	5−500	0.9972	88 (13)	101 (4)	103 (5)	5	50
fenamiphos	5−500	0.9995	115 (6)	97 (11)	97 (10)	10	50
fenamiphos sulfoxide	10−500	0.9983	86 (8)	92 (10)	91 (6)	20	50
fenamiphos sulfone	5−500	0.9988	86 (14)	102 (6)	107 (4)	10	50
fenchlorphos	5−500	1.0000	103 (3)	91 (4)	96 (9)	5	50
fenitrothion	5−500	0.9993	84 (10)	99 (11)	99 (11)	5	50
fensulfothion	5−500	0.9997	78 (8)	75 (12)	85 (5)	5	50
fenthion	5−500	0.9993	88 (9)	93 (6)	95 (9)	5	50
fenthion sulfoxide	5−500	0.9971	78 (17)	96 (10)	94 (8)	10	50
fenthion sulfone	5−500	0.9979	78 (21)	64 (9)	73 (11)	10	50
Σ fenvalerate	5−500	0.9998	87 (12)	87 (5)	86 (7)	5	50
Σ flucythrinate	5−500	0.9999	90 (17)	103 (8)	98 (4)	5	50
flumetralin	5−500	0.9984	78 (10)	92 (11)	96 (5)	5	50
folpet	10 - 500	0.9952	50 (15)	65 (4)	66 (6)	20	NG
fonofos	5−500	0.9972	71 (14)	87 (14)	102 (9)	5	50
formothion	5−500	0.9996	97 (11)	82 (5)	82 (13)	5	50
HCH (α-)	5−500	0.9998	88 (12)	89 (10)	93 (10)	10	50
HCH (β-)	5−500	0.9998	79 (11)	101 (11)	104 (9)	5	50
HCH (δ-)	5−500	1.0000	101 (12)	90 (6)	95 (13)	5	50
γ-HCH (lindane)	5−500	0.9997	105 (12)	94 (9)	104 (11)	5	50
heptachlor	5−500	0.9999	95 (12)	91 (8)	96 (10)	5	50
heptachlor epoxides (*cis*-)	5−500	0.9993	81 (14)	88 (7)	93 (8)	5	50
heptachlor epoxides (*trans*-)	5−500	0.9996	92 (18)	84 (4)	92 (9)	10	50
heptenophos	5−500	0.9998	89 (14)	81 (11)	87 (13)	5	50
hexachlorobenzene	5−500	0.9999	72 (14)	93 (9)	95 (10)	5	50
indoxacarb	5−500	0.9999	78 (10)	103 (8)	101 (8)	5	50
iprobenfos	5−500	1.0000	108 (5)	89 (6)	93 (10)	5	50
iprodione	5−500	0.9994	79 (20)	90 (12)	90 (7)	5	50
isazophos	5−500	0.9964	120 (8)	108 (10)	107 (13)	5	50
isopropalin	5−500	0.9987	83 (6)	92 (6)	98 (6)	5	50
isoprothiolane	5−500	0.9996	77 (10)	71 (10)	82 (7)	5	50
leptophos	5−500	0.9989	86 (6)	87 (5)	88 (3)	5	50
malathion	5−500	0.9998	116 (8)	99 (7)	104 (11)	5	50
metalaxyl	5−500	0.9978	71 (16)	84 (8)	86 (7)	5	50
methamidophos	5−500	0.9998	82 (15)	73 (6)	83 (9)	10	50
methidathion	5−500	1.0000	74 (15)	89 (10)	88 (5)	5	50
methiocarb	5−500	0.9997	100 (11)	98 (9)	102 (10)	5	50
methiocarb sulfone	5−500	0.9991	96 (11)	93 (9)	96 (8)	5	50
methomyl	5−500	0.9960	103 (12)	110 (11)	91 (13)	5	50
methoprene	10−500	0.9994	69 (8)	84 (13)	91 (9)	10	50
methoxychlor	5−500	0.9994	111 (5)	96 (5)	95 (4)	5	50
metolachlor	5−500	1.0000	105 (6)	93 (5)	96 (9)	5	50
mevinphos	5−500	1.0000	104 (11)	90 (4)	105 (8)	5	50
mirex	5−500	0.9999	82 (9)	97 (2)	100 (2)	2	50

dx.doi.org/10.1021/jf400887x | J. Agric. Food Chem. 2013, 61, 5746−5757

Table 3. continued

pesticide	linear range (μg/L)	R^2	recovery, % (RSD, %) 50 μg/kg	250 μg/kg	500 μg/kg	LOD (μg/kg)	LOQ (μg/kg)
monocrotophos	10−500	0.9996	103 (9)	85 (17)	89 (14)	20	50
myclobutanil	5−500	0.9994	86 (9)	97 (5)	97 (4)	5	50
napropamide	5−500	0.9993	89 (3)	76 (11)	82 (7)	10	50
nitrofen	5−500	0.9985	94 (8)	98 (7)	102 (2)	5	50
omethoate	5−500	0.9999	88 (12)	73 (6)	79 (9)	5	50
oxadixyl	5−500	0.9999	101 (6)	105 (3)	99 (4)	10	50
oxamyl	25−500	0.9947	ND	85 (11)	89 (10)	20	250
parathion (-ethyl)	5−500	0.9984	86 (8)	98 (10)	103 (8)	10	50
parathion-methyl	5−500	0.9999	83 (11)	97 (12)	98 (7)	5	50
penconazole	5−500	0.9998	72 (10)	91 (10)	95 (3)	5	50
pendimethalin	5−500	0.9984	82 (9)	83 (8)	86 (9)	5	50
permethrin (*cis*-)	5−500	0.9989	111 (9)	102 (4)	102 (3)	10	50
permethrin (*trans*-)	5−500	0.9998	112 (6)	100 (4)	99 (3)	10	50
phorate	5−500	0.9997	108 (9)	89 (6)	94 (10)	5	50
phosalone	5−500	0.9999	100 (9)	107 (1)	105 (3)	5	50
phosphamidon (*E*)	5−500	0.9998	110 (8)	83 (13)	85 (12)	5	50
phosphamidon (*Z*)	5−500	0.9998	84 (11)	89 (13)	92 (11)	5	50
piperonyl butoxide	5−500	1.0000	97 (15)	99 (8)	96 (5)	5	50
pirimicarb	5−500	0.9999	99 (9)	95 (7)	99 (8)	5	50
pirimiphos-methyl	5−500	0.9984	81 (13)	84 (10)	87 (12)	10	50
profenofos	5−500	0.9998	97 (12)	95 (8)	95 (4)	5	50
propoxur	5−500	0.9999	100 (8)	87 (8)	90 (9)	5	50
prothiofos	5−500	0.9999	80 (10)	97 (8)	101 (3)	5	50
pyrazophos	5−500	0.9994	104 (6)	98 (4)	92 (3)	5	50
quinalphos	5−500	0.9999	96 (14)	98 (8)	101 (5)	10	50
quizalofop-*P*-ethyl	5−500	0.9999	84 (12)	85 (6)	86 (3)	5	50
schradan	5−500	0.9992	97 (17)	85 (7)	98 (12)	5	50
teflubenzuron	5−500	0.9923	84 (12)	95 (14)	92 (7)	5	50
tefluthrin	5−500	0.9992	92 (17)	101 (8)	100 (10)	5	50
terbufos	5−500	0.9999	98 (18)	93 (6)	96 (9)	5	50
terbufos sulfone	5−500	0.9954	105 (11)	112 (8)	96 (8)	5	50
tetrachlorvinphos	5−500	0.9997	82 (11)	89 (7)	90 (5)	5	50
tetradifon	5−500	0.9992	52 (13)	84 (9)	85 (6)	5	250
thiamethoxam	5−500	0.9981	84 (6)	101 (4)	97 (4)	5	50
thionazin	25−500	0.9950	ND	71 (18)	75 (16)	20	250
triadimefon	5−500	0.9994	81 (11)	88 (8)	92 (8)	5	50
triadimenol	5−500	0.9992	90 (16)	92 (8)	90 (3)	5	50
triazophos	5−500	0.9997	111 (12)	98 (6)	97 (3)	5	50
trichlorfon	5−500	0.9993	98 (27)	75 (22)	74 (18)	10	50
triflumuron	5−500	0.9975	74 (22)	98 (5)	106 (7)	5	50
trifluralin	5−500	0.9992	89 (19)	85 (12)	93 (9)	10	50
uniconazole	5−500	0.9996	69 (10)	83 (10)	92 (5)	5	50
vamidothion	10−500	0.9996	ND	71 (9)	80 (8)	10	250

[a]NG, not given [b]ND, not detected.

increase of PSA. However, in the recovery test, some pesticides, such as formothion, gave poor performance when the PSA was at a concentration of 75 mg/1 mL acetonitrile extract. C18 did not influence pesticide recovery, but it did improve the efficiency of purification. Therefore, the optimized sorbents for sample cleanup consisted of a mixture of PSA and C18 each at a concentration of 50 mg/mL in the extract.

Solvent Exchange versus Dilution. Considering the high vapor volume and polarity of acetonitrile, it is not a good solvent for sample introduction in GC. As it is the most popular extraction solvent in multiresidue analytical methods, either a dilution or solvent exchange step is generally performed prior to GC analysis. In this work, a spike recovery test at 0.1 mg/kg was used to evaluate the two approaches. Sample dilution was as described under Sample Treatment. For solvent exchange, approximately 2 g of pulverized tobacco was weighed into a 50 mL centrifuge tube. Then, 10 mL of ultrapure water was added to hydrate the tobacco sample, which was then shaken in a vortex for 30 s. The sample was then left to stand for 10 min. After that, 10 mL of acetonitrile and 100 μL of internal standard solution (TPP at 20 mg/L) were added, and the tube was vortexed for 2 min. The tube was frozen at −20 °C for at least 10 min. Then a salt mixture consisting of 4 g of anhydrous magnesium sulfate ($MgSO_4$), 1 g of sodium chloride (NaCl), 1 g of trisodium citrate dihydrate ($Na_3Cit{\cdot}2H_2O$), and 0.5 g of disodium hydrogen citrate sesquihydrate ($Na_2HCit{\cdot}.1.5H_2O$) was added, and the mixture was vortexed for 2 min and centrifuged for 5 min. One and a half milliliters of the upper

dx.doi.org/10.1021/jf400887x | J. Agric. Food Chem. 2013, 61, 5746−5757

Table 4. Pesticides Detected in the Real Tobacco Samples (n = 118)

pesticide	no. of positive samples	concentration range (mg/kg)	no. of postive samples > GRLs[a]
azoxystrobin	64	<0.05−2.25	−
bifenthrin	19	<0.05−0.32	−
butralin	70	<0.05−4.82	−
carbaryl	11	<0.05−3.20	3
chlorantraniliprole	25	0.09−1.25	−
cyhalothrin	65	<0.05−1.42	1
cypermethrin	32	<0.05−0.18	−
difenoconazole	8	<0.05−0.09	−
dimetachlone	26	0.19−2.65	−
dimethomorph	4	<0.05−1.46	−
diphenamid	1	<0.05	−
α-endosulfan	1	0.19	3[b]
β-endosulfan	12	<0.05−3.40	
endosulfan-sulfate	12	<0.05−0.65	
fenamiphos sulfoxide	2	<0.05−0.16	−[c]
fenamiphos sulfone	6	<0.05−0.10	
Σ fenvalerate	19	<0.05−1.30	1
flumetralin	65	<0.05−2.70	−
indoxacarb	5	<0.05−0.10	−
iprodione	3	<0.05−0.75	1
metalaxyl	31	<0.05−0.85	−
myclobutanil	9	<0.05−0.04	−
pendimethalin	71	<0.05−2.23	−
triadimefon	26	0.08−0.33	−
triadimenol	26	0.44−3.43	−

[a]GRLs established by CORESTA 2008. [b]Sum of α- and β-isomers and endosulfan-sulfate expressed as endosulfan. [c]Sum of fenamiphos, fenamiphos sulfoxide and fenamiphos sulfone expressed as fenamiphos.

layer was placed in a 2 mL centrifuge tube containing 150 mg of $MgSO_4$, 40 mg of PSA, and 40 mg of C18. The mixture was then vortexed for 2 min and centrifuged for 5 min. After centrifugation, 1 mL of the cleaned upper layer extract was evaporated under a stream of nitrogen, and the residue was reconstituted in 1 mL of n-hexane/acetone (9:1, v/v).

Most of the pesticides exhibited good recoveries of between 70 and 120% for the two treatments. Figure 2 shows comparison data for selected pesticides. As can be seen, some low-boiling pesticides with retention times of <15 min such as acephate, dimefox, methamidophos, and omethoate yielded poor results by solvent exchange, which might be caused by loss of compound in the evaporation stage. The recoveries for dieldrin, captan, folpet, and napropamide, obtained from the solvent exchange method, were better than those observed in the dilution method, average recoveries being 70−90 and 50−70%, respectively.

Taking advantage of the solvent exchange method, we participated in the CORESTA 2012 joint experiment, which was organized by the Food Analysis Performance Assessment Scheme. Some 5 months later, we also tested the same tobacco sample by the dilution method. Table 2 shows that the results from the two methods, and the CORESTA data have good consistency for most of the detected pesticides. The low results for bifenthrin and carbofuran, obtained for the "dilution" comparing to the "solvent exchange" methods, might be caused by sample degradation and analyte loss during storage.

The dilution method gave better results for some pesticides such as acephate and dimefox. In addition, this method was simpler and faster than the solvent exchange method because of the omission of the solvent exchange step. Some pesticides, including dieldrin and folpet, however, gave poor recoveries for the dilution method, but recovery levels of 50−70% and RSDs of <20% were deemed acceptable.[22] Therefore, the dilution method was chosen for the GC introduction, and it was used in the method validation stage.

Method Validation. To ensure that the developed method was suitable for routine analysis, basic analytical performance parameters such as linearity, dynamic range, recovery (trueness), and precision as well as limits of detection (LOD) and limits of quantification (LOQ) were determined.

Calibration curves were established from the matrix-matched standard calibration solutions, which were prepared as described under Sample Treatment. Linearity of the calibration was confirmed from analysis of the seven multistandards that covered the concentration range of 5−500 μg/L for all pesticides. All compounds gave correlation coefficients (R^2) of >0.99 except for acetamiprid and methyl-azinphos (Table 3).

For the recovery study, blank tobacco samples were spiked with the corresponding volume of the standard solution and left standing for 2 h at room temperature before extraction. Six replicate spikes each at 50, 250, and 500 μg/kg were prepared and processed. The recovery data calculations were based on comparing the concentration levels of the spiked samples, which were calculated from the standard curves, to the theoretical values of the corresponding levels of matrix-matched standards. The RSDs were calculated by the analysis of six replicate samples at each spiked level. As shown in Table 3, recoveries ranged from 69 to 141%, but for most cases, values were between 70 and 120%. Notable exceptions were chinomethionate and folpet, for which recoveries ranged between 30 and 70%. The RSDs ranged from 2 to 27%.

LODs were evaluated by injecting matrix-matched standard solutions at the 0.5, 1, 2, and 5 ng/mL concentration levels. The LOD was determined as the minimum concentration of analyte providing a spectrum in which the qualifier transition had a signal-to-noise ratio (S/N) of 3. The LOQs were defined as the minimum concentration of the analyte that can be quantified with acceptable accuracy and precision, as described in Document No. SANCO/12495/2011.[22] As shown in Table 3, most of pesticide compounds exhibited LODs of <10 μg/kg. For LOQs, most pesticides could be quantified with recoveries between 70 and 120% and repeatability RSDs of <20% at 50 μg/kg. It could be estimated that for part of the pesticides, for example, mirex, LOQs in the range of 5 and 50 μg/kg could probably be achieveable.

Application to Real Samples. The validated method was applied to the analysis of 118 tobacco samples. To ensure the measurement process was under control, a recovery QC sample at 0.1 mg/kg and a matrix blank were analyzed in each batch of samples. The protocol for identification of pesticide residues was based on the following factors: the retention time, two transitions, and the intensity ratio of the two transitions. Analyses of blank samples were also performed to check for false-positive results. Samples that were positively identified were quantified with reference to the matrix-matched standard curves that employed TPP as internal standard.

The pesticides detected in the real samples are listed in Table 4. Of the 159 confirmed pesticides, 25 were detected in the concentration range <0.05−4.82 mg/kg. Ninety-eight percent

(116 of 118) of the tested samples gave positive results, which indicated that the samples analyzed had at least one pesticide with concentrations above the LODs. Eight of 118 samples exceeded the GRLs set by the CORESTA ACAC. The most frequently detected pesticides were azoxystrobin, butralin, cyhalothrin, cypermethrin, flumetralin, metalaxyl, and pendimethalin, with a percentage occurrence ranging from 25 to 60%. Other pesticides detected were bifenthrin, carbaryl, chlorantraniliprole, difenoconazole, dimetachlone, dimethomorph, diphenamid, α-endosulfan, β-endosulfan, endosulfan-sulfate, fenamiphos sulfoxide, fenamiphos sulfone, Σ fenvalerate, indoxacarb, iprodione, myclobutanil, triadimefon, and triadimenol, which were present in <22% of the analyzed samples. Carbaryl, cyhalothrin, endosulfan, Σ fenvalerate, and iprodione pesticide residues were found at concentrations exceeding the GRLs in eight samples. These results demonstrated that the developed method could be applied to the analysis of pesticides in tobacco samples.

■ ASSOCIATED CONTENT

Supporting Information

GC-MS/MS parameters optimization; stability of instrument and standard solutions; results of matrix effects under different instrument conditions. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*(G.T.) Phone: +86-371-67672613. Fax: +86-371-67672625. E-mail: tglcttc@163.com. (Q.H.) Phone: +86-371-67672601. Fax: +86-371-67672601. E-mail: huqy@ztri.com.cn.

Author Contributions

‖G.T. and Q.H. equally contributed in accomplishing this work.

Funding

We acknowledge funding support from the Presidental Foundation of Zhengzhou Tobacco Research Institute (No. 512012CA0080).

Notes

The authors declare no competing financial interest.

■ ABBREVIATIONS USED

AOAC, Association of Official Analytical Chemists; ACAC, Agro-Chemical Advisory Committee; CEN, European Committee for Standardization; CORESTA, Cooperation Center for Scientific Research Relative to Tobacco; DBCP, dibromochloropropane; d-SPE, dispersive-solid phase extraction; EI, electron impact; EPN, ethyl para-nitro-phenyl; FAPAS, Food Analysis Performance Assessment Scheme; GC, gas chromatography; GCB, graphitized carbon black; GRLs, guidance residue levels; HCH, hexachlorocyclohexane; LC, liquid chromatography; LLE, liquid−liquid extraction; LOD, limit of detection; LOQ, limit of quantification; MRLs, maximum residue limits; MRM, multiple reaction monitoring; MS/MS, tandem mass spectrometry; PLE, pressurized liquid extraction; PSA, primary secondary amine; QuEChERS, quick, easy, cheap, effective, rugged and safe; RSD, relative standard deviation; S/N, signal-to-noise; TPP, triphenyl phosphate

■ REFERENCES

(1) Beseler, C. L.; Stallones, L.; Hoppin, J. A.; Alavanja, M. C.; Blair, A.; Keefe, T.; Kamel, F. Depression and pesticide exposures among private pesticide applicators enrolled in the Agricultural Health Study. *Environ. Health Perspect.* **2008**, *116*, 1713.

(2) Jeyaratnam, J. Acute pesticide poisoning: a major global health problem. *World Health Stat. Q.* **1990**, *43*, 139−144.

(3) CORESTA. Guide No. 1: *The concept and implementation of agrochemical guidance residue levels*, 2008; http://www.coresta.org/Guides/Guide-No01-GRLs- (2nd-Issue-June08-Addendum-June10).pdf (accessed Feb 22, 2013).

(4) Lehotay, S. J.; Son, K.; Kwon, H.; Koesukwiwat, U.; Fu, W.; Mastovska, K.; Hoh, E.; Leepipatpiboon, N. Comparison of QuEChERS sample preparation methods for the analysis of pesticide residues in fruits and vegetables. *J. Chromatogr., A* **2010**, *1217*, 2548−2560.

(5) Lozano, A.; Rajski, Ł.; Belmonte-Valles, N.; Uclés, A.; Uclés, S.; Mezcua, M.; Fernández-Alba, A. R. Pesticide analysis in teas and chamomile by liquid chromatography and gas chromatography tandem mass spectrometry using a modified QuEChERS method. Validation and pilot survey in real samples. *J. Chromatogr., A* **2012**, *1268*, 109−122.

(6) Walorczyk, S. Development of a multi-residue method for the determination of pesticides in cereals and dry animal feed using gas chromatography-tandem quadrupole mass spectrometry. *J. Chromatogr., A* **2008**, *1208*, 202−214.

(7) Steiniger, D.; Lu, G.; Butler, J.; Phillips, E.; Fintschenko, Y. Determination of multiresidue pesticides in green tea by using a modified QuEChERS extraction and ion-trap gas chromatography/mass spectrometry. *J. AOAC Int.* **2010**, *93*, 1169−1179.

(8) Walorczyk, S. Application of gas chromatography/tandem quadrupole mass spectrometry to the multi−residue analysis of pesticides in green leafy vegetables. *Rapid Commun. Mass Spectrom.* **2008**, *22*, 3791−3801.

(9) 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.

(10) Camino-Sánchez, F.; Zafra-Gómez, A.; Oliver-Rodriguez, B.; Ballesteros, O.; Navalón, A.; Crovetto, G.; Vilchez, J. UNE-EN ISO/IEC 17025: 2005-accredited method for the determination of pesticide residues in fruit and vegetable samples by LC-MS/MS. *Food Addit. Contam.* **2010**, *27*, 1532−1544.

(11) Gilbert-López, B.; García-Reyes, J. F.; Molina-Díaz, A. Sample treatment and determination of pesticide residues in fatty vegetable matrices: a review. *Talanta* **2009**, *79*, 109.

(12) Radišić, M.; Grujić, S.; Vasiljević, T.; Laušević, M. Determination of selected pesticides in fruit juices by matrix solid-phase dispersion and liquid chromatography−tandem mass spectrometry. *Food Chem.* **2009**, *113*, 712−719.

(13) Cai, J.; Liu, B.; Zhu, X.; Su, Q. Determination of pyrethroid residues in tobacco and cigarette smoke by capillary gas chromatography. *J. Chromatogr., A* **2002**, *964*, 205−211.

(14) Haib, J.; Hofer, I.; Renaud, J.-M. Analysis of multiple pesticide residues in tobacco using pressurized liquid extraction, automated solid-phase extraction clean-up and gas chromatography−tandem mass spectrometry. *J. Chromatogr., A* **2003**, *1020*, 173−187.

(15) Mayer-Helm, B.; Hofbauer, L.; Müller, J. Method development for the determination of selected pesticides on tobacco by high-performance liquid chromatography−electrospray ionisation-tandem mass spectrometry. *Talanta* **2008**, *74*, 1184−1190.

(16) Mayer-Helm, B. Method development for the determination of 52 pesticides in tobacco by liquid chromatography−tandem mass spectrometry. *J. Chromatogr., A* **2009**, *1216*, 8953−8959.

(17) Lee, J.-M.; Park, J.-W.; Jang, G.-C.; Hwang, K.-J. Comparative study of pesticide multi-residue extraction in tobacco for gas chromatography−triple quadrupole mass spectrometry. *J. Chromatogr., A* **2008**, *1187*, 25−33.

(18) Lehotay, S. J.; de Kok, A.; Hiemstra, M.; Bodegraven, P. V. Validation of a fast and easy method for the determination of residues from 229 pesticides in fruits and vegetables using gas and liquid

chromatography and mass spectrometric detection. *J. AOAC Int.* **2005**, *88*, 595−614.
(19) Lehotay, S. J. Determination of pesticide residues in foods by acetonitrile extraction and partitioning with magnesium sulfate: collaborative study. *J. AOAC Int.* **2007**, *90*, 485−520.
(20) Cajka, T.; Sandy, C.; Bachanova, V.; Drabova, L.; Kalachova, K.; Pulkrabova, J.; Hajslova, J. Streamlining sample preparation and gas chromatography−tandem mass spectrometry analysis of multiple pesticide residues in tea. *Anal. Chim. Acta* **2012**, *742*, 51−60.
(21) EN 15662 (2008). Foods of plant origin − Determination of pesticide residues using GC-MS and/or LC-MS/MS following acetonitrile extraction/partitioning and clean-up by dispersive SPE − QuEChERS-method; http://esearch.cen.eu/esearch/ (accessed Feb 23, 2013).
(22) SANCO/12495/2011 (2012). Method validation and quality control procedures for pesticide residues analysis in food and feed; http://www.eurl-pesticides.eu- /docs/public/tmplt_article.asp?CntID=727&LabID=100&Lang=EN (accessed Feb 22, 2013).