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Development and validation of multi-residue analysis of 110 pesticides in brown rice using GC-MS/MS with compound based quantitation

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Abstract

The Quick Easy Cheap Effective Rugged and Safe (QuEChERS) multi-residue method has been validated for the extraction of 110 pesticides in brown rice. A mixture of 110 pesticides amenable to gas chromatography (GC) was quantitatively recovered from spiked brown rice and determined using gas chromatography-tandem mass spectrometry (GC-MS/MS). The method employed involved initial extraction in a acetonitrile system, an extraction/partitioning step after the addition of salt, and a cleanup step utilizing dispersive solid-phase extraction (d-SPE); this combination ensured that it was a rapid, simple and cost-effective procedure. The matrix-matched calibration results have demonstrated good reproducibility, robustness and linearity. The spiking levels for the recovery experiments were 0.010, and 0.1 mg/kg for GC-MS/MS analyses. Mean recoveries mostly ranged between 70 and 120 %, and RSD were generally below 20%. For almost all compounds limit of detections (LOD) were 0.001 to 0.005 mg/kg and limit of quantitation (LOQ) were 0.005 to 0.020 mg/kg. Correlation coefficients of the calibration curves were >0.991. Based on these results, the methodology has been proven to be highly efficient and robust and thus suitable for monitoring the Maximum Residual Limit (MRL) compliance of a wide range of commodity/pesticide combinations.

Keywords: brown rice, pesticides, QuEChERS sample preparation, method validation, GC-MS/MS

INTRODUCTION

Pesticides are widely used in food production to eliminate pests such as insects, rodents, weeds, bacteria, mold and fungus.(EPA, Food and Pesticides) Some pesticides can be extremely toxic and harmful to humans. More than 1000 pesticides are used worldwide and, along with their metabolites and degradation products, are present in food and the environment.(Czapiewski, 2010) Therefore, it is necessary to develop a method for simultaneous determination of the pesticides present in food.

High performance liquid chromatography(HPLC) and gas chromatography(GC) are currently the most widely used method for detecting pesticides. But both methods need complicated sample preparation and cleaning. With the development of simple sample preparation(QuEChERS), Determination of pesticides by mass spectrometry has been under spotlight. Moreover mass spectrometry has more selectivity. Especially tandem mass spectrometry technology expands the number of pesticides that can be analyzed in a single injection. But most of multi-residue methods are only for screening.

In this study, we developed and validated multi-residue analysis of 110 pesticides in Brown Rice Using GC-MS/MS with compound based quantitation. The ability to quantitate 110 pesticides simultaneously via a method with QuEChERS sample preparation is expected to play an important role in food safety.

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MATERIALS AND METHODS

Reagents, solvent and standards

Analytical reagent grade water and acetonitrile (ACN) were obtained from Fisher scientific (Fair Lawn, NJ, USA). Water was purchased from J.T. Baker (Phillipsburg, NJ, USA). Standards were purchased from ULTRA-Scientific.

Sample preparation

Organic brown rice was purchased from market for recovery experiment. 5g of Sample was weighed in 50 mL centrifuge tube. 100 µL of 10 mg/kg standard mixture was added for recovery of high, 100 µL of 1 mg/kg for low concentration. 10 mL of water was added. Then, a 10 mL of Acetonitrile, 100 µL internal standard, 4 g MgSO₄, 1 g NaCl, 1 g Trisodiumcitrate dehydrate, 0.5 Disodium hydrogencitrate sesquihydrate was added. Sample tubes were capped and shaken for 1minutes with an SPEX 2000 Geno grinder(1000 stroke/min) and Centrifuged for 5 minutes(3000 rpm). 1 mL of upper Acetonitrile layer was transferred to Bond Elut EN dispersive SPE tubes. The tubes were Vortexed for 30 second, Centrifuged for 5 minutes. A 0.405 mL portion of the extract was Transferred to 2 mL vial and then 15 µL of analyte protectants and 5 µL of 0.5 % formic acid was added.

Chromatographic conditions

The GC/MS/MS systems used for these experiments are described in Table 1.

Table1. Instrumentation and Analytical Conditions for GC/MS/MS System

GC	Bruker 456
Autosampler	CTC combi PAL
Inlet	Multimode inlet
Carrier gas	Helium
PTV Mode Inlet parameters	
Temperature	80°C (0.5min), 200°C/min, 280°C(29min)
Injection volume	4µℓ
Oven temperature program	90 °C (3 min), 20 °C/min to 120°C, 8 °C/min to 300°C(3min) Total : 30min
Analytical column	DB-5MS(30 m × 0.25 mm(i.d), 0.25 µm)
Triple Quadrupole Mass	Bruker SION TQ
Mode	Electron impact
Transfer line temperature	280 °C
Source temperature	230 °C
Manifold	40°C

Method validation

Method validation is universally recognized as an important part of a comprehensive system of quality assurance in the analytical chemistry. Validation experiments were performed using a GC/MS/M instrument. The validation was conducted according to the EURL SANCO/12495/2011 guidelines.

RESULTS AND DISCUSSION

Limit of detection (LOD), limit of quantification (LOQ) and Recovery

Linearity was determined using calibration curves spiked into brown rice matrix. Levels ranged from 0.005mg/kg to 0.1mg/kg. The linearity for all compounds was R² > 0.991. Limits of detection (LOD) and quantitation (LOQ) were determined by preparing matrix spikes at low concentration. Concentrations of 0.01 mg/kg were analyzed in seven matrix samples, and the LOD and LOQ were calculated from these results by multiplying the standard deviation of the calculated amounts by 3 and 9 respectively. The average LOD of the 110 pesticides was

0.002 mg/kg and the average LOQ was 0.006 mg/kg. Method precision was demonstrated by analyzing seven matrix samples spiked at 0.01 and 0.1 mg/kg. Samples had average recoveries of 96.5% at low concentration, 101.5% at high concentration and relative standard deviation (RSD) of 8.8% at low concentration, 4.1% at high concentration.

Table 2. Recovery, RSD, LOD and LOQ data for 110 Compounds

NO.	Compound	10ppb Recovery%	Low RSD	100ppb Recovery%	High RSD	LOD ($\mu\text{g/kg}$)	LOQ ($\mu\text{g/kg}$)
1	Acrinathrin	111.1	6.3	107.3	6.1	2.1	6.3
2	Alachlor	94.3	5.7	103.1	2.8	1.6	4.8
3	Aldrin	89.7	7	94.1	3	1.9	5.6
4	Ametoctradin	90.8	3.7	97.9	3.2	1	3
5	Azaconazole	83	6.6	85.7	2.9	1.6	4.9
6	Benfuresate	104.3	10.5	108.4	4.4	3.3	9.8
7	BHC(alpha)	98.6	4.4	100	3.2	1.3	3.9
8	BHC(beta)	98.8	5.3	100.8	4	1.6	4.7
9	BHC(delta)	95	7.2	103.3	2.7	2.1	6.2
10	BHC(gamma)	95.1	4.5	103.5	2.7	1.3	3.8
11	BifenoX	105.8	6.8	101.4	4.5	2.1	6.4
12	Bifenthrin	95.7	7	101.9	4.1	2	6
13	Bromobutide	94.2	5.4	111.4	1.3	1.5	4.5
14	Bromopropylate	96	6	95.9	3.4	1.7	5.2
15	Butachlor	104.1	9.2	108.8	4.1	2.9	8.6
16	Butafenacil	103.9	5.3	104.1	2.9	1.7	5
17	Carbophenothion	89.9	6.6	91.1	4.1	1.8	5.4
18	Chlorantraniliprole	109.6	6.3	123	5.5	2.1	6.2
19	Chlordane(cis)	88.2	6.8	91.5	3.9	1.8	5.4
20	Chlordane(trans)	89.1	6.7	91.9	3.2	1.8	5.4
21	Chlorfenapyr	77.6	17.1	87.2	4.1	4	11.9
22	Chlorfenvinphos	96.6	6.6	102.4	3.6	1.9	5.8
23	Chlorobenzilate	106.4	9.3	100.6	3.7	3	8.9
24	Chlorpropham	102.2	6.5	106.5	3.8	2	5.9
25	Chlorpyrifos-methyl	99.8	6.7	104.4	2.5	2	6
26	Cyfluthrin	103	16.1	105.9	4.4	5	15
27	Cyhalothrin	100.4	16.6	117.2	5.8	5	15
28	Cypermethrin	100.8	9.8	106.1	3.9	3	8.9
29	Cyprodinil	84.3	6.9	103.9	3	1.8	5.3
30	Deltamethrin	114.3	7.1	119.6	5.9	2.4	7.3
31	Dichlobenil	92.6	5.7	100.1	2.1	1.6	4.8
32	Dieldrin	73.8	6	75.4	2.8	1.3	4
33	Difenoconazole	78.6	6.6	97.1	4	1.6	4.7
34	Dimethachlor	99.7	8.2	105.9	2.8	2.4	7.3
35	Dimethoate	102.7	16.6	110.2	3	5.1	15.3
36	Dimethylvinphos	105.8	8.6	119.8	3.5	2.7	8.2
37	Disulfoton	91.2	6.4	101.3	2.3	1.8	5.3
38	Endosulfan(alpha)	96.8	7.7	101.3	3.7	2.2	6.7
39	Endosulfan-sulfate	95.5	9.2	115.9	3.7	2.6	7.9
40	Endrin	85.8	6.1	84.6	3.2	1.6	4.7
41	EPN	102.5	7.1	107	3.7	2.2	6.5
42	Epoxiconazole	87.4	8.8	95.9	5	2.3	6.9
43	Ethalfuralin	117.5	9.8	110.1	6.8	3.4	10.3
44	Ethion	89	7.1	87.5	3.4	1.9	5.7
45	Etridiazole	99.4	5.6	104.5	2.9	1.7	5
46	Fenclorim	101.4	7.2	93.8	4.5	2.2	6.5

47	Fenitrothion	97.2	5.8	110.6	1.9	1.7	5.1
48	Fenothiocarb	103.1	8.6	107.8	3.1	2.7	8
49	Fenoxanil	86.1	7.4	86.8	1.9	1.9	5.7
50	Fenpropathrin	82.9	11.6	96.3	4.4	2.9	8.7
51	Fenthion	97.3	9.5	102.9	3.9	2.8	8.4
52	Fenvalerate	94.2	5.4	104.9	3.7	1.5	4.6
53	Fipronil	100	6.6	97.8	3	2	6
54	Flucythrinate	96.2	7	104.3	3.2	2	6
55	Flumioxazine	97.1	6.3	103.4	3.5	1.8	5.5
56	Fluopyram	100.6	5.7	103.9	2.3	1.7	5.1
57	Fonofos	104.2	6.6	104	3.2	2.1	6.2
58	Fthalide	92.2	5.1	102.3	2	1.4	4.2
59	Halfenprox	87.2	4.6	97.5	3.3	1.2	3.6
60	Heptachlor	95.1	5.6	97.3	3.5	1.6	4.8
61	Heptachlor-epoxide	95.4	6.1	99.5	2.4	1.7	5.2
62	Imibenconazole	87.3	5.5	90.1	10.7	1.4	4.3
63	Iprodione	104.3	8.2	105.3	3.4	2.6	7.7
64	Isazofos	94.7	7.5	105.4	2.1	2.1	6.4
65	Isofenphos	102.1	7.4	100.3	3.3	2.3	6.8
66	Mecarbam	86.8	10.5	101.2	2.9	2.7	8.2
67	Methidation	89.6	7	108.1	3	1.9	5.6
68	Methoxychlor	96.5	5.5	103.1	3.2	1.6	4.8
69	Metolachlor	107.2	6.6	113.1	3	2.1	6.3
70	Metribuzin	95.8	7.3	102.9	3	2.1	6.3
71	o,p-DDT	82.4	6.6	87.4	2.8	1.6	4.9
72	Oxyfluorfen	80.3	4.5	74.1	4.5	1.1	3.3
73	p,p-DDD	80.3	5	87.8	2.7	1.2	3.6
74	p,p'-DDE	92.2	6	91.8	4.1	1.7	5
75	p,p-DDT	92.7	4.7	99	3.2	1.3	4
76	Parathion-ethyl	99.8	6.8	104.9	3.7	2	6.1
77	Parathion-methyl	101.3	2.9	105.7	3.9	0.9	2.7
78	Pendimethalin	101.3	9.1	98	5.2	2.8	8.3
79	Pentachloroaniline	92.7	6.8	91.2	2.8	1.9	5.7
80	Penthiopyrad	93.6	9.1	93.2	6.4	2.6	7.7
81	Phorate	119.7	5	112.2	3.7	1.8	5.4
82	Phosalone	113.1	8.5	115.8	3	2.9	8.6
83	Picoxystrobin	100.8	10	96.5	5.5	3	9.1
84	Piperonyl bitoxide	92.1	6.2	98.8	6	1.7	5.1
85	Pirimiphos-ethyl	98.7	4.7	103.1	3	1.4	4.2
86	Prochloraz	103.3	13.4	117.7	4	4.1	12.4
87	Procymidone	94.8	9.7	104	3.9	2.8	8.3
88	Promecarb	112.3	3.8	115.3	2.4	1.3	3.9
89	Prometryn	102.8	8.3	98	4.9	2.6	7.7
90	Propachlor	107	6	105.9	3.7	1.9	5.8
91	Propazine	96.4	3.1	112	3.8	0.9	2.7
92	Propiconazole	91.9	5.9	104.1	3.4	1.6	4.9
93	Propisochlor	100.7	8	106.9	3	2.4	7.2
94	Propyzamide	101.8	5.6	104	2.7	1.7	5.2
95	Pyridalyl	99.4	12.1	103.9	2.5	3.6	10.8
96	Quintozene	101	5.2	99.5	5.9	1.6	4.7
97	Simazine	89.8	9.8	105.9	5.7	2.6	7.9
98	Simeconazole	88.6	3.3	100.9	4.5	0.9	2.6
99	Simetryn	94.4	7.6	91	7.8	2.1	6.4
100	Spiromesifen	92.1	8.8	105.7	5	2.4	7.3
101	Tebupirimfos	99.4	7.6	100.2	3.3	2.3	6.8

102	Tefluthrin	99.2	6.3	101.6	2.6	1.9	5.7
103	Terbufos	105.6	4.8	104.9	2.6	1.5	4.5
104	Terbutryn	94.5	6.8	104.1	2	1.9	5.8
105	Tetradifon	92.2	6.6	99.4	3.9	1.8	5.5
106	Thifluzamide	80.6	6.7	94.5	3.4	1.6	4.9
107	Tolclofos-methyl	101.5	7	105.9	3.6	2.1	6.4
108	Triadimenol	97.7	8.7	99.9	3.1	2.6	7.7
109	Tri-allate	92.2	6.1	99.9	2.7	1.7	5.1
110	Trifluralin	107.9	5.6	107.7	5.7	1.8	5.4
	Average	96.6	7.2	101.7	3.7	2.1	6.3

CONCLUSIONS

The following conclusions can be drawn from the study:

- A qualitative and quantitative method for simultaneous determination of 110 pesticides compounds by GC-MS/MS was successfully developed and validated. All recovery of compounds are 70% ~ 120%, RSD < 20%.
- The proposed method showed appropriate accuracy and precision, and was successfully used to analyze 110 pesticides simultaneously. The developed method could be employed as a valuable tool during routine analysis for pesticides in agricultural foods, as the ability to simultaneously determine the levels of these compounds in agricultural foods will play an important role in research.

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