Journal of Chromatography A, 1218 (2011) 334–342

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Determination of 195 pesticide residues in Chinese herbs by gas chromatography–mass spectrometry using analyte protectants

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ARTICLE INFO

Article history:
Received 3 September 2010
Received in revised form
17 November 2010
Accepted 18 November 2010
Available online 24 November 2010

Keywords:
Analyte protectants
Matrix-induced enhancement effect
Pesticides multi-residue analysis
Gas chromatography–mass spectrometry (GC–MS)
Chinese herbs

ABSTRACT

The phenomenon known as "matrix-induced enhancement effect" is not only observed in the analysis of pesticides in food, but also in Chinese herbs. Several approaches have been proposed to overcome the matrix-induced effect, but each method has serious limitations. Compared with standard calibration methods, the procedure with adding analyte protectants offers a more convenient and effective route to solve the problem. In the current study, we have analyzed 195 types of pesticides in Chinese herbs by gas chromatography–mass spectrometry (GC–MS), and the compounds that are susceptible to matrix effect were picked up and confirmed. In addition, several analyte protectants were evaluated and the most effective combination was determined. D-Ribonic acid-γ-lactone (2 mg/ml) and D-sorbitol (1 mg/ml) were shown to be the best analyte protectants for the analysis of most pesticides.

1. Introduction

In the trace analysis with gas chromatography (GC), such as analysis of pesticides in food, the accuracy is often seriously affected by a phenomenon known as "matrix-induced chromatographic response enhancement effect" [1]. It was first described by Erney et al. [2]. According to the definition of European guidelines [3], the matrix effect is that the measurement of an analyte concentration or mass is influenced by one or more undetected components from the sample. In the GC detection, interactions of pesticides with matrix were often observed, as indicated by the increased detector responses and good chromatographic shape in samples, compared with the same residues in simple solvent solutions. However, not all the pesticide compounds are affected by matrix-induced enhancement effect. Some factors may affect sample matrix enhancement such as the nature of pesticide, the nature of the matrix and the GC system [4]. The compounds that are thermally unstable at their vaporizing temperature or tend to adsorb on the surfaces of the GC system are easily affected by the matrix [5,6]. Currently, several approaches have been proposed to overcome the matrix effect, such as (1) use of the standards in residue-free matrix spiked with standards (matrix-matched standards); (2) sample purification; (3) the use of deuterated internal and/or surrogate standard and (4) the use of calibration correction factors [7]. The most widely used approach in laboratories is the matrix-matched standards, which requires enough blank matrix, and extra time, label, expense for preparing the blank extracts. It is impossible to control the quality of routine analysis. Additional sample pre-treatment could reduce the matrix effect by partly removing the matrix components, but this procedure is unfeasible in multi-pesticide analysis because of the wide polarity range of the analytes and the potential analyte losses, which may lead to low recovery. Alternatively, analyte protectants can be used. Analyte protectants were firstly introduced by Erney and Poole [8]. And this method was re-introduced by Lehotay et al. in 2003. The benefits of using analyte protectants in quantitative analysis include: (1) improvement of the shape and intensity of chromatographic peaks, especially the susceptible analytes; (2) lower detection limits; (3) less maintenance for GC system and (4) simpler procedure and lower cost. Since then, the analyte protectants method has been extended and widely studied.

The matrix effect occurs not only in food analysis, but also in the quality control process of Chinese herbs. In this study, we have studied 195 types of pesticides by gas chromatography–mass spectrometry (GC–MS) analysis, and selected some typical compounds that are susceptible to matrix-induced enhancement in Chinese herbs. Furthermore, we have evaluated seven potential analyte protectants [9,10,11], and determined the best combination of analyte protectants. Our work is aiming to develop a combination of analyte protectants that can effectively diminish the matrix-induced response enhancement effect in the analysis of pesticide residues

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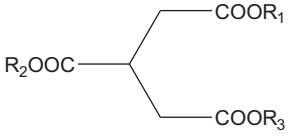
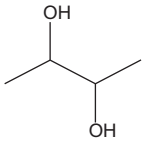
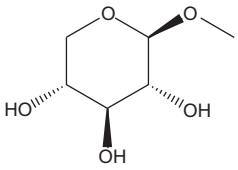
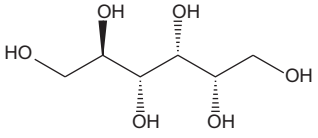
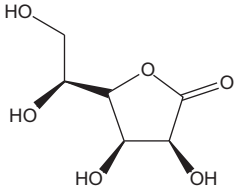
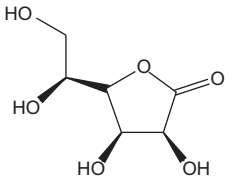
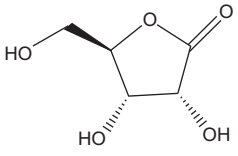
doi:10.1016/j.chroma.2010.11.036

Table 1
List of compounds evaluated as protectants in the study.

Code	Compound name	CAS no.	Structure	t_R (min)
a	Olive oil	8001-25-0	 R1: oleic acid; R2: Palmitic acid; R3: Linoleic acid	14.6–15.1 27.0–27.8
b	2,3-Butanediol	513-85-9		6.4–7.2
c	1-O-Methyl-β-D-xylopyranoside	612-05-5		7.7–8.7
d	D-Sorbitol	50-70-4		10.9–13.8
e	Gulonic acid-γ-lactone	1128-23-0		7.6–8.4 13.2–18.2
f	3-Eththoxy-1,2-propanediol	1874-62-0		5.6–5.8 9.7–10.2
g	D-Ribonic acid-γ-lactone	5336-8-3		7.8–8.5 9.2–11.6

in Chinese herbs, such as Common yam rhizome, Milkvetch, and Dried Tangerine Peel.

2. Experimental

2.1. Chemicals and reagents

Table 1 lists the compounds that were evaluated as analyte protectants. All the compounds were commercially available, which were bought from Sigma or other sources, and the pesticide reference standards were obtained from the National Pesticide Standard Repository of PR China, Dr. Ehrenstorfer GmbH and Chemservice. Stock solution of 100 μg/ml for each pesticide was stored at −35 °C. A standard stock solution of 1 μg/ml containing 195 types of pesticide was prepared in acetonitrile (MeCN). Ethyl acetate, cyclohexane, MeCN and acetone of pesticide grade were purchased from Dikma.

2.2. Samples

Common yam rhizome (Rhizoma Dioscoreae), Milkvetch (Radix Astragali), Dried Tangerine Peel (Pericarpium Citri Reticulatae) were purchased from Pharmacies in Beijing. The three samples were analyzed by the procedure described below and those samples showing the absence of target analytes were used as blank samples to evaluate analyte protectants.

2.3. Instrumentation

An SHIMADZU gas chromatograph-mass (QP-2010) equipped with an automatic split–splitless injector (AOC i+s) were used in

Table 2
Retention time (t_R), target ion (T), qualifier ions (Q_1, Q_2,), LOQ of pesticides.

Code	Name	LOQ (μg kg^{-1})	LOQ* (μg kg^{-1})	t_R (min)	T	Q_1	Q_2	C (%)	M (%)	D (%)
SIMA										
1	Dichlorvos	5	2.5	5.455	109	185	79	104.5	107.2	113.6
2	Methamidophos	50	5	6.418	94	141	95	99.7	88.8	109.4
3	Acephate	50	5	8.838	136	94	125	102.8	87.5	102.1
4	Tecnazene	10	5	9.566	261	203	215	102.4	103.9	109.1
5	Mecoprop	100	25	9.566	169	107	214	110.5	108.4	113.9
6	Hexachlorbenzene	2.5	2.5	10.497	284	286	282	99.7	102.7	97.9
7	Pentachloroanisole	7.5	5	10.646	265	280	237	89.3	92.1	106.2
8	α-BHC	5	5	10.929	219	181	221	93.4	102	115.8
9	Omethoate	50	4	11.085	156	110	109	83.7	80.7	113.1
10	Dichlorprop	100	10	11.15	234	162	164	103.9	104.8	111.3
11	Diazinon	10	5	11.54	179	137	304	106.1	105.9	114.7
12	Quintozene	7.5	5	11.688	237	249	295	104.3	103.1	112.1
13	γ-BHC	7.5	5	12.1	219	181	109	102.1	94.1	109.1
14	Monocrotophos	50	2.5	12.255	127	192	97	97.4	91.3	108
15	β-BHC	7.5	5	12.838	219	181	254	96.3	103	118.1
16	Phosphamidon	40	15	12.925	264	127	138	92.5	95.5	112.4
17	Dimethoate	50	5	12.932	229	93	87	114.6	109.8	110.6
18	2,4-D-butylate	50	10	13.007	276	220	185	90	99	119.8
19	Heptachlor	7.5	7.5	13.018	272	237	337	96	99.9	103.2
20	Vinclozolin	15	7.5	13.234	212	285	198	110.2	116.7	114.1
21	Pentachloroaniline	15	7.5	13.54	265	263	230	100.3	93.9	113.5
22	δ-BHC	15	7.5	13.906	219	181	217	94.7	87.3	96
23	Chlorothalonil	25	10	13.95	266	264	268	97.2	92.3	104
24	Chlorpyrifos-methyl	7.5	3	14.086	286	125	288	104.6	109.3	111.5
25	Aldrin	10	10	14.152	263	293	193	101.3	102	111.7
26	Pirimiphos-methyl	7.5	3	14.72	290	276	305	101.3	104.1	119
27	MPCPS	10	5	14.8	296	263	246	95.4	94	107.3
28	Metalaxyl	15	7.5	14.9	206	146	234	108.3	99.6	113.5
29	Chlorthal-dimethyl	5	2.5	15.244	301	332	299	96	95.7	115.9
30	Triadimefon	10	5	15.322	208	57	181	113.1	104.8	105.9
31	Chlorpyrifos	10	5	15.34	314	286	258	103.8	107.2	99.3
32	Malathion	10	5	15.64	173	143	158	101	94.4	106.2
33	Fenitrothion	15	5	15.742	277	125	260	101.6	102.6	100.2
34	Parathion	20	7.5	15.924	291	155	235	103.2	103.4	98.1
35	Pendimethalin	7.5	5	16.624	252	281	162	101.5	94.2	91.5
36	Heptachlor-epoxide	3	3	16.69	353	355	351	100	100	98.4
37	Triadimenol	15	7.5	17.438	112	168	128	108.1	109.2	110.6
38	Chlordane	10	10	17.629	373	375	377	96.4	100.4	84.3
39	Procymidone	7.5	5	18.15	283	285	255	103.5	111.3	119.9
40	Endosulfan	15	15	18.282	241	339	265	95.8	113.8	107.1
41	Paclobutrazol	10	5	18.657	236	238	167	99.9	105.3	112.1
42	Pretilachlor	4	2.5	19.009	238	162	262	95.9	102.4	117.9
43	Bentazone	50	10	19.227	198	161	119	93.6	82.3	104.5
44	p,p′-DDE	5	4	19.926	246	318	248	98.3	107.2	112.1
45	Dieldrin	12.5	12.5	20.175	263	277	345	116.7	111.6	111.6
46	Methidathion	7.5	3	21.234	145	93	125	98.6	102.6	238
47	Endrin	10	10	22.225	263	281	345	96.1	92.8	111.7
48	o,p′-DDT	5	3	23.086	235	165	199	98.8	99.2	113.8
49	p,p′-DDD	5	3	23.654	235	165	199	92.7	100.4	112.4
50	Ethion	5	3	23.823	231	153	384	97.5	103.5	119.5
51	p,p′-DDT	10	7.5	25.383	235	165	199	104.7	98.3	115.8
52	Propiconazol	10	5	25.658	259	173	261	94.5	102.6	114.6
53	RH-5849	20	10	25.91	240	105	77	93.9	102.2	111.7
54	Endosulfan sulfate	10	10	26.242	272	387	389	94.1	106.6	113.7
55	Iprodione	20	10	26.967	187	244	246	94.4	97.1	114.9
56	Dicofol	100	25	27.3	139	251	141	105.1	95.5	107.2
57	Methoxychlor	4	2.5	27.551	227	212	228	92.6	96.9	117.5
58	Mirex	7.5	7.5	27.599	272	237	274	94.3	100	100
59	Tetradifon	15	7.5	27.933	227	159	356	107	105.6	112.1
60	Phosalone	20	10	28.058	182	154	367	100	108.3	116.9
61	Acetamiprid	100	50	28.992	126	152	166	88.3	107.9	101.4
62	Coumaphos	50	25	29.585	362	226	210	94.7	94.6	118
SIMB										
63	Vernolate	10	5	6.874	128	203	86	88.4	87.1	94.9
64	Methacriphos	10	5	8.292	208	180	240	87.1	88.1	104.5
65	Metolcarb	25	2.5	8.47	108	107	109	92.7	99.2	106.2
66	Molinate	4	2.5	8.975	126	187	55	96.3	105.6	103.2
67	Isoprocarb	5	2.5	9.11	121	136	122	91.8	91.5	109.8
68	Ethoprophos	5	2.5	9.91	158	200	139	98	105.1	107.6
69	Diphenylamine	7.5	2.5	10.284	169	168	167	106.3	107.1	110.5
70	Sulfotep	2.5	2.5	10.436	322	202	237	103.3	111.2	105.2
71	Phorate	15	7.5	11	260	121	75	101.1	103.8	106.6
72	Bromoxynil	25	2.5	11.557	277	275	279	95.2	117.5	108.8

Table 2 (*Continued*)

Code	Name	LOQ (μg kg^{-1})	LOQ* (μg kg^{-1})	t_R (min)	T	Q_1	Q_2	C (%)	M (%)	D (%)
73	Propetamphos	10	5	11.608	138	194	236	95.2	89.4	110.5
74	Dicloran	20	7.5	11.992	206	124	176	92.9	110.3	117.2
75	Etrimfos	5	2.5	12.296	292	181	153	111.2	117	109.6
76	Isazofos	12.5	7.5	12.974	119	161	257	103.7	107.4	110.8
77	Fenchlorphos oxon	15	5	13.467	269	109	271	116.4	112.6	117.4
78	Alachlor	7.5	7.5	13.707	160	188	237	112.6	115.8	111.3
79	Paraoxon methyl	30	10	13.716	247	230	109	102.1	111.8	112.5
80	Fenchlorphos	5	2.5	14.08	285	287	125	106.8	112.6	109.3
81	Propanil	50	10	14.367	161	217	163	81.9	89	109.6
82	Prometryn	15	10	14.483	184	241	226	99.2	118.7	109.5
83	Parathion-methyl	15	5	14.73	263	125	109	114.6	87.4	110.6
84	Tolclofos-methyl	4	2.5	14.771	265	267	200	102.5	111.6	110.6
85	Ametryn	12.5	7.5	14.85	212	227	170	102.2	106.8	102.7
86	Metolachlor	7.5	7.5	14.975	238	162	240	107.5	108.7	111.6
87	Paraoxon-ethyl	25	15	15.01	275	149	220	117.4	109.5	118
88	Pirimiphos-ethyl	5	5	15.907	333	318	168	119.7	102.5	108.7
89	Dichlofluanid	100	15	16.05	123	167	224	104.8	103.5	103.9
90	Carbaryl	12.5	2.5	16.226	144	115	201	92.7	87.7	112.8
91	Bromophos-methyl	10	2.5	16.637	331	329	125	101.5	102.7	110.5
92	Isofenphos	10	7.5	17.116	213	185	255	99.5	102.4	111.3
93	Butachlor	5	5	17.548	176	160	188	103.2	110.7	115.8
94	Bromophos-ethyl	10	5	17.766	359	303	331	94.6	89.1	106.3
95	Chlorfenvinphos	7.5	5	18.071	267	269	323	105.1	113.7	106.8
96	Mecarbam	15	15	18.255	131	159	296	108.1	116.3	118.4
97	Tolylfluanid	100	10	18.274	137	238	240	108.1	117.8	113.2
98	Anilazine	50	10	18.348	239	178	143	116	87.9	82.8
99	Quinalphos	10	7.5	18.644	146	298	157	104.8	118.9	109.2
100	Dimethachlon	20	10	18.718	243	245	187	107.3	99	112.7
101	Phenthoate	7.5	7.5	19.151	274	121	246	107.1	117.3	116.8
102	Oxadiazon	10	10	19.224	302	258	302	99.5	101.3	105
103	Chinomethionat	25	5	20.211	234	206	116	92	87.3	116.1
104	Buprofezin	5	10	20.523	172	105	119	97.1	99.3	113
105	Profenofos	12.5	10	20.716	337	339	207	98.8	112.5	113.7
106	Uniconazole-P	10	7.5	20.897	234	236	131	90.1	89.9	101.8
107	Imazalil	25	10	20.99	215	172	217	115.5	118.2	114
108	Thiabendazole	50	5	21.893	201	174	129	103.2	88.9	104.6
109	Diniconazole	10	5	22.675	268	270	232	93.1	107.5	101.8
110	Primiphos-methyl1	50	25	22.166	360	313	289	89.3	99	101.3
111	Myclobutanil	12.5	10	22.408	179	150	152	100.1	117.6	105.8
112	Isoprothiolane	7.5	7.5	23.74	118	204	290	92.4	102.7	98.4
113	Fludioxonil	15	5	23.926	248	127	154	90.6	100.2	107.5
114	Primiphos-methyl	10	5	23.949	360	313	289	98.1	81.4	117
115	Carbophenothion	2.5	1.5	25.501	342	125	157	104.7	115.7	109.6
116	Piperonyl butoxide	4	2.5	25.654	176	177	149	103.1	113.2	109.7
117	Tebuconazole	15	7.5	26.092	250	163	252	85.9	100.1	107
118	Tricyclazole	45	15	26.12	189	162	118	93.6	86.5	176.6
119	Triazophos	10	5	26.69	161	172	257	91.3	102.5	111.2
120	Bromopropylate	7.5	7.5	26.75	341	183	339	90.6	100.4	100.1
121	Phenothrin	10	5	27.202	183	123	350	98.1	92.9	107.7
122	EPN	20	10	27.437	157	323	169	90.7	104.2	106.1
123	Phosmet	5	2.5	28.167	160	161	317	87.3	98	115.8
124	Pyridaben	7.5	7.5	28.933	147	117	309	94	103.4	116.2
125	Azinphos methyl	20	10	29.108	132	160	104	88.9	108.5	117.5
126	Prochloraz	50	25	29.132	180	308	70	102.8	85.6	107.9
127	Azinphos methyl	15	7.5	29.416	132	160	77	90.1	108	111.6
128	Etofenprox	20	10	30.25	163	376	135	85.3	87.9	105.4
129	Quizalofop-ethyl	25	5	30.365	299	372	243	81	95.8	110.9
SIMC										
130	Mevinphos	5	2.5	9.16	127	192	109	87.3	89	104.4
131	Demeton	25	10	11.38	88	171	60	102.9	112.6	117.6
132	Trifluralin	5	2.5	9.691	335	306	264	96.5	95.7	107.8
133	Cadusafos	50	25	10.108	159	127	213	91.5	103.5	110.3
134	Terbufos	2.5	2.5	11.25	231	153	186	95.4	101.8	104.1
135	Dicrotophos	20	10	11.58	127	237	193	110.5	106.4	115.2
136	Atrazine	20	5	11.85	200	215	173	105.3	92.6	114.2
137	Fonofos	5	2.5	12.158	137	246	109	97.8	98.2	106.8
138	Dichlofenthion	50	5	12.846	279	251	223	105.7	105.6	110.8
139	Procymidone	10	5	13.108	321	333	279	104.7	89.5	116.7
140	Octachlorodipropyl ether	25	10	13.242	130	131	79	97.8	103.7	87
141	Pirimicarb	10	5	13.873	166	238	72	91.4	93.8	108.1
142	Fluazinam	20	10	14.066	418	372	337	110.4	96.5	115.8
143	Ethiofencarb	20	5	14.493	107	168	77	92.9	110.1	91.9
144	Malaoxon	20	10	14.641	127	268	195	114.5	112.5	115.1
145	Butralin	20	10	14.659	266	295	224	100.5	91.3	115.9

Table 2 (*Continued*)

Code	Name	LOQ (μg kg^{-1})	LOQ* (μg kg^{-1})	t_R (min)	T	Q_1	Q_2	C (%)	M (%)	D (%)
146	Metribuzin	15	5	15.175	198	144	103	91.9	85.8	116.4
147	Fipronil	4	2.5	15.613	367	369	213	109.6	115.6	109.9
148	Allethrin	5	2.5	15.645	123	136	107	83.1	86.3	110
149	Methiocarb	6	2	16.199	168	225	153	95	91.2	112.5
150	Flumetralim	12.5	10	16.677	143	404	145	102	90.1	114
151	Fenthion	2.5	1.5	16.808	278	125	169	106.6	111.1	108.8
152	Isofenphos-methyl	10	5	16.846	199	58	241	102.1	100.4	112.6
153	Haloxyfop	15	5	17.017	375	316	288	83.8	77.3	106.8
154	Cyprodinil	10	5	17.503	224	225	210	91	88.6	105.7
155	Isocarbophos	10	5	17.678	136	230	289	107.6	109.2	117.9
156	Fosthiazate	50	25	19.438	195	283	97	85.3	87	110.4
157	Prothiophos	10	5	19.339	309	267	162	97.9	98.6	110.1
158	Dimepiperate	12.5	10	19.355	119	145	91	101	106.6	108.6
159	Hexaconazole	10	5	19.623	214	231	88	88.9	94.4	114.2
160	Fluazifop-p-butyl	7.5	2.5	19.769	282	383	254	89.6	83.7	105.6
161	Thiamethoxam	50	10	20.024	212	247	182	85.6	86.8	88.1
162	Flutolanil	7.5	2.5	20.251	323	145	173	88.5	92.8	109
163	Napropamide	5	2.5	20.87	72	128	271	97.7	94.4	107.5
164	Fenamiphos	10	5	21.023	303	154	288	97.6	114.6	103.9
165	Chlorfenapyr	5	5	21.18	247	328	59	110.6	101.7	84.8
166	Flusilazole	10	5	21.44	233	206	315	89.7	97	103.4
167	Nitrofen	50	10	22.97	283	202	139	107.4	94.4	115.1
168	Phosfolan	50	10	24.04	140	255	92	98.9	98.7	111.9
169	Mepronil	25	5	25.498	119	269	91	91.1	97.2	108.2
170	Propargite	25	10	25.8	135	350	173	93.4	102.3	172
171	Fensulfothion	5	2.5	25.76	292	293	308	88.1	93.3	78.4
172	Benalaxyl	5	4	25.913	148	206	234	87.8	87.4	93.4
173	Bifenthrin	12.5	5	26	181	165	166	96.4	100.2	105.3
174	Fenthion sulfoxid	7.5	2.5	26.309	279	278	294	85.9	102.7	112.4
175	Fenthion sulfone	10	5	26.395	310	136	231	82.8	91.8	91.4
176	Oxadixyl	25	25	26.73	163	132	105	101.6	81.7	109.6
177	Edifenphos	10	5	26.83	310	173	201	86.7	86.4	109.9
178	Fenpropathrin	7.5	7.5	26.941	265	181	249	88.5	101	115.5
179	Tetramethrin	20	10	27.24	164	165	123	90.3	91.7	107.5
180	Cyhalothrin	10	10	27.29	181	197	208	96.6	97.3	114.1
181	Hexazinone	20	10	27.549	171	252	128	89.4	91.8	104.8
182	Furathiocarb	25	20	27.582	163	194	325	93.7	94.8	109.2
183	Cyhalofop-butyl	20	5	27.767	357	256	229	104.9	81.6	106
184	Fluoroglycofen-ethyl	25	7.5	28.033	344	447	417	87.6	97.5	100.6
185	Permethrin	25	25	28.77	183	163	184	95.2	98.9	106.5
186	Fenarimol	10	5	28.7	330	251	219	88.3	82.8	106.3
187	Bitertanol	7.5	2.5	28.75	170	112	141	83.9	87.5	107.8
188	Cyfluthrin	10	10	29.3	163	206	226	115.8	117.5	117
189	Flucythrinate	20	15	29.99	199	157	225	86.6	108.5	114.8
190	Cypermethrin	25	25	29.868	163	181	209	109.6	98.7	112.9
191	Tau-fluvalinate	20	15	30.38	250	252	181	88.7	103.3	117.2
192	Fenbuconazole	25	15	30.49	129	198	125	88.6	96.8	103.4
193	Fenvalerate	7.5	7.5	31.119	167	125	225	99.2	329.9	113.8
194	Deltamethrin	25	25	32.843	181	253	172	109.8	103.2	97.3
195	Difenoconazole	20	10	33.16	323	325	265	86.3	97.1	96.3

LOQ: limit of quantification of pesticides in simple solvent; LOQ*: limit of quantification of pesticides using analyte protectants; C: Common yam rhizome; M: Milkvetch; D: Dried Tangerine Peel.

the chromatographic analysis. GPC ULTRA (LC Tech) were used for clean up.

2.4. Sample preparation

Extraction: 10 g of fine powder sample and 1 g of sodium chloride (NaCl) were weighted and put into a 150 ml conical flask. Then 100 ml acetone was added, and the mixture was ultrasonicated for 30 min, cooled down, and centrifuged. The supernatant was transferred to another conical flask, in which 1 g of sodium sulfate has been added, and kept for 30 min.

According to the property of each sample, different procedures were chosen to clean up the samples.

Common yam rhizome: 40 ml extract was concentrated in vacuum to almost dryness at 55 °C. The residue was dissolved with cyclohexane–ethyl acetate (1:1), transferred to a volumetric flask, and diluted with cyclohexane–ethyl acetate (1:1) to 10 ml. Then it was cleaned by gel permeation chromatography (GPC) at the flow rate of 5 ml/min. The GPC eluate collected from 16 min to 26 min was concentrated to 5 ml. Then 5 ml solution was concentrated to almost dryness at 65 °C, transferred to a graduated flask, and finally diluted with MeCN to 1 ml.

Milkvetch: 20 ml extract was concentrated in vacuum to almost dryness at 55 °C. The pear-shaped flask was rinsed with 3× 1 ml acetone–ethyl acetate (1:1), which were loaded onto A NH_2 column. The cartridge was conditioned with 5 ml acetone–ethyl acetate (1:1) before being added with the sample. The pesticides were eluted with 15 ml acetone–ethyl acetate (1:1), and the eluate was evaporated to about 1 ml using a rotary evaporator at 65 °C.

Dried Tangerine Peel: 20 ml extract was treated following the same procedure that used in the cleaning up of Milkvetch. The only difference is that CARB/NH_2 was used for cleaning.

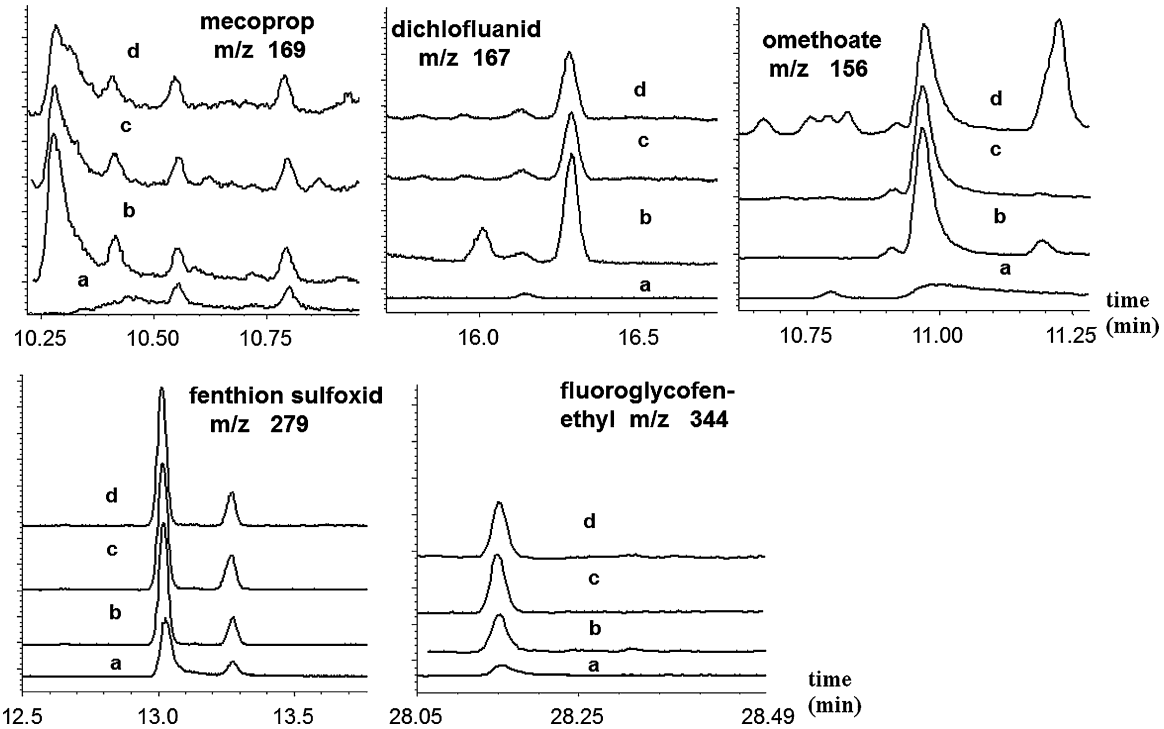


Fig. 1. The representative compounds that are seriously affected by matrix effect. (a) Blank solvent; (b) blank matrix of Common yam rhizome; (c) blank matrix of Milkvetch and (d) blank matrix of Dried Tangerine Peel (each at 200 ng/ml).

2.5. Determination of pesticides by GC–MS

The chromatographic conditions were DB-17ms capillary column of 0.25 mm i.d., 30 m, and 0.25 μm film thickness, He constant flow of 1.3 ml/min, inlet temperature at 230 °C, pulsed split (pulsed pressure 250 kPa for 1 min), injection volume 1 μl, MS transfer line temperature 250 °C. The column temperature program was as follows: 60 °C(1.5 min) → 30 °C/min → 120 °C → 10 °C/min → 200 °C → 20 °C/min → 230 °C(10 min) → 30 °C/min → 300 °C(7 min). Total run time was 37.63 min. Full scan analysis (40–450 *m/z*) was used in the experiments to determine the chromatographic and MS traits of the different compounds. Quality control was performed with selected ions monitoring (SIM) mode with one target and two or three qualifier ions. The SIM mode was selected because it allows increased peak response by concentrating on ions specific to the compounds under investigation [12]. Sometimes, a few target ions of the 195 selected pesticides were affected by the analyte protectants. In those cases, we choose the ions that were not affected to ensure the accuracy of analysis. Table 2 gives the final SIM condition including the retention times, the target ion and two qualifier ions.

3. Results and discussion

3.1. Compounds susceptible to matrix effect

The matrix effects were extensively studied by researchers [13–15]. The majority of matrix effect studies are related to the analysis of vegetable, honey, or meat and there is no similar study that has been carried out in Chinese herbs. Matrix effect can be observed by comparing the response from the analyte in the residue-free matrix with that prepared in solvent alone. We have studied 195 different pesticides covering a wide range of polarity, volatility and other physicochemical properties, and pointed out the compounds which are susceptible to the matrix-induced enhancement effect in Chinese herb samples, including Common yam rhizome, Milkvetch and Dried Tangerine Peel. Through our study, we have obtained the compounds that are thermally unstable [16], or have the adsorption interactions in hot injectors, both of which are susceptible to matrix effect, as summarized in Table 3.

Fig. 1 shows the representative compounds that are seriously affected by the matrix effect, such as mecoprop, dichlofluanid, omethoate, fenthion sulfoxid and fluoroglycofen-ethyl. Some pesticides (mecoprop, dichlofluanid, omethote, fluroglycofen-ethyl) were not almost detected in neat solvent, but they gave narrow and tall peaks in the three matrices. Some pesticides, such as fenthion sulfoxid, demonstrated three times higher peaks if analyzed in matrices.

In fact, the pesticides with phosphate (–P=O), hydroxyl (–OH), azoles (–N=), amino groups (–R–NH–), imidazole, benzimidazole, carboxyl (–COOH), carbamate (–O–CO–NH–) and urea (–NH–CO–NH–) are the most susceptible type of analytes to matrix effect. Organochlorine pesticides (OC) were compounds that presented low matrix effect because they are less polar and less adsorb on the surface of liner.

3.2. Evaluation of different analyte protectants

3.2.1. The category of analyte protectants

Matrix effect is based on the assumption that the analye has interacted with the active sites in the GC system, such as silanol groups and metal ions present at the glass surface, which results in losses and distorted peak shapes. Ideally, analyte protectant is a substance that could competitively interact with active sites in the liner and column, so that the response enhancement from analytes can be maximized. We have studied seven potential compounds in this work. These compounds were at 0.2–20 mg/ml, which were prepared in MeCN or MeCN:water solutions containing 50 ng/ml of 195 pesticides. After adding analyte protectants, the percentage of water present in the final pesticide standard solutions is given in Table 4.

Table 3
Typical compounds susceptible to matrix enhancement.

Category	Compound name
Orgnaophosphorus	Dichlorvos, methamidophos, omethoate, monocrotophos, phosphamidon, chlorpyrifos-methyl, parathion-methyl, pirimiphos-methyl, fenitrothion, parathion, methidathion, phosalone, coumaphos, ethoprophos, phorate, etrimfos, fenchlorphos oxon, paraoxon methyl, fenchlorphos, malaoxon, tolclofos-methyl, paraoxon-ethyl, pirimiphos-ethyl, fenthion, mecarbam, quinalphos, fenamiphos, carbophenothion, triazophos, phosmet, azinphos methyl, mevinphos, demeton, cadusafos, Dicrotophos, dichlofenthion, isazofos, primiphos-methyl, phosfolan, edifenphos, EPN, isofenphos-methyl, fosthiazate, ethion, sulfotep, terbufos, isazofos, bromophos-ethyl, prothiophos, phenthoate, profenofos
Organochlorine	Dicofol, chlorothalonil, captan, quintozene, PCA
Pyrethroid	Fenvalerate, cyhalothrin, cyfluthrin, tau-fluvalinate, flucythrinate, etofenprox, tetramethrin, deltamethrin, cyhalofop-butyl, phenothrin
Azoles	Triadimefon, triadimenol, propiconazol, paclobutrazol, imazalil, fenbuconazole, difenoconazole, diniconazole, tebuconazole, tricyclazole, myclobutanil, hexaconazole
Carbamates	Carbaryl, ethiofencarb, dimepiperate, furathiocarb, metolcarb, methiocarb, molinate, isoprocarb, pirimicarb
Dinitroaniline	Fluazinam, procymidone, pendimethalin, trifluralin, butralin, flumetralim
Amide	Alachlor, metolachlor, butachlor
Phenoxyacetic acid	2,4-D-Butylate, haloxyfop
Others	Acetamiprid, mecoprop, dichlorprop, vinclozolin, fipronil, dichlofluanid, tolylfluanid, chlorfenvinphos, piperonyl butoxide, fenthion sulfoxid, fenthion sulfone, pyridaben, fluoroglycofen-ethyl, octachlorodipropyl ether, chinomethionat, flutolanil, nitrofen, hexazinone, fenarimol, bitertanol

Table 4
List of pesticide standard solution (50 ng/ml) containing different contents of protectants.

Code	Concentration	% Water
a	0.2	–
	0.5	–
	1	–
	2	–
b	5	–
	10	–
	20	–
c	1	0.2
	2	0.4
	5	1
	10	2
d	0.5	1
	1	2
	2	4
	5	10
e	1	2
	2	4
	5	10
f	5	–
	10	–
	20	–
g	0.4	–
	1	–
	2	–
	5	–
	10	–

Fig. 2 shows the degree of effects observed within different analyte protectants at the peak shapes and intensities of 50 ng/ml.

Acephate and omethoate are the compounds known to be susceptible to matrix effect. As shown in Fig. 2, D-ribonic acid-γ-lactone (2 mg/ml) displayed the best quantification results for acephate and omethoate and other early eluting pesticides. D-Sorbitol gave good results for late-eluting pesticide such as fenitrothion and methidathion. 2,3-Butanediol showed the lowest chromatographic response enhancement effect in most of the pesticides. Olive oil was effective for some pesticide, but it had interferences in the SIM analysis of other pesticides. 3-Eththoxy-1,2-propanediol that aligned with previous findings is a highly effective compound for early eluting pesticides, especially for methamidophos, but seems to be a less effective coverage compared with D-ribonic acid-γ-lactone. If 3-eththoxy-1, 2-propanediol was used, a high concentration (10 mg/ml) was required so that too much substance was injected into GC system. L-Gulonic acid-γ-lactone is considered as a good agent that can provide good protection for many pesticides. However, this agent lacks the ability of late-eluting. Overall, none of the studied compounds can be used as an analyte protectant for all the pesticides. Therefore, we need to figure out a suitable combination of analyte protectants that can completely compensate for the matrix-induced enhancement in GC multi-residue analysis in Chinese herbs matrixes. The best result is a mixture of D-ribonic acid-γ-lactone and D-sorbitol.

In our study, we also found that the concentration of the analyte protectant in the standard solution is an important factor. At lower concentration, no effect was observed for some pesticides. But the response of pesticides remains relatively stable if the concentration of protectant is above a certain value. Therefore, we believe that the number of active sites in the GC systems is limited, and the matrix effect could be effectively compensated if the active sites were masked completely by the analyte protectants. Upon

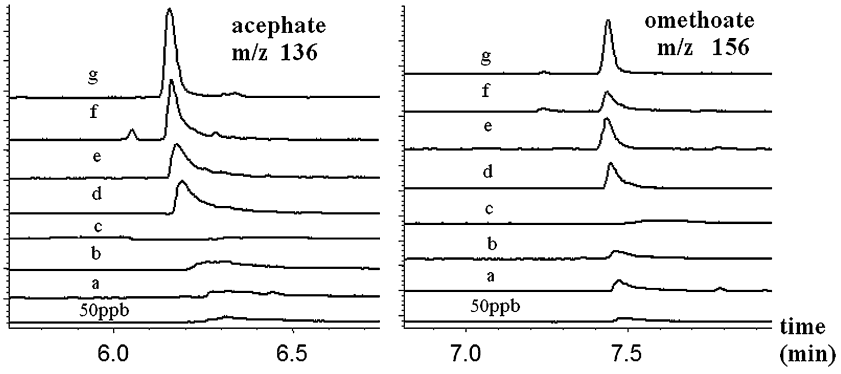


Fig. 2. Effect of different analyte protectants that were evaluated at the peak shape and intensity of 50 ng/ml acephate and omethoate.

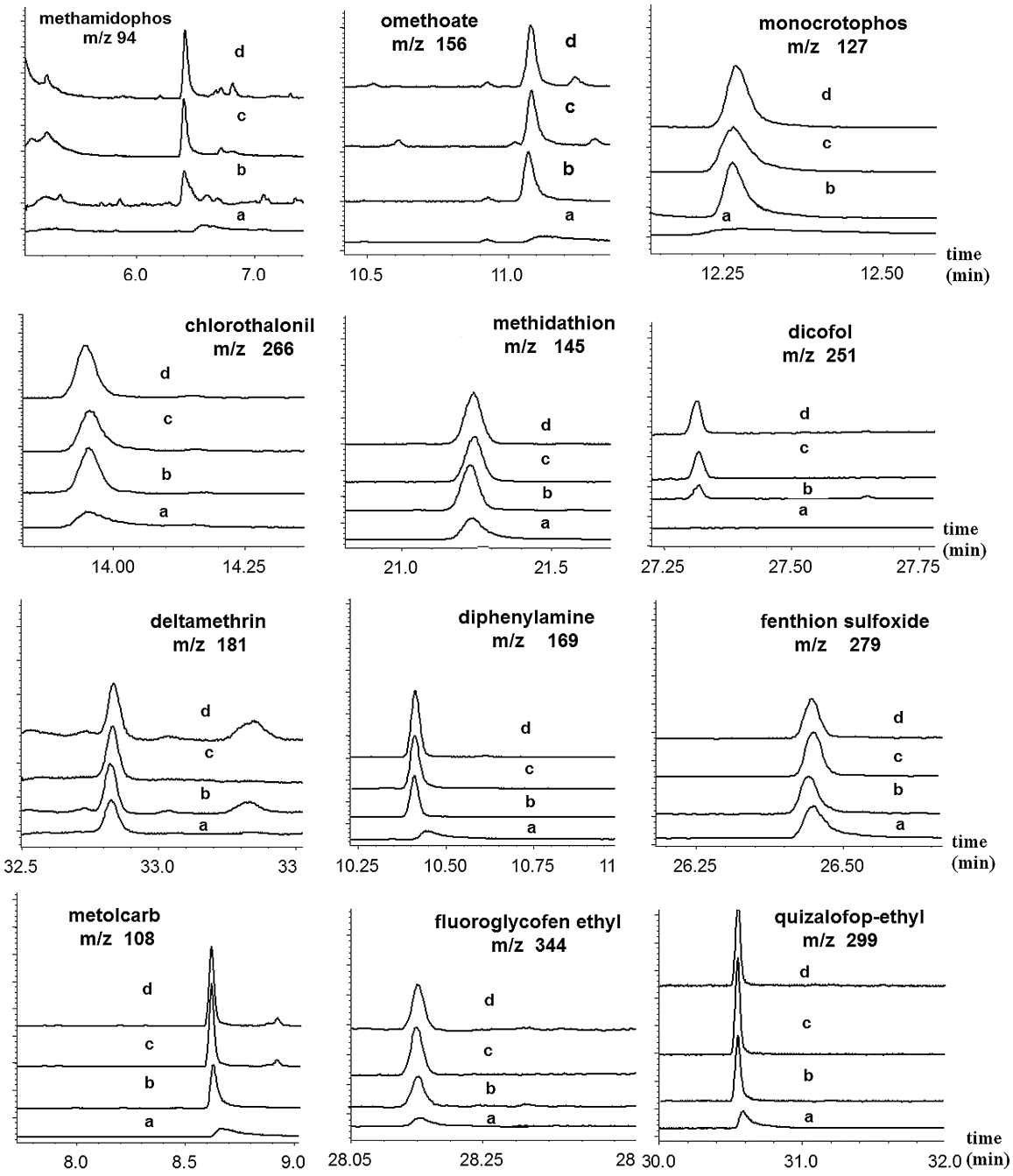


Fig. 3. The peak shape and intensity have been improved using D-ribonic acid-γ-lactone (2 mg/ml) and D-sorbitol (1 mg/ml)—GC/MS at a mixture of pesticide at 200 ng/ml. (a) Pesticide in solvent only; (b) matrix extracts; (c) analyte protectants and (d) matrix extracts + analyte protectants.

optimization, we have selected the D-ribonic acid-γ-lactone at 2 mg/ml and D-sorbitol at 1 mg/ml.

3.2.2. Determination of pesticide residue with analyte protectants

In order to evaluate the compensation effect of analyte protectants, we have studied three pesticide-free matrices that have different ingredients. The studied matrices were extracted following the procedure described in Section 2.4. Take Common yam rhizome as example, the analyte protectants have significantly improved the intensity and chromatographic shape of the peaks for most pesticides, as shown in Fig. 3. Some organophosphorus pesticides (methamidophos, omethoate, monocrotophos, etc.) were not detected at 200 ng/ml if injected in simple solvent. But narrow and tall peaks appeared if analyte protectants were added. Other pesticides, such as chlorothalonil, methidathion, gave four times higher peaks when they were injected with analyte protectants. Organochlorine pesticides were slightly affected. Absolute recoveries were evaluated in order to assess the efficiency of the analyte protectants to compensate the matrix-induced response enhancement [4]. The absolute recoveries were estimated by comparing peak areas between spiked samples and the standards in acetonitrile with analyte protectants. Table 2 lists the quantification results of all pesticides. If the ratio is close to 100%, it indicates that the protectants can effectively compensate matrix-induced enhancement effect, and reduce the quantitative error as well. Table 2 shows the recovery percentage of all pesticides in the three matrices, most of which fall into the reasonable range (80%, 120%) except for methidathion,

tricyclazole, propargite in Dried Tangerine Peel and fenvalerate in Milkvetch, respectively. All samples were spiked at level of approximately 200 ng/ml. Moreover, the change of quantification limit after adding analyte protectants is also illustrated in Table 2.

3.2.3. Maintenance of GC–MS

The maintenance of GC–MS is important, especially in the trace analysis. The maintenances of liner, column, ion source are the key factors to affect the sensitivity and reproducibility of the GC–MS method. The method of matrix-spiked standard calibration is the most widely used approach to compensate for the matrix effect. However, if this method was used, it generally leads to more frequent maintenance of the GC system because it allowed more samples injected into the GC–MS system. We showed that adding analyte protectant is an effective method to overcome matrix effect which is consistent with other reports [9–11]. In our study, the contamination of the GC–MS system by high concentrations of analyte protectant was examined. We found that there was no obvious residue of analyte protectant detected when air was analyzed with the same column temperature program right after an injection of analyte protectants. Actually, we found that the contamination by an analyte protectants was less than that by the samples. In addition, we have not found that the use of analyte protectants shortened the lifetime of liner. Actually, sensitivity decreasing problems are most likely associated with the pollution of GC system (liner and column). In general, we replaced the liner and about 50 cm column was cut off about 50 cm when the sensitivity was decreased.

4. Conclusions

In this study, we have analyzed 195 types of pesticides in three Chinese herbs: Common yam rhizome, Milkvetch and Dried Tangerine Peel. By comparing the standards prepared in simple solvent with that prepared in blank matrix extracts, we have determined the compounds that are susceptible to matrix effect. The matrix-induced enhancement effect depends on the category and concentration of pesticides, the type and amount of matrix, injection technique and other factors.

In addition, we have studied seven potential analyte protectants to eliminate the matrix-induced enhancement effect in the GC–MS analysis of pesticides. A mixture of D-ribonic acid-γ-lactone (2 mg/ml) and D-sorbitol (1 mg/ml) was found to be the best combination, which could be applied in multi-pesticide analysis, and reduce the quantitative error by overcome matrix effect in GC–MS. Moreover, the utilization of analyte protectants could improve the chromatographic peak shape, and lower the quantification limit. Further work is being conducted to evaluate the performance of analyte protectants in determining other pesticides in Chinese herbs.

Acknowledgment

This research was funded by “Major Drug Discovery” science and technology special program “Technical standards platform on detection of harmful residues in TCM” (2009ZX09308-006)

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