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Interlaboratory study of identification and quantitation of multiresidue pyrethroids in agricultural products by gas chromatography–mass spectrometry

Gou-Fang Pang[a,*], Yan-Zhong Can[a], Chun-Lin Fan[a], Jin-Jie Zhang[a], Xue-Min Li[a], Jun Mu[b], Da-Ning Wang[b], Sheng-Mei Liu[c], Wen-Bin Song[c], Hong-Ping Li[d], Sue-Sun Wong[d], Robert Kubinec[e], Jozef Tekel[e], Sona Tahotna[e]

[a]*Qinghuangdao Import and Export Commodity Inspection Bureau, Qinhuang-dao, P.C. 066002, China*

[b]*JiLin Import and Export Commodity Inspection Bureau, ChangChun, P.C. 130062, China*

[c]*LiaoNing Import and Export Commodity Inspection Bureau, DaLian, P.C. 116001, China*

[d]*Taiwan Agricultural Chemicals and Toxic Substances Research Institute, 11 Kung-Ming Road, We-feng, Taichung, Hsien, Taiwan*

[e]*Chemical Institute, Food Research Institute, Faculty of Natural Sciences, Comenius University, Bratislava, Slovak Republic*

Abstract

This paper deals with the different GC–MS analytical conditions adopted by four laboratories in an attempt to confirm the accuracy of the GC–electron-capture detection (ECD) analytical results during the international collaborative study for the establishment of the AOAC Official Method 998. 01. What is especially noted is that two laboratories have conducted comparative analysis of the respective 12 blind samples with both methods of GC–ECD and GC–MS, and the analytical results of the two methods turn out to be basically identical. This fully demonstrates that GC–MS is not only an effective confirmation tool in the analysis of the pyrethroid residues but also of sufficient sensitivity regarding the maximum residue limit of determination prescribed by FAO/WHO. Moreover, its selectivity is better than GC–ECD. © 2000 Elsevier Science B.V. All rights reserved.

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1. Introduction

GC–MS is, during the analysis of the synthetic pyrethroid pesticides, mainly used for verification of the target pesticide residues [1–4], multiresidue screening [5–7], the study of pyrethroid metabolite or degradation products [8–10] and examination of the relationship of pesticide structures and activity [11]. Particularly when false-positive results needs to be confirmed, GC–MS shows its unique advantage which cannot be replaced by other techniques. As far as the quantification analysis of the residue is concerned, GC–MS has drawn more and more public attention in recent years. At the time of developing AOAC Official Method 998. 01 [12], we organized 15 laboratories from six countries and regions to participate in the international collaborative study, not only did five laboratories of which at least conducted GC–MS identification on the target pesticide detected in their own blind samples but also two laboratories implemented quantification analysis with GC–MS towards these samples after determination by GC–electron-capture detection (ECD) per the stipulation of the international collaborative study

*Corresponding author.

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protocol of the AOAC official method. Through the comparison of the analytical results of the same lot of blind samples of GC–MS and GC–ECD, it can be seen that GC–MS technique is not only an effective confirmation tool in terms of pyrethroid multiresidue analysis but also of sufficient sensitivity regarding the maximum residue limits (MRLs) of the pyrethroids in agricultural products prescribed by FAO/WHO. The accuracy of the method can also be compared to that of GC–ECD and the selectivity is better than it. The said paper is a summary based on the GC–MS study data of pyrethroid residues supplied to us by four laboratories during the international collaborative study of the AOAC Official method 998. 01.

2. Experimental

2.1. Test material

The test material preparation was based on Youden's matched pairs principle [13] and a third fortification level that differs from one of the first two by approximately 10% was also included as shown in Table 1.

One unfortified test portion and three fortified test portions of each agricultural product were analyzed. The fortification levels of eight synthetic pyrethroid pesticides were from 0.091 to 2.000 mg/kg depending on the target pesticide and product evaluated. The MRL prescribed by FAO/WHO is contained in one Youden pair. The samples prepared were sent to our invited laboratories which did not know the fortification concentrations of the samples, each of the laboratories analyzed a total of 12 samples of wheat, oranges and tomatoes. We call them blind samples in this paper.

2.2. Reagents

Neat pesticide standards used in this study were obtained from Guangzhou Nanfang Scientific Instrument, (Guangzhou, China). Synthetic pyrethroid standard solution were prepared from target pesticides dissolved in hexane. All solvents were pesticide grade, or redistilled in all-glass apparatus and checked for interferences by GC–ECD. Florisil 60–100 mesh was pesticide grade and was deactivated and standardized by the AOAC Official Method 998.01. Eluting solvent was prepared by mixing 60 ml of diethyl ether and 940 ml of hexane.

2.3. Gas chromatography and mass spectrometry (GC–MS)

A Fisons MD800 GC–MS system with a DB-5MS capillary column (30×0.25 mm I.D., 0.25-μm film thickness) (J&W Scientific, Folsom, CA, USA); a Shimadzu QP-5000 GC–MS system with a DB-17 capillary column (30 m×0.25 mm I.D., 0.25-μm film thickness) (J&W Scientific); a HP-5890 II-5971 GC–MS system with a DB-5 capillary column (30 m×0.32 mm I.D., 0.25-μm film thickness) (J&W Scientific) and an HP-5890 II-ENGIN GC–MS system with a DB-5 capillary column (30 m×0.25 mm I.D., 0.10-μm film thickness) (J&W Scientific) were used.

Table 1
Fortification levels of Youden-matched pairs for the test material (mg/kg)

Item	Wheat				Oranges				Tomatoes			
	1	2	3	4	5	6	7	8	9	10	11	12
Biphenthrin	0.100	0	0.105	0.110	0	0.100	0.105	0.110	0.100	0.105	0	0.110
Fenpropathrin	0.200	0	0.210	0.220	0	0.200	0.210	0.220	0.909	0.954	0	1.000
Cyhalothrin	0.100	0	0.105	0.110	0	0.100	0.105	0.110	0.100	0.105	0	0.110
Permethrin	1.818	0	1.909	2.000	0	0.909	0.954	1.000	0.909	0.954	0	1.000
Cypermethrin	0.364	0	0.382	0.400	0	1.818	1.909	2.000	0.454	0.477	0	0.500
Fenvalerate	1.818	0	1.909	2.000	0	1.818	1.909	2.000	0.909	0.954	0	1.000
Fluvalinate	0.909	0	0.954	1.000	0	1.818	1.909	2.000	0.909	0.954	0	1.000
Deltamethrin	0.909	0	0.954	1.000	0	0.091	0.095	0.100	0.182	0.191	0	0.200

2.3.1. Sample preparation

The sample extraction and clean-up procedure employed in this study is the AOAC Official Method 998.01. The procedure is as follows: For extraction from high-moisture (≥75%) products such as fruits and vegetables, weigh 50.0 g (accurate to 0.1 g) chopped sample into an homogenizer jar, add 120 ml of acetone, and homogenize for 3 min at high speed. Filter with suction through a 12-cm perforated Buchner funnel with filter paper into a 500-ml suction flask. Rinse the homogenizer jar with two 25-ml portions of acetone and use the washes to rinse the residues in the Buchner funnel. Transfer the filtrate to a 500-ml separating funnel and rinse the suction flask with two 10-ml portions of acetone. Add the washes to the separating funnel containing filtrate. For dry or low-moisture products such as grains, weigh 20.0 g (accurate to 0.1 g) into an homogenizer jar, add 150 ml of acetonitrile–water (2:1), and homogenize at high speed for 5 min. Proceed as for high-moisture products except use acetonitrile–water (2:1) instead of acetone.

Measure 60 ml of hexane and pour into the separating funnel containing the filtrate. Shake the funnel contents vigorously for 5 min, with frequent venting. Add 200-ml portions of 4.0% (w/v) aqueous NaCl and mix vigorously for 30 s. Allow layers to separate and discard aqueous layer. Pass hexane layer through glass funnel containing glass wool plug and ca. 15 g anhydrous sodium sulfate. Collect the extracts into a 250-ml round-bottom flask. Rinse the separating funnel with two 20-ml portions of hexane, pass washes through glass funnel containing anhydrous sodium sulfate, and collect them in the round bottom flask with extracts. Evaporate the contents of the round-bottom flask to dryness on a rotary evaporator at 40°C. Redissolve residue in 10 ml of hexane and transfer to a 125-ml separatory funnel. Rinse the round-bottom flask with two 5-ml portions of hexane and transfer washes to the same separatory funnel. Add 30 ml of acetonitrile saturated with hexane and shake vigorously for 5 min. Allow layers to separate and drain the acetonitrile phase into a 250-ml round-bottom flask. Add 30 ml of acetonitrile saturated with hexane to the hexane phase in the separatory funnel and shake vigorously for 5 min. Allow phase separation and drain the acetonitrile layer into the same 250-ml round-bottom flask. Repeat the 30-ml acetonitrile extraction and collect acetonitrile. Evaporate acetonitrile extract to dryness on a rotary evaporator at 60°C. Dissolve residue in 5 ml of hexane.

Prepare Florisil column (40 cm×22 mm I.D.). Place a small plug of glass wool at the bottom of the glass column and add a 1-cm layer of anhydrous Na_2SO_4, Pour ca. 50 ml of hexane into the column, introduce 10 g of deactivated Florisil (5% water) and tap the sides of column to produce even packing. Top with a 1-cm layer of anhydrous Na_2SO_4. Prewash column with 50 ml of hexane, do not allow the Florisil column to go to dryness until after the last eluent fraction is added. Transfer the concentrated sample extracts to the column and allow the level to fall until just above the Florisil packing. Rinse the 250-ml round-bottom flask with two 10-ml portions of hexane, add each washes to column, and allow to run though column. Elute pyrethroid residues with a volume of 6% eluting solvent determined in standardization of the Florisil batch, collecting the eluate at 3 ml/min in a 250-ml round-bottom flask. Evaporate the eluate to less than 50 ml on a rotary evaporator at 40°C and transfer to a 50-ml volumetric flask. Dilute to volume with hexane so that the final concentration is 1.0 g/ml for fruits and vegetables or 0.4 g/ml for grains. A 1–3 μl of portion of the solution was analyzed by GC–ECD and GC–MS.

3. Results and discussion

3.1. Selection of the GC–MS confirmation condition

During the international collaborative study for the AOAC Official Method 998.01, 14 laboratories of six countries and regions analyzed a total of 168 blind samples by GC–ECD method and obtained 1344 analytical data. For the purpose of accurately identifying the target pesticides in these blind samples, certain laboratories in different countries and regions conducted a detailed study on the GC–MS confirmation condition respectively and executed verification towards their own analytical results of the GC–ECD method. The analytical conditions studied by four laboratories for the pyrethroid pes-

Table 2
Analytical conditions of the GC–MS system

Instrument	Shimadzu QP-5000	Fisons MD 800	HP-5890 II -5971A	HP-5890 II -ENGIN
Injection mode	Splitless mode, opening splitter 1.2 min after injection	Splitless mode, opening splitter 1.2 min after injection	Splitless mode, opening splitter 1.5 min after injection	Splitless mode
Injection volume	1 μl	3 μl		
Injector temperature	270°C	280°C	280°C	280°C
Column model	HP-17	DB-5MS	DB-5	DB-5
Column length	30 m	30 m	30 m	30 m
Column diameter	0.25 mm I.D.	0.25 mm I.D.	0.32 mm I.D.	0.25 mm I.D.
Column film	0.25 μm	0.25 μm	0.25 μm	0.10 μm
Carrier gas	He	He	He	
Column pressure	100 (kPa)	5 psi	200 (kPa)	
Ionization mode	Electron impact	Electron impact		Electron impact
Source temperature		200°C	175°C	
Interface temperature	250°C	280°C	280°C	250°C
Electron energy	70 eV	70 eV	70 eV	70 eV
Scanning range		50–520 u		

ticides is shown in Table 2 and the temperature program of the three chromatographic columns adopted by them is seen in Table 3. The main fragment ions of the eight pesticides determined by these four laboratories and their selected monitored ions are given in Table 4.

On the basis of the study, Lab-1 chose six ions, Lab-2 chose one, Lab-3 chose two, Lab-4 chose nine, all of which conducted identification of the multiresidue pyrethroid in the 12 blind samples respectively. Eventually, laboratories of different countries and regions reached the same conclusion as if by prior agreement: i.e. of the 12 blind samples analyzed by each laboratory, three are found absent of any variety of pyrethroid pesticide among which wheat, oranges and tomatoes account for one each and the balance of nine samples are detected to contain pyrethroid pesticides in varying degrees. This conclusion coincides with the actual situation of test materials supplied by us. This explicitly demonstrates that the confirmation results of these laboratories are correct.

3.2. Comparison of two methods of GC–MS and GC–ECD

In the international collaborative study for AOAC Official Method 998. 01, Lab-1 constructed calibration curves for each of the eight pyrethroids by GC–MS. Their linear range and linear regression

Table 3
Four temperature programs of three capillary column

Column temperature program	Initial (°C)	Initial hold time (min)	Temperature ramp (°C min)	Final (°C)	Hold time (min)
DB-17	50	1	30	200	0
	–	–	5	280	15
DB- 5MS	50	1	30	220	0
	–	–	1.5	260	5
DB-5	50	3	30	205	2
	–	–	2	280	–
DB-5	50	–	30	200	0
	–	–	3	260	4

Table 4
GC–MS acquisition parameters by four laboratories

Compound	Main fragments *m/z*				Target ion, *m/z*			
	Lab-1	Lab-2	Lab-3	Lab-4	Lab-1	Lab-2	Lab-3	Lab-4
Biphenthrin	181,166,182 165,180,179	181,166,165 182,141,152	181,166	165,166,181	166	181	165 181	181
Fenpropathrin	181,349,265 125,208,97	97,55,181 125,83,141	97,181,125	97,125,141 152,181,265	181	181	181 265	181
Cyhalothrin	181,197,208 141,152,209	181,197,208 141,77,199	181,197,77	141,181,197	181	181	181 197	181
Permethrin	183,163,184 165,127,165	183,163,165 184,77,91	183	163,165,183	183	181	165 183	183
Cypermethrin	163,181,209 165,127,208	163,181,165 91,127,77	163,165,181,91	91,152,163 165,181	163	181	165 181	181
Fluvalinate	250,252,251, 181,208,206	250,252,181, 55,251,208,77	250,181,55	181,250,252	225	181	181 250	181
Fenvalerate	225,167,125 152,127,181	125,167,225 152,181,169	125,167,181,225	125,152,167 181,225,479	250	181	167 225	181
Deltamethrin	181,252,250 209,127,254	181,253,251 77,172,255,174	181,77,253	181,251,253,255	181	181	181 253	181

coefficient and ours with GC–ECD are both shown in Table 5. For the convenience of comparison, the limits of detection (LODs) of both methods are also shown respectively in Table 5.

It can be seen that the linear range and linear regression coefficient of GC–ECD are slightly better than those of GC–MS. In terms of the LODs of the two methods, the sensitivity of GC–ECD is slightly better than that of GC–MS for the latter four varieties of pyrethroids, cypermethrin, fluvalinate fenvalerate and deltamethrin with a longer retention time, but the sensitivity of GC–MS is much higher than that of GC–ECD for permethrin adjacent to cypermethrin in retention time. In the international collaborative study, Lab-1 and Lab-2 also conducted analysis on the identical samples respectively with GC–MS in the selected-ion monitoring mode after determination by GC–ECD as stipulated in the international collaborative study protocol. The analytical results of the two methods by these two laboratories are shown in Tables 6 and 7. Total ion chromatograms of the orange samples and controlled samples analyzed with GC–MS by Lab-1 are shown in Fig. 1.

Through the comparison of the analytical results of the 12 blind samples with the two methods by the

Table 5
Linear range and LOD of pyrethroid pesticides by two methods

Insecticide	Linear range (ng)		r^2		LOD (mg/kg)	
	ECD	MS	ECD	MS	ECD	MS
Biphenthrin	0.005–1.000	0.008–0.800	0.996	0.995	0.02	0.02
Fenpropathrin	0.005–1.000	0.016–1.600	0.999	0.996	0.02	0.02
Cyhalothrin	0.002–0.500	0.008–1.000	0.998	0.987	0.01	0.02
Permethrin	0.020–4.000	0.008–0.800	0.998	0.993	0.08	0.02
Cypermethrin	0.012–2.500	0.040–2.000	0.999	0.987	0.04	0.10
Fluvalinate	0.010–2.000	0.032–1.600	0.996	0.989	0.04	0.08
Fenvalerate	0.010–2.000	0.032–1.600	0.998	0.992	0.04	0.08
Deltamethrin	0.012–2.500	0.032–1.600	0.992	0.988	0.05	0.08

Table 6
Results of analysis with GC–ECD and GC–MS by Lab-1 (mg/kg)

Pesticide	1. ECD 2. MSD	No. sample of wheat				No. sample of oranges				No. sample of tomatoes			
		1	2	3	4	5	6	7	8	9	10	11	12
Biphenthrin	1	0.101	ND[a]	0.106	0.104	ND	0.108	0.097	0.110	0.101	0.100	ND	0.114
	2	0.106	ND	0.102	0.109	ND	0.105	0.105	0.106	0.094	0.108	ND	0.117
Fenpropathrin	1	0.201	ND	0.208	0.206	ND	0.219	0.213	0.232	0.920	0.901	ND	1.024
	2	0.202	ND	0.201	0.211	ND	0.214	0.228	0.208	0.883	0.917	ND	1.017
Cyhalothrin	1	0.101	ND	0.106	0.103	ND	0.110	0.098	0.112	0.098	0.096	ND	0.113
	2	0.105	ND	0.104	0.104	ND	0.107	0.107	0.106	0.094	0.099	ND	0.117
Permethrin	1	1.873	ND	1.952	1.952	ND	0.985	0.888	1.022	0.922	0.892	ND	1.010
	2	1.842	ND	1.984	1.886	ND	0.964	0.997	0.975	0.867	0.910	ND	0.990
Cypermethrin	1	0.380	ND	0.384	0.391	ND	2.001	1.702	1.958	0.451	0.451	ND	0.505
	2	0.361	ND	0.368	0.401	ND	2.000	1.953	2.015	0.441	0.460	ND	0.525
Fenvalerate	1	1.938	ND	1.938	1.934	ND	2.018	1.711	1.989	0.910	0.894	ND	1.012
	2	1.760	ND	1.905	1.886	ND	1.974	2.014	2.008	0.868	0.915	ND	1.040
Fluvalinate	1	0.971	ND	0.960	0.947	ND	2.015	1.719	1.986	0.900	0.914	ND	1.028
	2	0.932	ND	0.880	1.000	ND	1.968	1.904	1.912	0.894	0.898	ND	1.041
Deltamethrin	1	0.978	ND	0.971	0.960	ND	0.108	0.095	0.110	0.182	0.188	ND	0.203
	2	0.917	ND	1.025	0.947	ND	0.096	0.094	0.100	0.185	0.183	ND	0.196

[a] ND: no detection.

Table 7
Results of analysis with GC–ECD and GC–MS by Lab-2 (mg/kg)

Pesticide	1. ECD 2. MSD	No. sample of wheat				No. sample of oranges				No. sample of tomatoes			
		1	2	3	4	5	6	7	8	9	10	11	12
Biphenthrin	1	0.095	ND[a]	0.103	0.096	ND	0.102	0.091	0.094	0.096	0.097	ND	0.100
	2	0.100	ND	0.105	0.114	ND	0.095	0.082	0.088	0.108	0.106	ND	0.109
Fenpropathrin	1	0.192	ND	0.212	0.198	ND	0.206	0.205	0.198	0.872	0.879	ND	0.909
	2	0.214	ND	0.225	0.238	ND	0.210	0.199	0.199	0.976	0.933	ND	0.985
Cyhalothrin	1	0.098	ND	0.108	0.098	ND	0.104	0.090	0.094	0.095	0.096	ND	0.099
	2	0.102	ND	0.107	0.113	ND	0.097	0.076	0.081	0.106	0.104	ND	0.110
Permethrin	1	1.733	ND	1.916	1.786	ND	0.953	0.849	0.857	0.883	0.914	ND	0.917
	2	1.718	ND	2.054	2.274	ND	0.936	0.741	0.802	1.030	1.012	ND	1.062
Cypermethrin	1	0.338	ND	0.383	0.342	ND	1.866	1.567	1.657	0.432	0.442	ND	0.452
	2	0.361	ND	0.394	0.422	ND	1.762	1.286	1.380	0.510	0.485	ND	0.511
Fenvalerate	1	1.755	ND	1.968	1.764	ND	1.866	1.570	1.658	0.877	0.900	ND	0.921
	2	1.601	ND	1.778	1.998	ND	1.639	1.190	1.228	0.929	0.886	ND	0.930
Fluvalinate	1	0.820	ND	0.902	0.850	ND	1.735	1.403	1.457	0.780	0.793	ND	0.835
	2	0.882	ND	0.981	1.080	ND	1.756	1.274	1.337	0.999	0.954	ND	0.986
Deltamethrin	1	0.870	ND	0.964	0.868	ND	0.096	0.086	0.085	0.171	0.182	ND	0.183
	2	0.851	ND	0.961	1.080	ND	0.085	0.070	0.068	0.201	0.200	ND	0.199

[a] ND: no detection.

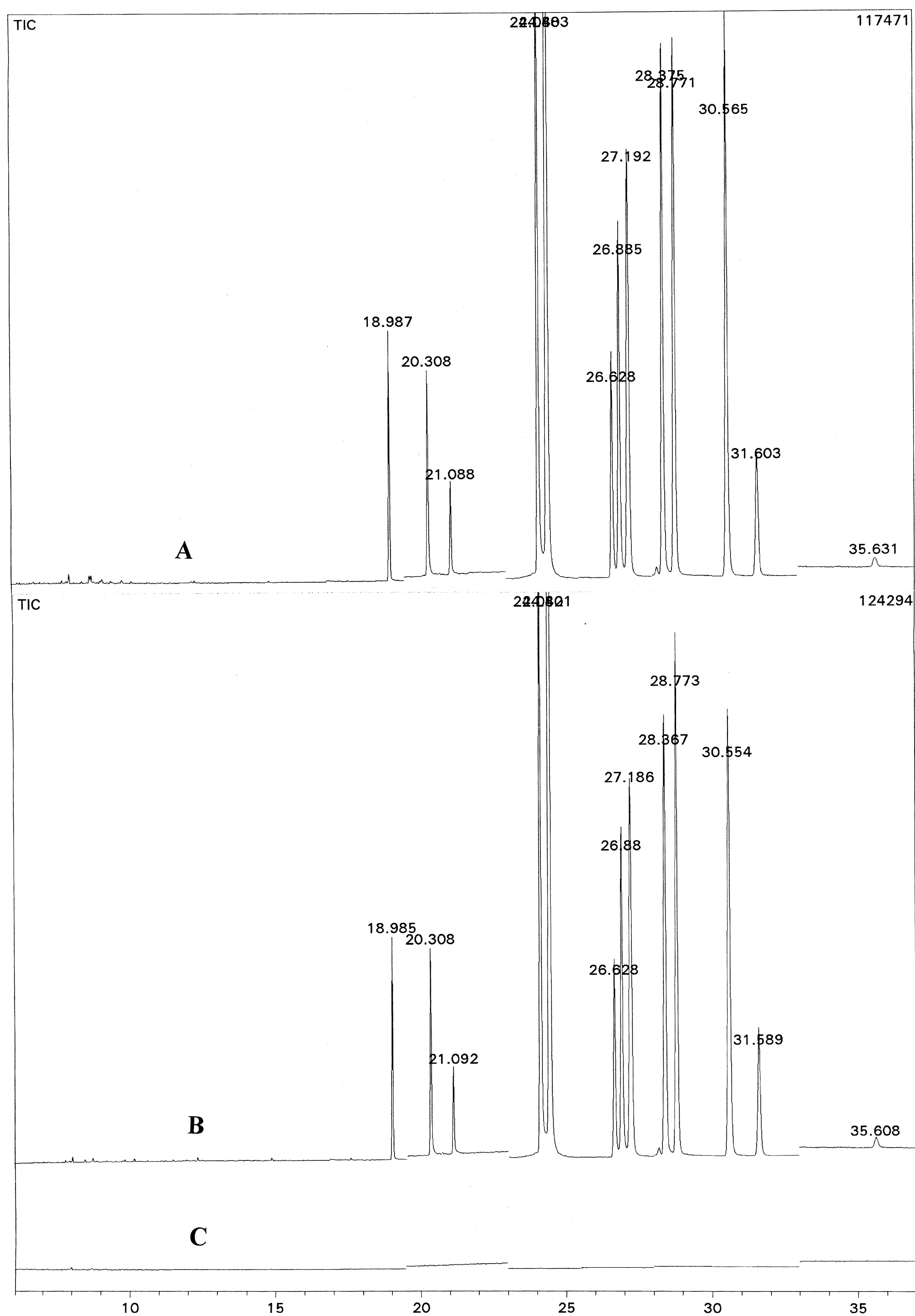

Fig. 1. Total ion chromatograms of pyrethroid standard (A), and fortified orange sample (B), and controlled orange sample (C). Peak No. from left to right: 1, biphenthrin, 2, fenpropathrin, 3, cyhalothrin, 4–5, permethrin, 6–8, cypermethrin, 9–10, fluvalinate, 11–12, fenvalerate, 13, deltamethrin.

two laboratories, it can be clearly seen that both analytical results are basically identical. It was found out from a comparison between Tables 6 and 7 and Table 1 that the analytical results of GC–MS and GC–ECD all are correct. Of these 12 blind samples, no interfering matters have been found affecting the determination of the pyrethroid pesticides. With regard to the quantification analysis of MRL of pyrethroid pesticides in the agricultural products prescribed by FAO/WHO, GC–MS is as good as GC–ECD in respect of sufficient sensitivity and accuracy. Moreover, its selectivity is better than that of GC–ECD. This amply illustrates that GC–MS is not only an effective confirmation tool but also a more reliable quantification analysis method of the multiresidue pyrethroid pesticides. It was also found that certain GC–MS systems still failed to meet these requirements.

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