

Short communication

Response surface optimization for determination of pesticide multiresidues by matrix solid-phase dispersion and gas chromatography

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Abstract

An optimized multiresidue analysis method based on matrix solid-phase dispersion (MSPD) and gas chromatography (GC) is proposed for the determination of organochlorines and pyrethroids in the tea samples. Response surface methodology (RSM) was used to optimize the extraction conditions of MSPD, such as the sorbent type, eluent composition, dichloromethane concentration and eluting volume. Desirability function approach was employed to optimize the pesticide recoveries and matrix cleanup. Compromising the recoveries and cleanup degree, MSPD was carried out with Florisil as the sorbent and *n*-hexane–dichloromethane (1:1, v/v) as the eluent. The pesticide recoveries in tea samples were better than 80% spiked in the concentration range of 0.01–0.05 mg/kg and the relative standard deviations were lower than 7%. The quantification limits of the pesticides were in the range of 0.002–0.06 mg/kg, which were lower than the maximum residue limits of the pesticides in tea samples established by the European Union.

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1. Introduction

Tea is one of the most popular drinks in the world. However, the pesticide multiresidues in it are a potential threat to the health of tea drinkers. In recent years, more and more attention has been paid to this issue and many countries have established more rigid maximum residue limits (MRLs) for the pesticide in tea. However, since tea samples contain complex components including pigments, alkaloids and polyphenols, etc. the analysis of pesticide multiresidue in tea is usually difficult owing to matrix interference and complicated extraction procedures. Some attempts [1,2] had been made to analyse pesticide multiresidue in tea, but sample throughput or solvent consumption was dissatisfactory. Therefore, development of fast and sensitive methods for pesticide multiresidue analysis in tea is of great significance. Matrix solid-phase dispersion (MSPD) [3] has proved to be an effective pretreatment technique and has been adopted for various samples [4,5] because it is simple, rapid and sol-

vent saving. However, the application of MSPD for tea samples containing high pigment content has not been reported yet.

In the performance of MSPD, the pesticide recoveries and matrix cleanup depend on the pesticide solubility in the eluent and the interaction among the analyte, matrix, sorbent and eluent. In general, optimal conditions can be obtained by the classical method called one-variable-at-a-time, but this method cannot solve the dependence of multi-variables [6]. Response surface methodology (RSM), an effective mathematical statistics method for establishing models, evaluating the relative significance of variables and determining optimal conditions of desirable responses [7,8], successfully overcomes the limitation of the classical method. Furthermore, both discrete and continuous factors can be evaluated by RSM. Up to now, RSM has been used in chemical engineering [9,10] for the optimization of producing conditions. Several papers have been reported using RSM for optimization of the analytical conditions, such as for fluorometry [11], micellar electrokinetic chromatography [12], flow injection analysis [13] and solid-phase extraction (SPE) [14].

In the present work, a fast and sensitive determination method based on MSPD and gas chromatography (GC) was proposed

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for pesticide multiresidue analysis in tea and RSM was taken for the optimization of the process parameters. Desirability function approach was employed to optimize the responses of the pesticide recoveries and pigment cleanup degree. The analytical characteristics obtained by MSPD were compared with those of SPE used as a purification procedure in terms of the limit of quantification (LOQ) and pesticide recovery.

2. Experimental

2.1. Reagents and standards

Standard pesticides with purities of 95–99% were purchased from the Environmental Protection Institute of the Agriculture Ministry (Tianjin, China). Single and mixed standard solutions were prepared in *n*-hexane. Chromatography-grade acetone, dichloromethane (DCM) and ethyl acetate (EA), and pesticide-grade *n*-hexane were purchased from Merck (Darmstadt, Germany). The sorbent material of Florisil PR (60–100 mesh) was purchased from Fluka (Buchs, Switzerland), neutral alumina (70–230 mesh, activity I) and silica gel 60 (230–400 mesh) were ordered from Merck. All the sorbents need not be deactivated. SPE columns packed with 500 mg Florisil, 500 mg silica or 1.0 g neutral alumina were purchased from Supelco (Bellefonte, PA, USA).

2.2. Apparatus

An Agilent-6890 gas chromatography system equipped with a DB-1701 capillary column (30 m × 0.52 mm I.D., 0.33 μm film thickness), an auto-sampler and a ⁶³Ni electron-capture detection (ECD) system was employed in this work. Nitrogen was used as the carrier and make-up gas at the flow rate of 15.6 and 30 ml/min, respectively. One microlitre sample volume was injected with splitless mode. The temperature of the injector and detector were 270 and 300 °C, respectively. The column temperature was initially maintained at 60 °C for 1 min, increased at 10 °C/min to 200 °C, kept for 4 min, then enhanced at 2 °C/min to 210 °C and held for 4 min, being finally increased at 10 °C/min to 270 °C and held for 8 min.

Pesticides residues were confirmed by an Agilent 6890GC/5973MSD system equipped with a DB-5 MS column (30 m × 0.25 mm I.D., 0.25 μm film thickness). Scan acquisition mode and selected ion monitoring (SIM) mode were used. The temperature of electron impact ionization source was 250 °C. The injector and column temperature were the same as those in GC–ECD.

2.3. MSPD procedure

After milling by a Cyclotec 1093 Mill (Foss-Tecator, Hoganas, Sweden), 0.500 g homogeneous tea sample was transferred into a glass mortar and blended gently with 2.00 g Florisil. For the spiked sample, the mixed standard solution was added to the tea sample. Then the homogeneous mixture was transferred into a glass cartridge underlaid with a glass filter paper (Whatman GF/A). Another filter paper was placed on the top of the sample mixture. The sample mixture was gently pressed and the cartridge was connected to a SPE vacuum manifold. Five millilitres of *n*-hexane–DCM (1:1, v/v) was introduced to elute the pesticides from the cartridge directly, 1.0 ml additional eluent was adopted to wash the mortar and pestle, and then transferred into the cartridge. The eluents were collected, concentrated to dryness under N₂ flow and dissolved in 0.5 ml *n*-hexane for GC analysis.

2.4. SPE procedure

Sample powder 0.500 g was transferred to a 10 ml centrifugal tube and extracted with 4.0 ml *n*-hexane–DCM (1:1, v/v) by vortex agitator (MS1 Minishaker, Guangzhou, China) at full speed for 2 min. The mixture solution was then centrifuged at 3000 rpm for 5 min and the supernatant solution was transferred to a test tube. Four millilitres of additional *n*-hexane–DCM (1:1, v/v) was added into the precipitate and the extraction procedure was repeated once. The supernatant solutions were merged and introduced into the Florisil column preconditioned with *n*-hexane. An extra 2.0 ml *n*-hexane–DCM (1:1, v/v) was used to elute the pesticide residues from the column. The eluents were collected, concentrated to dryness under N₂ flow and dissolved in 0.5 ml *n*-hexane for GC analysis.

Table 1
Experimental matrix of eluent system and solid sorbent and responses of R_{OC} , R_P and A

Run number	X_1 code	X_2 code	X_1 eluent system	X_2 solid sorbent	R_{OC} (%)	R_P (%)	A
1	–3	–1	<i>n</i> -Hexane	Florisil	72.3	12.5	0
2	–1	–1	<i>n</i> -Hexane:DCM (3:1)	Florisil	80.8	46.7	0.01
3	1	–1	<i>n</i> -Hexane:EAT (3:1)	Florisil	89.9	95.0	1.55
4	3	–1	<i>n</i> -Hexane:acetone (3:1)	Florisil	76.6	83.6	2.40
5	–3	0	<i>n</i> -Hexane	Silica	74.0	19.5	0.03
6	–1	0	<i>n</i> -Hexane:DCM (3:1)	Silica	89.0	36.4	0.89
7	1	0	<i>n</i> -Hexane:EAT (3:1)	Silica	90.7	97.0	1.70
8	3	0	<i>n</i> -Hexane:acetone (3:1)	Silica	75.5	78.4	3.00
9	–3	1	<i>n</i> -Hexane	Netural alumina	73.0	32.0	0.01
10	–1	1	<i>n</i> -Hexane:DCM (3:1)	Netural alumina	82.9	49.7	0.59
11	1	1	<i>n</i> -Hexane:EAT (3:1)	Netural alumina	91.2	96.0	1.68
12	3	1	<i>n</i> -Hexane:acetone (3:1)	Netural alumina	83.3	84.0	2.70

2.5. Experimental design

In this paper, full factorial design (FFD) and full factorial central-composition design (CCD) [15] of RSM were adopted to optimize the MSPD conditions. The responses included the average recovery of organochlorines (R_{OC}) and pyrethroids (R_P), and the absorbance (A) of the sample extract, which represents the pigment absorbance of extractive solution at 550 nm, i.e., the purification degree of the sample extract. The discrete factors of sorbent type and eluent composition and the continuous factors of DCM concentration in the eluent and eluting volume were used as the variables in the experimental design.

The quadratic equations of R_{OC} , R_P and A with respect to the discrete and continuous factors were regressed by MATLAB 6.5. Meanwhile, three-dimensional response surfaces or two-dimensional contour curves were constructed to give the visual responses of R_{OC} , R_P and A . The optimized variables can be obtained by the partial differential of the responses.

3. Results and discussion

3.1. Effect of eluent system and sorbent type on R_{OC} , R_P and A

To evaluate the influence of eluent system X_1 , sorbent type X_2 and their interaction on R_{OC} , R_P and A , a FFD matrix is listed in Table 1. The tea sample was spiked with the standard pesticides in the concentration range of 0.04–0.25 mg/kg.

The results of the regression analysis demonstrate that the influence of eluent composition (X_1) on R_{OC} and R_P is more significant than that of sorbent type (X_2). The mixed solvents of *n*-hexane–DCM (3:1) and *n*-hexane–EA (3:1) yield higher R_{OC} than others, while R_P increases with the polarity of the eluting solvent and *n*-hexane–EA (3:1) provides the highest value. In addition, the influence of X_1 on the response A is

significant. However, the interaction between X_1 and X_2 has slight effect on the responses of R_{OC} , R_P and A . To reduce the pigment concentration, i.e., the response A , in the sample extract, *n*-hexane or *n*-hexane–DCM (3:1) can be chosen as the eluent.

Since the purification effect is affected obviously by the discrete factors, it can be considered as the main response in this step. To obtain satisfying pesticide recovery and matrix cleanup degree in a single step, *n*-hexane–DCM and Florisil were selected for the following studies owing to their high cleanup degree and satisfactory eluting capability for the organochlorines. Although the pyrethroid recovery was not satisfying, it may be improved by selecting a suitable eluting volume and DCM concentration.

3.2. Effect of DCM concentration and eluting volume on R_{OC} , R_P and A

To improve pesticide recoveries, continuous factors of DCM concentration of the eluent (X_3) and eluting volume (X_4) were also optimized by RSM, which were coded according to Ref. [8]. The corresponding design matrix based on CCD is listed in Table 2 and the two-dimensional contour curves of R_{OC} , R_P and A are presented in Fig. 1. It is seen that R_{OC} and R_P increase clearly when the DCM concentration increases to 50% and the eluting volume increases to 5.0 ml. Then, R_{OC} and R_P change slightly with further increase of the variables. The corresponding contour curves of R_{OC} (Fig. 1A) and R_P (Fig. 1B) possess a considerable curvature, which implies that the influence of the interaction between X_3 and X_4 on R_{OC} and R_P is significant. Fig. 1C illustrates that the absorbance A becomes small enough as DCM concentration is reduced to 50%. In contrast, it increases significantly with concentration higher than 60%.

Fig. 1 reveals that the optimization of DCM concentration can not only improve the recoveries of the pesticides but also

Table 2
Experimental matrix of DCM concentration and eluting volume and responses of R_{OC} , R_P and A

Run number	X_3 code	X_4 code	DCM concentration (%)	Eluting volume (ml)	R_{OC} (%)	R_P (%)	A
1	−2	−2	30	1	30.0	6.7	0
2	−2	0	30	5	68.3	26.3	0
3	−2	2	30	9	80.8	46.7	0.004
4	−1	−2	40	1	44.7	40.1	0
5	−1	−1	40	3	77.9	73.8	0
6	−1	1	40	7	90.7	87.3	0.009
7	−1	2	40	9	91.6	90.0	0.018
8	0	−2	50	1	51.4	54.4	0
9	0	0	50	5	93.3	91.9	0.018
10	0	2	50	9	96.0	93.0	0.036
11	1	−1	60	3	86.6	92.9	0.046
12	1	1	60	7	96.7	94.2	0.155
13	2	−2	70	1	58.3	59.1	0.187
14	2	0	70	5	96.8	94.4	0.444
15	2	2	70	9	97.4	94.8	0.678
16	0	0	50	5	93.5	91.2	0.027
17	0	0	50	5	92.9	91.5	0.018
18	0	0	50	5	93.7	92.4	0.022
19	0	0	50	5	94.1	92.7	0.013

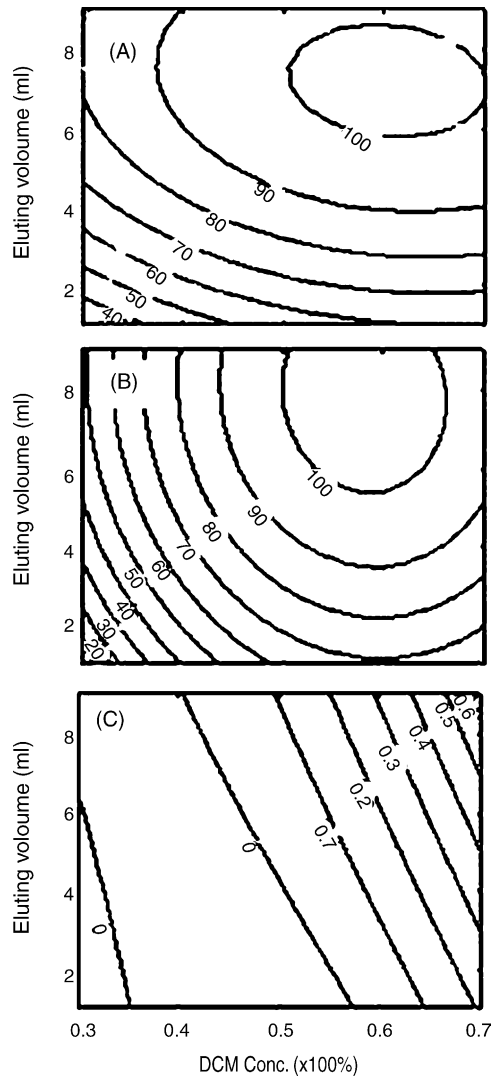


Fig. 1. Contour curve plots of R_{OC} (A), R_P (B) and A (C) as functions of DCM concentration and eluting volume.

ameliorate the pigment cleanup degree in the sample extract. However, the eluting volume has no significant effect on the absorbance A , which is reflected by the slight curvature of the contour curves in Fig. 1C.

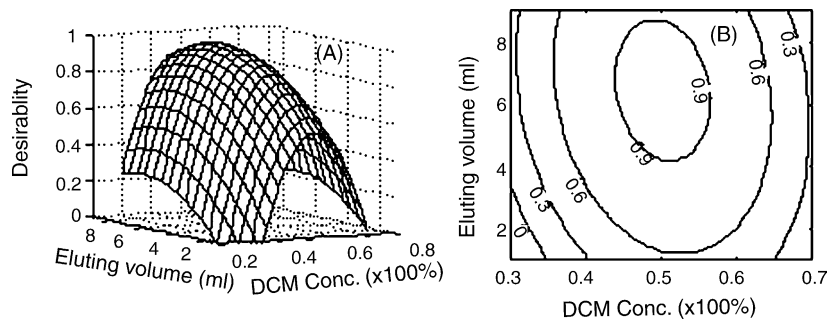


Fig. 2. Three-dimensional response surface plot (A) and two-dimensional contour plot (B) for desirability function approach as a function of DCM concentration and eluting volume.

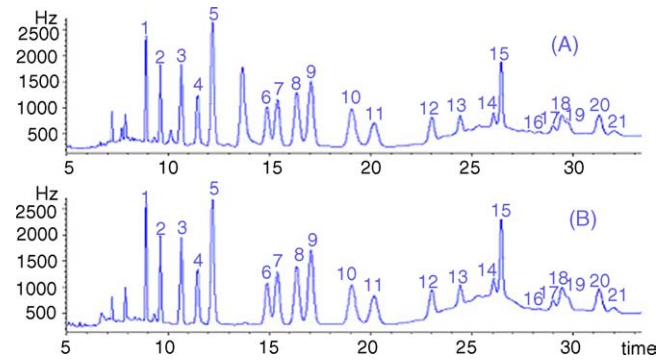


Fig. 3. Comparison of chromatograms obtained by MSPD–GC (A) and SPE–GC (B) (1) α -BHC; (2) γ -BHC; (3) aldrin; (4) β -BHC; (5) δ -BHC; (6) p,p' -DDE; (7) dieldrin; (8) endrin; (9) p,p' -DDD; (10) p,p' -DDT; (11) o,p' -DDT; (12) bifenthrin; (13) fenpropathrin; (14) cyfluthrin I; (15) cyfluthrin II; (16) cypermethrin I; (17) cypermethrin II; (18) cypermethrin III; (19) cypermethrin IV; (20) fenvalerate I and (21) fenvalerate II.

3.3. Optimization for multiple responses

In this work, desirability function approach [7,15] was used to optimize multiple responses of R_{OC} , R_P and A , in order to obtain clean extracts and high recoveries simultaneously. The completely desirable and undesirable values of the response R_{OC} and R_P were set at 95% and 50%, and 0.001 and 0.4 were assigned to those of response A , respectively.

The response surface and contour curve plots of the desirability value D as a function of DCM concentration and eluting volume are illustrated in Fig. 2. According to the results shown in Fig. 2, the optimal conditions for desirability function D can be obtained with X_3 at 50% and X_4 at 6.0 ml, respectively, and the corresponding desirable value D of 97% is achieved. The considerably large curvatures of the contour curve indicate that the influence of the interaction between X_3 and X_4 on D is significant.

3.4. Comparison MSPD with SPE

The SPE method with *n*-hexane–DCM (1:1) eluent and a different sorbent was also evaluated. It can be found that pesticides recoveries are similar by using Florisil, silica or neutral alumina. However, the sample extract contains less pigment interference with Florisil or neutral alumina than that of

Table 3
Comparison of analysis results obtained by MSPD–GC and SPE–GC

Pesticides	Average recoveries (%) \pm RSD ^a (%)				LOQ (mg/kg)		MRL (mg/kg) EU	Council directive
	High level ^b		Low level ^c		MSPD	SPE		
	MSPD	SPE	MSPD	SPE				
α -BHC	96 \pm 3	94 \pm 2	91 \pm 2	84 \pm 5	0.005	0.013	–	–
β -BHC	94 \pm 1	92 \pm 2	85 \pm 3	79 \pm 4	0.01	0.01	0.2 ^d	93/58/EEC
γ -BHC	96 \pm 2	95 \pm 4	89 \pm 1	83 \pm 5	0.005	0.005	0.2 ^d	93/58/EEC
δ -BHC	89 \pm 3	91 \pm 3	83 \pm 4	76 \pm 4	0.006	0.007	–	–
Aldrin	90 \pm 1	86 \pm 3	92 \pm 2	85 \pm 4	0.005	0.008	0.02	93/57/EEC
Dieldrin	95 \pm 4	92 \pm 4	89 \pm 4	80 \pm 5	0.004	0.004	0.02	93/57/EEC
Endrin	94 \pm 2	94 \pm 2	83 \pm 3	75 \pm 4	0.002	0.005	0.01	93/58/EEC
<i>p,p'</i> -DDE	87 \pm 4	83 \pm 5	82 \pm 4	71 \pm 2	0.01	0.020	–	–
<i>o,p'</i> -DDT	88 \pm 5	89 \pm 3	80 \pm 6	69 \pm 5	0.01	0.02	0.2 ^d	92/58/EEC
<i>p,p'</i> -DDD	91 \pm 3	91 \pm 4	92 \pm 4	83 \pm 4	0.01	0.02	–	–
<i>p,p'</i> -DDT	86 \pm 3	90 \pm 2	81 \pm 3	67 \pm 6	0.005	0.007	–	–
Bifenthrin	96 \pm 2	90 \pm 4	90 \pm 2	82 \pm 3	0.02	0.02	5.0	98/82/EC
Fenpropathrin	92 \pm 1	85 \pm 3	82 \pm 4	74 \pm 5	0.02	0.02	0.02	–
Cyfluthrin	97 \pm 2	94 \pm 5	83 \pm 3	84 \pm 4	0.02	0.03	0.1 ^d	00/42/EC
Cypermethrin	92 \pm 5	96 \pm 6	91 \pm 4	79 \pm 7	0.04	0.09	0.5 ^d	98/82/EC
Fenvalerate	91 \pm 4	93 \pm 3	80 \pm 2	75 \pm 5	0.06	0.09	0.1 ^d	00/42/EC

^a Relative standard deviation ($n = 5$).

^b Sample spiked with pesticides at concentration from 0.08 to 0.40 mg/kg.

^c Sample spiked with pesticides at concentration from 0.01 to 0.05 mg/kg.

^d Sum of the total isomers.

silica. Using Florisil sorbent, the analytical characteristics of MSPD are compared with those of SPE in terms of recovery and LOQ, and the results are listed in Table 3. Here, LOQ is defined as the pesticide concentration of 10-times the signal-to-noise ratio. Tea samples were spiked with 0.08–0.40 mg/kg and 0.01–0.05 mg/kg pesticides, respectively. The average recoveries were statistically evaluated by *t*-test. No significant differences are observed between the two methods at the high spiking level ($t < t_{0.05,16}$). In contrast, pesticide recoveries obtained by MSPD are better than those by SPE and significant differences exist between the two methods at the low spiking level

($t > t_{0.05,16}$), for which the reason may be that MSPD simplifies the sample preparation. Fig. 3 illustrates the chromatograms of a tea sample spiked with 0.04–0.25 mg/kg pesticides and detected by MSPD–GC and SPE–GC, respectively. The chromatograms show that the matrix cleanup with SPE is slightly better than that of MSPD. On the other hand, the LOQs for most of the pesticides obtained by MSPD–GC are lower than 0.05 mg/kg, a little better than those by SPE. In sum, both MSPD–GC and SPE–GC can be employed in the pesticide residue analysis, because their LOQs are lower than the MRLs of pesticide residues in tea samples established by EU. MSPD can be an acceptable

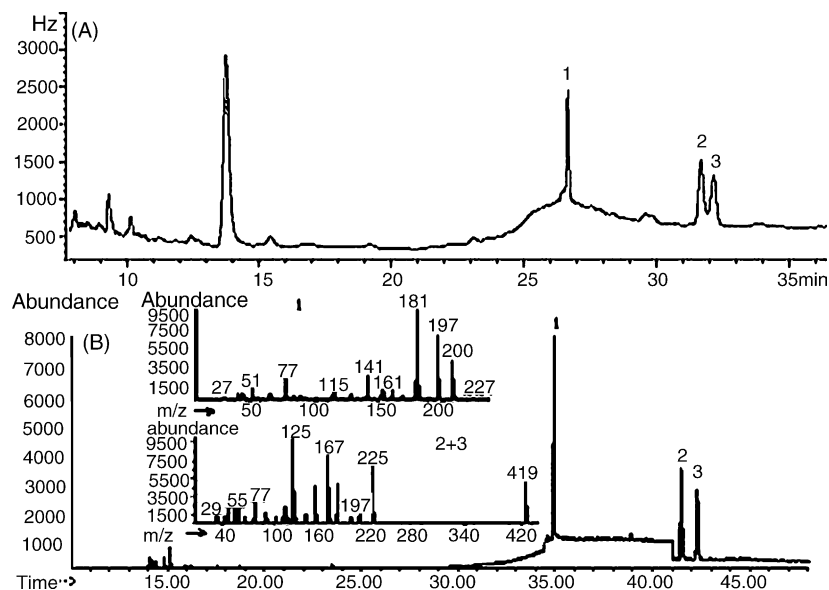


Fig. 4. Pesticide residue analysis for a tea sample by GC–ECD (A) and confirmation by GC–MS (B): (1) Cyfluthrin; (2) fenvalerate I and (3) fenvalerate II.

alternative owing to its high sample throughput and low solvent consumption.

3.5. Quantitative analysis

The quantitative analysis was carried out by the external standard method. The calibration curves and linear regression equations were obtained with five mixture standard solutions in the concentration range from 0.01 to 0.40 $\mu\text{g/ml}$. The proposed method was applied to the determination of the pesticide residues in 20 tea samples collected from different regions. Two pesticide residues of 0.28 mg/kg cyfluthrin and 0.32 mg/kg fenvalerate were detected in one sample by GC–ECD, and confirmed by GC–MS in SIM mode, as shown in Fig. 4.

4. Conclusions

A simple and sensitive multiresidue analysis method based on MSPD and gas chromatography was developed for the determination of organochlorines and synthetic pyrethroids in tea samples. The analyte extraction and matrix cleanup were carried out in a single step without additional purification. RSM was used to optimize the parameters of MSPD and to investigate the interaction effects of different factors. With a balanced consideration of the pesticide recoveries and pigment cleanup degree, optimal conditions of MSPD were established using Florisil as the sorbent and 6.0 ml *n*-hexane–DCM (1:1, v/v) as the eluent. The optimized conditions obtained in the present work might

provide useful information for the analysis of pesticide multiresidues in tea samples with high pigment content.

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