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Selective sample cleanup by immunoaffinity chromatography for determination of fenvalerate in vegetables

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ABSTRACT

This paper describes the establishment of an immunoaffinity chromatography (IAC) for selective extraction of fenvalerate from vegetable samples. The IAC column was constructed by covalently coupling monoclonal antibody (mAb) against fenvalerate to CNBr-activated Sepharose 4B and packed into a cartridge. The extraction conditions were carefully optimized, including loading, washing and eluting solutions. Under the optimal conditions, the IAC column was able to capture fenvalerate with the maximum capacity of 4000 ng. An average recovery of 94.5% and a RSD of 8.8% were obtained with six IAC columns prepared on six different days. Three vegetable samples spiked with fenvalerate at four different concentrations were extracted with IAC column and determined by gas chromatography with electron capture detection (GC–ECD). Chromatograms of final extracts were clean and fenvalerate could be easily detected without the interferences. The extraction recoveries and RSD were 74.7–96.5% and 2.5–5.2%, respectively, and the calculated limit of detection of the whole method was 0.008–0.012 ng g^{-1}.

1. Introduction

Fenvalerate, a synthetic pyrethroid, is one of the most widely used pesticides because of its excellent insecticidal properties and low mammalian toxicity [1]. Nevertheless, fenvalerate residues in vegetables still pose potential risks to public health. The European Union (EU) enacted a regulatory limit for its levels ranging from 0.02 mg kg^{-1} to 0.2 mg kg^{-1} depending on the food [2]. Therefore, fast and reliable analytical methods for the monitoring of fenvalerate in food are required.

The conventional method used for the identification of fenvalerate is based on gas chromatography and electron capture detection (GC–ECD) [3], and more recently GC–EI-MS or GC–EI-CID-MS/MS [4,5] is being used for identification and confirmation purposes. Other methods such as thin-layer chromatography [6,7], gas–liquid chromatography [8,9], enzyme immunoassays [10,11], as well as high-performance liquid chromatography with either fluorescence [12,13] or mass spectrometry detection [14] for the analysis of fenvalerate in different samples have been reported.

Considering the complexity of matrix and the low concentration of fenvalerate in real samples analysis, these methods are usually associated with a sample preparation step for extraction or enrichment of the analytes. Liquid–liquid extraction and solid phase extraction (SPE) are the most common used methods. Extraction solution such as acetonitrile [15], hexane–acetone [16], hexane–dichloromethane (1:1) [17], hexane–acetonitrile (1:1) [18] and hexane–acetone (1:1) [19] have been employed for fenvalerate analysis. Solid phase extraction (SPE) with different solid supports such as florisil, alumina, graphitized carbon black, or mixtures alumina/C18 has been shown increased method sensitivity. However, due to the large consumption of organic solvents in these methods, the development of new SPE sorbents which are environmental friendly and less toxic is a subject of interest, such as immunoaffinity supports.

Immunoaffinity chromatography (IAC) is a separation method that takes advantage of the specific interaction between antibody and antigen [20]. Antibodies raised against the analyte are immobilized on a solid support. By loading the sample extract onto the IAC column, the immobilized antibodies will specifically retain the analytes. Bond analytes can finally be eluted by breaking the antibody–antigen bond. IAC offers a rapid and highly effective clean-up of extract from different matrixes and has been widely employed for the determination of toxins [21–25], hormones [26–29] and other contaminants. However, to our knowledge, unlike microtoxins and veterinary drugs [27], there are no

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commercially available IAC columns for pesticides, although related researches have been published [30–33].

In our previous study, we have successfully produced monoclonal antibody (mAb) against fenvalerate and developed a corresponding enzyme-liked immunosorbent assay (ELISA) for the determination of fenvalerate. By using the mAb we produced which displayed a high sensitivity (the IC_{50} value is 19.2 $\mu g\,L^{-1}$) and low cross-reactivity (below 1%), our purpose is to establish an IAC column for the extraction of fenvalerate by combining anti-fenvalerate mAb with CNBr-activated sepharose. The extraction conditions of the IAC column for fenvalerate were optimized and the IAC column was characterized in terms of binding capacity, extraction recovery and reproducibility. To our knowledge, it is the first report for the extraction of fenvalerate with IAC column.

2. Materials and methods

2.1. Regents and materials

CNBr-activated Sepharose 4B was purchased from GE Healthcare (Sweden). Fenvalerate standards were obtained from Dr. Ehrenstorfer GmbH (Augsburg,Germany). Methanol (MeOH) and acetonitrile (ACN) (HPLC grade) were from Honeywell Burdick & Jackon (Muskegon, USA). Sodium acetate (NaAc), sodium chloride (NaCl), sodium dihydrogen phosphate, disodium hydrogen phosphate, glacial acetic acid, tris(hydroxymethyl)aminomethane and sodium azide (NaN_3) was from Sinopharm Chemical Reagent (Shanghai, China). Hydrochloric acid (HCl), caprylic acid, hexane, ammonium sulfate, sodium bicarbonate and potassium dihydrogen phosphate were obtained from Kermel Chemicals (Tianjin, China). All other inorganic chemicals and organic solvents were of analytical reagent grade. Water was obtained from a MilliQ purification system (Millipore). Medium-speed qualitative filter paper was from Xinhua Paper Ltd. (Hangzhou, China). Florisil columns were purchased from Agilent Technologies Inc. (USA). Nonspecific rabbit immunoglobulins were produced by our laboratory.

Stock solutions of fenvalerate (1000 $\mu g\,mL^{-1}$) was prepared in acetonitrile and hexane and stored at −20 °C until use.

2.2. Instrumentation

GC analysis was carried out on an Agilent 6890 GC system (Agilent Technologies Inc., USA) equipped with a 7683B autosampler and an Agilent μ-ECD detector. Verification of the identity of fenvalerate was accomplished with Agilent 7890 GC equipped with 5975C mass spectrometer. Lyophilizer (Thermo Savant, England) was used to freeze dry the antibodies. Shaker (Chinese Academy of Sciences Scientific Instrument, Wuhan) was used to prepare the immunosorbent. Centrifugation of the vegetable samples was performed on a centrifuge (Hitachi CF 16RX). And a homogenizer (IKA Laboratory Equipment, Germany) was used in the sample preparation.

2.3. GC condition

The extracts were analyzed using an Agilent 6890 series GC equipped with HP-5 column (30 m × 250 μm × 0.25 μm film thickness; Agilent) and an μ-ECD. A 1 μL sample was injected in splitless mode at 250 °C with an Agilent 7683 autosampler. Nitrogen was used as carrier gas at a flow-rate of 1.0 mL min^{-1}. The oven was set at 180 °C and held for 5 min, heated to 230 °C at 5 °C min^{-1} increment and held for 5 min, then to 280 °C at 5 °C min^{-1} increment and held at 280 °C for 13 min. The detector temperature was kept at 320 °C.

2.4. GC–EI-MS condition

The extracts were analyzed using an Agilent 7890 series GC equipped with 5975C mass spectrometer. Separation of the analytes was achieved using a DB-5MS column (30 m × 250 μm × 0.25 μm). A 1 μL sample was injected in a splitless mode at 280 °C operated by an Agilent 7683B autosampler. Helium was used at the carrier gas at a flow-rate of 1.2 mL min^{-1}. The temperature program was: initial temperature 50 °C held for 2 min, 10 °C min^{-1} ramp to 180 °C held for 1 min, and finally 3 °C min^{-1} to 270 °C, held for 14 min; injection temperature of 280 °C; ionization temperature of 150 °C. The mass range in scan mode was 100–500 *m*/*z*.

2.5. Production and purification of monoclonal antibodies

Monoclonal antibodies against fenvalerate were produced according to procedures described in our previous study [10]. In brief, hybridoma cells were injected into Balb/c mice which previously had been given 0.4 mL of Freund's incomplete adjuvant. The obtained ascites were further purified based on the caprylic acid–ammonium sulfate method with a little modification [28]. Acsites were filtrated through double filter paper, mixed with two volumes acetate buffer (0.06 M, pH 4.8) and adjusted to pH 4.5–4.8 with 0.1 M HCl. A certain volume of caprylic buffer (33 μL per milliliter mixed solution) was added drop wise under stirring. After 30 min reaction, the solution was left at 4 °C for 3 h. Then the solution was centrifuged at 12,000 rpm for 5 min. The supernatant was collected, mixed with 1/10 volume phosphate buffer (0.01 M, pH 7.4), adjusted to pH 7.4 with 2 M NaOH and cooled down at 4 °C. A certain mass of ammonium sulfate (0.277 g per milliliter solution) was slowly added under magnetic stirring and ice-cooling. After standing at 4 °C for 2 h, the turbid solution was centrifuged at 12,000 rpm for 30 min. The supernatant was discarded and the precipitation was dissolved in 1/10 ascites volume of PBS (0.01 M, pH 7.4). The aqueous solution was dialyzed against PBS for 24 h and H_2O for 48 h at 4 °C. The purified antibody solution was then freeze-dried and stored at −20 °C until use.

2.6. Preparation of IAC columns

Immunosorbent was prepared following the manufacturer's instructions and related literature [34]. 0.3 g CNBr-activated Sepharose 4B was swelled and washed with 50 mL 1 mM HCl for more than 15 min to remove the protecting groups. The gel was then mixed with 5 mL coupling buffer (0.1 M $NaHCO_3$, 0.5 M NaCl, pH 8.3) containing 10 mg purified antibodies. The coupling reaction proceeded in a shaker at a speed of 150 rpm for 1 h at RT. Then the solution was transferred to a sand funnel (pore size 2–5 μm) and the unbound antibodies were removed by washing with the coupling buffer for no less than 5 medium volume. Capping the remained active group in the gel was accomplished with blocking buffer (0.1 M Tris–HCl, pH 8.0) for 2 h at RT without shaking. To remove excess of uncoupled ligand after coupling, the adsorbent was washed with high and low pH buffer (0.1 M HAc–NaAc buffer, pH 4.0, containing 0.5 M NaCl and 0.1 M Tris–HCl buffer, pH 8.0, containing 0.5 M NaCl) solutions at least three times. Finally, the gel was equilibrated with 0.01 M PBS and stored in 0.01 M PBS containing 0.02% NaN_3 at 4 °C until use.

2.7. Sample preparation

Vegetable samples were grounded in a mechanical mortar and frozen in 50 g batches at −70 °C. Before use, samples were thawed, mixed with 100 mL ACN, and homogenized at high speed for 3 min. The mixture was filtered through double filter paper. Dissolved the

residue by 25 mL ACN and used the washes to rinse the residues on the filter paper. The filtrate was collected in a 250 mL capped graduated cylinder with 15 g sodium chloride and vortexed for 60 s. After the layers were separated, the acetonitrile layer was transferred to a centrifuge tube and centrifuged at 5000 rpm for 10 min. The supernatant was gathered for further treatment.

2.8. IAC extraction

A volume of 0.35 mL wet gel was accurately measured and packed in a 1 mL column. The column was preconditioned by 10 mL PBS prior to sample application and always kept under buffer during the experiment. The sample extract, diluted 5 times with PBS, was loaded on the column at a flow-rate of 2 mL min^{-1}, followed by a washing step with 10 mL MeOH–water (5:95, v/v). Fenvalerate was eluted using 1 mL MeOH at a flow-rate of 2 mL min^{-1}. The eluate was collected in a 10 mL spherical flask and evaporated until dryness under a stream of nitrogen at 50 °C. The residues were dissolved in 1 mL hexane and injected to GC system to determine the concentration of fenvalerate.

3. Results

The affinity interaction between antigen and antibody is generally thought to be a comprehensive combination of various noncovalent bonding, including electrostatic attraction, van der Waals' attraction force, hydrogen bonding, hydrophobicity, etc. [29]. The binding capacity of antibodies can be influenced by the properties of solvents introduced on the columns, such as polarity, pH, ionic strength. For the analyte must be introduced on the columns using solvent, it is essential that the solvent do not influence the antibody–antigen interaction. As a result, the main task in developing operation procedures for IAC columns consists finding a loading medium which allows retention of all analytes by interaction with the antibodies and a suitable elution medium without irreversibly changing the affinity of antibody [22]. In this paper, characters of loading, washing and elution solvents were all carefully investigated.

3.1. Retention mechanism

In order to investigate whether fenvalerate is retained in the IAC columns by specific interactions with the anti-fenvalerate antibodies, we carried out breakthrough experiment with three different columns. One column contained 3.3 mg of anti-fenvalerate antibodies in 0.35 mL gel, the second one contained the same amount of non-specific anti-rabbit immunoglobulins and the third one is 0.35 mL pure gel. A fenvalerate stand solution (100 ng mL^{-1} in 20% ACN–PBS) was applied to each column and the eluates were collected in 1 mL fractions. Due to the incompatibility of the eluate solution and the GC separation, an additional step had to be included to change the solvent. Each dilute was evaporated till almost dryness under a stream of nitrogen at 50 °C and resuspended using 1 mL hexane. One microliter of the aliquot was injected to GC to determine the concentration of fenvalerate. In the cases of cartridges without antibodies, analyte was entirely recovered in the effluent and was therefore not retained by the columns (Fig. 1). In contrast, breakthrough of fenvalerate was not observed before the ninth eluate fraction (0.03 ng mL^{-1}), which helped prove the specificity of retention of the analyte.

3.2. Loading condition

Fenvalerate, which is low-polar, must be extracted by organic solvent, usually ACN or MeOH. However, the organic solvent can damage antibody and interfere with the antibody–antigen interaction. So it is necessary to reduce the solvent concentration to a rather low level which allows all analyte to be extracted without influencing the antibody–antigen interaction before being introduced to the columns. In our experiment, different loading solvents, including PBS, ACN–PBS (5:95, v/v), ACN–PBS (10:90, v/v) and ACN–PBS (20:80, v/v) were tested and compared. The columns were loaded with 10 mL fenvalerate standard (10 ng mL^{-1}) in the four kinds of loading solvent above respectively. After washing the columns with 10 mL water, fenvalerate was eluted with 1 mL MeOH. The elute was evaporated till almost dryness under a stream of nitrogen at 50 °C and redissolved using 1 mL hexane. One microliter of the solution was injected into GC to determine the fenvalerate concentration. As is shown in Table 1, the analyte recovery gradually improved as the concentration of ACN increased, and 20% ACN/PBS achieved the highest recovery (105.3%). Additional experiment was carried out using 40% ACN/PBS as loading medium. The recovery decreased to a rather low value (33%). The reason may be that too low concentration of organic solvent may not guarantee complete dissolution of fenvalerate while too high concentration may denature the antibody. As a result, we used the loading buffer containing 20% ACN throughout this study.

In order to investigate the influence of sample loading flow rate on analyte recovery, a 10 mL aliquot of a fenvalerate standard

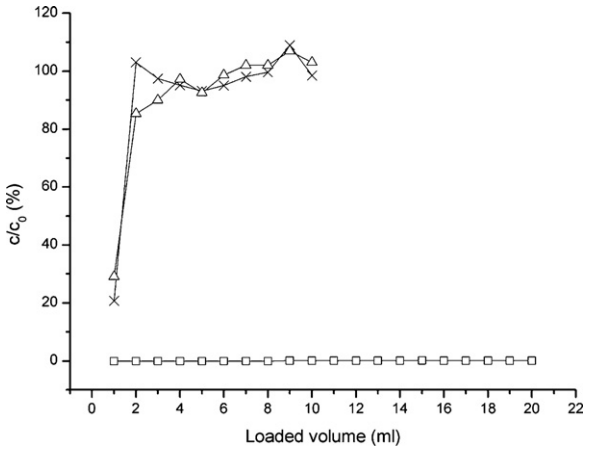

Fig. 1. Breakthrough curves obtained with a column packed with 0.35 mL sepharose containing either 3.3 mg anti-fenvalerate antibodies (□), 3.3 mg non-specific anti-rabbit immunoglobulin (×) or no antibodies (△). Loading solution: 100 ng fenvalerate/mL in 20% ACN/PBS (v:v, 20:80). c_0: fenvalerate concentration in the loading solution, c: fenvalerate concentration in the eluate.

Table 1
Influence of loading condition on fenvalerate recovery.[a]

Loading medium	Recovery ± SD (%) (n = 3)
PBS	62.3 ± 2.3
ACN/PBS (5:95, v/v)	65.3 ± 3.7
ACN/PBS (10:95, v/v)	80.3 ± 1.2
ACN/PBS (20:95, v/v)	105.3 ± 4.2

[a] Values correspond to three independent determination.

Table 2
Influence of washing condition on fenvalerate recovery.[a]

Washing medium	Recovery ± SD (%) (n = 3)
Water	105.8 ± 2.5
ACN/water (5:95, v/v)	100.1 ± 1.7
ACN/water (10:95, v/v)	62.2 ± 1.5
Tween 20/water (1:99, v/v)	72.6 ± 3.1

[a] Values correspond to three independent determination.

solution (10 ng mL^{-1}) in 20% ACN/PBS was loaded onto a column applying flow rates of 0.5, 1, 1.5, 2 mL min^{-1}. After the column was washed by 10 mL water, 1 mL MeOH was used to elute the fenvalerate. The results indicated that a fenvalerate standard solution could be loaded up to a flow rate of 2 mL min^{-1} without reducing analyte recovery.

3.3. Washing condition

In the previous study, sample matrixes always cause high background in the chromatogram. In the IAC extraction, sample extracts were 5 times diluted before being applied to the columns, which decreased the matrix effect to some extent. However, we hope to further remove interference of complex matrixes by washing the columns after the loading procedure to get a better chromatogram and increase sensitivity. The following washing media were tested: water, MeOH–water (5:95, v/v), MeOH–water (10:90, v/v), and Tween 20–water (1:99, v/v). Tween 20 is a nonionic detergent, which was commonly used to minimize nonspecific binding and improve sensitivity in immunoassays.

In order to investigate whether low concentration of MeOH or surfactants interfered with antigen–antibody reaction, experiments with the washing mediums above were first carried out with fenvalerate standard solutions (Table 2). The results showed that using water or MeOH–water (5:95, v/v) as washing medium can give an acceptable fenvalerate recovery. The recovery of analyte decreased significantly when using MeOH–water (10:90, v/v) or Tween 20–water (1:99, v/v).

To determine the column's efficiency in removing nonspecifically bound matrix components, additional experiments were carried out with blank cabbage samples spiked with 150 ng of fenvalerate per milliliter of extract. A diluted portion of the extract (1/5 PBS) was passed through the column and a washing step was performed with 10 mL MeOH–water (5:95, v/v) followed by 10 mL pure water before applying 1 mL MeOH to dilute the analyte. The comparison between the chromatograms of a spiked cabbage sample (300 ng mL^{-1}) and that of a fenvalerate standard solution (150 ng mL^{-1}) was reported in Fig. 2. The chromatogram obtained from the spiked sample (Fig. 2a) was as clean as that of a standard solution (Fig. 2b). No significant interference was observed. This result demonstrated the possibility of the IAC method to remove interference from sample matrix.

3.4. Elution condition

The analyte is eluted from the columns by breaking the antibody–antigen bond. For small molecules, this is always achieved with a small volume of methanol or acetonitrile [27]. In this experiment, methanol of different concentration was evaluated. After loading 10 mL of fenvalerate standard solutions (10 ng mL^{-1}), the columns were washed with 10 mL water and eluted with 10 mL MeOH–water (100%, 80:20, 60:40, 40:60) collecting 1 mL eluate fractions. Each of the eluate fractions was evaporated under a stream of nitrogen at 50 °C until almost dryness and redissolved using 1 mL hexane before injecting to the GC. Fig. 3 displays the elution profiles of fenvalerate by using four kinds of elution solvents. As shown in Fig. 3, a recovery rate of 100% can be obtained when using 1 mL MeOH or 80% MeOH in water or 2 mL 60% MeOH. The 40% MeOH elution buffer was found to be unable to quantitatively remove fenvalerate with 10 volumes. Elution medium with low methanol concentration which may cause less damage to the antibodies can increase the reusable times of the columns. However, due to the evaporation step in the method, water contained in the eluate makes the clean-up procedure more time-consuming. A sample cleanup procedure including a time-consuming step is, however, not expected for consumers even if the columns could be reused many times. Thus, 1 mL MeOH was selected as the elution solvent.

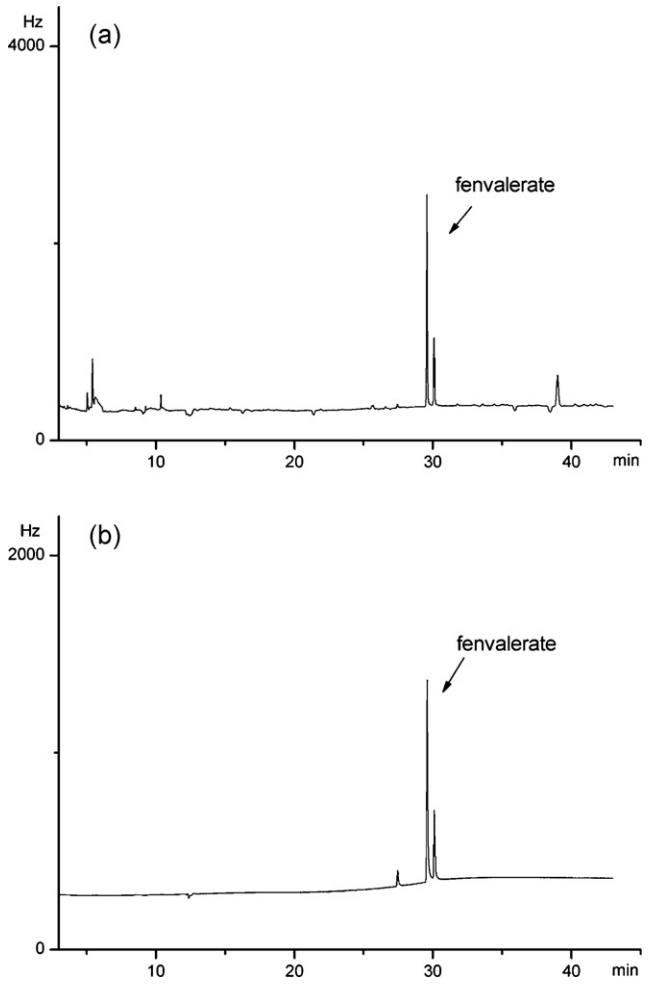

Fig. 2. Chromatograms obtained by injecting aliquots of a purified extract of a cabbage sample spiked with 150 ng of fenvalerate/mL (a) and a fenvalerate standard solution (150 ng mL^{-1} in hexane) (b) into GC system.

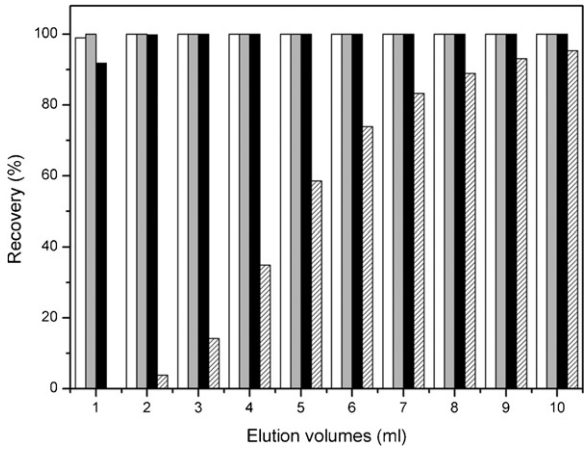

Fig. 3. Distribution of fenvalerate in the eluate fractions of the immunoaffinity column, depending on the MeOH concentration in the elution medium. (white columns: pure MeOH, grey columns: 80% MeOH, black columns: 60% MeOH, white columns packed with lines: 40% MeOH). Fenvalerate recovery rates are the mean values of three times of determination.

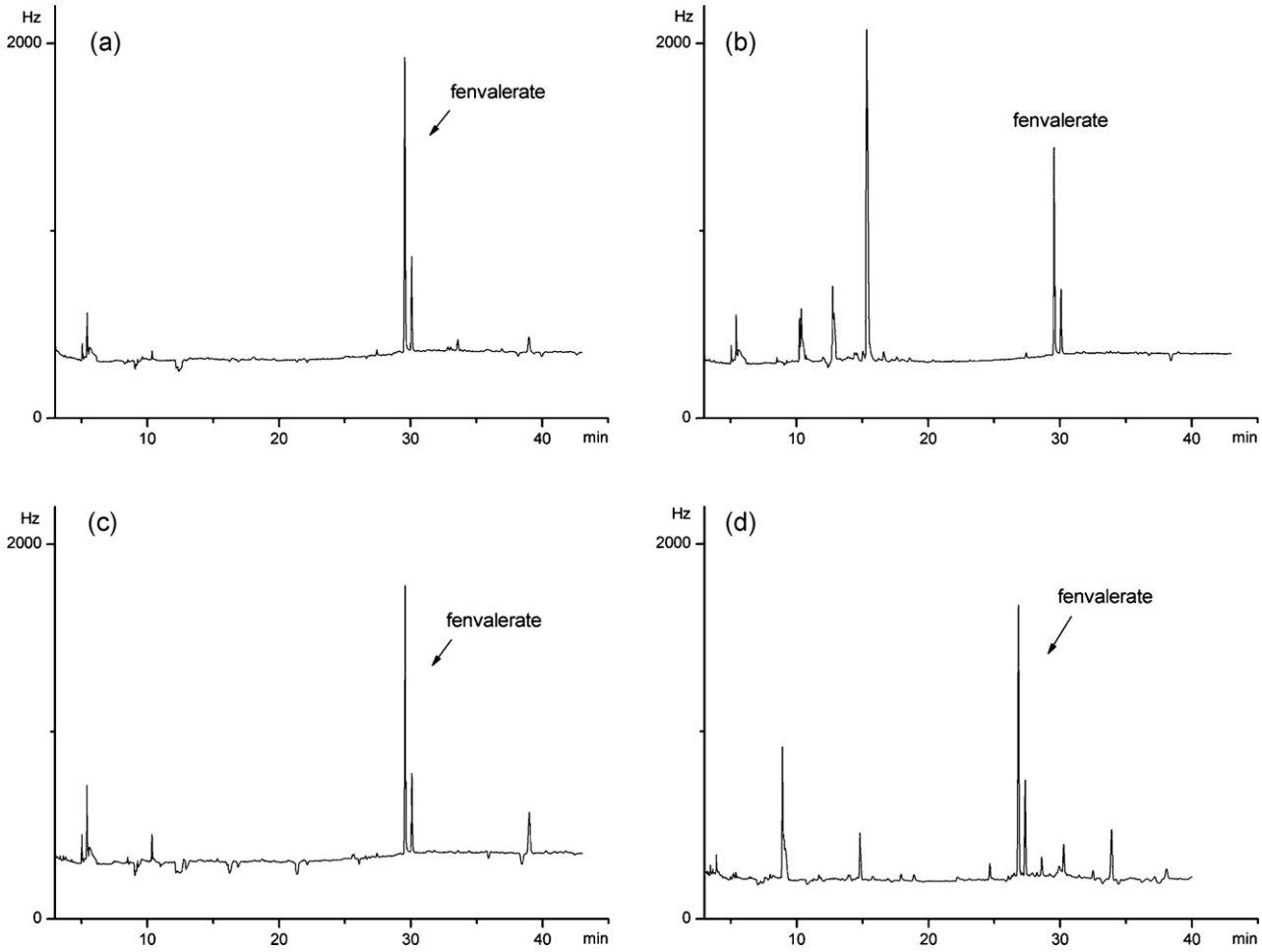

Fig. 4. Chromatograms obtained by injecting aliquots of extracts purified either by an immunoaffinity column (a–c) or a florisil SPE column (d). (a) Cucumber sample spiked with 125 ng fenvalerate/g. (b) Carrot sample spiked with 125 ng fenvalerate/g. (c) Cabbage sample spiked with 125 ng fenvalerate/g. (d) Cucumber sample spiked with 100 ng fenvalerate/g.

3.5. Binding capacity

The binding capacity was determined by overloading the IAC columns with 100 mL of a fenvalerate standard solution (100 ng mL^{-1}) in ACN–PBS (20:80) using a flow-rate of 2 mL min^{-1}. After washing the columns with 10 mL water, fenvalerate was eluted with 1 mL MeOH. The results indicated that the columns containing 3.3 mg antibody showed a binding capacity of 4000 ng fenvalerate.

3.6. Column to column reproducibility

In order to determine batch to batch reproducibility, six IAC columns prepared on six different days were subjected to recovery tests with fenvalerate standard solution (Table 3). The mean recovery was found to be 94.5% with a standard deviation of 8.8%. These results indicated that the IAC columns can be carried out highly reproducibly.

3.7. Sample preparation

The whole analytical method was applied to determine fenvalerate in vegetable samples. Vegetables were extracted with acetonitrile. After filtration and dilution 5-fold with PBS, the extract was loaded onto the IAC column. Representative chromatograms of purified vegetable samples were showed in Fig. 4, (a) cucumber, (b) carrot, (c) cabbage, each of them spiked with 125 ng of fenvalerate per gram of sample, corresponding to 100 ng fenvalerate in the loading solution. In order to further determine the efficiency of the IAC column in removing matrix interference, a cucumber sample spiked with 100 ng of fenvalerate per gram of sample (100 ng fenvalerate in the loading solution) was purified by a commercially available florisil SPE column. The comparison between the chromatogram and those of IAC columns shown in Fig. 4 demonstrated that the cleanup procedure was fit for purpose.

Table 3
Fenvalerate recoveries obtained with immunoaffinity columns from different production batches.

Production batch	Recovery ± SD[a] (%) (n = 3)
I	104.5 ± 2.4
II	104.3 ± 1.8
III	97.8 ± 0.9
IV	94.2 ± 1.2
V	84.3 ± 0.7
VI	82.2 ± 2.5
Mean recovery	94.5
Standard deviation	8.8

[a] SD values correspond to three independent determination.

3.8. Verification of the identity of fenvalerate

In order to confirm the identity of the columns of the extracted compounds, the purified cucumber sample extracts were analyzed

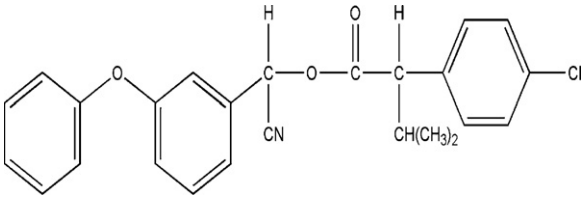

Fig. 5. Molecular structure of fenvalerate.

by 70 eV electron impact GC–EI-MS. The chemical structure of fenvalerate was shown in Fig. 5. Fenvalerate was eluted at around 44 min (the peak retention time: 44.43 min, 44.77 min) (Fig. 6a). The full scan MS spectrum shows a signal at *m*/*z* 419 ion (Fig. 6b), which was assigned to the molecular ion $[M]^{+*}$. There are two useful fragments at *m*/*z* 167 and 211 assigned as $[M\text{-}C_{15}H_{10}O_3N^*]^+$ and $[M\text{-}C_{14}H_{10}ON^*]^+$ [35].

3.9. Quantitative determination of fenvalerate in real samples

The GC system was calibrated by injecting seven standard solutions in the concentration range from 5 to 1000 ng mL^{-1} in hexane. A linear relationship was obtained between fenvalerate concentration and peak area over the whole concentration range. The correlation coefficient was 0.9999 ($n = 7$).

Finally, in order to assess the whole method, we have measured non-spiked and spiked cucumber, cabbage and carrot (0, 50, 100, 150 ng mL^{-1}). The results (Table 4) demonstrated that both high recovery rates and a good reproducibility using the method described above. The limits of detection (S/N = 3) were found to be 0.012, 0.008, 0.009 ng g^{-1}, respectively.

4. Discussion

In a conclusion, the use of IAC column for the purification of fenvalerate from vegetables followed by GC–ECD analysis was validated for the determination of fenvalerate in vegetable samples. A mean percentage of recovery of 83.5% was achieved for cucumber, cabbage and carrot with a limit of detection lower than 15 ng fenvalerate per gram for all tested samples. This method provided good sensitivity towards fenvalerate and required low organic consuming. However, due to the use of 100% methanol as elution solution, the mean recovery rate of fenvalerate falls to 60% at the third time use of the same column.

The application of immunoaffinity to analytical sciences has received more attention in recent years. With the aid of IAC columns as a pretreatment method, the contaminations and non-target compounds in the complex matrix such as pigment could be removed and the target analyte fenvalerate was collected specifically. From the chromatograms obtained from cucumber, cabbage and carrot samples, some other peaks (retention time: 5 min, 10 min, 39 min) besides those of fenvalerate (29.7 min, 30.1 min) were also found at the same time in all the three chromatograms. In the chromatogram of carrot, high peaks were always found at the retention time of about 15 min. These interferents may be co-extractives structurally similar to fenvalerate. These unknown chemicals, however, having no influence with the quantification of fenvalerate, will be further investigated in our following study.

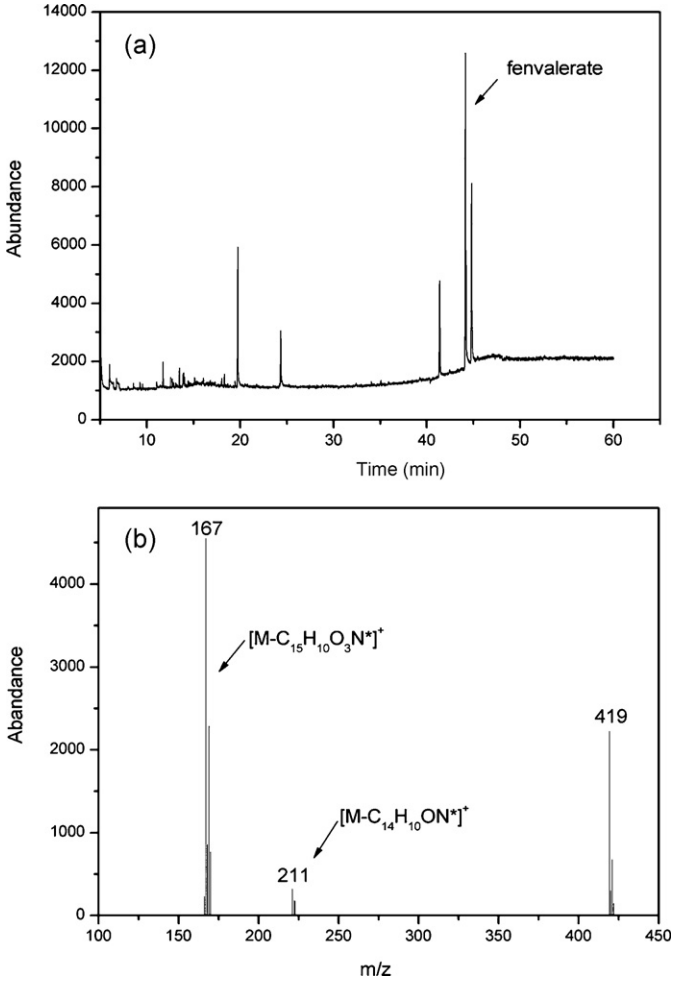

Fig. 6. (a) Total ion chromatogram of fenvalerate and (b) mass spectra of fenvalerate obtained by injecting an aliquot of a purified cucumber sample extract into GC–MS system.

In the current study of analytical chemistry, multiple-analyte IAC for the extraction of several compounds from different matrix has been getting great attention. The multiple-analyte IAC was usually achieved by co-immobilizing several antibodies or one

Table 4
The recovery rates and LOD of the IAC-GC method for the detection of fenvalerate in three vegetable samples.

Sample	Spiking value (ng mL^{-1})	Measured value (ng mL^{-1})	Recovery (%) ($n = 3$)	Mean recovery ± SD (%)	Limit of detection (mg kg^{-1})
Cucumber	0	0		89.8 ± 5.1	0.012
	50	44.4	88.9		
	100	96.5	96.5		
	150	126.1	84.1		
Cabbage	0	0		79.7 ± 2.5	0.008
	50	40.9	81.8		
	100	81.1	81.1		
	150	114.3	76.7		
Carrot	0	0		81.1 ± 5.2	0.009
	50	40.6	81.3		
	100	74.7	74.7		
	150	131.0	87.3		

kind of broad-specificity antibody on the solid support. Such pre-analytical cleanup technique has been reported in the areas of mycotoxins and cytokines. Further application of IAC for the simultaneous analysis of pyrethroids is at present under study by our group. The application of GC–EI-MS as a quantitative method is also under examination to further confirm the purification effect and to enhance the sensitivity.

Acknowledgements

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