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Simultaneous determination of two acute poisoning rodenticides tetramine and fluoroacetamide with a coupled column in poisoning cases

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ABSTRACT

A coupled column system was developed for the simultaneous determination of both rodenticides fluoroacetamide and tetramine in this paper by gas chromatography/mass spectrometry (GC/MS). A short length of strong polar column (1.5 m of Innowax) was coupled to the top of a 30 m of DB-5ms with a quartz capillary column connector. Peak width at half height (W_h) was used to evaluate the band broadening of the coupled column system. The length of the short couple column and oven temperature program were discussed according to W_h. The precisions of the coupled column were analyzed with peak area and retention time. Good linear correlations were found for both rodenticides. Typical samples were discussed for each rodenticide and some poisoning cases were presented.

1. Introduction

Tetramine (tetramethylene disulfotetramine, CAS No. 80-12-6, Fig. 1) is a lethal rodenticide. The median lethal dose of mammals (LD50) is 0.1–0.3 mg kg^{-1}. A dose of 7.0–10.0 mg was considered lethal for human beings [1,2]. It is potentially 100 times more toxic to humans than potassium cyanide and might be a more powerful human convulsant than strychnine [3]. Mild symptoms include headache, dizziness, fatigue, anorexia, nausea, vomiting, abdominal pain, numbness of lips and listlessness. Severe clinical features include reduction of consciousness, seizures (grand mal epilepsy type, or may last 1–6 min and occur repetitively after several minutes), foaming at the mouth, urinary incontinence, coma (with ECG/EEG abnormalities) and death from respiratory failure [3]. Fluoroacetamide (CAS No. 640-19-7, Fig. 1) is another lethal rodenticide to human beings. It blocks the tricarboxylic acid cycle and is extremely toxic with the approximate lethal oral dose of 30 mg for adults [4]. The poisoning symptoms of fluoroacetamide are similar to those of tetramine [5]. The route of exposure for both rodenticides to humans and animals includes suicide, ingestion of contaminated food and homicide. Both can be powerful tools to cause chemical terror events due to their colorlessness, tastelessness, simple synthesis and extremely toxicity. Some acute poisoning cases involved in both ones were reported in USA, Hongkong and mostly, in China [1,3,6–13]. Vomitus (or stomach contents), blood, urine and the first time gastric lavage fluid should be sampled for the measurement of tetramine and fluoroacetamide.

Individual determination methods for each rodenticide have been reported in literatures [9–11]. Wu et al. reported a simultaneous detection method with polyethylene glycol column FFAP and nitrogen–phosphorous detector [12]. However, 1 and 5 μg mL^{-1} of detection limits for tetramine and fluoroacetamide, respectively, were too high to measure the concentrations in blood or urine. Ma et al. established a gas chromatography/mass spectrometry (GC/MS) method with a (5%-phenyl)-methylpolysiloxane column (DB-5ms) for the simultaneous detection of both rodenticides [13]. The detection limits of fluoroacetamide was 2 μg mL^{-1} and also not suitable to blood or urine samples for its much less poisoning concentration according to the approximate lethal oral dose. A simultaneous detection method with enough sensitivity is necessary.

Tetramine can be detected with weak polar columns (such as (0–5%-phenyl)-methylpolysiloxane column) for its lipophicity and symmetrical molecular structure (Fig. 1) [10]. As to the detection of fluoroacetamide with strong polarity and hydrophilicity, weak polar columns are not compatible for the band broadening and peak tailing. It is necessary that strong polar columns (such as polyethy-

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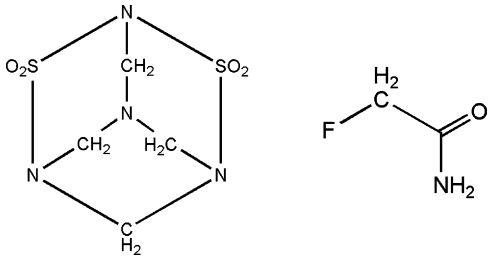

Fig. 1. Structures of tetramine and fluoroacetamide.

lene glycol column) were needed [12]. However, these kinds of columns are not suitable to GC/MS for the high column bleeding. These kinds of columns are also not compatible to detection of tetramine for the band broadening.

In this paper, a coupled column system was developed at the first time for the simultaneous detection of both rodenticides in blood, urine and vomitus samples. The purpose was to find a condition, under which, the strong polar compound fluoroacetamide can be detected directly and sensitively with weak polar column DB-5ms. A short length of strong polar column, polyethylene glycol column Innowax, was coupled to the top of a 30 m of DB-5ms with a quartz capillary column connector. Band broadening is unavoidable for the coupled column since each rodenticide was only compatible to one column (tetramine to DB-5ms and fluoroacetamide to Innowax). According to the simplified calculation formula that peak area equals to peak width at half height (W_h) multiplying peak height, it is obvious that peak height would be increased and sensitivity improved with W_h decreasing at the same concentration. So W_h was introduced as an evaluation parameter of band broadening for the coupled column system. The purpose was to find an optimal condition, under which, W_h of both rodenticides in the coupled column system would be as near as possible to that of each compatible column by changing the length of the short column and the oven temperature program.

2. Experimental

2.1. Chemicals and reagents

All reagents and solvents were analytical grade unless specified. All glassware was treated at 120 °C for 4 h.

Fluoroacetamide was purchased from Chem Service, Inc. (West Chester, PA, USA) and 100 μg mL^{-1} of tetramine in ethyl acetate from National Poison Control Center (Beijing, China). Acetonitrile and hexane were HPLC grade and were purchased from TEDIA (Fairfield, OH, USA). Ultra-pure water was obtained from a Millipore system (Bedford, MA, USA). Supelclean ENVI-Carb 120/400 was bought from Supelco (Bellefonte, PA, USA). Sodium chloride and anhydrous sodium sulphate were purchased from Huadong Medicine (Hangzhou, China) and baked at 500 °C for 4 h before use.

2.2. Preparation of standards

Stock standard solution of fluoroacetamide was prepared at concentration of 1 mg mL^{-1} in acetonitrile by dissolution of the neat chemicals in quantitative amounts of solvent. It was diluted to 100 μg mL^{-1} with acetonitrile.

Mixed working standard solutions were prepared for calibration by mixing a quantitative amount of each of the stock standard solution and serially diluting the mixture with acetonitrile to give the concentrations of 0.01–30.0 μg mL^{-1}.

2.3. Instrumentations

All samples were analyzed with an Agilent 6890N GC and 5973i MSD in election impact ionization mode. Gas chromatographic separations were performed on a 30 m of DB-5ms capillary column (0.25 mm i.d., 0.25 μm df) coupled with a 1.5 m of Innowax capillary column (0.32 mm i.d., 0.25 μm df) by a quartz capillary column connector (Agilent). The oven was maintained at the initial temperature of 50 °C for 5 min, heated to 245 °C at the rate of 40 °C min^{-1}, and then held at 245 °C for 5 min. The inlet temperature was at 220 °C and the transport line at 250 °C. Helium gas was used as carrier gas at a flow rate of 1.0 mL min^{-1}. Injection volume was 1 μL with splitless mode. Ion source and quadrupole temperatures were 230 and 150 °C, respectively. Electron energy was 70 eV. The full scan MSD was operated at a rate of 3.46 scan s^{-1} over the range of 30–450 amu for identification of each rodenticide. Selected ion monitor (SIM) was used for quantitative measurement of fluoroacetamide at *m*/*z* 44, 77and of tetramine at 240, 212 with a solvent delay of 5 min.

2.4. Sample preparation

Blood, vomitus (or stomach contents) and urine were detected in this article. About 2 g of homogenised sample, 0.5 g of sodium chloride, 0.2 g of ENVI-Carb were weighed, and 4 mL of acetonitrile were added to a 10 mL test tube. The sample was then mixed with a vortex for 1 min and centrifugated at 4000 rpm for 5 min. 2 mL of acetonitrile was dried with 0.2 g anhydrous sodium sulphate and concentrated to 1 mL with nitrogen gas at 45 °C. 1 mL of hexane was added to the concentrated solution. After mixed with a vortex for 0.5 min and centrifugated at 4000 rpm for 2 min, the acetonitrile phase was ready to injection.

3. Results and discussion

3.1. Chromatography and mass spectrum of tetramine and fluoroacetamide with DB-5ms and Innowax columns, respectively

The total ion current chromatogram (TIC) of tetramine and fluoroacetamide is shown in Fig. 2. A tailed peak of fluoroacetamide was detected as to the column DB-5ms at the concentration of 10 μg mL^{-1}. It is not suitable for this kind of column to simultaneously detect both rodenticides. Although both were detected in terms of the column Innowax, band broadening was found for tetramine compared to DB-5ms. In addition, the column bleeding was high and not compatible to MSD.

Two characteristic ions were found for fluoroacetamide, i.e. *m*/*z* 77 and 44 (Fig. 3). *m*/*z* 77 was used as the quantitative ions. *m*/*z* 212 and 240 were used as the characteristic ions for tetramine (Fig. 3) and *m*/*z* 212 as the quantitative ions. As to the qualitative analysis, the ion ratio of *m*/*z* 77 to *m*/*z* 44 and *m*/*z* 212 to *m*/*z* 240 must be controlled at the deviation range of 20% in samples compared to that of standard solution. A full scan mass spectrum would be required for positive samples of each rodenticide since only two characteristic ions were found.

3.2. A coupled column system

Since DB-5ms only suitable for tetramine and Innowax for fluoroacetamide, a coupled column system was introduced at the first time in this paper to simultaneously detect both rodenticides. A 30 m of DB-5ms capillary column (0.25 mm i.d., 0.25 μm df) was selected as the main column and the length was fixed. Some length of strong polar column Innowax (0.32 mm i.d., 0.25 μm df) was coupled to the top of DB-5ms with a quartz

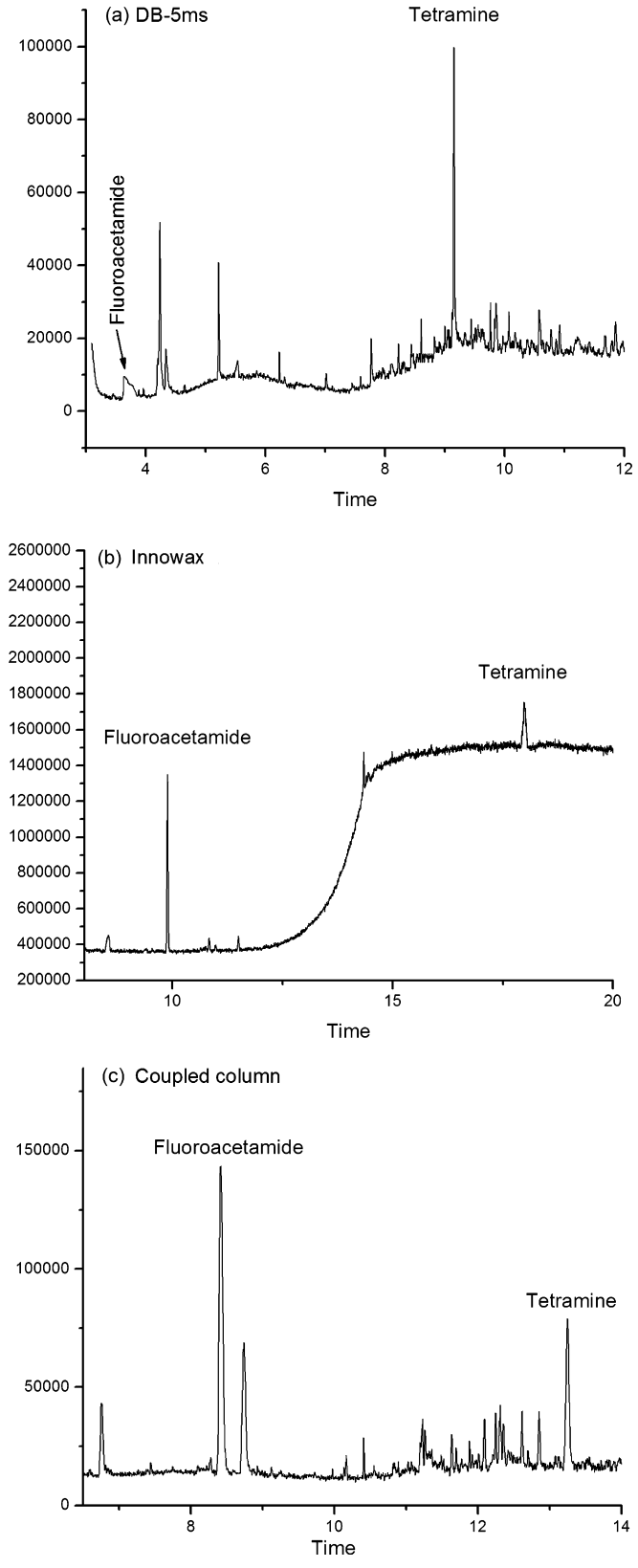

Fig. 2. Total ion current chromatogram (TIC) of tetramine and fluoroacetamide with column of (a) DB-5, (b) Innowax and (c) coupled column, respectively.

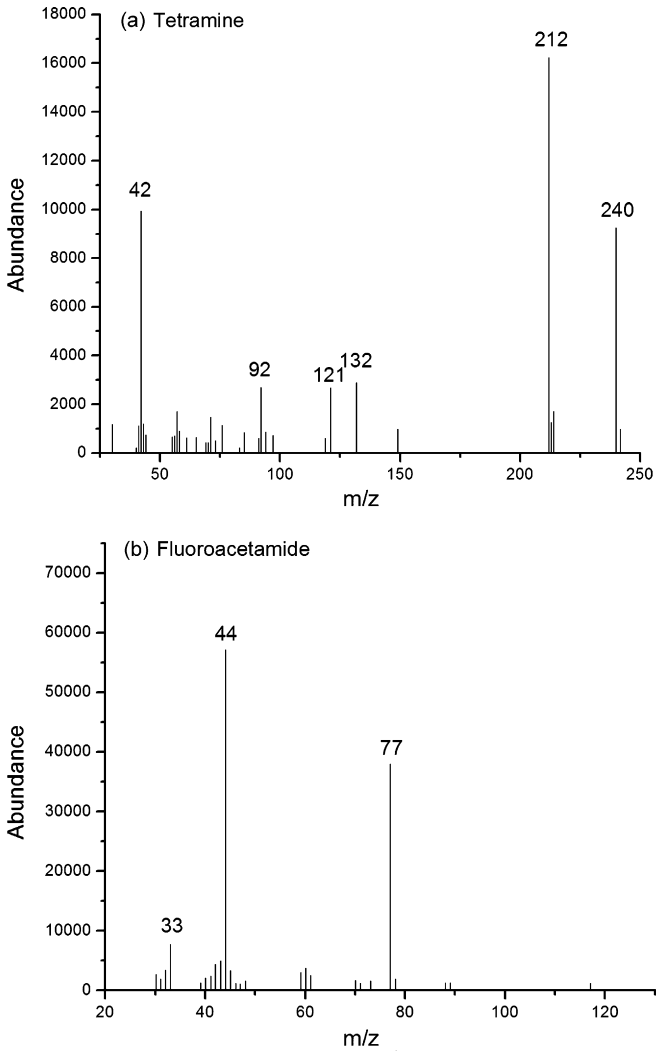

Fig. 3. Full scan mass spectrum of (a) tetramine and (b) fluoroacetamide.

capillary column connector. Band broadening is unavoidable for the coupled column since each rodenticide was only compatible to one column (tetramine to DB-5ms and fluoroacetamide to Innowax). W_h was introduced to measure this item. W_h would be as near as possible to that of each compatible column by changing the length of the coupled column and the oven temperature program. W_h can be directly obtained from the Data Analysis software.

3.2.1. Length of the short coupled column

W_h was detected to be 0.022 for tetramine and 0.261 for fluoroacetamide when a single DB-5ms column was used. W_h of fluoroacetamide was too big to give enough sensitivity with DB-5ms column. As to Innowax, W_h was 0.080 and 0.020, respectively. Band broadening was unavoidable for tetramine since four times of W_h was found between both rodenticides as to Innowax column. Under the conditions mentioned in Section 2.3, the length of the short coupled column Innowax was studied at 0.6, 1.2, 1.5, 2 and 3 m, respectively. The relationship between the length of the short coupled column and W_h of fluoroacetamide and tetramine is shown in Fig. 4. From 0 to 1.2 m, W_h of fluoroacetamide was decreased extremely. With the length of the short coupled column increased from 1.2 m (W_h was 0.042 at 1.2 m (Fig. 4)), W_h decreased and approached to 0.020 (at a single Innowax) gradually. So the length

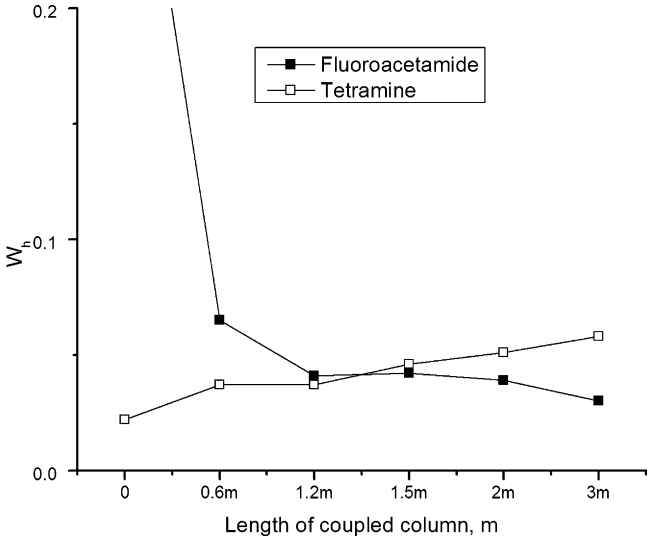

Fig. 4. Relationship between the length of the short coupled column and W_h of fluoroacetamide and tetramine.

of the short coupled column was the longer the better if only W_h of fluoroacetamide was considered. The change of W_h of tetramine to the length of the short coupled column was reversed to that of fluoroacetamide. They were crossed between 1.2 and 1.5 m. According to both change of W_h of fluoroacetamide and tetramine, the length of the short coupled column between 1.2 and 1.5 m would be better. In this paper, the length of 1.5 m was adopted.

3.2.2. Oven temperature program

In addition to the length of the short coupled column, the relationship between W_h and the initial oven temperature program was also studied at 40, 50, 60, 70 and 80 °C, respectively (Fig. 5). From the above temperature sequence, W_h of fluoroacetamide became to decrease firstly with the selected 5 coupled column lengths. A bottom was found at about 50 °C and then to increase. So 50 °C would be the best initial oven temperature for fluoroacetamide. W_h of tetramine was as the same trend at the length of 0.6 and 1.2 m. W_h was changed slightly from 40 to 70 °C at 1.5, 2 and 3 m. So considering the change of W_h for both rodenticides with temperatures, the preferable initial oven temperature was at 50 °C.

The total ion current chromatogram (TIC) of tetramine and fluoroacetamide is shown in Fig. 2c at the coupled column length 1.5 m and initial oven temperature 50 °C. Both peaks of tetramine and fluoroacetamide were obtained with sharp and symmetrical bands. Less column bleeding was found in this system.

3.3. Sample preparation

The concentration of rodenticide was relatively high in poisoning cases. Samples would be weighed as little as possible to simplify the operation steps so that the poison could be found as soon as possible. Acetonitrile was used to extract the hydrophilic compound fluoroacetamide and lipophilic one tetramine. To meet the requirement of emergent detection, only one step of extract operation was processed although the recovery of fluoroacetamide in blood was only about 55–60%. Some of the fat was removed with hexane for the purification of tetramine. ENVI-Carb was introduced to eliminate emulsification during centrifugal process and remove some of the interfering compound.

The full scan mass spectrum can be identified at about 0.1 μg mL^{-1} of each rodenticide under the coupled column system.

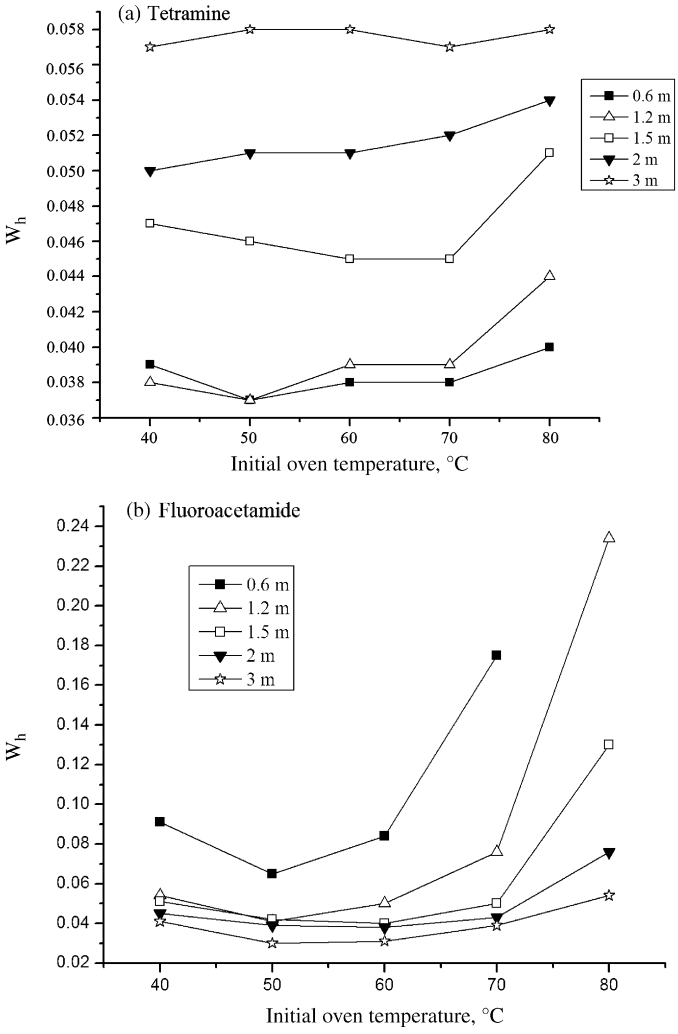

Fig. 5. Relationship between the initial oven temperature and W_h of (a) tetramine and (b) fluoroacetamide.

Therefore, further evaporation would be necessary for rodenticide concentrations of positive samples bellow that.

3.4. Methodology

3.4.1. Calibration curve and detection limit

The method showed linear regression with correlation coefficient $r = 0.997$ ranging from 0.03 to 30 μg mL^{-1} for fluoroacetamide and $r = 0.998$ from 0.01 to 20 μg mL^{-1} for tetramine. A standard solution with both rodenticides at the levels that produced distinguishable signals from the baseline noise was used to determine the limits of detection (LODs, based on signal-to-noise ratio of 3) and the limits of quantification (LOQs, based on signal-to-noise ratio of 10). According to 1 g of sample and 1 mL of condensation volume before the injection, the LODs and LOQs achieved were 0.01 and 0.03 μg g^{-1} for fluoroacetamide while 0.003 and 0.01 μg g^{-1} for tetramine, respectively. The LODs of fluoroacetamide was 60 times lower than the calculated deathful body concentration (0.6 μg g^{-1}) to a man weighed 50 kg according to the approximate lethal oral dose 30 mg for adults [4]. As to tetramine, it was 47–67 times lower according to a lethal dose of 7.0–10.0 mg in humans (0.14–0.2 μg g^{-1}) [2]. So the method has good sensitivity to meet the requirement of the simultaneous detection for poisoning cases.

Table 1
Precision of intra- and inter-day area and retention time (t_R) for both rodenticides.

Rodenticide	Spiked content ($\mu g\,g^{-1}$)	In urine (%)				In blood (%)			
		Intra-day, $n=6$		Inter-day, $n=5$		Intra-day, $n=6$		Inter-day, $n=5$	
		Area	t_R	Area	t_R	Area	t_R	Area	t_R
Fluoroacetamide	0.05	7.02	0.04	9.33	0.04	9.73	0.04	11.2	0.05
	0.50	6.89	0.04	8.97	0.04	7.72	0.04	9.13	0.04
	3.00	6.43	0.04	8.74	0.04	7.01	0.04	8.29	0.04
tetramine	0.05	6.12	0.02	6.98	0.04	6.59	0.02	7.09	0.03
	0.50	4.37	0.02	5.31	0.03	6.15	0.02	6.54	0.04
	3.00	4.22	0.02	4.69	0.03	5.32	0.02	6.44	0.03

3.4.2. Precision of peak area, variability of the retention time and recovery at different concentrations

The precision of the method was evaluated with spiked urine and blood samples. Samples were pre-treated with the procedure described in Section 2.4. The relative standard deviations (RSD) of peak area were examined for intra- ($n=6$) and inter-day ($n=5$) precisions. The RSD of retention time to both rodenticides was also measured to evaluate reliability of the coupled column. The results presented in Table 1 indicated that the coupled column system had good precision for the simultaneous detection of both rodenticides.

Three different spiked concentrations to three representative samples were adopted to examine the recovery of the method. The results are listed in Table 2. Satisfactory recoveries were obtained for tetramine. The recoveries of fluoroacetamide in urine were better than in blood and vomitus. The lowest recovery of fluoroacetamide was only 56.9% in blood after one step of extract operation. Although better recovery can be obtained with two (70.5–83.3%) or three (78.9–89.1%) repetitive steps of extract operations, only one step of extraction was processed in this paper to meet the requirement of fast detection in emergent cases.

3.5. Cases to real samples

Excess half of the poisoning cases managed in our laboratory were involved in rodenticides in recent years. Some of them were caused for ingestion of contaminated food, some for suicide and some for homicide, indeed. Fluoroacetamide is easily metabolized to fluoroacetic acid, another extremely poisonous rodenticidein in body [5] and a small quantity of fluoroacetamide would exist in blood and urine. So the most typical samples for fluoroacetamide would be vomitus or stomach contents before gastric lavage. Although high concentration, such as 25 $\mu g\,g^{-1}$, was ever found in vomitus of a poisoned patient with case involved in fluoroacetamide, a little amount (0.04 $\mu g\,g^{-1}$) was detected in blood and nothing in urine. In addition to vomitus, blood and urine were also typical samples with cases involved in tetramine. The change of the concentration for tetramine in blood and urine of a poisoned patient in about a month is shown in Fig. 6. The concentrations were 36.4, 1.81 and 1.11 $\mu g\,g^{-1}$ in vomitus, blood and urine, respectively, at the day the poisoning case happened. With the traditional detoxification method in the first 2 weeks, only one third was decreased in blood and even much less change in urine. The contents were decreased about a half from the fourteenth to fifteenth day in both samples after the treatment of hemodialysis at the fourteenth day. It was shown that hemodialysis would be a relatively efficient therapeutical method compared to medicine. Unfortunately, the concentration (0.65 $\mu g\,g^{-1}$ in blood) was still much higher than the lethal one until to the twenty-ninth day (0.21 $\mu g\,g^{-1}$ in blood) and the patient was still exanimation.

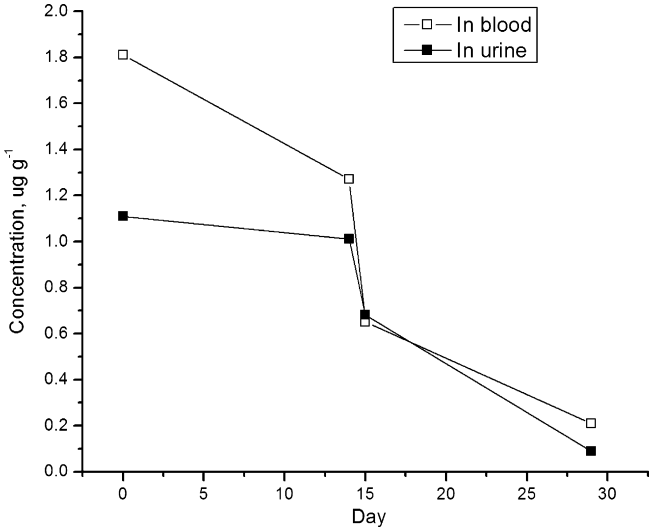

Fig. 6. Change of the concentration for tetramine in blood and urine of a poisoned patient in about a month.

Table 2
Recovery for the method validation of both rodenticides ($n=3$).

Sample	Spiked content ($\mu g\,g^{-1}$)		Recovery (%)	
	Fluoroacetamide	Tetramine	Fluoroacetamide	Tetramine
Blood	0.05	0.017	60.5	109
	0.50	0.17	56.9	98.4
	3.00	1.00	57.2	93.7
Urine	0.05	0.017	72.6	93.1
	0.50	0.17	71.7	91.8
	3.00	1.00	70.1	90.9
Vomitus	0.05	0.017	65.2	90.4
	0.50	0.17	66.4	88.9
	3.00	1.00	68.3	90.4

4. Conclusions

A short length of coupled column with Innowax to DB-5ms was proved to be quick, sensitive, selective and reliable for the simultaneous determination of fluoroacetamide and tetramine in emergent poisoning cases under the studied column length and oven temperature program. The coupled column system can improve band broadening of strong polar compound fluoroacetamide at weak polar column and bring relatively low column bleeding. W_h was validated as an effective evaluation parameter for the condition optimization to this kind of coupled system. A full scan mass spectrum would be required for positive sample of each rodenticide since only two characteristic ions were found. It was revealed that vomitus or stomach contents before gastric lavage would be the most typical samples for the determination of both rodenticides according to the present cases. Blood and urine were

found to be useful samples for the analysis of tetramine but not quite suitable for that of fluoroacetamide.

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