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Headspace solid-phase microextraction and capillary gas chromatographic-mass spectrometric determination of rivastigmine in canine plasma samples

Yunfei Sha [a], Chunhui Deng [b], Zhen Liu [a], Taomin Huang [a], Bei Yang [a], Gengli Duan [a,*]

[a] *Department of Pharmacy, Fudan University, Shanghai 200032, PR China*
[b] *Department of Chemistry, Fudan University, Shanghai 200433, PR China*

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Abstract

A simple, rapid and sensitive method for determination of rivastigmine in plasma samples was developed using headspace solid-phase microextraction (HS-SPME) and gas chromatography with mass spectrometry (GC–MS). The optimum conditions for the SPME procedure were: headspace extraction on a 65-μm polydimethylsiloxane/divinylbenzene (PDMS/DVB) fiber; 0.5 ml of plasma modified with 1.0 ml of sodium hydroxide-sodium carbonate solution (0.7 M:0.5 M); extraction temperature of 100 °C, with stirring at 2000 rpm for 30 min. The calibration curve showed linearity in the range from 0.2 to 80 ng/ml with regression coefficient corresponding to 0.9965 and coefficient of the variation of the points of the calibration curve lower than 10%. The quantification limit for rivastigmine in plasma was 0.2 ng/ml. The method was applied to determination of rivastigmine in canine plasma samples from animals after a single oral administration.

Keyword: Rivastigmine

1. Introduction

Rivastigmine (ENA713, Exelon®, Fig. 1A), (*S*)-*N*-ethyl-3-[(1-dimethyl-amino)ethyl]-*N*-methyl-phenylcarbamate hydrogen tartrate, is a reversible cholinesterase inhibitor indicated for the symptomatic treatment of mild to moderate dementia [1]. Cholinesterase inhibitors target the enzymes responsible for grading acetylcholine (Ach) in the brain, and have demonstrated significant benefits in patients with Alzheimer's disease (AD) [2,3]. Rivastigmine acts as a dual inhibitor of acetylcholinesterase (AchE) and butyrylcholinesterase (BuChE) [4], both of which are believed to co-regulate Ach [5].

Liquid–liquid extraction (LLE) followed by gas chromatography–mass spectrometry (GC–MS) analysis has been applied to determination of rivastigmine in plasma [6]. Recently, Pommier and Frigola [7] has developed a novel method for quantifying rivastigmine in human plasma by liquid chromatography with atmospheric pressure chemical ionization tandem mass spectrometry after pretreatment of the samples by LLE. As we know, LLE is a time-consuming procedure and requires large amounts of organic solvents to extract the drugs, which can cause harm to the human body and the surrounding environment. Therefore, developing a relatively simple, fast and solvent-free extraction method is a relevant task.

Solid-phase microextraction (SPME), introduced by Pawliszyn and his co-workers in 1990, is a relatively new sampling and concentration technique [8]. SPME combined with GC–MS is currently applied in several different areas, including analysis of environment, food, drink and drugs [9]. Recently, this novel technique has been developed for biological sample analysis [10–16].

In this paper, a HS-SPME coupled with GC–MS method was developed for the determination of rivastigmine in animal plasma. The SPME parameters were optimized by using drug-free plasma samples spiked with rivastigmine. To demonstrate the validation of the proposed method, the quantification limit, linearity and precision were investigated. The

* Corresponding author. Fax: 86-21-64707421.
E-mail address: glduan@shmu.edu.cn (G. Duan).

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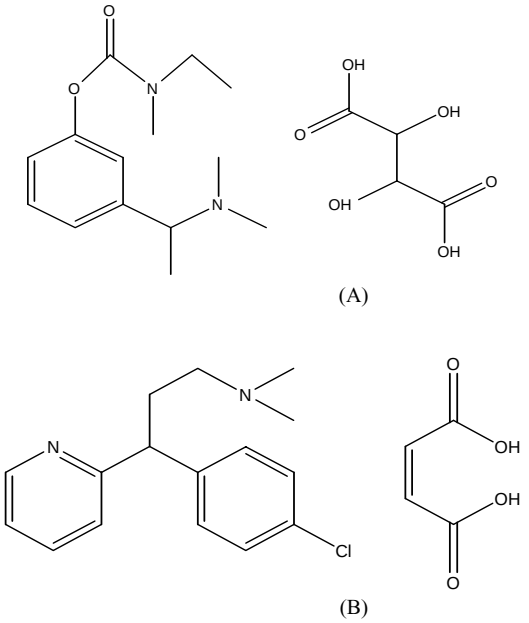

Fig. 1. Structures of rivastigmine tartrate (A) and chlorpheniramine maleate (B).

present method was applied to the analysis of rivastigmine in canine plasma samples from animals after a single oral rivastigmine administration.

2. Experimental

2.1. Reagents and analytical standards

Rivastigmine and chlorpheniramine (internal standard, Fig. 1B) were obtained from Shanghai Institute for Drug Control, with purity greater than 99%. Drug-free plasma was collected from healthy experimental animals and stored at −20 °C.

2.2. Preparation of calibration solutions

Firstly, 1.0 mg/ml rivastigmine stock solution was made. Rivastigmine solutions ranged from 0.01 to 4.0 μg/ml were prepared by diluting rivastigmine stock solution with distilled water. Chlorpheniramine solution with the concentration of 1.0 μg/ml was made by the same method. Calibration solutions were prepared by adding 10 μl rivastigmine solutions from 0.01 to 4.0 μg/ml and 10 μl chlorpheniramine solution (1.0 μg/ml) into 0.5 ml drug-free plasma. Thus, calibration solutions from 0.2 to 80 ng/ml were obtained.

2.3. SPME equipment

SPME fibers and a holder for manual sampling were purchased from Supelco, Bellefonte, PA, and USA. Fibers with 65-μm-thick polydimethylsiloxane/divinylbenzene (PDMS/DVB) or 100-μm-thick polydimethylsiloxane (PDMS) coatings were used. Both kinds of fiber were conditioned before use by heating in a gas chromatograph injection port (0.5 h at 250 °C). Samples were stirred during extraction using an 85-2 model hotplate/stirrer (Si Le instrumental company, Shanghai) and PTFE-coated stir bars 3 mm in diameter and 7 mm long.

2.4. SPME procedure

0.5 ml canine plasma, 10 μl chlorpheniramine (IS, 1.0 μg/ml), 1.0 ml sodium hydroxide-sodium carbonate (0.7 M:0.5 M) and a stir bar were placed into a 15 ml vial and quickly sealed with a silicone septum and an aluminum cap. The vial was heated on the hotplate with stirring at a rate of 2000 rpm. The needle of the SPME device was inserted through the septum of the vial and the extraction fiber was exposed to the headspace of the vial for 30 min. The needle was then removed from the vial and inserted into the injection port of the GC–MS system. The fiber was exposed in the injection port (250 °C) for 2 min and the analytes were desorbed into the GC column.

2.5. Optimization of the SPME conditions

In order to find out the optimal extraction conditions, 0.5 ml drug-free canine plasma samples spiked with 10 ng rivastigmine, 10 ng IS and 1.0 ml sodium hydroxide-sodium carbonate (0.7 M:0.5 M) were placed into a 15 ml vial. To select the optimum fiber, two commercial fibers of PDMS/DVB and PDMS were applied to extraction of the plasma samples at 100 °C for 30 min (stirring rate of 2000 rpm), respectively. Desorption of the analytes adsorbed on the fibers was performed at GC–MS injection port at 250 °C for 3 min. The optimum fiber was determined by peak areas of rivastigmine and IS.

Using the optimum fiber of PDMS/DVB, further work was performed on optimization of heating temperature, extraction time and desorption time. The spiked samples were extracted by PDMS/DVB fiber at varying heating temperatures (70, 90, 100 °C) for varying adsorption times (10, 20, 30, 40 min). Desorption was carried out at 250 °C for varying desorption times (1, 1.5, 2, 3, 5 min).

2.6. Instrumentation

GC–MS analyses were performed on HP 6890 GC system, coupled with a HP MD5973 quadrupole mass spectrometer. The compounds were separated on a HP-5MS capillary column (30 m × 0.25 mm i.d. × 0.25 μm film). Splitless injection was employed. The column oven temperature was programmed to rise from an initial temperature of 100 °C (1 min) to 280 °C at 20 °C/min and was maintained at 280 °C for 15 min. The injection temperature and ion source temperature were 250 and 230 °C, respectively. Helium with a flow rate of 1.0 ml/min was used as the carrier gas. The ionizing

energy was 70 eV. Selective ion monitoring (SIM) mode was used as follows: rivastigmine, *m*/*z* 235; chlorpheniramine (IS), *m*/*z* 203.

3. Results and discussion

3.1. Optimization of the SPME conditions

First of all, headspace extraction was used in this study to avoid possible contamination from plasma samples and damage to the fiber that might occur through direct liquid contact [17].

Rivastigmine was extracted from samples in the presence of sodium hydroxide-sodium carbonate to make rivastigmine non-ionic. This is related to the fact that unless ion-exchange coating is used, SPME can extract only neutral species from sample [18]. In this study, 1.0 ml sodium hydroxide-sodium carbonate (0.7 M:0.5 M) was added to 0.5 ml plasma sample. In addition, rapid mass transport from plasma to fiber was achieved by using stirring. Stirring rate of 2000 rpm was used in all the sample extraction.

The choice of a particular SPME fiber coating depends on the chemical structure of the analytes. In this work, consideration of the volatility and nonpolarity of rivastigmine and of fiber coating led to evaluation of two fiber types, one coated with 65-μm-thick PDMS/DVB and the other coated with 100-μm-thick PDMS. A comparative study of their extraction efficiencies obtained by different fiber is shown in Fig. 2. The results prove that the PDMS/DVB fiber performed better response to both rivastigmine and IS than the PDMS fiber.

Using the PDMS/DVB fiber, the equilibrium time and heating temperature were further optimized. The effect of heating temperature and extraction time on peak areas of rivastigmine is shown in Fig. 3. To our knowledge, low volatility may slow the mass transfer from the matrix to the headspace, resulting in the need for a higher temperature to give detectable amounts of analyte. The high boiling point and molecular weight of rivastigmine led to slow evaporation [19], which increases the extraction time. The results in Fig. 3 show that when extraction temperature reached 100 °C, the amounts extracted increased with time up to 30 min. This demonstrates that sampling time could be shortened by increasing the heating temperature. Because plasma was vulnerable to high temperature more than 100 °C, which might result in protein precipitation, temperature higher than 100 °C was not used. Based on the experimental results, extraction time of 30 min and temperature of 100 °C were selected as the optimum extraction conditions.

The spiked plasma sample was extracted by PDMS/DVB fiber at the optimum extraction conditions. Desorption of the analytes was carried out in the GC injector at 250 °C for varying times from 1.0 to 5.0 min. The results presented in Fig. 4 show that the optimum desorption time was 2 min.

In conclusion, the optimum conditions for the SPME procedure were as follows: headspace extraction with PDMS/DVB fiber (65-μm film thickness); 0.5 ml of

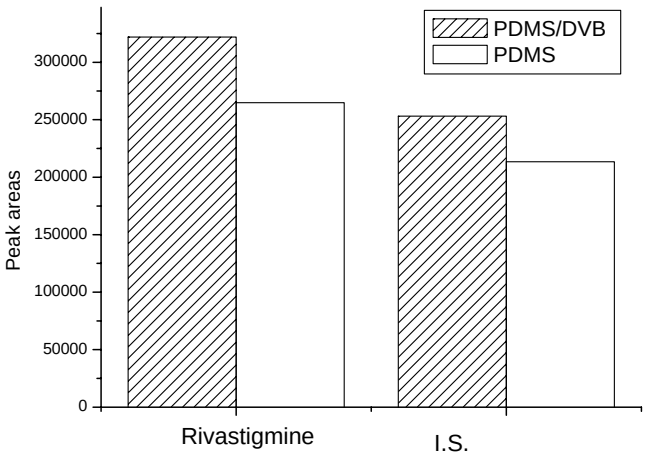

Fig. 2. Effect of extraction fiber on the SPME efficiency.

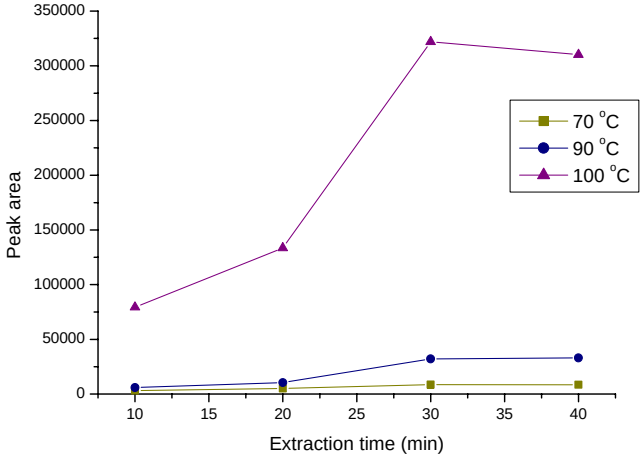

Fig. 3. Effect of extraction temperature and time on the SPME efficiency.

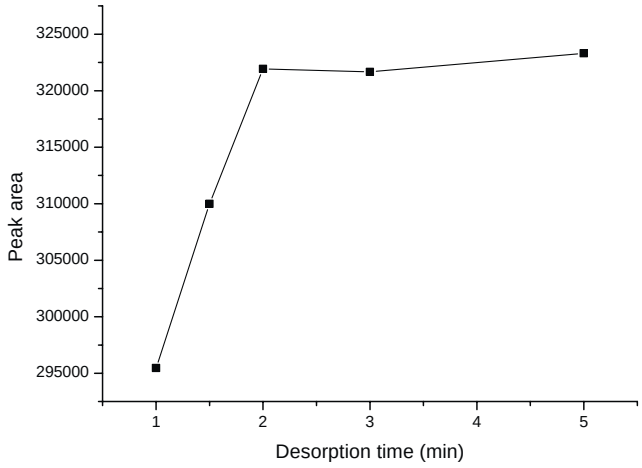

Fig. 4. Effect of desorption time on the rivastigmine peak area (desorption temperature: 250 °C).

sample plasma diluted with 1.0 ml sodium hydroxide-sodium carbonate (0.7 M:0.5 M) solution; extraction temperature at 100 °C with stirring at a rate of 2000 rpm for 30 min; desorption temperature at 250 °C for 2 min.

3.2. Validation of the method

In order to determine calibration curves, drug-free plasma spiked with rivastigmine at the concentration range from 0.2 to 80 ng/ml were prepared. The calibration curve was obtained by plotting the peak area ratio between rivastigmine and IS with correlation coefficient of 0.9965 and the linear regression equation was $y = 0.098801x + 0.057759$ with a slope standard deviation = 0.021725 and intercept standard deviation = 0.000744.

Table 1
Precision within and between days of the procedure with plasma sample spiked with rivastigmine

Concentration added (ng/ml)	Measured concentration (mean ± S.D.[a])	CV[b] (%)
Within-day ($n = 6$)		
1	0.91 ± 0.06	6.6
10	9.68 ± 0.48	5.0
80	79.11 ± 3.59	4.5
Between-day ($n = 6$)		
1	0. 93 ± 0.09	9.7
10	9.23 ± 0.74	8.0
80	79.65 ± 5.48	6.9

[a] S.D.: standard deviation.
[b] CV: coefficient of variation.

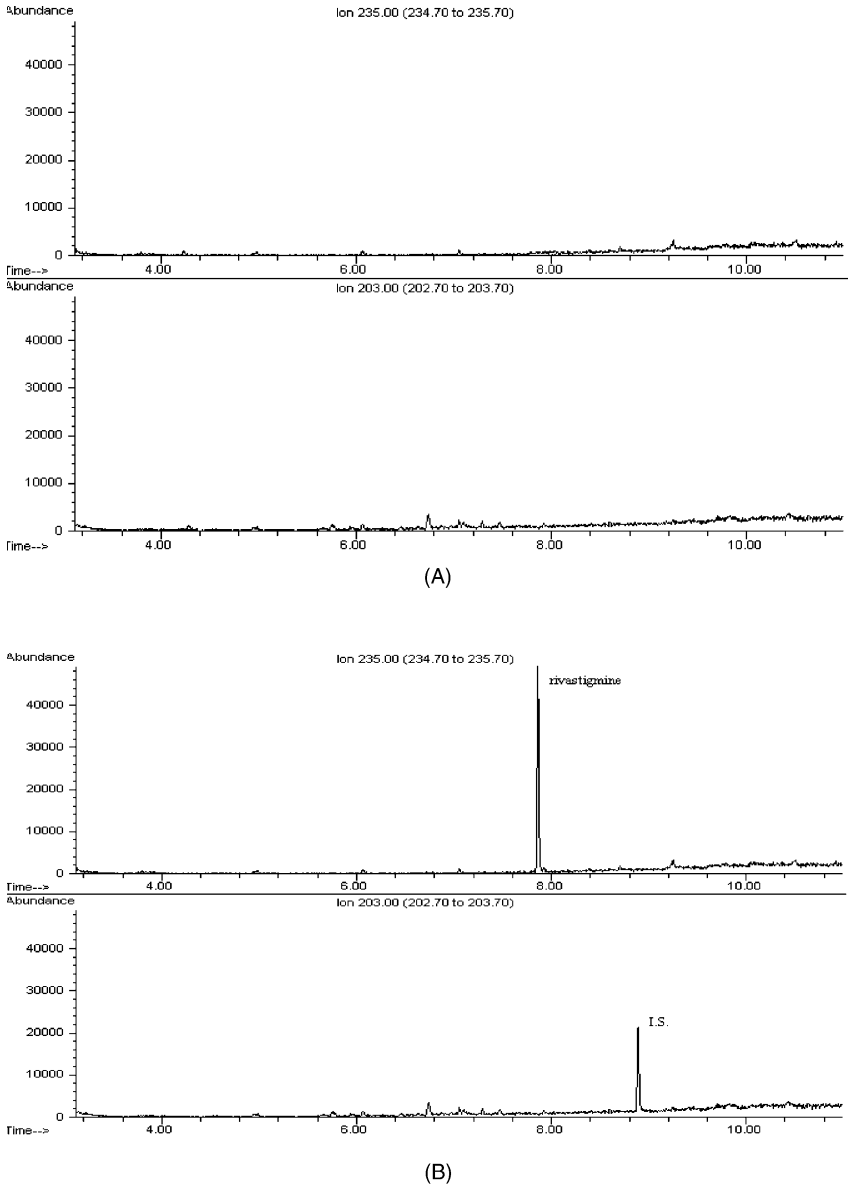

Fig. 5. Capillary GC–MS chromatogram for the SPME extracts of (A) blank plasma from healthy dog; (B) plasma from dog that was given a single oral rivastigmine administration, 0.9 mg/kg of body weight, resulting in plasma levels of 36.44 ng/ml. Internal standard (IS) = chlorpheniramine.

Table 2
Determination of rivastigmine in plasma samples from two dogs in treatment, both given a single oral rivastigmine administration of concentration 0.9 mg/kg body weight

Collected time (h)	Plasma rivastigmine concentration (ng/ml)	
	Dog no. 1	Dog no. 2
0.5	36.44	38.20
1	68.86	65.30
1.5	15.49	12.78
2	8.98	7.26
6	2.01	1.92

The precision was determined by the percentage coefficient variation of within and between day variations at three different concentrations (Table 1). The precision was ranged from 4.5 to 6.6% for within-day measurement, and for between-day variation was in the range 6.9–9.7%. Considering these CVs, the proposed method was found to be reproducible.

The limit of quantification (LOQ) of rivastigmine in plasma samples was 0.2 ng/ml. This LOQ was determined as the lowest concentration on the calibration curve in which the CV was lower than 10%.

The selectivity of the method was demonstrated by satisfactory separation of the compounds with no interfering peaks in the chromatograms of drug-free plasma from healthy dogs (Fig. 5).

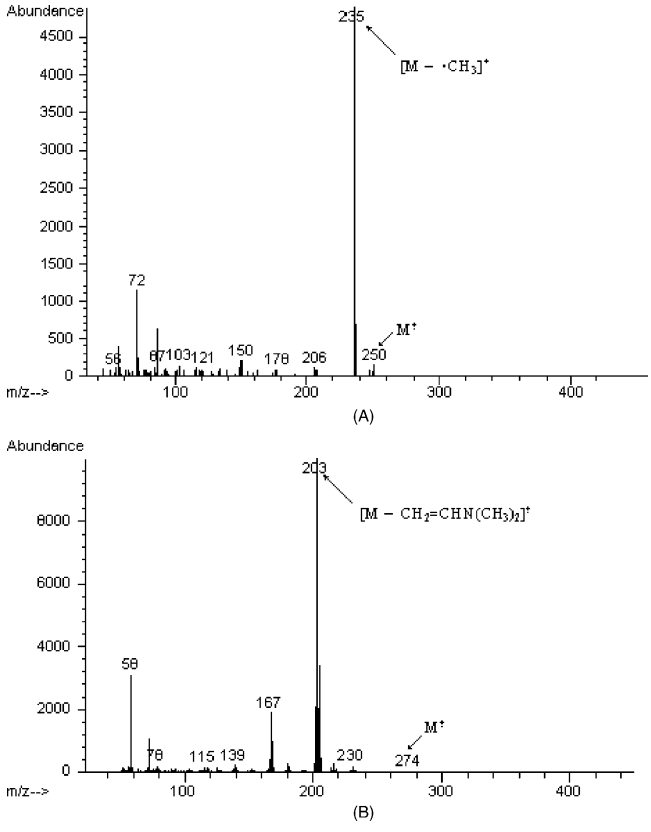

Fig. 6. GC–MS spectra of rivastigmine (A) and chlorpheniramine (B).

3.3. Determination of rivastigmine in canine plasma samples

The present method was applied to the determination of rivastigmine in plasma samples from two dogs after a single oral administration, at a concentration of 0.9 mg/kg of body weight. Fig. 5B shows the selective ion monitoring (SIM) chromatogram of the SPME extract of plasma from a dog, resulting in plasma levels of 36.44 ng/ml, collected 0.5 h after rivastigmine administration. As we know, SIM can improve analytical sensitivity and detection limit. The GC–MS spectra of rivastigmine and IS were shown in Fig. 6, which indicated that characteristic ion peaks at *m*/*z* 235 for rivastigmine and *m*/*z* 203 for IS were their base peaks. Using internal standard method, rivastigmine concentrations in canine plasma samples collected at different times after administration were calculated and presented in Table 2.

4. Conclusion

Compared with LLE, HS-SPME is a simple, rapid, sensitive and solvent-free method for determination of rivastigmine in plasma samples. In our lab, the present method will be applied to the study of rivastigmine metabolism in vivo.

Acknowledgements

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