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Rapid determination of tramadol in human plasma by headspace solid-phase microextraction and capillary gas chromatography–mass spectrometry

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Abstract

A simple, rapid and sensitive method for determination of tramadol 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 0.5 mL of sodium hydroxide (0.1 M); extraction temperature of 100 °C, with stirring at 2000 rpm for 30 min. The calibration curve showed linearity in the range of 1–400 ng mL^{-1} with regression coefficient corresponding to 0.9986 and coefficient of the variation of the points of the calibration curve lower than 10%. The detection limit for tramadol in plasma was 0.2 ng mL^{-1}. The proposed method was successfully applied to determination of tramadol in human plasma samples from 10 healthy volunteers after a single oral administration.

Keywords: Headspace solid-phase microextraction; Gas chromatography–mass spectrometry; Tramadol; Human plasma

1. Introduction

Tramadol hydrochloride, (±)-trans-2-[(dimethyl-amino)methyl]-1-(3-methoxyphenyl)cyclo-hexanol (Fig. 1a), is a centrally acting opioid analgesic agent marketed for the treatment of moderate to severe pain [1]. It binds weakly but effectively to opioid μ-receptors [2]. Nevertheless, a non-opioid mechanism is involved in tramadol analgesia [3], that is, it lacks the tolerance or abuse potential of pure opioids. Furthermore, excessive sedation, respiratory depression and urinary retention are rare complications [4]. Hence, tramadol appears to be an exciting addition to the analgesic armamentarium.

The methods described for the determination of tramadol in biological samples involve high performance liquid chromatography and gas chromatography with the sample preparation of liquid–liquid extraction (LLE) [5–7] and solid-phase extraction (SPE) [8–10]. As we know, LLE can produce emulsions, and large amounts of organic solvents are often needed to extract the drugs; SPE techniques often introduce artifacts in the sample extracts and can be lengthy, with a series of stages including washing, conditioning, eluting and drying of the process [11]. Therefore, it is a relevant task to develop a relatively simple, fast and solvent-free extraction method.

Solid-phase microextraction (SPME), introduced by Pawliszyn in 1990, is a relatively new sampling and concentration technique [12]. SPME combined with GC–MS is currently applied in several different areas, including analysis of environment, food, drink and drugs [11]. Recently, this novel technique has been developed for biological sample analyses [13–17].

In this paper, a HS-SPME coupled with GC–MS was developed for the determination of tramadol in human plasma. The SPME parameters were optimized by using drug-free plasma samples spiked with tramadol. To demonstrate the

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validation of the proposed method, the detection limit, linearity and precision were investigated. The present method was applied to the analysis of tramadol in real plasma samples from 10 healthy volunteers after a single oral tramadol administration.

2. Experimental

2.1. Reagents and analytical standards

Tramadol hydrochloride and chlorpheniramine maleate (internal standard, Fig. 1b) were obtained from Shanghai Institute for Drug Control, with purity greater than 99%. Stock solutions (1.0 mg mL^{-1}) for both tramadol and chlorpheniramine were prepared in distilled water. Tramadol working analytical standard solutions from 0.05 to 20 μg mL^{-1} were made by diluting the stock solution with water. Internal standard working solution was prepared at the final concentration of 40 μg mL^{-1}. They were stored at 4 °C. Drug-free plasma was collected from healthy volunteers and stored at −20 °C. Spiked plasma samples were made by adding 10 μL of standard working solutions of tramadol to 0.5 mL of drug-free plasma to a final concentration from 1 to 400 ng mL^{-1}.

2.2. 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), 100-μm-thick polydimethylsiloxane (PDMS) or 85-μm-thick polyacrylate (PA) coatings were used. All kinds of fiber were conditioned before use by heating in a gas chromatographic injection port (0.5 h at 250 °C). Samples were stirred during extraction using a 85-2 model hotplate/stirrer (Si Le instrumental company, Shanghai) and PTFE-coated stir bars 3 mm in diameter and 7 mm long.

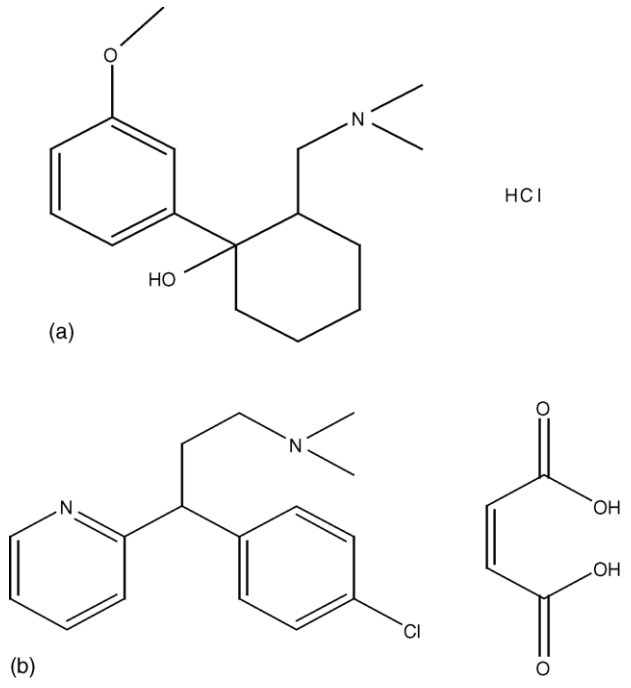

Fig. 1. Structures of tramadol hydrochloride (a) and chlorpheniramine maleate (b).

2.3. SPME procedure

Half milliliter human plasma, 10 μL chlorpheniramine (I.S. 40 μg mL^{-1}), 0.5 mL sodium hydroxide (0.1 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.

2.4. Optimization of the SPME conditions

In order to find out the optimal extraction conditions, 0.5 mL drug-free human plasma sample spiked with 40 ng tramadol and 400 ng I.S. and 0.5 mL sodium hydroxide (0.1 M) were placed into a 15 mL vial. To select the optimum fiber, three commercial fibers of PDMS/DVB, PDMS and PA 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 2 min. The optimum fiber was determined by peak areas of tramadol.

Using the optimum fiber of PDMS/DVB, further work was performed on optimization of heating temperature, extraction time, dilution factor and desorption time. The spiked samples were extracted by PDMS/DVB fiber using varying dilution factors (1:1, 2:1, 3:1, V_{NaOH}/V_{plasma}) 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).

2.5. 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^{-1} 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^{-1} was used as the carrier gas. The ionizing energy was 70 eV. All data were obtained by collecting the full-scan mass spectra within the scan range 40–500 amu.

Afterwards, *m*/*z* 58 and *m*/*z* 203 were extracted for quantitative determination of tramadol [18] and chlorpheniramine (I.S.), respectively.

3. Results and discussion

3.1. Optimization of the SPME conditions

The HS-SPME mode was adapted for the analysis of tramadol and I.S. in plasma to prevent direct fiber contact with the sample thus lowering background noise [19,20] and prolonging the fiber lifetime. Analytes were adsorbed onto fiber coating based on the principle "like dissolves like". Thus, theoretically, the mixed phase PDMS/DVB containing apolar PDMS and polar DVB was more suitable to extract these semi-volatile pharmaceuticals while PDMS only extracts nonpolar analytes and PA is mainly for polar compounds. The peak areas of tramadol were presented in Fig. 2. As is expected, PDMS/DVB was a more effective coating and was selected for further studies. Additionally, by comparison of the total ion chromatogram obtained from the above three fibers, PA is supposed not to be competent for biological extraction without pretreatment due to its ability to adsorb too many substances in plasma except the drug.

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 tramadol 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 tramadol led to slow evaporation [21],

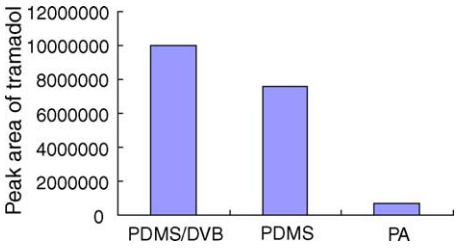

Fig. 2. Effect of extraction fiber on the SPME efficiency.

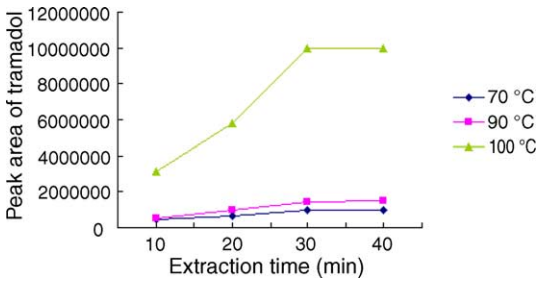

Fig. 3. Effect of extraction temperature and time on the SPME efficiency.

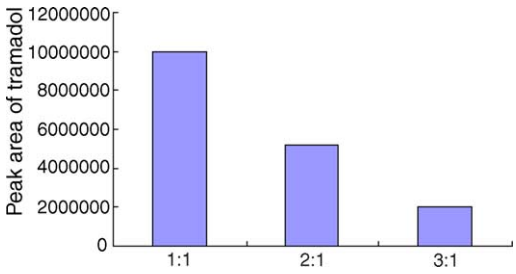

Fig. 4. Effect of dilution factor on the SPME efficiency.

which increases the extraction time. The results in Fig. 3 show that when it achieved to 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 outcome, extraction time of 30 min and temperature of 100 °C were selected as the optimum extraction conditions.

Tramadol was extracted from samples in the presence of sodium hydroxide to make it non-ionic. This is related to the fact that unless ion-exchange coating is used, SPME can extract only neutral species from sample [22]. In this study, the effect of its dilution factor was investigated in the range 1:1, 2:1 and 3:1 (V_{NaOH}/V_{plasma}) under above conditions. The results in Fig. 4 demonstrate that there was an inverse relationship between the extracted amount and the added quantity of sodium hydroxide. However, dilution factor less than 1:1 was not compared in this work due to serous facility in coagulation at high temperature if not diluted enough. Therefore, 0.5 mL of sodium hydroxide was added to 0.5 mL plasma sample. Besides, 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 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. 5 show that a time of 2 min performed well enough.

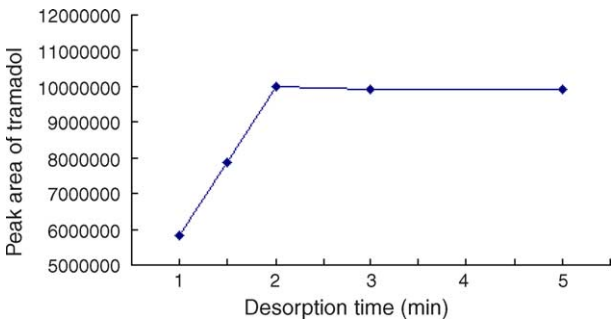

Fig. 5. Effect of desorption time on the tramadol peak area (desorption temperature: 250 °C).

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 sample plasma diluted with 0.5 mL sodium hydroxide (0.1 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 tramadol at the concentration range from 1 to 400 ng mL^{-1} were prepared. The calibration curve was obtained by plotting the peak area ratio between tramadol and I.S. with correlation coefficient of 0.9986 and the linear regression equation was $y = 0.009072x + 0.012522$ ($n = 6$) with a slope standard deviation = 0.003144 and intercept standard deviation = 0.009652.

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

Concentration added (ng mL^{-1})	Measured concentration (mean ± S.D.)[a]	C.V.[b]	Accuracy (%)
Within-day ($n = 6$)			
10	9.58 ± 0.46	4.8	95.8
80	77.54 ± 3.58	4.6	96.9
400	381.26 ± 14.32	3.8	95.3
Between-day ($n = 6$)			
10	9.34 ± 0.73	7.8	93.4
80	77.63 ± 4.47	5.8	97.0
400	369.55 ± 17.25	4.7	92.4

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

The precision was determined by the percentage coefficient variation of within- and between-day variations at three different concentrations (Table 1). The precision ranged from 3.8 to 4.8% for within-day measurement, and for between-

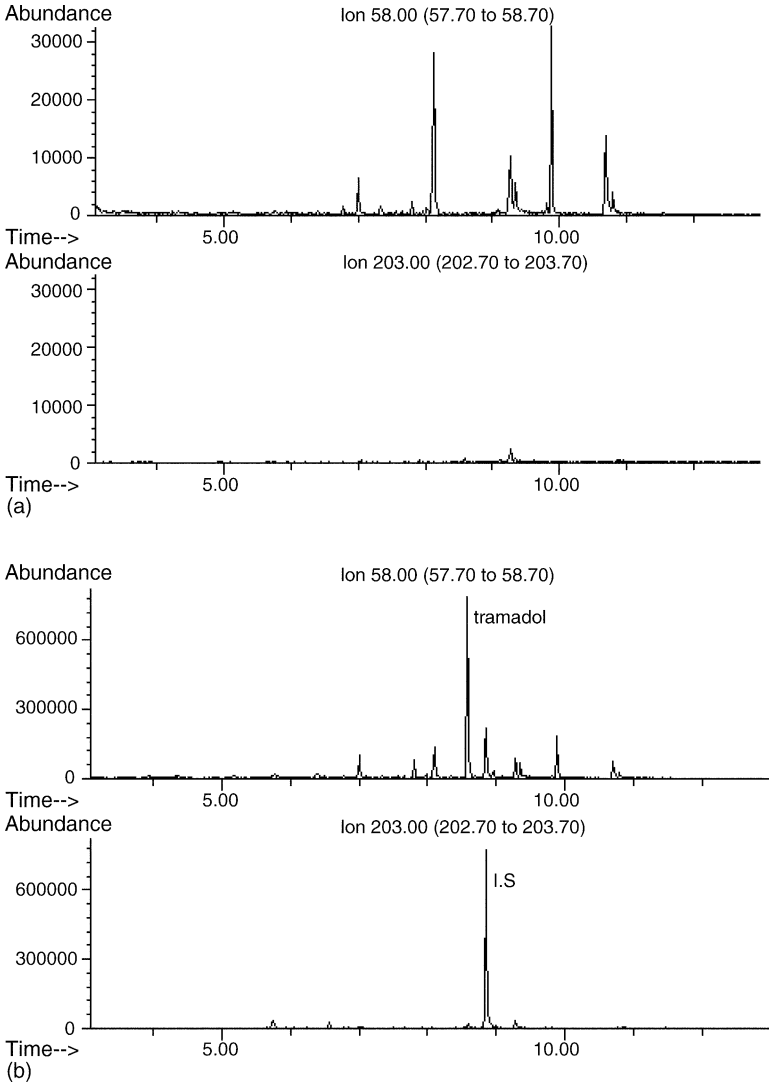

Fig. 6. Capillary GC–MS chromatogram for the SPME extracts of (a) blank plasma from healthy volunteer; (b) plasma from one of the volunteers that were given a single 100 mg oral tramadol administration, resulting in plasma levels of 78.32 ng mL^{-1}. Internal standard (I.S.): chlorpheniramine.

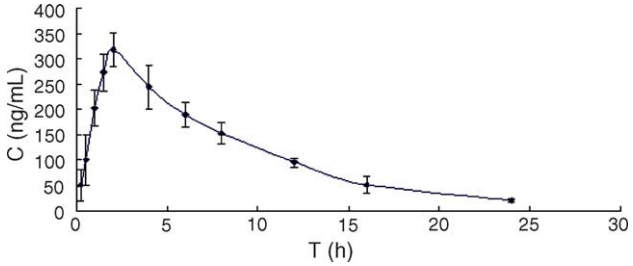

Fig. 7. Mean plasma concentration–time profile of 10 volunteers that were given a single 100 mg oral tramadol administration.

day variation was in the range 4.7–7.8%. The proposed method was found to be reproducible.

The corresponding accuracy data were also given in Table 1.

The limit of detection (LOD) of tramadol in plasma sample was 0.2 ng mL^{-1}, in which the C.V. was lower than 10%.

The selectivity of the method was demonstrated by satisfactory separation of the compounds with no interfering peaks in the chromatogram of drug-free plasma from healthy volunteer (Fig. 6a).

3.3. Determination of tramadol in human plasma samples

The present method was applied to the determination of tramadol in plasma samples from 10 healthy volunteers after a single oral administration, at a dosage of 100 mg. Fig. 6b shows the capillary GC–MS chromatogram of the SPME extract of plasma from one of the volunteers, resulting in plasma level of 78.32 ng mL^{-1}, collected 0.25 h after tramadol administration. Fig. 7 gives the mean plasma concentration-time profile of tramadol in these 10 volunteers.

4. Conclusion

In principle, SPME is an alternative to traditional liquid–liquid and solid-phase extraction for the sample preparation for GC–MS analysis of tramadol drug in human plasma. However, compared with LLE and SPE, the advantages for HS-SPME are: (1) no use of organic solvent, (2) easy handling, (3) rapid method, (4) good linearity and high sensitivity. In our lab, the present method (HS-SPME-GC–MS) will be applied to the study of tramadol in human hair.

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