Journal of Chromatography A, 1218 (2011) 2521–2527

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Quantitative determination of phenothiazine derivatives in human plasma using monolithic silica solid-phase extraction tips and gas chromatography–mass spectrometry

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ARTICLE INFO

Article history:
Received 6 January 2011
Received in revised form 24 February 2011
Accepted 25 February 2011
Available online 4 March 2011

Keywords:
Solid-phase extraction
Monolithic silica
Pipette tip
Phenothiazine
Gas chromatography
Mass spectrometry

ABSTRACT

Solid-phase extraction (SPE) using micropipette tips is a useful technique to prepare samples prior to mass spectrometry. However, most commercial SPE tips have loading capacities that are insufficient for quantitative determination. In this paper, we describe a rapid method for quantitative microanalysis of five phenothiazine derivatives, chlorpromazine, levomepromazine, promazine, promethazine and trimeprazine, using a recently introduced C_{18} monolithic silica SPE tip, the MonoTip C_{18}, for extraction from human plasma. The drugs could be extracted within 5 min from 0.1-mL plasma samples, eluted with methanol, and the eluate injected directly into a gas chromatograph prior to mass spectrometry analysis. Only 0.7 mL of solvent was required for each step of the extraction process. The recoveries of the five phenothiazines spiked into plasma were 91–95% and the limits of quantification for each drug were between 0.25 and 2.0 ng/0.1 mL. The maximum intra- and inter-day coefficient of variation was 11%. The validated method was successfully used to quantify the plasma concentration of levomepromazine in a human subject after oral administration of the drug. This new method is expected to have wide applications as a pretreatment for the rapid, quantitative determination of drug concentrations in plasma samples.

1. Introduction

Solid-phase extraction (SPE) is a sample clean-up and preconcentration technique that has been used widely to isolate target analytes due to its operational simplicity, high selectivity, and good reproducibility. Recent trends in SPE techniques focus on miniaturizing the process, reducing sample and solvent consumption, and increasing sample throughput [1]. Miniaturization has been achieved by loading packing materials into plastic micropipette tips. These SPE tips have quickly become an attractive format for microvolume sample preparation, especially when interfacing with matrix assisted laser desorption ionization or nanoelectrospray mass spectrometry (MS) [2,3]. Several manufacturers have developed SPE tips packed with silica particles, including the ZipTip (Millipore, Billerica, MA, USA), NuTip (Glygen, Columbia, MD, USA), HyperSep Tip (Thermo Fisher Scientific, Waltham, MA, USA), and StageTip (Proxeon Biosystems, Odense, Denmark). Although these devices are commercially available and can be used successfully to facilitate protein or peptide binding, desalting, detergent removal, and sample elution for direct MS analysis, their sorbent capacity is very small (several micrograms) so the tips are used only for qualitative studies [2–5].

Recently, a new SPE tip using a monolithic silica gel packing material, the MonoTip C_{18} tip (GL Sciences, Tokyo, Japan), has been developed for proteomic and metabolomic analyses [6]. These are highly porous, containing throughpores (macropores) that serve as channels for liquid flow (Fig. 1). In addition to the throughpores, there are also small pores inside the silica stationary phase skeleton, called mesopores. The monolithic silica is directly attached to the inner surface of 200 μL pipette tips (Fig. 1) and the silanol moiety present on the monolithic silica surface is chemically modified with a C_{18} phase. We recently demonstrated, for the first time, the feasibility of using MonoTip C_{18} tips by applying them to the extraction of drugs in human specimens for forensic analysis [7,8]. The procedure for drug extraction with the MonoTip C_{18} tip is essentially the same as that for the conventional C_{18} SPE (conditioning, sample loading, washings, drying, and elution) but differs in that all manipulations are carried out by aspirating and dispensing through a sin-

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doi:10.1016/j.chroma.2011.02.070

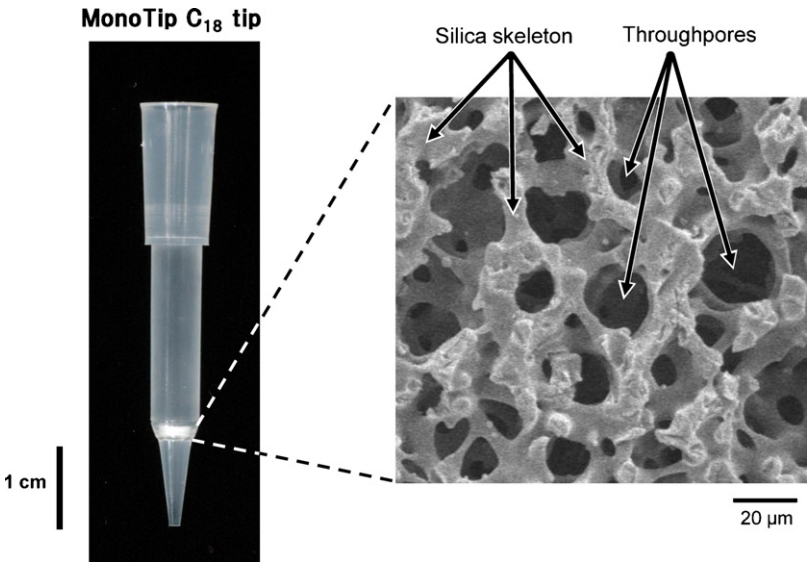

Fig. 1. Appearance of a MonoTip C_{18} tip and electron micrograph of the monolithic silica gel.

gle pipette tip using a manual or electronic motorized micropipettor. An advantage of using MonoTip C_{18} tips for sample preparation is that extraction can be carried out more easily and rapidly than by using conventional SPE cartridges. The small bed volume and sorbent mass within the MonoTip C_{18} tip enables the use of a reduced solvent volume, smaller elution volume, reduced time for the evaporation step, clean extract solution, and higher throughput.

Phenothiazine derivatives have long been used as antipsychotics (major tranquillizers), antiparkinsonism drugs, and antihistaminines. The common chemical structure of phenothiazines consists of a three-ring structure in which two benzene rings are joined by a sulfur and a nitrogen atom at nonadjacent positions. These drugs are frequently encountered in clinical toxicological practice [9–15] because of their relatively narrow safe therapeutic dose ranges (oral dose range: 200–800 mg daily for chlorpromazine hydrochloride [16]). Therefore, the blood concentrations of these drugs must be monitored for clinical and toxicological analysis to ensure optimum dosages.

Phenothiazines with light aliphatic side chains such as chlorpromazine, levomepromazine, and promazine can be measured by gas chromatography (GC) with MS or nitrogen–phosphorus detection [12,14,17–24]. Both GC methods are relatively simple and have sufficient sensitivity to detect phenothiazine derivatives in biological samples. Most GC techniques use extraction methods to remove impurities contained in various matrices including liquid–liquid extraction (LLE) [12,14,19,20,23] or conventional SPE [17,18,20–22]. However, there are several disadvantages associated with extraction procedures such as LLE and conventional SPE. These procedures are time-consuming and tedious prior to instrumental analysis. Moreover, the large amounts of organic solvents required cause problems with regard to health and the environment. Therefore, a new microscale sample preparation method that can be used widely for the extraction of phenothiazine derivatives from human matrices is required.

The purpose of this work was to develop and validate a new SPE method using MonoTip C_{18} tips for simultaneous quantitative determination of five phenothiazine derivatives, chlorpromazine, levomepromazine, promazine, promethazine, and trimeprazine, in human plasma samples, for therapeutic drug monitoring and clinical toxicology. To the best of our knowledge, no micropipette tip-based SPE technique for quantitative analysis of major tranquilizers has been reported in literature. The proposed method was successfully applied to simple and rapid extraction of the five phenothiazines from human blood samples, followed by GC–MS quantification.

2. Experimental

2.1. Chemicals and materials

Chlorpromazine hydrochloride, promazine hydrochloride, promethazine hydrochloride, and triflupromazine hydrochloride as internal standard (IS) were purchased from Sigma Chemicals (St. Louis, MO, USA). Levomepromazine maleate was provided by Yoshitomi Pharmaceutical Ind. (Osaka, Japan) and trimeprazine tartrate by Daiichi Seiyaku (Tokyo). MonoTip C_{18} tips (lot no. KM050; pipette tip volume, 200 μL; C_{18}-bonded monolithic silica gel with diameter 2.8 mm, thickness 1 mm, weight 2 mg, mesopore size 15 nm, throughpore size 15–25 μm, and surface area 200 m^2/g) were purchased from GL Sciences. Other common chemicals were of the highest purity, and are commercially available.

2.2. Preparation of plasma samples

Drug-free whole blood samples from healthy volunteers were taken intravenously in the presence of EDTA-2Na as an anticoagulant. Samples were centrifuged at 1700 × *g* for 10 min at 4 °C and the plasma supernatant was decanted into clean tubes and stored at −80 °C until use.

2.3. Preparation of standard solutions and quality control samples

Individual stock standard solutions of the five phenothiazine derivatives and IS were prepared separately by dissolving appropriate amounts of each compound in methanol to achieve a concentration of 1 mg/mL using a 4 mL volumetric flask. Working standard solutions from 0.25 to 128 ng/10 μL (0.25, 0.50, 1.00, 2.00, 4.00, 5.00, 8.00, 10.0, 16.0, 32.0, 50.0, 64.0, and 128 ng/10 μL) in methanol were prepared by appropriate dilution of stock standard solutions. A series of 10 μL standard solutions were evaporated to dryness under a gentle stream of nitrogen in 0.75 mL Screen-Mates Tubes (Matrix Technologies, Hudson, NH, USA). Residues were reconstituted in 0.1 mL drug-free pooled plasma, obtained from 5 healthy individuals, to prepare calibration standards containing 0.25–128 ng/0.1 mL for the five drugs, and 5.00 ng/0.1 mL

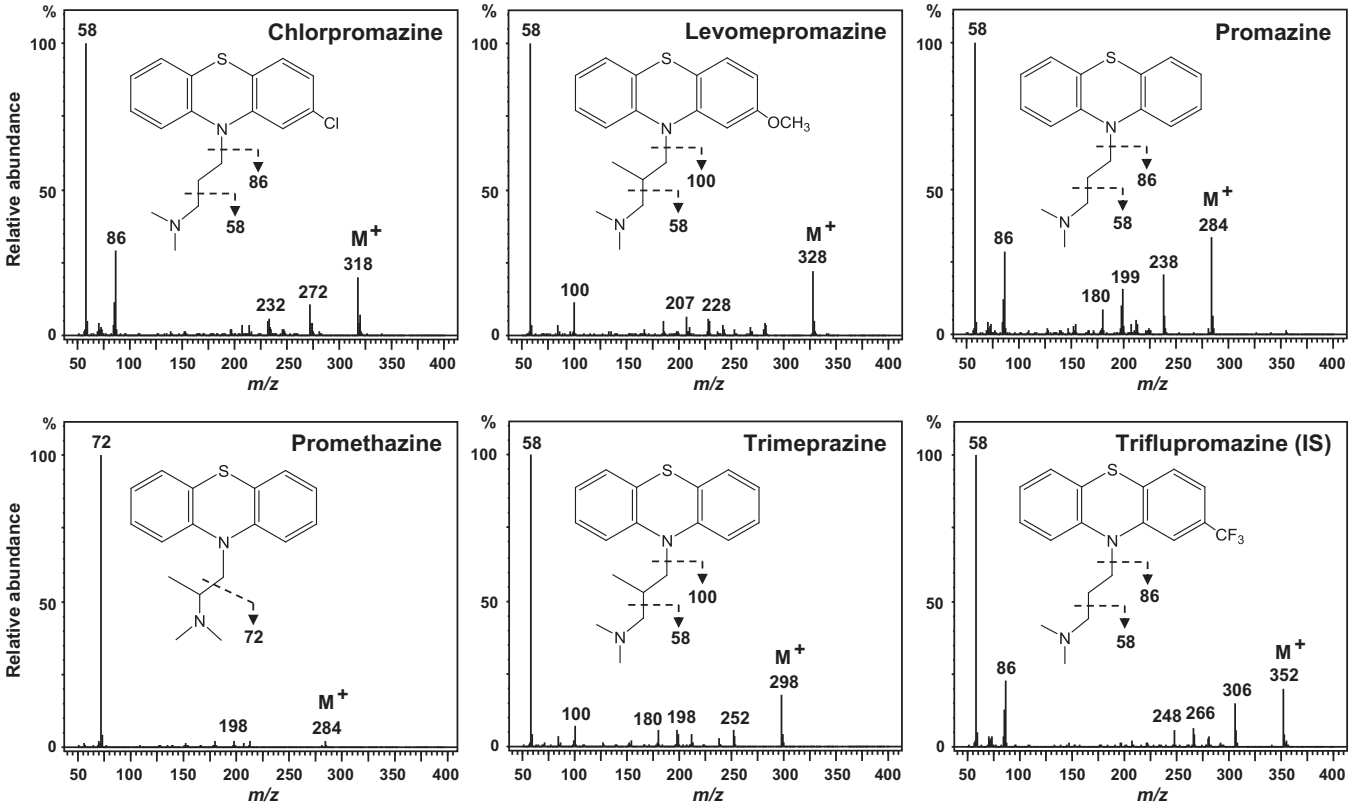

Fig. 2. GC–MS spectra of the five phenothiazine drugs and triflupromazine (IS) used in this study.

for IS. Quality control (QC) samples were also prepared using the same procedure, at concentrations of 0.25–50.0 ng/0.1 mL for the five drugs, and 2.00–50.0 ng/0.1 mL for IS. All stock solutions and working standard solutions were stored at 4 °C in silanized brown glass vials.

2.4. Extraction procedure using MonoTip C_{18} tip

Extraction of the phenothiazine derivatives from human plasma was achieved using a MonoTip C_{18} tip. After attaching the tip onto a Pipetman P200 pipette (Gilson SAS, Villiers-le-Bel, France), conditioning of the tip was achieved by aspirating and dispensing (to waste) 200 μL methanol, followed by 200 μL distilled water through the tip. To 0.1 mL of a plasma sample containing the five drugs and IS were added 375 μL of distilled water and 25 μL of 1 M glycine–NaOH solution (pH 11) in a 0.75 mL ScreenMates Tube. A 200 μL aliquot of the sample was aspirated into the conditioned MonoTip C_{18} tip and dispensed back into the same sample tube. These two steps are referred to as one aspirating/dispensing cycle. Extraction of the phenothiazine derivatives onto the C_{18} phase of the tip was performed by 25 aspirating/dispensing cycles. The tip was then washed by aspirating 200 μL of 10% methanol in distilled water and dispensing the eluate as waste. After washing, the tip was placed on a vacuum manifold and dried under vacuum for 30 s to remove all traces of water. Finally, analytes were eluted from the tip with 100 μL of methanol into a vial (1.5 mL) using 5 aspirating/dispensing cycles. A 2 μL aliquot of the eluate was then subjected to GC–MS analysis.

2.5. GC–MS conditions

All analyses were performed using a Shimadzu GC-2010 gas chromatograph interfaced with a Shimadzu QP-2010 quadrupole mass spectrometer (Shimadzu Corp., Kyoto, Japan). The GC–MS was operated with an interface temperature of 300 °C and an ionization source temperature of 250 °C. The mass spectrometer was tuned daily, using perfluorotributylamine. A solvent delay of 4.0 min was set to protect the filament from oxidation. Chromatographic separation was achieved using an Equity-5 fused silica capillary column (30 m × 0.32 mm i.d., film thickness, 0.25 μm; Supelco, Bellefonte, PA, USA). Helium, with a minimum purity of 99.99995%, was used as a carrier gas at a flow rate of 2 mL/min. The gas chromatograph was equipped with a split/splitless injection port, operated at 200 °C. Samples were injected in the splitless mode, at a column temperature of 120 °C, and the splitter was then opened after 1 min. The gas chromatograph oven temperature was initially held at 120 °C for 1 min and then programmed to ramp up at 20 °C/min to 300 °C. The mass spectrometer was operated in the positive-ion electron ionization (EI) mode. EI mass spectra were obtained at an ionizing energy of 70 eV and an emission current of 60 μA. To select the monitoring ion for the five phenothiazines and IS, GC–MS spectra were obtained from injections of analyte standard working solutions (Fig. 2). Full scan data were obtained over the mass range *m*/*z* 50–400. Quantification was performed by selected ion monitoring (SIM) using each molecular ion (M^+) at *m*/*z* 318 for chlorpromazine, *m*/*z* 328 for levomepromazine, *m*/*z* 284 for promazine, *m*/*z* 298 for trimeprazine, and *m*/*z* 352 for IS. The molecular ions chosen were those with the highest *m*/*z* value for each analyte and having the minimum background interference or chemical noise compared with their corresponding lower mass fragments. However, the molecular ion for promethazine was barely detectable and thus was of little quantitative use, at *m*/*z* 284. For promethazine, therefore, a base peak ion at *m*/*z* 72 was used for quantification.

2.6. Evaluation of recovery, quantification, and linearity

Recoveries were calculated by comparing chromatographic peak areas obtained from extracts of QC samples with those

obtained by direct GC injection of non-extracted standard compounds dissolved in methanol. Recoveries were determined at three different concentrations of each compound. Regression equations for the five phenothiazines extracted from human plasma were obtained by fitting a plot of the ratio of the peak area of the analyte to that of the IS (5.00 ng) versus concentration of analytes. Intra-day precision and accuracy were carried out by analyzing QC samples spiked with the five phenothiazine derivatives at three to five different concentrations in five replicate samples on the same day. The same procedure was repeated on five different days to determine the inter-day precision and accuracy. The concentration of the analytes in the QC samples was calculated using the calibration curves. The precision was determined by calculating the coefficient of variation (CV), while the accuracy was expressed as a percentage of the mean of measured concentrations against the nominal concentration. The evaluation of precision and accuracy were based on previously published criteria [25]. The acceptance criteria for precision (% CV) and accuracy (% of nominal) were ≤15% and 100 ± 15%, respectively. The limit of detection (LOD) was defined as the lowest concentration of analyte spiked in plasma that could be detected with a signal-to-noise ratio of at least 3. The limit of quantification (LOQ) was defined as the lowest concentration on the calibration curve that could be measured with intra-day and inter-day CV of ≤20% and an accuracy of 100 ± 20%. Both parameters were determined as the averages of five replicate analyses of QC samples. The acceptance criterion for the correlation coefficient was >0.9990.

2.7. Stability

The stability of stock and working standard solutions of the five phenothiazine compounds was tested after refrigeration (4 °C) for 3 months and 2 weeks, respectively. The analyte stability test using QC samples (0.50, 1.00, 2.00, 10.0, and 50.0 ng/0.1 mL) was performed for short-term storage stability (kept at 4 °C for 8 h) and long-term storage stability (kept at −80 °C for 4 weeks). Freeze–thaw stability was also verified by freezing freshly prepared QC samples at −80 °C and thawing them at room temperature (23 °C), then assaying the QC samples after 3 freeze–thaw cycles. To estimate the stability of the analyte in the prepared samples, aliquots of prepared QC samples were kept in the autosampler and maintained at 23 °C for 12 h and then analyzed. The stabilities of the analytes in the QC samples were expressed as the percentage remaining of the initial values that had been determined immediately after sample preparation. The analytes were considered stable in plasma when 100 ± 15% of the initial amount remained [26,27].

2.8. Administration of levomepromazine to a volunteer

A therapeutic dose of levomepromazine maleate (50 mg) was administered orally to a 29-year-old female volunteer (body weight, 48 kg). Informed consent was obtained from the subject. Whole blood samples were collected pre-dose (0 h) and 2 h after drug administration and transferred to centrifuge tubes containing EDTA-2Na. To prepare plasma samples, the EDTA-treated whole blood was centrifuged at 1700 × *g* for 10 min at 4 °C and plasma was decanted into an Eppendorf tube. Plasma samples were stored at −80 °C until analysis.

3. Results and discussion

3.1. Optimization of extraction conditions for MonoTip C_{18} tips

The number of aspirating/dispensing cycles is a critical parameter for extraction recovery via the monolithic silica SPE method using MonoTip C_{18} tips. Extraction profiles of various amounts of

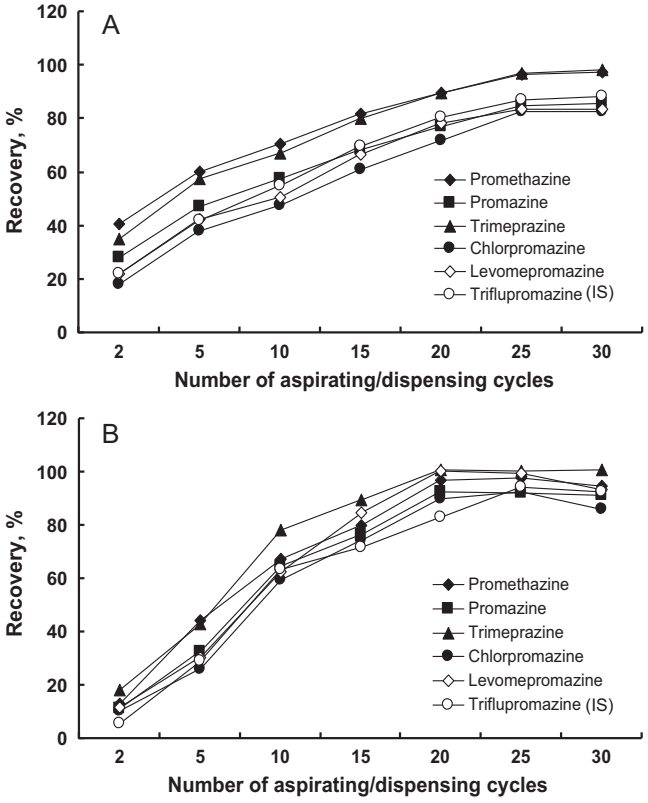

Fig. 3. Effect of number of aspirating/dispensing cycles on extractions of the five phenothiazine derivatives and IS in human plasma using MonoTip C_{18} tips. 100 ng and 2.50 ng of each compound was spiked into 0.1 mL plasma in graphs A and B, respectively. Each data point represents mean of duplicate determinations.

the five phenothiazines and IS were examined by plotting analyte recovery from plasma samples versus aspirating/dispensing cycles (Fig. 3). Extraction of the compounds reached equilibrium after 25 aspirating/dispensing cycles at both high (100 ng/0.1 mL) and low (2.50 ng/0.1 mL) concentrations. Therefore, we decided that the number of aspirating/dispensing cycles of extraction should be 25 cycles (approximately 1.5 min). In the elution process, analytes were eluted from the MonoTip C_{18} tip into a vial (1.5 mL) by aspirating/dispensing 100 μL methanol through the tip several times. Results showed that the number of aspirating/dispensing cycles for desorption was not significant for the compounds tested. However, to achieve sufficient recovery within a short period of time, 5 repeated aspirating/dispensing cycles (approximately 10 s) with 100 μL methanol were used in the elution step.

The entire MonoTip C_{18} tip extraction process, including conditioning, sample loading, washing, drying, and elution required approximately 5 min. In contrast, the time required to manually perform conventional cartridge SPE was reported to be >20 min [28–30]. The eluate from the MonoTip C_{18} tips was directly injected into the GC without evaporation and reconstitution steps, which is particularly important for rapid and simple analysis. Therefore, the use of MonoTip C_{18} tips is recommended for rapid extraction of phenothiazine derivatives from human plasma.

The total solvent volume used for each step of the extraction process was 0.7 mL, which is lower than that required by conventional SPE cartridges (around 5.6–65 mL) [18,20,22,31]. Furthermore, the required plasma sample volume was reduced to 0.1 mL, which is approximately 4.5–20 times less than that previously reported for phenothiazine analysis in plasma samples [17–20,22,31,32]. Thus, the small volumes of solvent and human plasma used in this study are a significant advance in sample preparation miniaturization and

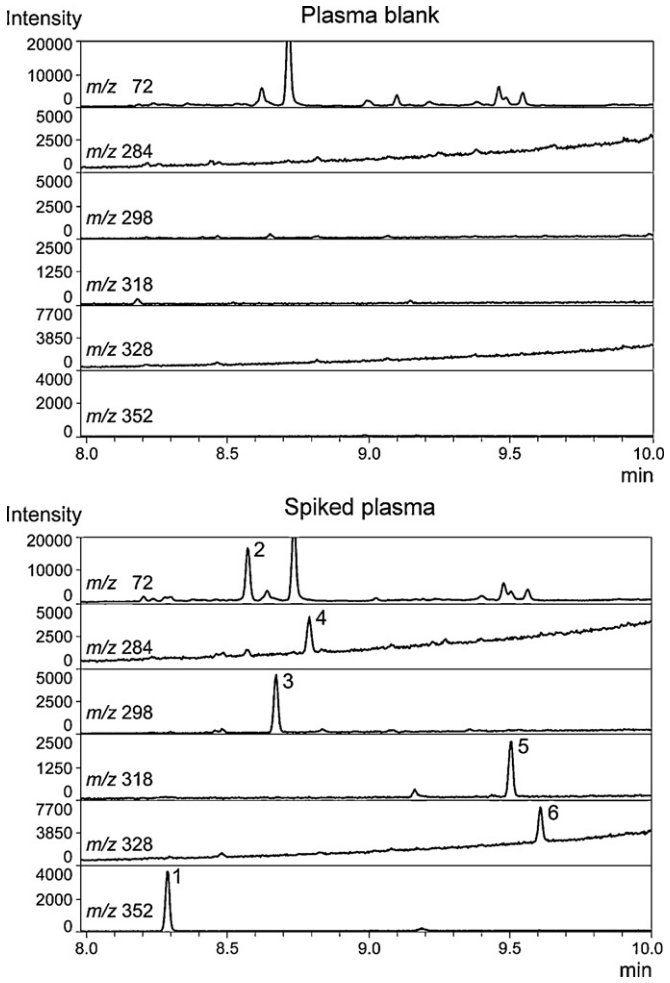

Fig. 4. SIM chromatograms for the five phenothiazine derivatives and IS extracted from human plasma using MonoTip C_{18} tips. 5.00 ng of each compound was spiked into 0.1 mL plasma. Peaks: 1 = IS; 2 = promethazine; 3 = trimeprazine; 4 = promazine; 5 = chlorpromazine; 6 = levomepromazine.

the overall procedure can be considered to be "green" because it requires little solvent and produces little waste.

3.2. Method validation

Fig. 4 shows SIM chromatograms obtained for extracts from 0.1 mL human plasma in the presence (5.00 ng of each compound) or absence of test compounds. Distinct peaks appeared for the six analytes and the retention times for IS, promethazine, trimeprazine, promazine, chlorpromazine, and levomepromazine were 8.29, 8.57, 8.68, 8.79, 9.50, and 9.61 min, respectively (Fig. 4, lower panel). Although small impurity peaks were observed for blank plasma, no interfering peaks were found around the peaks of the test compounds (Fig. 4, upper panel).

Recoveries of the six analytes of interest from plasma samples using the present method are presented in Table 1. Recoveries of chlorpromazine, levomepromazine, promazine, promethazine, trimeprazine, and IS were 88–95%. The reduction in recovery (<100% level) is probably due to a loss of analyte during overall sample preparation steps. However, the reduction was not problematic, because satisfactory quantification was achieved using the method described, as shown in Tables 2 and 3. Table 2 shows the regression equations, LOD, and LOQ obtained by the present method. The equations for these compounds had good linearity in the ranges shown in the table, with correlation coefficients of at least 0.9991. The LODs of the five phenothiazine derivatives under the optimal conditions were 0.08–0.60 ng/0.1 mL (0.8–6.0 ng/mL) for plasma. The LOQ, which corresponds to the lowest level of the concentration range, was 0.50 ng/0.1 mL (5.0 ng/mL) in plasma for chlorpromazine and levomepromazine, while for promazine, promethazine, and trimeprazine, the LOQs were 1.00 ng/0.1 mL (10.0 ng/mL), 2.00 ng/0.1 mL (20.0 ng/mL), and 0.25 ng/0.1 mL (2.5 ng/mL), respectively. The therapeutic blood concentrations of chlorpromazine, levomepromazine, promazine, promethazine, and trimeprazine are reported to be 30–100, 5–25, 10–50, 50–200, and 50–400 ng/mL, respectively [33]. Therefore, the present method can be used in therapeutic drug monitoring of phenothiazine derivatives.

Table 1
Recovery data for five phenothiazines and IS from human plasma.

Compound	Concentration added (ng/0.1 mL)	Recovery[a] (%)
Chlorpromazine	2.00	93 ± 9
	10.0	92 ± 9
	50.0	91 ± 7
Levomepromazine	2.00	91 ± 6
	10.0	93 ± 7
	50.0	92 ± 7
Promazine	2.00	95 ± 7
	10.0	92 ± 7
	50.0	91 ± 7
Promethazine	2.00	94 ± 3
	10.0	91 ± 5
	50.0	93 ± 6
Trimeprazine	1.00	95 ± 1
	10.0	93 ± 7
	50.0	95 ± 8
Triflupromazine (IS)	2.00	88 ± 4
	10.0	91 ± 8
	50.0	88 ± 7

[a] Values are mean ± SD of 5 experiments.

Intra-day and inter-day CVs and accuracy were evaluated by assessing QC samples prepared in human plasma, and these are summarized in Table 3. Intra-day and inter-day CVs at all concentrations examined were less than 10 and 11%, respectively. Accuracy was in the range of 88–99% for all concentrations.

3.3. Stability

Stability testing is very important for validated methods in biological samples. The results are shown in Table 4. All analytes in plasma were found to be stable for at least 8 h at 4 °C and for at least 4 weeks at −80 °C. The freeze–thaw stability of the QC samples following storage at −80 °C indicates that the five analytes of interest were stable after 3 freeze–thaw cycles. Furthermore, post-preparative stability experiments showed that all analytes were fairly stable for at least 12 h in the autosampler. The five compounds were reliably stable in human plasma, as shown by the finding that the QC samples met the criteria. The stock standard solutions containing 1 mg/mL of the five phenothiazines and IS prepared in methanol were stable for at least 3 months at 4 °C in the dark. The working standard solutions of the compounds were also investigated over a period of 2 weeks at 4 °C in the dark, and no significant changes were observed.

3.4. Actual measurements of levomepromazine in human plasma after oral administration

In addition to spiked human plasma, the present method was applied to samples of human plasma collected following oral administration of levomepromazine maleate. The amount of triflupromazine added as an IS was 5.00 ng to 0.1 mL plasma. Typical SIM chromatograms obtained from a female volunteer are shown

Table 2
Data on regression equations for five phenothiazines extracted from human plasma.

Compound	Equation[a]	Correlation coefficient	Correlation range (ng/0.1 mL)	LOD (ng/0.1 mL)	LOQ (ng/0.1 mL)
Chlorpromazine	$y = 0.158x + 0.032$	0.9992	0.50–128	0.20	0.50
Levomepromazine	$y = 0.329x + 0.055$	0.9993	0.50–128	0.30	0.50
Promazine	$y = 0.235x + 0.030$	0.9991	1.00–128	0.50	1.00
Promethazine	$y = 1.416x + 0.036$	0.9994	2.00–128	0.60	2.00
Trimeprazine	$y = 0.392x + 0.025$	0.9995	0.25–128	0.08	0.25

[a] The linear regression was obtained by fitting peak area ratios (y) of each compound to the IS against the spiking concentrations (x). Responses from 7 to 10 different concentrations for each compound were used to obtain the equations.

Table 3
Intra- and inter-day coefficients of variation (CV) and accuracy for five phenothiazines in human plasma.[a]

Compound	Concentration added (ng/0.1 mL)[b]	Intra-day			Inter-day[c]		
		Concentration detected (ng/0.1 mL)	CV (%)	Accuracy (%)	Concentration detected (ng/0.1 mL)	CV (%)	Accuracy (%)
Chlorpromazine	0.50	0.49 ± 0.03[d]	7	99	0.46 ± 0.05[d]	11	92
	2.00	1.92 ± 0.05	3	96	1.96 ± 0.17	8	98
	10.0	9.84 ± 0.53	5	98	9.23 ± 0.62	7	92
	50.0	49.4 ± 4.87	10	99	47.7 ± 5.03	10	95
Levomepromazine	0.50	0.45 ± 0.02	4	91	0.49 ± 0.04	9	98
	2.00	1.89 ± 0.18	10	95	1.94 ± 0.17	9	97
	10.0	9.40 ± 0.13	1	94	9.45 ± 1.06	11	94
	50.0	49.5 ± 3.14	6	99	47.0 ± 2.43	5	94
Promazine	1.00	1.78 ± 0.14	8	89	1.90 ± 0.19	10	95
	10.0	8.86 ± 0.53	6	89	9.23 ± 1.04	11	92
	50.0	44.6 ± 1.52	3	89	46.2 ± 4.08	9	92
Promethazine	2.00	1.91 ± 0.09	4	95	1.82 ± 0.15	8	91
	10.0	9.22 ± 0.25	3	92	9.48 ± 0.68	7	95
	50.0	46.8 ± 3.01	6	93	46.8 ± 3.66	8	94
Trimeprazine	0.25	0.23 ± 0.01	4	93	0.22 ± 0.03	11	89
	0.50	0.46 ± 0.04	9	93	0.49 ± 0.04	7	97
	2.00	1.76 ± 0.10	5	88	1.98 ± 0.20	10	99
	10.0	9.01 ± 0.47	5	90	9.26 ± 0.91	10	93
	50.0	44.2 ± 1.55	3	88	47.3 ± 4.53	10	95

[a] All data were obtained using IS (see the legend in Table 2 and the text).
[b] Intra-day CVs were calculated from measurements of five spiked samples on the same day.
[c] Spiked plasma were analyzed on five separate days, with one sample each day.
[d] The values are mean ± SD.

Table 4
Stability of five phenothiazines in human plasma at different QC levels.

Compound	Concentration added (ng/0.1 mL)	Stability (% of initial)[a]			
		Short-term stability	Long-term stability	Freeze–thaw stability	Postpreparative stability
Chlorpromazine	0.50	95 (8)[b]	90 (11)[b]	99 (4)[b]	96 (7)[b]
	2.00	100 (7)	97 (11)	97 (3)	99 (6)
	10.0	100 (5)	91 (8)	100 (5)	99 (4)
	50.0	96 (7)	104 (5)	100 (1)	99 (7)
Levomepromazine	0.50	98 (7)	106 (9)	100 (6)	94 (4)
	2.00	98 (4)	97 (10)	99 (8)	98 (2)
	10.0	98 (10)	90 (10)	99 (4)	100 (6)
	50.0	101 (7)	105 (5)	99 (1)	97 (3)
Promazine	1.00	98 (9)	106 (8)	98 (5)	99 (6)
	10.0	96 (6)	90 (7)	100 (4)	98 (3)
	50.0	101 (6)	107 (9)	104 (2)	98 (6)
Promethazine	2.00	92 (4)	104 (12)	92 (6)	99 (4)
	10.0	91 (10)	94 (8)	92 (9)	93 (7)
	50.0	91 (10)	100 (10)	99 (2)	99 (2)
Trimeprazine	0.50	99 (9)	93 (10)	101 (6)	94 (4)
	2.00	100 (7)	97 (6)	99 (4)	96 (5)
	10.0	94 (10)	93 (7)	99 (5)	99 (9)
	50.0	102 (4)	102 (1)	104 (2)	100 (9)

[a] Each freshly prepared sample was analysed before being of storage and was set at 100%.
[b] The values are mean percentage and CV in parentheses.

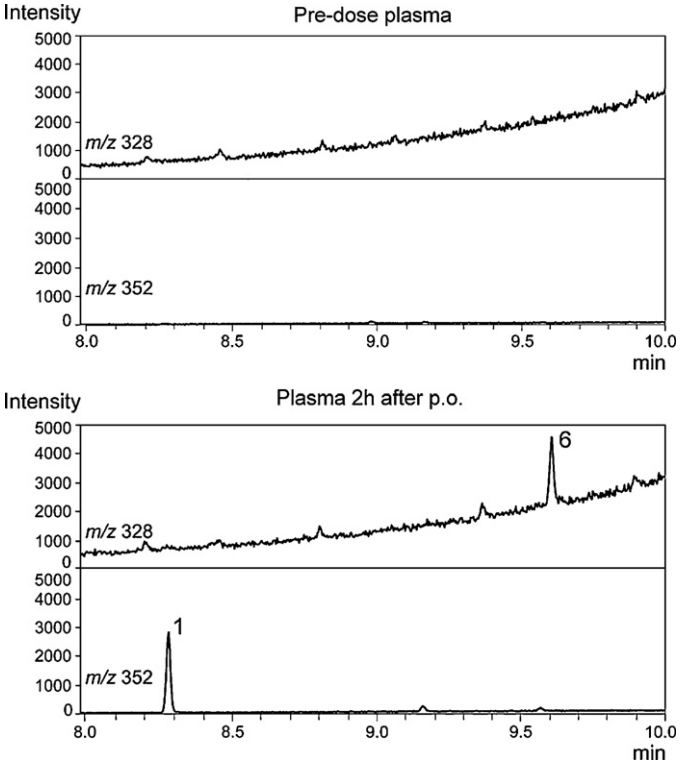

Fig. 5. SIM chromatograms obtained from extracts of a plasma sample of a female volunteer 2 h after oral administration of 50 mg of levomepromazine maleate. The amount of triflpromazine used as IS was 5.00 ng for 0.1 mL of plasma. The key numbers are the same as those specified in Fig. 4.

in Fig. 5. The drug concentration in plasma calculated by internal calibration was 2.39 ng/0.1 mL (23.9 ng/mL) at 2 h after administration of levomepromazine. This concentration in plasma was within therapeutic levels [33].

4. Conclusions

The use of monolithic silica SPE tips is an ideal sample preparation technique because of the simple extraction method, the rapid high-throughput extraction, and the minimization of sample and solvent requirements. The monolithic silica SPE method demonstrated in this study can continuously perform extraction of phenothiazine derivatives, chlorpromazine, levomepromazine, promazine, promethazine and trimeprazine, from human plasma, followed by GC–MS analysis. The recoveries of the five drugs in plasma were 91–95% and the LOQs were 0.25–2.00 ng/0.1 mL. The intra-and inter-day CVs for all drugs in plasma were less than 11%. We believe that this method will provide a useful tool for the screening and quantitative determination of phenothiazine tranquilizers in clinical analysis and expect that it will become more popular as a pretreatment of method for human blood samples before GC–MS.

Acknowledgements

We thank Dr. Shota Miyazaki of GL Sciences, Tokyo, Japan, for kindly providing an electron micrograph of the monolithic silica gel. This study was supported in part by a grant-in-aid for Scientific Research (C) of the Japan Society for the Promotion of Science.

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