Journal of Chromatography B, 872 (2008) 51–57

Contents lists available at ScienceDirect

Journal of Chromatography B

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

Development and validation of a gas chromatographic–mass spectrometric method for quantitative determination of perphenazine in rabbit plasma after sublingual administration

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ARTICLE INFO

Article history:
Received 18 January 2008
Accepted 6 July 2008
Available online 15 July 2008

Keywords:
Perphenazine
GC–MS
Rabbit plasma
Sublingual administration

ABSTRACT

Perphenazine is a phenothiazine-type antipsychotic that is a potential candidate for sublingual administration due to its extensive first-pass metabolism. In this study, a gas chromatographic–mass spectrometric method was developed for quantification of perphenazine in rabbit plasma after sublingual administration. The plasma samples were purified by mixed-mode solid phase extraction with good recovery (>83%). The method was linear ($r^2 > 0.99$) over a range of 2–64 ng/ml, with a lower limit of quantification of 2 ng/ml. The accuracy was 100 ± 4%, and the within-day and between-day precisions were <6.8% R.S.D. and <14% R.S.D., respectively. Perphenazine was stable in stock solutions and plasma. The method was successfully applied for analysing perphenazine in plasma after sublingual administration to rabbits.

1. Introduction

Perphenazine (Fig. 1) is a potent phenothiazine-type antipsychotic used in the treatment of, e.g. schizophrenia, anxiety and severe nausea or vomiting [1]. The oral bioavailability of perphenazine is relatively poor due to extensive hepatic first-pass metabolism [1,2]. Intraoral drug delivery via the sublingual and buccal membranes is an effective means to bypass the first-pass effect and thus increase the drug's bioavailability [3]. The intraoral administration route offers also many other benefits for drug delivery, such as rapid onset of action, ease of use and improved patient compliance.

Sublingual and buccal drug absorptions are often investigated using rabbits, as the oral cavities of rabbits and humans are histologically similar [4]. In these absorption studies, a sensitive method of analysis is required if one wishes to quantify drugs from plasma, since the volume of plasma that can be collected from rabbits is relatively small and the drug concentrations expected in the samples are often low. Several methods are available for determining perphenazine and other phenothiazine-type antipsychotics from human plasma, urine or oral fluid, e.g. liquid chromatography with ultraviolet [5–8], coulometric [9–11], fluorescence [12] or mass spectrometric detection [13], gas chromatography with electron capture [14,15], nitrogen [16,17] or mass spectrometric detection [18–21], as well as capillary electrophoresis coupled with chemiluminescence [22] or ultraviolet-visible detection [23]. Radioimmunoassays have also been applied in the determination of phenothiazines from plasma [24,25]. In addition to sensitivity, the United States Food and Drug Administration's (FDA) guideline on bioanalytical method validation stresses the importance of selectivity in quantitative analytical methods [26]. Most of the above-mentioned analytical methods are very sensitive, but their ability to differentiate the analytes of interest from the other sample components cannot be undoubtedly proved. Mass spectrometric detection allows simultaneous quantification and reliable identification of the analytes based on the monitoring of target and qualifier ions in single ion monitoring (single quadrupole MS) or multiple reaction monitoring mode (MS/MS). Furthermore, mass spectrometry is also one of the most sensitive analytical methods available.

Plasma samples need to be adequately purified before they are injected into a GC–MS instrument. Previous studies have reported several sample preparation techniques for perphenazine and related substances, including liquid–liquid extraction (LLE) [8–11,14–18,21] and solid phase extraction (SPE) [5,6,12,13,19,20,22,23]. Being a multi-step process, LLE is often extremely time-consuming and laborious. It also requires large quantities of organic solvents and frequently results in poor extraction recovery and incomplete sample purification [8,27,28]. The use of SPE usually improves analyte recovery and also results in cleaner extracts compared to LLE. When perphenazine or related substances have been extracted from plasma, urine or oral fluid

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doi:10.1016/j.jchromb.2008.07.010

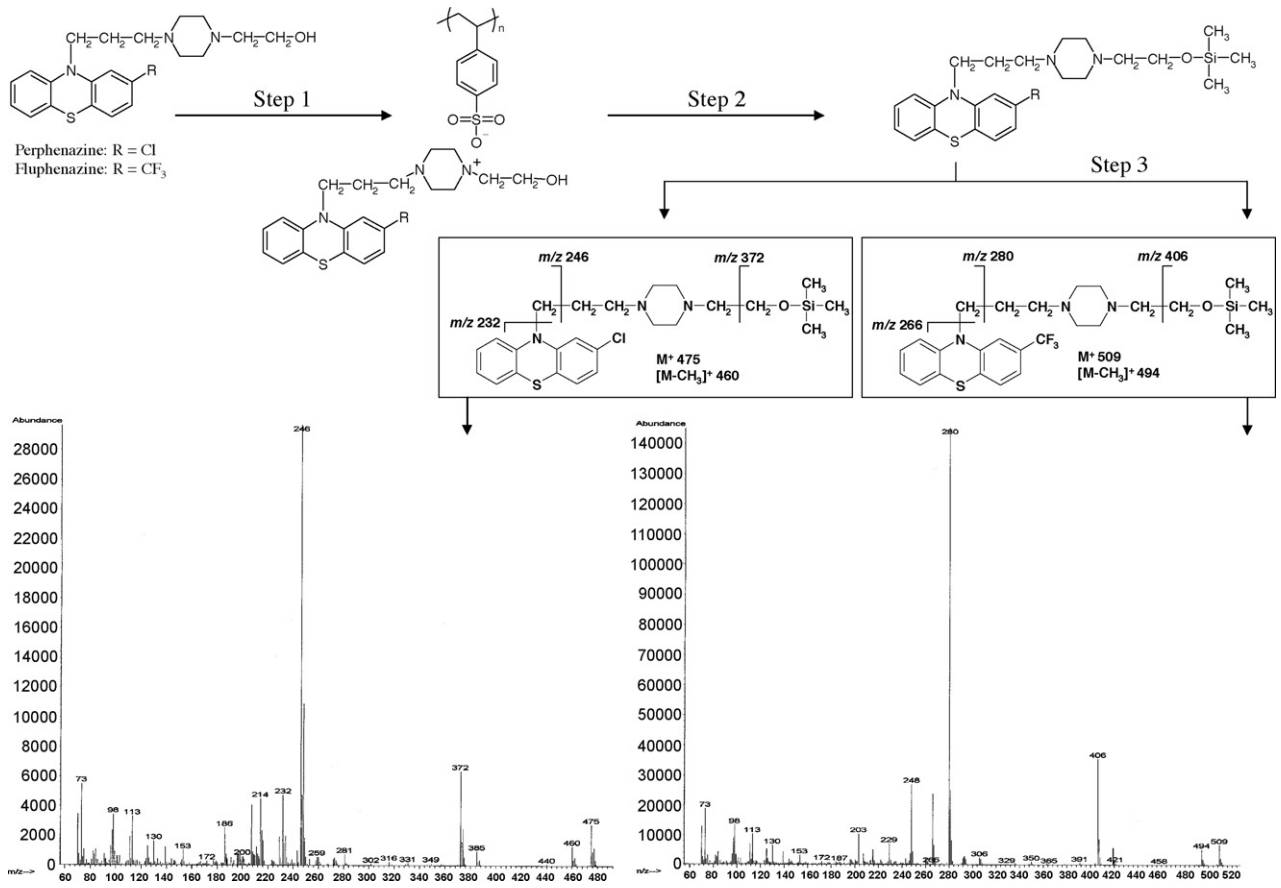

Fig. 1. Sample preparation and GC–MS analysis of perphenazine and I.S.: solid phase extraction (Step 1), silylation (Step 2) and fragmentation in MS (Step 3).

using SPE, recoveries ranging from 71% to 107% have been reported [5,6,12,13,19,20,22,23], whereas the recoveries after LLE have varied between 30% and 93% [9–11,14,16–18]. Moreover, the time required for sample purification by SPE is often shorter than for LLE, and thus a larger number of samples can be processed during a single working day.

In the present study, a GC–MS method with electron impact ionization (EI) was developed for the quantification of perphenazine in rabbit plasma, as GC–MS–EI is known to offer excellent chromatographic separation as well as a high degree of sensitivity and selectivity. The validity of the analytical method was confirmed according to the FDA guideline [26]. In addition, the suitability of mixed-mode SPE for preparing very clean extracts with good recovery was demonstrated for the first time for perphenazine in both human and rabbit plasma samples. The present method has been used in the development of novel fast-disintegrating formulations of perphenazine intended for sublingual delivery, which is a new route of administration for this drug.

2. Experimental

2.1. Materials

Perphenazine (≥99%) and fluphenazine dihydrochloride (≥99%) were purchased from Sigma–Aldrich (Steinheim, Germany). Pyridine (≥99.8%, GC-grade), 1-trimethylsilylimidazole (TMSI, 97%), trifluoroacetic anhydride (TFAA) and bis(trimethylsilyl)trifluoroacetamide (BSTFA; 1% TMCS) were supplied by Sigma–Aldrich (Steinheim, Germany). Medetomidine (Domitor®) was purchased from Orion (Espoo, Finland) and ketamine (Ketaminol®) was purchased from Intervet International B.V. (Boxmeer, The Netherlands). All other reagents were of analytical grade. Strata™ X-C cation mixed-mode polymer SPE cartridges were purchased from Phenomenex (Torrance, California, USA).

Human plasma was obtained from The Finnish Red Cross Blood Service (Helsinki, Finland). New Zealand white rabbits were purchased from The National Laboratory Animal Center (Finland). The rabbits were allowed to eat commercial food pellets and drink water *ad libitum*, except during the first 5 h of each test, when they were under anaesthesia. All procedures with animals were reviewed and approved by the Animal Ethics Committee of the University of Kuopio.

2.2. Standard solutions

Stock solutions (1 mg/ml) of perphenazine and fluphenazine dihydrochloride (internal standard, I.S.) were prepared using methanol as a solvent. Working solutions of perphenazine were diluted in methanol at concentrations of 20–640 ng/ml. Fluphenazine dihydrochloride was diluted in methanol to a working solution of 100 ng/ml. All solutions were stored at −20 °C.

Calibration and quality control (QC) standards were freshly prepared on the day of analysis. A total of 50 μl of the I.S. working solution and 50 μl of one of the calibration or QC standard working solutions were mixed in a test tube and evaporated to dryness under a nitrogen stream at 40 °C, after which 500 μl of analyte-free human plasma was added. Standards were then mixed for 20 s, equilibrated at room temperature (22 ± 2 °C) for 30 min and treated similarly as the plasma samples (Section 2.3). The calibration standards were prepared at six perphenazine concentrations: 2, 4, 8, 16,

32 and 64 ng/ml of plasma. At least four calibration standards were used for the calibration curve. The quality control samples were prepared at concentrations of 2, 10 and 40 ng/ml of plasma.

Reference standards were prepared by mixing 50 μl of the I.S. working solution and 50 μl of one of the QC working solutions in a test tube. The samples were evaporated to dryness, derivatized and analyzed. The concentrations of the reference standards were 2, 10 and 40 ng/ml. When the extraction recoveries were determined, reference standards (10 ng/ml) were also prepared using an alternative method, in which extracted analyte-free plasma was spiked with perphenazine and I.S.

2.3. Sample preparation

Frozen plasma samples were first thawed unassisted to room temperature (22 ± 2 °C). A total of 50 μl of the I.S. working solution was evaporated to dryness under a nitrogen stream at 40 °C, after which 500 μl of plasma sample was added. Samples were mixed for 20 s and equilibrated for 30 min at room temperature. After equilibration, they were acidified with 20 μl of 2 M HCl, diluted using 500 μl of 100 mM potassium dihydrogen phosphate buffer (pH 5.5) and centrifuged at 3000 rpm for 5 min.

SPE cartridges (Strata™ X-C) were preconditioned with 2 ml of methanol and equilibrated with 2 ml of water. The samples were then loaded under vacuum and washed with 1 ml of 2% (v/v) formic acid and 2 ml of methanol. After the washes, the solid phase material was aspirated to dryness for 30 s. Finally, the samples were eluted with 2 ml of 5% (v/v) ammonia in methanol.

2.4. Derivatization procedures

The standard solutions or methanolic extracts obtained from SPE were evaporated to dryness under a nitrogen stream at 40 °C. The trimethylsilyl derivatives were prepared using 50 μl of TMSI and 50 μl of pyridine. The samples were then kept at 70 °C for 30 min and analyzed after they had cooled to room temperature.

During the method development, two alternative derivatization methods were also investigated: trimethylsilylation by bis(trimethylsilyl)trifluoroacetamide (BSTFA) and acetylation by trifluoroacetic anhydride (TFAA). Trimethylsilylation by BSTFA was performed by treating the evaporation residues with 50 μl of BSTFA and 50 μl of pyridine. The samples were kept at 70 °C for 60 min, evaporated to dryness and dissolved in 100 μl of toluene. The acetylated derivatives were prepared using 200 μl of a derivatizing mixture that contained 2 ml of dichloromethane, 200 μl of TFAA and 60 μl of pyridine. The solutions were mixed for 10 s, equilibrated for 30 min and then evaporated to dryness. The evaporation residues were dissolved in 1 ml of toluene, 1 ml of water was added, and the solutions were mixed. After 1 min equilibration, the toluene layer was transferred to another test tube and the water phase was washed with 1 ml of toluene. Finally, the combined toluene phases were evaporated to dryness, and the evaporation residues were dissolved in 200 μl of toluene.

2.5. Instrumentation

The Agilent GC–MS system consisted of a gas chromatograph 6890N, autosampler 7683 and mass detector 5973N (Palo Alto, California, USA). Agilent Enhanced ChemStation software (version C.00.01.08) was used for data processing. A cross-linked 5% phenyl methyl siloxane capillary column (HP-5MS; 30 m × 0.25 mm i.d. × 0.25 μm film thickness) (Agilent Technologies; Palo Alto, California, USA) was used with helium as the carrier gas (1.0 ml/min, column head pressure 14.99 psi). Sample injection (1 μl) was performed in the pulsed splitless mode, in which an inlet pressure of 30 psi was maintained for 1.5 min. The initial oven temperature of 180 °C was held for 1 min, increased by 30 °C/min to 300 °C which was then maintained for 11 min (total run time 16 min). The temperatures at the inlet, interface, ionization source and quadrupole were 250, 290, 230 and 150 °C, respectively. Electron impact ionization was used with an ionization energy of 70 eV. MS was operated in the selected ion monitoring (SIM) mode with a dwell time of 50 ms. The ions monitored were as follows: perphenazine m/z 246.0, 372.2 and 475.2, fluphenazine dihydrochloride m/z 280.0, 406.2 and 509.2, respectively. The area ratio of 246.0/280.0 was used for quantification, whereas the other ions were used as qualifiers.

2.6. Validation of the method

The validity of the analytical method was confirmed according to the FDA guideline by investigating selectivity, linearity, accuracy, precision, recovery and stability of the analyte [26]. All statistical tests were performed using Microsoft Excel® 2002 software.

The selectivity of the method was confirmed by analysing analyte-free human and rabbit plasma for interfering peaks at the retention times of perphenazine and I.S. Rabbit plasma was collected prior to perphenazine administration as well as from rabbits that did not receive perphenazine. Linearity was determined using calibration standards at concentrations of 2, 4, 8, 16, 32 and 64 ng/ml. Three replicate analyses were performed at each concentration. The lower limit of quantification (LLOQ) was defined as the lowest concentration at which the determination was accurate and precise. The suitability of human plasma for the preparation of calibration curve and QC standards was investigated by comparing peak areas of quality control samples at concentrations of 5 and 40 ng/ml with analyte-free human or rabbit plasma as sample matrix. The similarity between the two plasma matrices was investigated by F-test followed by t-test ($p = 0.05$).

Within-day precision and accuracy were studied at concentrations of 2, 10 and 40 ng/ml by comparing five parallel samples at each concentration. To determine the between-day precision, three similar determinations were performed on different days of analysis. Precision was defined at each concentration level as the relative standard deviation (%R.S.D.) of calculated sample concentrations. Accuracy was determined by comparing the calculated concentrations to the nominal concentrations of the analyte. Extraction recoveries of perphenazine and I.S. were determined by comparing the analyte peak areas in quality control standards (10 ng/ml, $n = 4$) and in corresponding reference standards prepared either by spiking extracted analyte-free plasma with perphenazine and I.S. or by mixing the standard solutions, evaporating them to dryness and derivatizing the evaporation residues (Section 2.2).

The stability of perphenazine in plasma was evaluated by determining the short-term stability (24 h at room temperature, 22 ± 2 °C), freeze and thaw stability (three freeze-thaw cycles) and long-term stability (3 months at −80 °C). The stock solution stability (2 weeks at −20 °C) as well as the post-preparative stability of the analyte (24 h in the autosampler of the GC–MS) was also studied. Quality control samples ($n = 3$) at concentrations of 2 and 40 ng/ml were analyzed after each storage period, and the concentrations were compared to the corresponding results before storage. The short-term stability was also determined at 5 ng/ml.

2.7. In vivo absorption of perphenazine

The rabbits were anaesthetized using ketamine (25 mg/kg) and medetomidine (0.5 mg/kg). Perphenazine was administered to rabbits sublingually (1 mg/kg) as a micronized (<15 μm) powder. Blood samples were withdrawn either from a central artery or from a marginal vein of the ear before perphenazine administration and

10–480 min after administration. Blood samples were centrifuged (3300 × *g* for 5 min) at room temperature (22 ± 2 °C), after which the plasma layer was collected and frozen immediately. The samples were stored at −80 °C until analysis.

3. Results and discussion

3.1. Optimization of the method

In gas chromatography, derivatization is used to improve the chromatographic properties, volatility, detectability and stability of analytes and sample matrix components [29]. In GC–ECD and GC–MS analyses, perphenazine and fluphenazine have typically been analyzed as trimethylsilylated (*O*-TMS) derivatives (Fig. 1) prepared by a reaction of the analytes with, e.g. *N*-methyl-*N*-trimethylsilyl trifluoroacetamide (MSTFA) [19,20], *N*,*O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA) [18] or *N*,*O*-bis(trimethylsilyl)acetamide (BSA) [14,15,21]. Derivatization of perphenazine and fluphenazine by acetylation has also been reported [17].

In the present study, two derivatization methods were investigated: trimethylsilylation by TMSI or BSTFA and acetylation by trifluoroacetic acid (TFAA). Acetylated derivatives of perphenazine and I.S. were found to be unstable as they were readily hydrolyzed in acidic conditions. Moreover, the peak areas of the acetylated derivatives were too small to detect low perphenazine concentrations, and therefore trimethylsilylation was chosen for analyte derivatization. TMSI was found to be a superior silylating agent because of the higher stability of the derivatives and the lower variation between parallel samples in comparison to derivatives prepared using BSTFA (data not shown). In addition, the peak areas were somewhat larger when TMSI was used. TMSI reacts readily with hydroxyl groups but leaves aliphatic amino groups unaffected, whereas BSTFA is able to silylate both hydroxyl and amino groups [30]. As the molecular structures of perphenazine and fluphenazine contain only one hydroxyl group but several tertiary amino groups which BSTFA may attack, the derivatization reaction between TMSI and the analytes is more quantitative and repeatable than the silylation reaction with BSTFA.

Fig. 1 shows the fragment ions observed after electron impact ionization of the *O*-TMS derivatives of perphenazine and I.S. The base peaks (*m*/*z* 246 for perphenazine-*O*-TMS and *m*/*z* 280 for fluphenazine-*O*-TMS) resulted from the cleavage of the bond between the first two methylene groups adjacent to the phenothiazine ring ($[M\text{-}C_{13}H_9ClNS]^+$ and $[M\text{-}C_{14}H_9F_3NS]^+$ for perphenazine-*O*-TMS and fluphenazine-*O*-TMS, respectively). Ions corresponding to the breakdown of the silyl group with one methylene group ($[M\text{-}C_4H_{11}OSi]^+$) were also abundant (*m*/*z* 372 for perphenazine-*O*-TMS and *m*/*z* 406 for fluphenazine-*O*-TMS). In addition, other fragments with lower relative intensities were detected, corresponding to the cleavages depicted in Fig. 1. Molecular ions were present at *m*/*z* values of 475 (perphenazine-*O*-TMS) and 509 (fluphenazine-*O*-TMS) [19–21]. All the ions selected for SIM detection have relatively high masses, which reduces the background noise and thus improves method specificity.

In order to maximize the sensitivity and robustness of the method, dynamic mass calibration was performed to define the exact *m*/*z* values monitored in the SIM mode. Data was acquired using ions spaced 0.1 amu apart around the expected nominal masses of the ions used in the quantification and as qualifiers. The most abundant masses (*m*/*z* 246.0, 372.2 and 475.2 for perphenazine-*O*-TMS and *m*/*z* 280.0, 406.2 and 509.2, for fluphenazine-*O*-TMS) were then selected for SIM detection.

During the early phases of method development, inlet liners packed with silanized glass wool were used. However, low peak areas and carryover were observed as the analytes mostly remained in the glass wool material and did not appear to reach the GC column. With these liners, the relative peak areas of perphenazine and I.S. were only 11% and 33%, respectively, when compared to the peak areas obtained using inlet liners without glass wool packing. In addition to the increase of analyte peak areas, the use of empty inlet liners also reduced analyte carryover. In earlier studies, the glass wool material has been reported to decrease the detectability of, e.g. theophylline [31] and nitroaromatic compounds [32] as the glass wool either adsorbs or decomposes the analytes. In the present study, the use of empty inlet liners substantially increased the sensitivity of the method. Therefore, the analytes could be precisely quantified at lower concentrations, since analyte retention in the glass wool packing did not occur. On the other hand, the absence of glass wool packing clearly affected method robustness, and careful sample purification and instrument maintenance were thus needed to ensure reliable results.

3.2. Selectivity

The selectivity of the method was studied using analyte-free human plasma and analyte-free rabbit plasma collected prior to perphenazine administration or from rabbits that had not received perphenazine. The chromatograms were free of interfering peaks at the retention times of I.S. (9.6 min) and perphenazine (13.8 min). Fig. 2 shows representative chromatograms of analyte-free plasma, QC and reference standards (10 ng/ml) and a rabbit plasma sample (14.7 ng/ml) collected after sublingual administration of micronized perphenazine.

3.3. Precision, accuracy and extraction recovery

The data on precision and accuracy are summarized in Table 1. The within-day and between-day precisions of the determinations were acceptable (<14% R.S.D.) at all concentrations studied. The accuracy of the analyses was also well within the acceptance limits as all calculated concentrations were within 4% of the nominal concentrations.

Table 1
Within-day and between-day precision and accuracy

The nominal concentration of perphenazine (ng/ml)	Measured concentrations of perphenazine			Precision[a]		Accuracy[b,c]
	Day 1	Day 2	Day 3	Within-day[c]	Between-day[d]	
2	2.1	2.1	2.2	6.8	14	104
10	11	11	10	4.8	6.6	96
40	38	44	40	6.7	4.9	100

[a] Percent relative standard deviation (%R.S.D.).
[b] Percent of the nominal concentration.
[c] $n = 5$.
[d] $n = 15$; three similar determinations ($n = 5$) on different days.

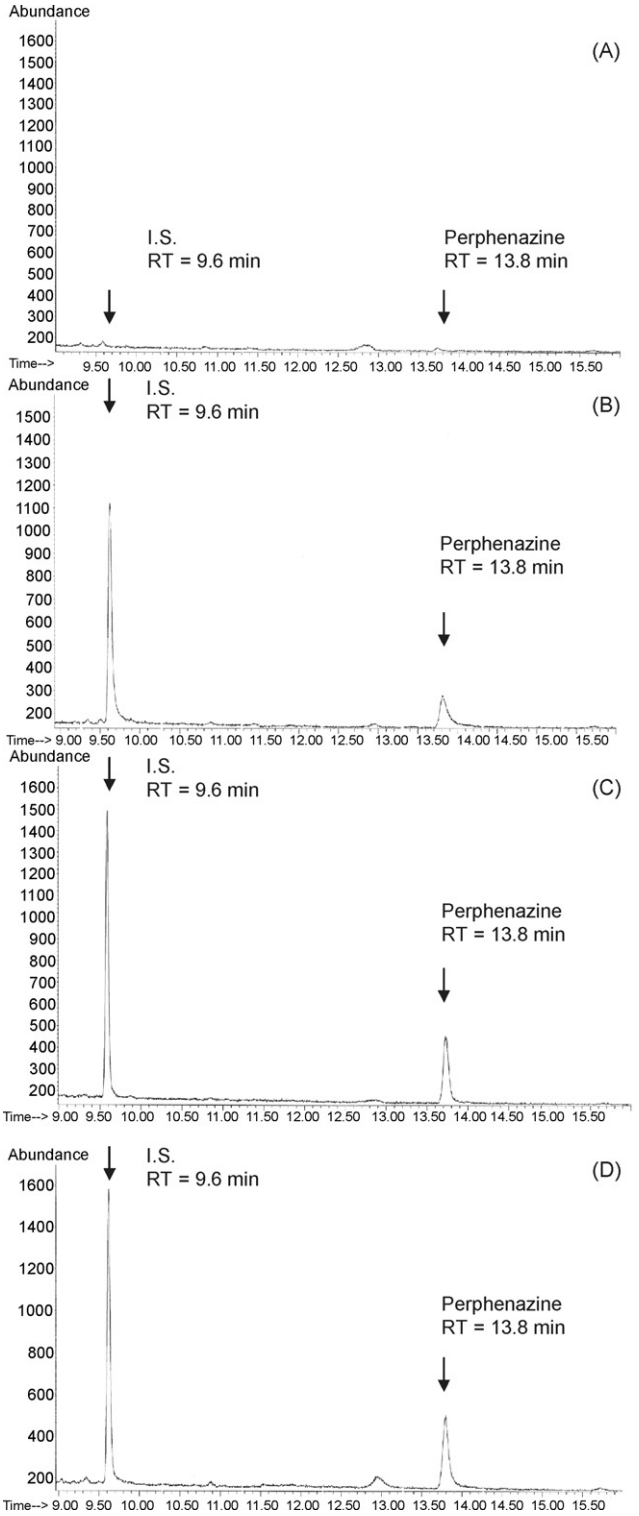

Fig. 2. Chromatograms from analyte-free plasma (A), a reference standard prepared by mixing the standard solutions, evaporating them to dryness and derivatizing the evaporation residues (10 ng/ml) (B), analyte-free plasma spiked with I.S. and perphenazine (10 ng/ml) (C), and a rabbit plasma sample (14.7 ng/ml) collected after sublingual administration of perphenazine (D).

Extremely clean extracts are required to ensure the sensitivity of the GC–MS analyses at low drug concentrations, especially when inlet liners without glass wool packing are used. SPE is an efficient sample preparation method that is known to produce very pure extracts with excellent recoveries. The stationary phase materials used in extracting perphenazine or other phenothiazine drugs from different biological matrices include reversed phase sorbents of different alkyl chain lengths [5,6], hydrophilic–lipophilic balanced polymeric sorbents [22,23] and mixed-mode polymeric stationary phases that combine cation exchange and reversed phase properties [13,19,20]. In this study, mixed-mode SPE was chosen as the sample preparation method. Mixed-mode phase materials have been reported to offer superior selectivity and sample purity in comparison to standard reversed phase sorbents. In addition to reversed phase interactions, they act via cationic retention mechanisms that allow efficient elution of interfering substances using strong organic solvents [33–35]. This clearly improves the robustness and usability of the analytical method, as cleaner samples reduce the need for instrument maintenance procedures and thus make longer analysis periods possible.

In the present study, a pronounced matrix effect was observed, leading to extraction recovery values that exceeded 100%. In order to compare the extraction recoveries, reference standards were prepared in two different ways: either by simply mixing, evaporating and derivatizing the methanolic standard solutions, or by spiking extracted analyte-free plasma with perphenazine and I.S., followed by evaporation and derivatization. In the reference standards prepared in methanol, the analyte peak areas were significantly smaller than those observed in the reference standards prepared in extracted analyte-free plasma. A similar difference was observed when extracted QC standards in phosphate buffer (pH 5.5) were compared to corresponding extracted QC standards in human or rabbit plasma. The mean calculated extraction recoveries ($n = 4$) for the reference standards prepared in methanol were 190% (7% R.S.D.) for I.S. and 140% (6% R.S.D.) for perphenazine. In contrast, with the reference standards prepared in extracted analyte-free plasma, the mean extraction recoveries were 88% (7% R.S.D.) for I.S. and 83% (6% R.S.D.) for perphenazine. The effect of plasma matrix components on the chromatographic properties was clearly observed especially for perphenazine, as the reference standards prepared in methanol showed increasing peak tailing (Fig. 2B) and decreasing detector response after a few days of analysis. These results indicate that the plasma matrix components improve the chromatographic properties the analytes and facilitate their evaporation and ionization during the GC–MS analysis [36,37].

3.4. Linearity and lower limit of quantification

The calibration curves were linear ($r^2 > 0.99$) over the entire concentration range of 2–64 ng/ml. The slope and intercept of the calibration curve ($n = 3$) were 0.476 (0.451–0.501) and −0.066 (−0.082–0.068), respectively (mean; 95% confidence interval). The LLOQ of the method was 2 ng/ml, which was suitable for monitoring plasma concentrations during the *in vivo* studies. The suitability of human plasma for the preparation of calibration curve and QC standards was investigated by comparing quality control samples with analyte-free human or rabbit plasma as sample matrix. No statistically significant differences were found between the peak areas of I.S. and perphenazine in rabbit and human plasma, and thus the use of human plasma in the calibration and QC standards was justified.

3.5. Stability

The stability of perphenazine during sample preparation and storage was investigated at 2, 5 and 40 ng/ml (Table 2). The con-

Table 2
Stability of perphenazine in plasma during sample preparation and storage ($n = 3$)

The nominal plasma concentration of perphenazine (ng/ml)	Short-term stability (24 h at RT)[a]	Long-term stability (3 months at −80 °C)[a]	Freeze-thaw stability (three freeze-thaw cycles)[a]	Post-preparative stability (24 h in the autosampler)[a,b]
2	90 (0.4)	104; 107[c]	89; 113[c]	100
5	105 (5.5)	N.D.	N.D.	N.D.
40	107; 117[c]	84 (13)	80 (15)	105

N.D. = not determined.
[a] Recovery (%) (%R.S.D.).
[b] $n = 1$.
[c] $n = 2$.

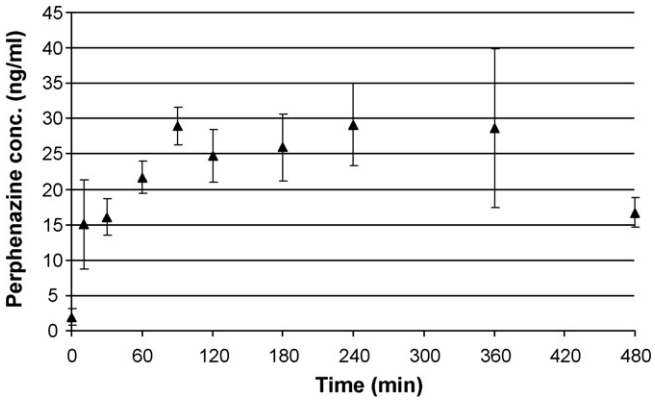

Fig. 3. Perphenazine concentrations in rabbit plasma after sublingual administration of plain micronized perphenazine (1 mg/kg) ($n = 3$–4; mean ± S.E.M.).

centrations of perphenazine remained within 20% of the initial concentrations under all conditions studied. Perphenazine was also found to be stable in stock solutions, as the QC standard concentrations remained above 98% of the initial concentrations during the 14-day storage at −20 °C. The stability of perphenazine was thus considered sufficient for the analytical application.

3.6. *In vivo* sublingual absorption of perphenazine

In the *in vivo* experiment, the absorption of perphenazine was investigated after its sublingual delivery, which is a new route of administration for this drug. Mean plasma concentrations ($n = 4$) of perphenazine after its sublingual administration as a solid, micronized powder are plotted in Fig. 3. The perphenazine concentrations of individual rabbit plasma samples ranged from 2.6 to 51 ng/ml, the highest concentrations being observed between 1.5 and 6 h after perphenazine administration. In every experiment almost all of the plasma concentrations were higher than 10 ng/ml, and concentrations lower than 5 ng/ml were only observed at the first time points in some experiments.

4. Conclusions

A sensitive GC–MS method was developed for the determination of perphenazine in rabbit plasma. The selectivity, linearity, accuracy and precision of the method as well as the stability of the analytes were found to be sufficient for plasma sample analyses. A new mixed-mode SPE procedure resulting in very clean extracts with good recovery was developed for perphenazine in human and rabbit plasma. The present method was successfully applied in measuring the concentrations of perphenazine in rabbit plasma after sublingual administration of the drug. The method can also be applied to the determination of perphenazine in human plasma.

Acknowledgements

The work was financially supported by the Finnish Funding Agency for Technology and Innovation. The authors thank Janne Mannila, Ph.D., for performing the animal studies and Mr. Heikki Pekonen and Mrs. Sari Ukkonen for skillful technical assistance. The National Laboratory Animal Center (Finland) is acknowledged for providing a pleasant working atmosphere and good care for the animals.

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