Short communication

Validated high-performance liquid chromatographic assay for the determination of promazine in human plasma Application to pharmacokinetic studies

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Abstract

A high-performance liquid chromatographic method for the determination of promazine in human plasma is described. The assay involves a single-step liquid–liquid extraction using pentane–2-propanol (98:2, v/v). The analyte of interest and the internal standard chlorpromazine were separated on a Spherisorb CN column using a mobile phase of acetonitrile–50 mM ammonium acetate (9:1, v/v). Electrochemical detection was achieved using an applied potential of +750 mV. The assay was validated according to international requirements prior to application to a pharmacokinetic study and was found to be specific, accurate and precise with a linear range of 0.25–25 ng ml^{-1}. © 1998 Elsevier Science B.V. All rights reserved.

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1. Introduction

Promazine (Fig. 1) is a phenothiazine neuroleptic agent with strong anticholinergic, hypotensive and sedative effects and moderate antiemetic effects. It is used mainly as an antipsychotic and antiemetic agent and additionally as an adjunct agent in the management of severe pain.

Existing methods for the determination of promazine in plasma make use of high-performance liquid chromatography (HPLC) with UV detection [1,2] and amperometric detection [1]. These methods involve multiple liquid–liquid extraction steps and relatively large volumes of plasma [1] or make use of solid-phase extraction and have a limit of quantification of 1 ng ml^{-1} [2]. Related compounds have been analysed by radioimmunoassay and gas chromatography with mass spectrometry [3] as well as by HPLC with coulometric detection [4].
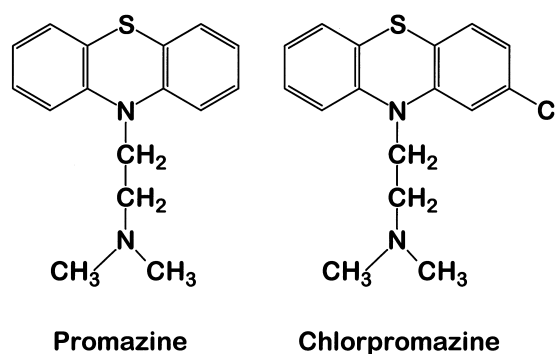

Fig. 1. Structural formulae.

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The method presented in this communication is rapid, straightforward, sensitive and selective, making use of a single extraction step and HPLC with electrochemical detection for the determination of promazine in human plasma. The method is based on an assay for the structurally similar compound fluphenazine [4], but has at least a four-fold lower limit of quantification compared to existing promazine methods [1,2]. The method was validated fully according to international requirements [5,6] in the range of 0.25–25 ng ml^{-1} prior to its application to the routine determination of samples from a pharmacokinetic study.

2. Experimental

2.1. Chemicals

Promazine hydrochloride and chlorpromazine hydrochloride (internal standard, I.S.) were reference substances obtained from the United States Pharmacopoeia (Rockville, MA, USA). Acetonitrile and methanol were of HPLC grade and were purchased from E. Merck (Darmstadt, Germany). All other chemicals were of analytical grade and were also acquired from E. Merck. Blank (drug free) human plasma was provided by the Hessen Blood Donor Service (Frankfurt, Germany). Distilled water, prepared from demineralised water, was used throughout.

2.2. Instrumentation and analytical conditions

The HPLC system consisted of a PU-980 pump, an 851-AS autosampler (both from Jasco, Groß-Umstadt, Germany) and an ESA Coulochem II electrochemical detector (supplied by Bischoff Analysentechnik, Leonberg, Germany). The detector was fitted with a 5020 guard cell operating at +900 mV and a 5011 high sensitivity analytical cell operating at +400 mV and +750 mV at the first and second electrodes, respectively. Chromatographic data was collected via Borwin chromatography software (Jasco, Groß–Umstadt, Germany).

Chromatography was carried out at 25°C using a Spherisorb CN, 3 μm, 150 mm×4.6 mm column and a mobile phase consisting of acetonitrile and 50 m*M* ammonium acetate (9:1, v/v) running at a constant flow-rate of 1.5 ml min^{-1}.

2.3. Sample preparation

Due to the light sensitivity of the analytes, liquid–liquid extraction of plasma samples was carried out away from direct sunlight. Frozen samples were thawed at room temperature in a water bath and 1 ml of sample was added to 1 ml of an aqueous I.S. solution (chlorpromazine 12.5 ng ml^{-1}) in a glass centrifuge tube fitted with a glass stopper. After vortexing for a few seconds, 0.5 ml of 0.65 *M* sodium carbonate were added and the sample was vortexed again prior to the addition of 4 ml of pentane–2-propanol (98:2, v/v). The samples were then shaken for 15 min on a flat-bed shaker and after centrifugation (5 min at 2700 *g*), the organic phase was decanted off and evaporated to dryness under a gentle stream of nitrogen. Thereafter, the residue was resuspended in 100 μl of mobile phase and a 20 μl aliquot was injected into the HPLC system.

For the preparation of standards, a methanolic stock solution (200 μg ml^{-1}) was made up and diluted appropriately with plasma to give a working standard of 1 μg ml^{-1}. This working standard was then diluted accordingly with plasma to yield the required standard concentrations. Thereafter, standards were treated as described above prior to HPLC analysis.

2.4. Quantification and assay validation

Plasma samples were quantified using the ratio of the peak height of promazine to that of I.S. as the assay parameter. Peak height ratios were plotted against theoretical concentrations (0.25–25 ng ml^{-1}) and promazine concentrations were calculated using weighted ($1/x^2$) least squares linear regression.

To evaluate linearity, plasma calibration curves (7 samples, 0.25–25 ng ml^{-1}) were prepared and assayed in triplicate on three separate days. Accuracy and precision were also assessed by determining three concentrations (0.25, 5 and 25 ng ml^{-1}, six samples each) over three days. Specificity was verified by analyzing six independent blank plasma samples and comparing these chromatograms to those obtained after spiking the plasma samples from the same source with promazine and I.S.. Recovery was determined by analyzing six spiked plasma samples of a midrange concentration (5 ng pro-

Table 1
Linearity data for HPLC assay of promazine in plasma

	Day 1	Day 2	Day 3
Correlation coefficient	0.9991	0.9991	0.9995
Slope (S.D.)	0.101919 (0.000462)	0.101296 (0.000467)	0.101808 (0.000355)
Intercept (S.D.)	0.001872 (0.002068)	0.001225 (0.001922)	−0.000255 (0.001463)
n	20	21	21

Table 2
Inter- and intra-day accuracy and precision data for HPLC assay of promazine in plasma

	Added concentration (ng ml^{-1})		
	0.25	5.00	25.00
Day 1			
Mean±S.D. (ng ml^{-1})	0.27±0.02	5.00±0.04	24.82±0.20
R.S.D. (%)	6.4	0.9	0.8
Bias (%)	9.3	0.0	−0.7
n	6	6	6
Day 2			
Mean±S.D. (ng ml^{-1})	0.29±0.01	4.98±0.06	24.95±0.29
R.S.D. (%)	4.8	1.3	1.2
Bias (%)	14.0	−0.4	−0.2
n	6	6	6
Day 3			
Mean±S.D. (ng ml^{-1})	0.27±0.02	4.98±0.08	24.79±0.35
R.S.D. (%)	9.1	1.7	1.4
Bias (%)	8.7	−0.5	−0.8
n	6	6	6
Overall			
Mean±S.D. (ng ml^{-1})	0.28±0.02	4.99±0.06	24.85±0.28
R.S.D. (%)	6.9	1.3	1.1
Bias (%)	10.7	−0.3	−0.6
n	18	18	18

Table 3
Stability data for promazine in plasma (n=6)

	Nominal concentration (ng ml^{-1})	Mean (ng ml^{-1})	R.S.D. (%)	Bias (%)
Processed samples at room temperature				
0 h	0.25	0.27	6.4	9.3
33 h	0.25	0.28	6.6	11.3
0 h	25.00	24.82	0.8	−0.7
33 h	25.00	24.65	0.7	−1.4
Freeze-thaw stability				
0 cycles	0.25	0.27	6.4	9.3
3 cycles	0.25	0.27	9.8	7.3
0 cycles	25.00	24.82	0.8	−0.7
3 cycles	25.00	25.02	1.6	0.1
Storage stability (≤−20°C)				
0 weeks	5.00	5.00	0.9	0.0
13 weeks	5.00	4.85	7.2	−3.1

mazine and 12.5 ng I.S. per ml plasma) and comparing the peak heights with the peak heights obtained from aqueous solutions of promazine and I.S. in mobile phase injected directly onto the column. The stability of promazine was assessed in processed samples after storage at room temperature for 33 h, in spiked plasma samples after three freeze and thaw cycles and after 13 weeks frozen storage below $-20°C$.

Over a period of three months, assay performance during routine analysis was monitored through the analysis of calibration and quality control (QC) samples (duplicate QC samples, 0.5, 5 and 20 ng ml^{-1}) with each run.

2.5. Volunteer study

After giving written informed consent, 24 young healthy male Caucasian volunteers took part in an approved study evaluating the relative bioavailability of promazine after oral administration of 100 mg promazine HCl as a sugar-coated tablet, a suspension or a solution. The study was conducted in an open, randomised, three-period changeover design with single dose administration and a wash-out phase of at least 14 days between periods. Plasma was obtained from blood samples collected prior to drug intake and up to 96 h post-dose. Plasma samples were stored frozen at less than $-20°C$ prior to analysis.

3. Results and discussion

3.1. Linearity and lower limit of quantification

Visual inspection of the plotted triplicate calibration curves and correlation coefficients >0.999 confirmed that the calibration curves were linear over the concentration range 0.25–25 ng ml^{-1}. Linearity data and relevant statistics as calculated by Microsoft Excel and WSA Statistik Software are given in Table 1. The lower limit of quantification, defined as the lowest concentration analysed with acceptable accuracy and precision, was 0.25 ng ml^{-1} (Table 2).

3.2. Accuracy and precision

The overall accuracy of the method, expressed in terms of bias (% deviation from the true values), lay

between -0.6% and 10.7% for the concentrations investigated (0.25, 5 and 25 ng ml^{-1}). The overall precision of the method, given by the relative standard deviations lay between 1.1% and 6.9%.
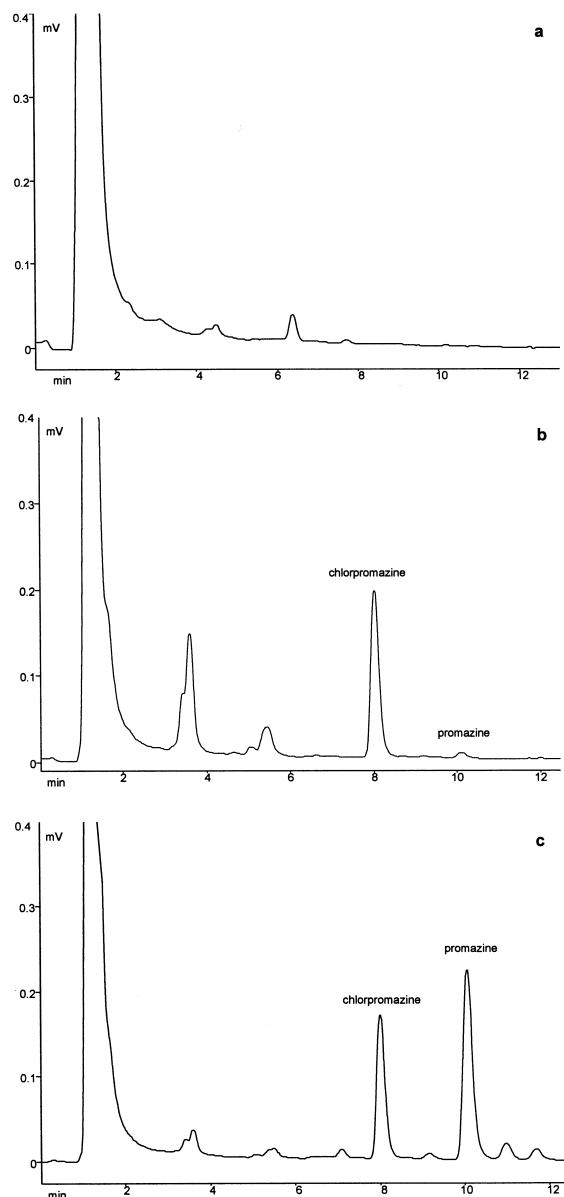

Fig. 2. Chromatograms of (a) a blank plasma sample, (b) a blank plasma sample spiked with the internal standard chlorpromazine (12.5 ng ml^{-1}) and promazine at the limit of quantification (0.25 ng ml^{-1}) and (c) a sample from a volunteer taken 2.5 h after the administration of 100 mg of promazine HCl as a sugar-coated tablet (13.87 ng ml^{-1}).

Inter- and intra-day statistics for accuracy and precision are given in Table 2.

3.3. Specificity

Representative chromatograms of a blank plasma sample, a blank plasma sample spiked with promazine and internal standard at the lower limit of quantification and a volunteer sample are shown in Fig. 2. No interferences from endogenous substances with either promazine or the internal standard chlorpromazine were detected.

3.4. Recovery

The recovery of promazine was $72.6\pm1.9\%$ (5 ng ml^{-1}, $n=6$) and the recovery of the internal standard chlorpromazine was $55.6\pm1.1\%$ at the concentration used in the assay (12.5 ng ml^{-1}, $n=6$).

3.5. Stability

The stablility of promazine in plasma was demonstrated after three freeze and thaw cycles and after 13 weeks frozen storage below $-20°C$. The stability of processed samples was confirmed after 33 h storage at room temperature. These data are summarised in Table 3.

3.6. Routine analysis

During routine analysis, correlation coefficients for the calibration curves ($n=30$) ranged between 0.9957 and 1.0000. The statistics compiled in Table 4 attest that quality control samples showed good precision and accuracy throughout the measurement of study samples. Typically, individual runs lasted 13 min and 75 samples, including calibration and qual-

Fig. 3. Representative plasma concentration vs. time curve obtained from a volunteer after the administration of 100 mg of promazine HCl as a sugar-coated tablet.

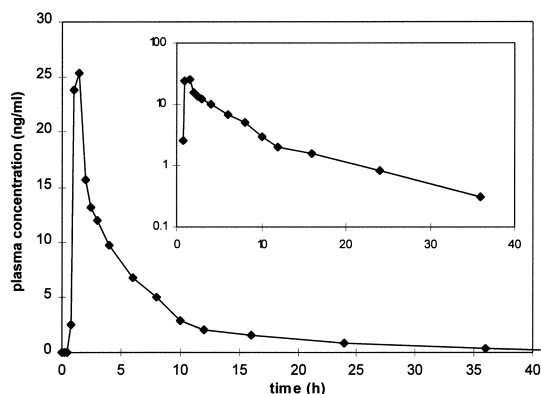ity control samples, could be processed and analysed in a 24 h period.

A representative plasma concentration vs. time curve obtained from a volunteer after the administration of 100 mg of promazine HCl as a sugar-coated tablet is shown in Fig. 3. Generally, plasma concentrations were below LLQ 36–48 h post-dose.

Acknowledgements

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Table 4
Accuracy and precision of quality control samples during routine analysis

	Nominal concentration (ng ml^{-1})		
	0.50	5.00	20.00
Mean (ng ml^{-1})	0.51	5.10	20.00
R.S.D. (%)	8.6	5.5	5.0
Bias (%)	2.1	2.0	0.0
n	56	56	56