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GAS CHROMATOGRAPHIC—MASS SPECTROMETRIC PROCEDURE FOR THE QUANTITATION OF CHLORPROMAZINE IN PLASMA AND ITS COMPARISON WITH A NEW HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC ASSAY WITH ELECTROCHEMICAL DETECTION

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SUMMARY

A gas chromatographic—mass spectrometric assay using selected ion monitoring is compared with a high-performance liquid chromatographic assay using an electrochemical detector for single-dose studies of the psychotherapeutic phenothiazine drug chlorpromazine. Measurements were made after extraction of chlorpromazine and the internal standard, prochlorperazine, from basified plasma with an isopropanol—pentane solvent mixture. Following evaporation of the organic solvents the residue was reconstituted in a small volume of methanol and subjected to gas chromatographic—mass spectrometric selected ion detection. The residual sample was then evaporated and made up in a larger volume of acetonitrile and analyzed by high-performance liquid chromatography using an electrochemical detector. These specific methods display excellent correlation for plasma concentration determinations in the range of 0.25—10 ng ml^{-1} and will allow for the study of the pharmacokinetics of chlorpromazine following single low doses of the drug.

INTRODUCTION

Chlorpromazine is the most widely used phenothiazine antipsychotic, and as such has been researched in the most detail with regard to analysis, metabolism, pharmacokinetics and plasma concentrations versus clinical response correlations. It is extensively metabolized to numerous metabolites, and several of these are psychoactive. The major metabolites include the sulfoxide, N-oxide, 7-hydroxy, N-desmethyl and N-didesmethyl compounds. The quantitative analysis of chlorpromazine in plasma has been performed by several methods; the first reported was the gas chromatographic—electrochemical detection (GC—ElCD) procedure of Curry [1]. This method has undergone several

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modifications to improve the sensitivity and ease of application, as well as to include the quantitation of metabolites such as the sulfoxide, 7-hydroxy and N-desmethyl compounds [2—5]. Other chemical methods of analysis include radioassay [6], fluorometry [7], gas chromatography—nitrogen—phosphorus detection (GC—NPD) [8, 9], high-performance liquid chromatography (HPLC) [10—13], and gas chromatography—mass spectrometry (GC—MS) [14—17]. Most of these methods have adequate sensitivity so as to quantitate chlorpromazine plasma concentrations following chronic therapeutic doses of the drug. However, except for GC—MS these chemical procedures generally lack the sensitivity needed for single-dose pharmacokinetic studies.

Furthermore, there is a special need for systematic comparison of the various methods proposed by different investigators for the determination of chlorpromazine, since it has been shown in the past that unexplained discrepancies were observed when the analytical results of different laboratories were compared with each other [18]. It is believed in the author's laboratory that the best quality control consists in comparing different sensitive and specific methods for the determination of chlorpromazine within the same laboratory. Once the valid comparisons are made, then, and only then can reliable pharmacokinetics and plasma levels—clinical response correlations be studied meaningfully.

Therefore, in the past a radioimmunoassay method [19] reported from this laboratory, which had the necessary sensitivity and reproducibility for single dose pharmacokinetic studies [20], was certified for its specificity by an HPLC—UV detection method [10]. This latter HPLC method, which was only sensitive down to 1 ng of chlorpromazine per ml of plasma, was recently improved by the use of an electrochemical detector and improved extraction recovery so that 0.25 ng ml^{-1} of the drug using a 2-ml plasma sample can be quantitated [21]. This HPLC—ElCD method is now compared with a newly developed GC—MS procedure, the latter method being reported here. In the GC—MS procedure the drug and the internal standard, prochlorperazine, following extraction from basified plasma are chromatographed on an OV-1 column. Use of a selected ion technique, monitoring the molecular ions for chlorpromazine at *m/z* 318 and prochlorperazine at *m/z* 373, provided sensitivity down to 0.25 ng ml^{-1} of plasma. For comparison of this procedure with the HPLC—ElCD method plasma samples were obtained from healthy volunteers administered single 50-mg oral doses of chlorpromazine.

EXPERIMENTAL

Materials

Heparinized evacuated tubes (Vacutainer®) were obtained from Becton Dickinson and Co. (Mississauga, Canada). All solvents were distilled in glass and all other chemicals were analytical grade used without further purification.

Chlorpromazine was obtained in the form of Thorazine® tablets, 25 mg, as chlorpromazine·HCl from Smith Kline and French Labs. (Philadelphia, PA, U.S.A.). Prochlorperazine mesylate was a gift from Rhone Poulenc Pharma (Montreal, Canada). All assays and sample manipulations were performed in subdued light. Standard solutions of chlorpromazine and prochlorperazine were

prepared by dilution in double distilled deionized water. Appropriate dilutions of the standard solutions were made in pooled fresh plasma obtained from blood collected from healthy volunteers.

Plasma level study

Five healthy, overnight fasted, male volunteers weighing between 56 kg and 86 kg were each given orally a single 50-mg dose of chlorpromazine, two 25-mg tablets (Thorazine SKF) with 250 ml tap water. Blood samples were collected at scheduled intervals over a 24-h period in evacuated glass tubes (Vacutainers), centrifuged, and the separated plasma was stored at −20°C until analysis. During collection of the venous samples care was taken to avoid contact of the blood with the rubber stopper of the evacuated tube. This precaution was taken in order to avoid distortions in plasma concentrations as reported for phenothiazines [22, 23].

Extraction of samples

To a 10-ml PTFE-lined screw-capped test tube were added 2 ml of plasma and 1 ml of aqueous internal standard solution (prochlorperazine, 100 ng ml^{-1}). The sample was gently mixed (Vortex Genie, Fisher Scientific Company, Edmonton, Canada) and 0.5 ml of saturated sodium carbonate solution was subsequently added. The mixing was then repeated and 5 ml of 3% isopropanol in *n*-pentane was added. The tube was tightly capped and mixed (Evapomix, Fisher Scientific) for 15 min at speed 6.5 and centrifuged (T-J6 centrifuge, Beckman Instruments, Toronto, Canada) at room temperature at 1720 *g* for 5 min. The upper organic layer was transferred by pasteur pipette to another 10-ml PTFE-lined screw-capped test tube. The aqueous layer was re-extracted with a further 5-ml of 3% isopropanol in *n*-pentane, which was again transferred by pasteur pipette into the test tube containing the first aliquot. After the addition of antibumping granules the organic solvents were evaporated in a dry bath at 65°C. The dried residue was reconstituted for GC—MS analysis with 30 μl of methanol by vortex mixing and subsequent centrifugation. Aliquots of 4 μl were injected for GC—MS analysis. After GC—MS analysis the residual sample was evaporated in a dry bath at 65°C, reconstituted in 200 μl of acetonitrile and a 100-μl aliquot was used for injection into the HPLC—ElCD system.

Instrumentation

The GC—MS mass fragmentography was performed on a V.G. Micromass MM 16F mass spectrometer interfaced via a single stage jet separator to a Hewlett-Packard 5711A gas chromatograph and equipped with a V.G. 2025 data system. The instrument was operated in the electron impact (EI) mode with the interface and ion source at 280°C and 220°C, respectively.

The chromatographic column was a coiled glass tube 1.22 m × 2 mm I.D. packed with 3% OV-1 on acid-washed, dimethyldichlorosilane treated Gas-Chrom Q, 100—120 mesh. The injection port and the column oven temperatures were 300°C and 280°C, respectively and the helium carrier gas flow-rate was 30 ml min^{-1}. Additional MS conditions were: emission current, 200 μA and electron multiplier 2.1 kV. A column bleed ion at *m/z* 281 was used as a

computer reference lock mass, in order to compensate for any instrumentational drift, and the molecular ions of chlorpromazine *m/z* 318 and the internal standard, prochlorperazine *m/z* 373 were alternately monitored by the computer with a dwell time on each ion of 200 msec.

HPLC—ElCD [21] was performed using a Waters M45 liquid chromatographic pump (Waters Assoc., Mississauga, Canada) fitted with a Rheodyne Model 7125 valve loop injection system utilizing a 500-μl loop (Technical Marketing Associates, Calgary, Canada). The column used was a 250 mm × 4.6 mm I.D. column packed with Spherisorb CN 10 μm (Beckman Instruments). The mobile phase consisted of 0.1 *M* ammonium acetate—acetonitrile (10:90), degassed before use by Millipore filtration. The column was maintained at ambient temperature with a flow-rate of 4 ml/min. Detection was achieved using an electrochemical detector (Bioanalytical Systems Model LC4A, Technical Marketing Associates). The detector was fitted with a glassy carbon electrode set at +0.9 V in the oxidation mode with a fixed 10-nA feed going to a Perkin-Elmer Model 056 recorder (Perkin-Elmer, Montreal, Canada). All changes in attenuation were made only with the recorder to avoid baseline stabilization problems.

RESULTS AND DISCUSSION

The low-resolution EI spectra of chlorpromazine and prochlorperazine (not shown) indicated that the best ions to monitor for single ion chromatograms of these two compounds, without significant interference or contribution to each other, were their molecular ions at *m/z* 318 and *m/z* 373 for chlorpromazine and prochlorperazine, respectively.

Using the GC—MS conditions as outlined in the Experimental section the chlorpromazine monitored ion at *m/z* 318 eluted with a retention time of 1 min 12 sec, while the monitored ion at *m/z* 373 for prochlorperazine eluted with a retention time of 2 min 58 sec.

Typical single ion chromatograms of chlorpromazine and the internal standard prochlorperazine are shown in Fig. 1. In order to obtain clean chromatograms, free of interfering peaks, such as those shown in Fig. 1, it is essential that the plasma used to prepare chlorpromazine standards, as well as the plasma used to dilute unknown plasma samples, be fresh plasma stored in glass containers. This requirement was made obvious when plasma stored in plastic was first employed as a diluent for patient plasma samples. Under these circumstances a large peak interfering with chlorpromazine was seen with a retention time of approximately 1 min 10 sec. This interfering peak may be some form of phthalate ester from the plastic, since fresh plasma stored only in glass did not contain this interfering peak. Fig. 1B shows a chromatogram of a spiked plasma sample containing 10 ng of chlorpromazine per ml of plasma. Fig. 1C is a chromatogram of a plasma sample taken 2 h after the oral administration of 50 mg of chlorpromazine to a healthy male volunteer; the chlorpromazine concentration was estimated to be 3.2 ng ml^{-1}. Fig. 1D shows a typical chromatogram for the internal standard at a concentration of 50 ng ml^{-1} of plasma.

The mean overall recovery for chlorpromazine at 1 and 5 ng ml^{-1} was 86% for both concentrations, while the recovery of prochlorperazine at 50 ng ml^{-1}

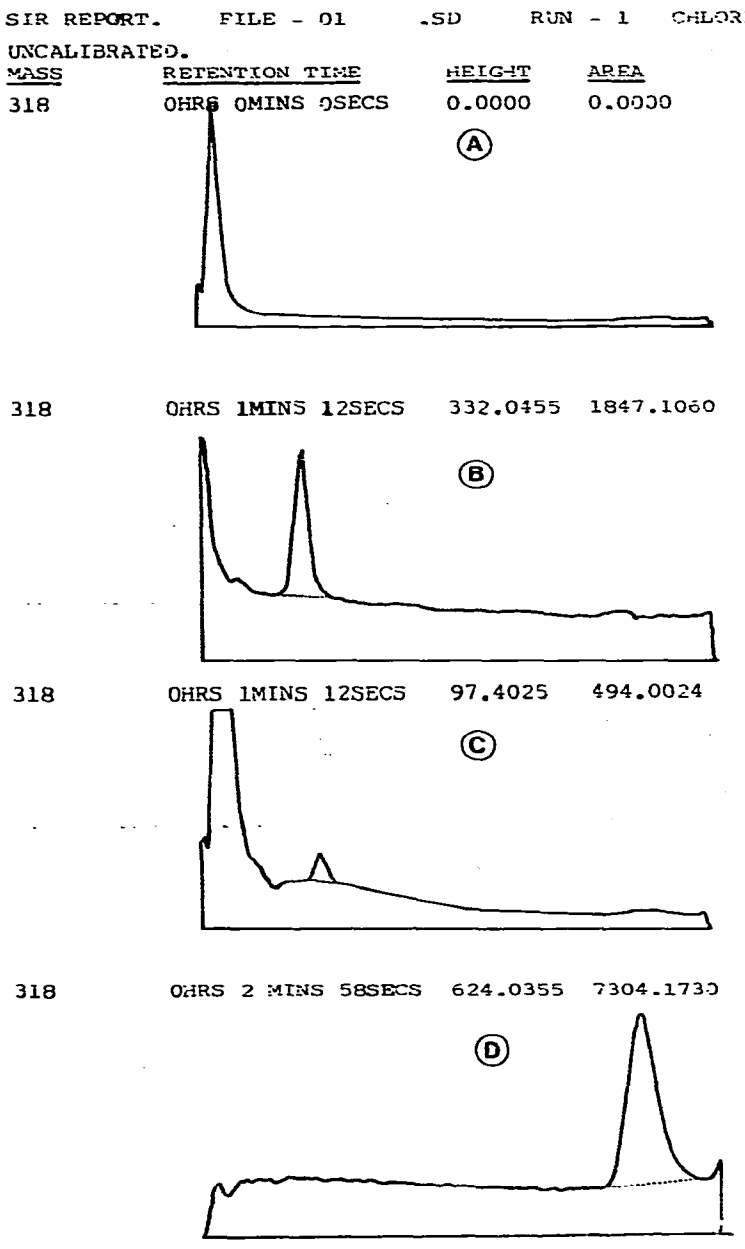

Fig. 1. Selected single ion chromatograms of chlorpromazine and prochlorperazine plasma samples. (A) Blank plasma sample; (B) spiked plasma for chlorpromazine at 10 ng ml^{-1}; (C) plasma from a healthy volunteer 2 h after receiving a single 50-mg oral dose of chlorpromazine, the concentration of chlorpromazine was estimated to be 3.2 ng ml^{-1}; (D) prochlorperazine peak obtained from the sample plasma used in (C), the concentration of prochlorperazine was 50 ng ml^{-1}.

was 86% [21]. Data obtained for the construction of a calibration curve for chlorpromazine analyzed using the described GC—MS procedure are shown in Table I. The calibration curve was linear from 0.25 to 10 ng ml^{-1}, with a coefficient of variation of 6.7% at 0.25 ng ml^{-1}, the lowest concentration analyzed. This coefficient of variation is comparable to that obtained by the HPLC—ElCD procedure (5.1% at 0.25 ng ml^{-1}) described earlier from these laboratories [21].

Both the GC—MS method described here and the earlier reported HPLC—ElCD procedure were applied to the pharmacokinetic analysis of plasma samples obtained from volunteers who had received a single 50-mg oral dose of chlorpromazine. A comparison of the plasma concentration versus time profile for a typical volunteer analyzed by both the GC—MS and HPLC—ElCD procedures is shown in Fig. 2. As can be seen, both methods, which are specific, demonstrate sufficient sensitivity to follow plasma concentrations of chlor-

TABLE I

CALIBRATION CURVE DATA FOR CHLORPROMAZINE GC—MS

Concentration (ng/ml)	n	Mean peak height ratio	Standard deviation	C.V. (%)
0.25	4	0.0161	0.0011	6.73
0.50	4	0.0293	0.0020	6.97
1.0	5	0.0583	0.0048	8.15
2.5	5	0.1579	0.0060	3.80
5.0	5	0.2787	0.0177	6.35
10.0	5	0.6205	0.0571	9.70

$y = 0.0614x - 0.0035; r^2 = 0.9970$

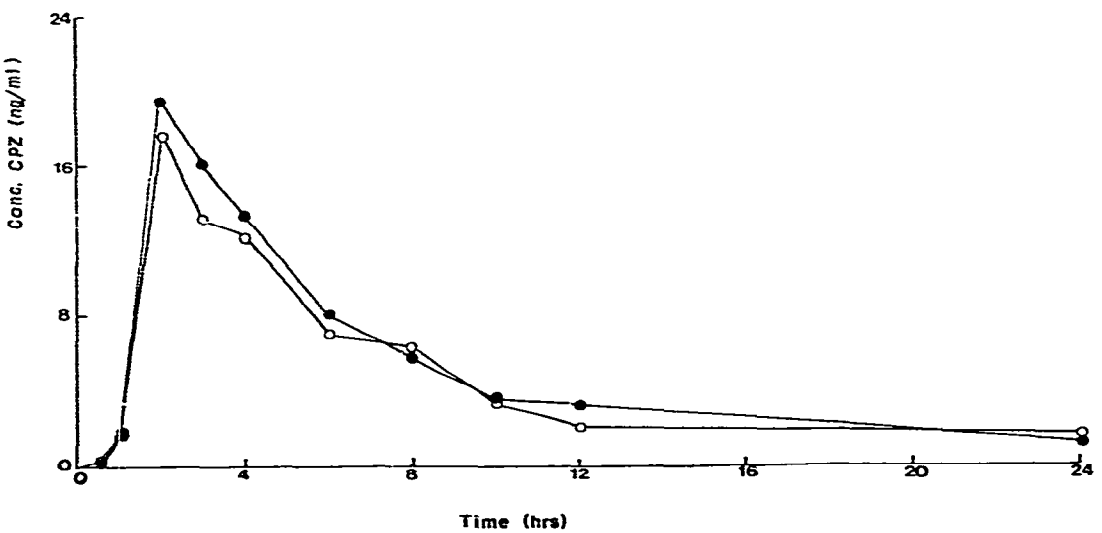

Fig. 2. Plasma concentration—time profiles for a single volunteer after ingesting 50 mg chlorpromazine (CPZ). Comparison of methods of analysis by HPLC—ElCD procedure [21] (●—●) and GC—MS procedure (present method) (○—○).

promazine to as late as 24 h following this low dose. The area under the curve from 0 to 24 h (AUC_0^{24}) based on the GC—MS procedure was determined to be 106.53 ng h ml^{-1} while the AUC_0^{24} for this same volunteer using the HPLC—ElCD procedure was 120.64 ng h ml^{-1}.

A further comparison of the HPLC—ElCD procedure and the described GC—MS procedure is shown in Fig. 3. In this figure the concentrations of chlorpromazine determined at various time intervals by both procedures is shown for the five volunteers who received a 50-mg oral dose of chlorpromazine. The slope of the regression line for this comparison had a value of 0.9713 with a correlation coefficient of 0.9617. These results indicate that the two methods compare favourably.

The described GC—MS method is precise, accurate and specific for chlorpromazine. The established sensitivity and specificity of GC—MS procedures for chlorpromazine and other drugs have made methods of analysis of this type the yardstick to which other methods are often compared. As such, the excellent agreement between the previously described HPLC—ElCD procedure [21] and the newer GC—MS procedure indicates that both methods accurately determine plasma chlorpromazine concentrations. In addition the results show that both methods have the necessary sensitivity to follow plasma concentrations

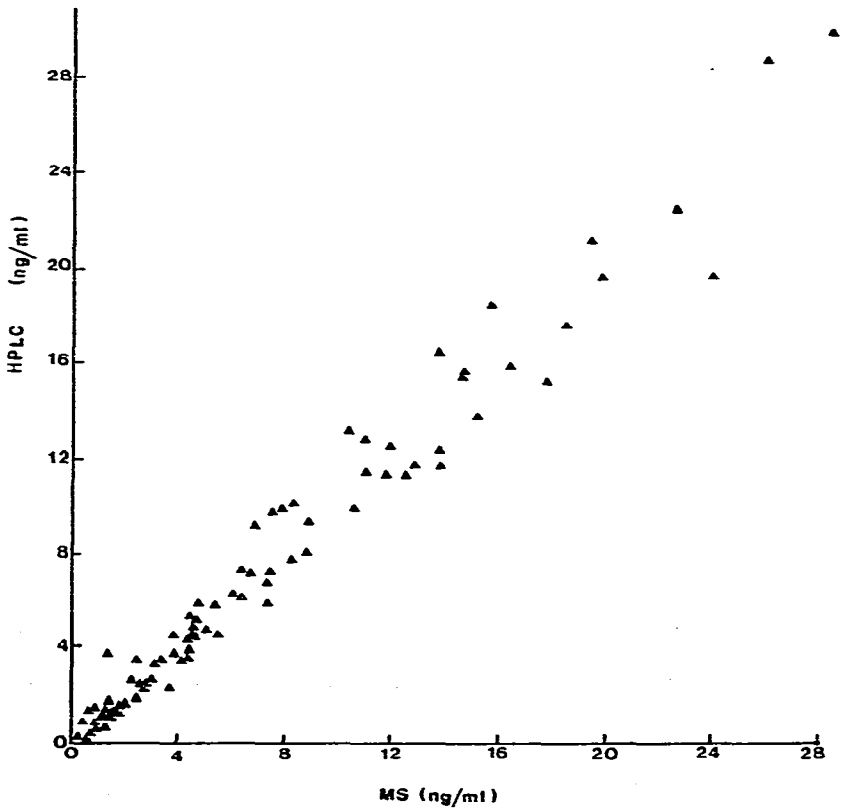

Fig. 3. Comparison of plasma concentrations determined for five volunteers after ingestion of 50 mg of chlorpromazine and analyzed by the described GC—MS procedure as well as by an HPLC—ElCD procedure [21]; $n=90$, $r^2 = 0.9617$, and slope = 0.9713.

of chlorpromazine in patients undergoing chronic treatment, as well as even for the assessment of bioavailability/pharmacokinetic parameters of chlorpromazine in healthy volunteers receiving low single oral doses. These methods will now be used to determine reliable and meaningful clinical pharmacokinetics of chlorpromazine, a drug for which studies of plasma levels—clinical response correlations have not been conclusive [2, 3, 5, 17, 24—27].

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