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## Note

# Plasma prochlorperazine assay by high-performance liquid chromatography—electrochemistry

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Prochlorperazine, 2-chloro-10-[3-(4-methyl-1-piperazinyl)propyl]phenothiazine, is a phenothiazine which is used clinically primarily to treat nausea and vomiting. Until the application of electrochemical detection [1] it was not possible to measure prochlorperazine in plasma samples following administration of a therapeutic dose. However, this assay method was not found to be reproducible in our hands. We therefore developed another method for the measurement of prochlorperazine in plasma samples. This has been used to measure prochlorperazine in plasma of both normal volunteers and patients.

## EXPERIMENTAL

### *Materials*

Prochlorperazine maleate was supplied by May and Baker (Dagenham, U.K.); chlorpromazine hydrochloride was obtained from Sigma (Poole, U.K.). All solvents were Fison's HPLC grade (Loughborough, U.K.). Sodium hydroxide, ammonium dihydrogen orthophosphate and EDTA were analytical-reagent grade (BDH, Poole, U.K.). Acetonitrile, chloroform and doubly glass-distilled water were filtered prior to use through 0.5-$\mu$m and 0.45-$\mu$m filters (Millipore, Bedford, MA, U.S.A.), respectively.

### *Chromatography*

A Waters Model 510 LC pump (Northwich, U.K.) in conjunction with a Bioanalytical Systems electrochemical cell (glassy carbon electrode) and LC-4B amperometric detector (West Lafayette, IN, U.S.A.) were used. The high-per-

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formance liquid chromatography (HPLC) column was a Spherisorb 5-$\mu$m nitrile normal phase, 25 cm × 4.6 mm I.D. (HPLC Technology, Macclesfield, U.K.).

The mobile phase was 0.1 *M* $NH_4H_2PO_4$ (containing 50 mg/l EDTA) adjusted to pH 6.5 with ammonia, and acetonitrile (40:60) which was degassed before use. The flow-rate was 2 ml/min and the system had an operating pressure of 167 bar. The detector voltage was set in oxidising mode at 0.85 V.

*Assay method*

Stock solutions of prochlorperazine and chlorpromazine (the internal standard), 100 $\mu$g/ml each, were made up in methanol and stored at 4°C. A standard concentration curve was constructed over the range 0, 5, 10, 20 and 50 ng/ml prochlorperazine in plasma.

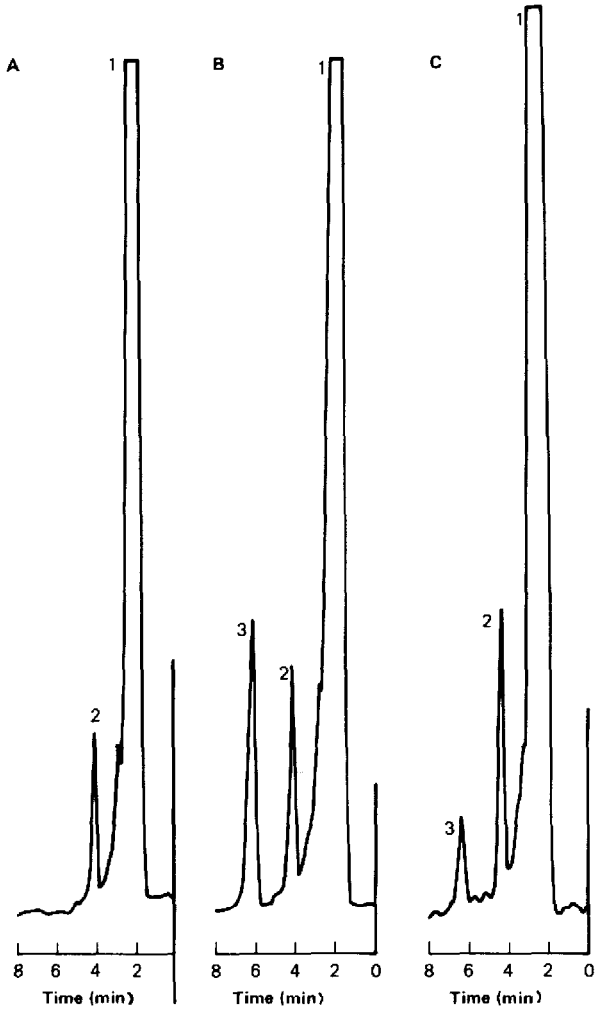


Fig. 1. Typical chromatograms of (A) blank plasma containing internal standard, (B) blank plasma containing 20 ng/ml prochlorperazine from standard curve and (C) plasma sample from normal volunteer, prochlorperazine measured at 5.3 ng/ml. Peaks: 1 = solvent front; 2 = chlorpromazine (internal standard); 3 = prochlorperazine.

The extraction was performed in 30-ml glass tubes with ground-glass stoppers (it had been found that unacceptable random contamination occurred when using PTFE-lined screw-top tubes). To 2 ml of plasma, chlorpromazine (to give a concentration of 15 ng/ml), 200 $\mu$l of 5 *M* sodium hydroxide and 10 ml of chloroform were added. The standard curve was constructed in a similar way using blank drug-free plasma. The tubes were shaken for 10 min, allowed to stand in an ice bath for at least 30 min and centrifuged for 10 min at 4°C, 2800 rpm (RCF = 1578). The aqueous layer was discarded, and the organic layer evaporated under nitrogen at 40°C. The residue was redissolved in 200 $\mu$l mobile phase and injected onto the HPLC system. Retention times were 4 min for chlorpromazine and 6 min for prochlorperazine.

*Analysis of clinical samples*

Samples from a study of the pharmacokinetics of prochlorperazine have been assayed. Normal volunteers had 12.5 mg prochlorperazine administered intravenously and blood samples were taken at specified intervals. In addition, samples obtained from patients receiving high-dose prochlorperazine for cytotoxic-induced nausea and vomiting have been analysed.

## RESULTS AND DISCUSSION

*Analytical method*

Extraction efficiency was only 60%, but this is probably accounted for by the number of steps in the extraction procedure. Despite the relatively poor extraction, the assay was found to be reproducible.

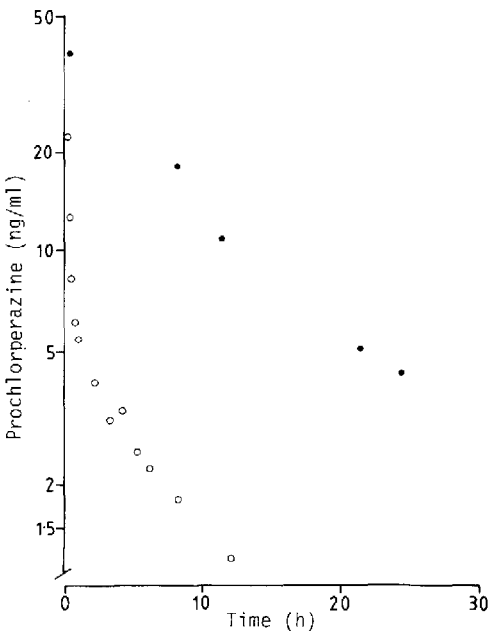


Fig. 2. Plasma concentration–time plots from a volunteer (○) and a patient (●) receiving 12.5 mg intravenously and an infusion of 50 mg prochlorperazine intravenously, respectively.

The standard curve used for sample concentration calculation was linear to 50 ng/ml prochlorperazine in plasma (mean correlation coeffient 0.9970 ± 0.002, $n$ = 21). The within-assay coefficient of variation at 2 ng/ml was 8% and at 5 ng/ml was 6%. The between-assay coefficient of variation for the quality control (15 ng/ml) was 6%. Minimum quantifiable level of prochlorperazine was 1 ng/ml of plasma.

Fig. 1 illustrates typical chromatograms. Fig. 2 shows plasma concentration–time profiles for prochlorperazine in a normal volunteer receiving 12.5 mg prochlorperazine intravenously and in a patient receiving 50 mg prochlorperazine for cytotoxic-induced vomiting.

## CONCLUSION

In summary a simple method has been devised for the assay of prochlorperazine in plasma samples. This method has proved useful for the measurement of prochlorperazine in clinical trials and patients; the results of these studies will be published elsewhere [2].

## ACKNOWLEDGEMENTS

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