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Note

Measurement of meptazinol in plasma by high-performance liquid chromatography with electrochemical detection

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Meptazinol, an effective analgesic agent for the treatment of moderate pain, is now available in both oral and parenteral formulations. Previous methods for the measurement of this drug in plasma have used the techniques of gas—liquid chromatography with flame ionisation detection [1] and high-performance liquid chromatography (HPLC) with fluorescence detection [2]. The method of Rosseel et al. [1] was not sufficiently sensitive for the measurement of therapeutic plasma concentrations, with a lower limit of detection of 30 μg/l. The method of Frost [2] improves upon this with a lower limit of detection of 3 μg/l from a 1-ml plasma sample. However, the sensitivity of detection of meptazinol by fluorescence is restricted by the similarity in the excitation (282 nm) and emission (300 nm) wavelengths. Sensitivity is further reduced if a high-resolution detector is not available.

This paper describes a method using the technique of electrochemical detection following HPLC separation with the advantages of increased sensitivity and general availability, a simple sample preparation procedure and a reduced sample volume requirement over previously published methods.

EXPERIMENTAL

Materials and reagents

Meptazinol [*m*-(3-ethyl-1-methylhexahydro-1H-azepin-3-yl] phenol (Fig. 1A) was obtained from Wyeth Labs. (Taplow, U.K.). The internal standard used was a 40 μg/l solution of fenethazine (supplied by Rhone-Poulenc, France) (Fig. 1B) in 2 *M* aqueous tris(hydroxymethyl)aminomethane (analytical-reagent grade) solution. Methanol was HPLC grade and the glacial acetic acid and ammonia solution (SG 0.88) were analytical-reagent grade.

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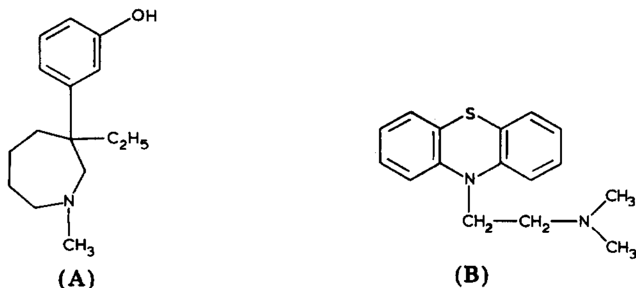

Fig. 1. Structural formulae of (A) meptazinol and (B) fenethazine, internal standard.

High-performance liquid chromatography

The solvent delivery system was a single, high-speed piston pulseless pump (Applied Chromatography Systems, Model 300/01/02) and sample injection was performed using a Rheodyne Model 7120 syringe-loading valve fitted with a 100-μl sample loop. Stainless-steel tubing (0.25 mm I.D.) was used to connect the outlet of the valve to the analytical column, a stainless-steel tube 125 mm × 5 mm I.D. packed with 5-μm silica (Spherisorb S5W: Hichrom, Woodley, U.K.), which was used at ambient temperature (normally 22°C). Detection was by electrochemical oxidation using a glassy carbon electrode LCA 15 (EDT Research, London, U.K.) in a flow cell and at a potential of +1.2 V applied against a silver/silver chloride reference electrode. The mobile phase was methanol—acetic acid—ammonia solution (996:3:1). The flow-rate was 2 ml/min.

Sample preparation

Plasma (200 μl) was pipetted into a small glass (Dreyer) test tube. Internal standard solution (100 μl) and methyl *tert.*-butyl ether (200 μl) were added using Hamilton repeating mechanisms fitted with 5-ml Hamilton gas-tight Luer fitting glass syringes. The contents of the tube were vortex-mixed for 30 sec and the tube was centrifuged at 9950 *g* for 2 min. A portion (ca. 110 μl) of the organic phase was taken to fill the sample loop of the injection valve.

To increase sensitivity where sufficient sample was available, plasma (1 ml) was pipetted into a 5-ml stoppered polypropylene tube (Elkay Products, Shrewsbury, MA, U.S.A.). Internal standard solution (300 μl) and methyl *tert.*-butyl ether (300 μl) were added. The contents of the capped tube were vortex-mixed (30 sec) after which the tube was centrifuged at 3000 *g* for 5 min. The organic phase was transferred to a Dreyer tube and recentrifuged at 9950 *g* for 2 min. A portion (110 μl) of the extract was taken to fill the sample loop of the injection valve. Analyses were performed in duplicate and mean results taken.

Instrument calibration

Standard solutions containing meptazinol at concentrations of 5, 20, 50 and 100 μg/l were prepared in bovine plasma. On analysis of these solutions the ratio of the peak height of meptazinol to the peak height of the internal standard when plotted against meptazinol concentration was linear and passed through the origin of the graph.

RESULTS AND DISCUSSION

The chromatogram obtained on analysis of an extract of analyte-free human plasma (200 μl) is illustrated in Fig. 2A. Fig. 2B shows the chromatogram obtained on analysis of an extract of a plasma standard (1 ml) containing 1 μg/l meptazinol, and Fig. 2C shows the chromatogram obtained on analysis of an extract (200 μl) of a plasma sample from a patient 6 h after receiving a single 25-mg intramuscular dose of meptazinol.

The recovery of the sample preparation was constant and virtually complete at over 98% (n = 5). The intra-assay coefficients of variation (C.V.) measured

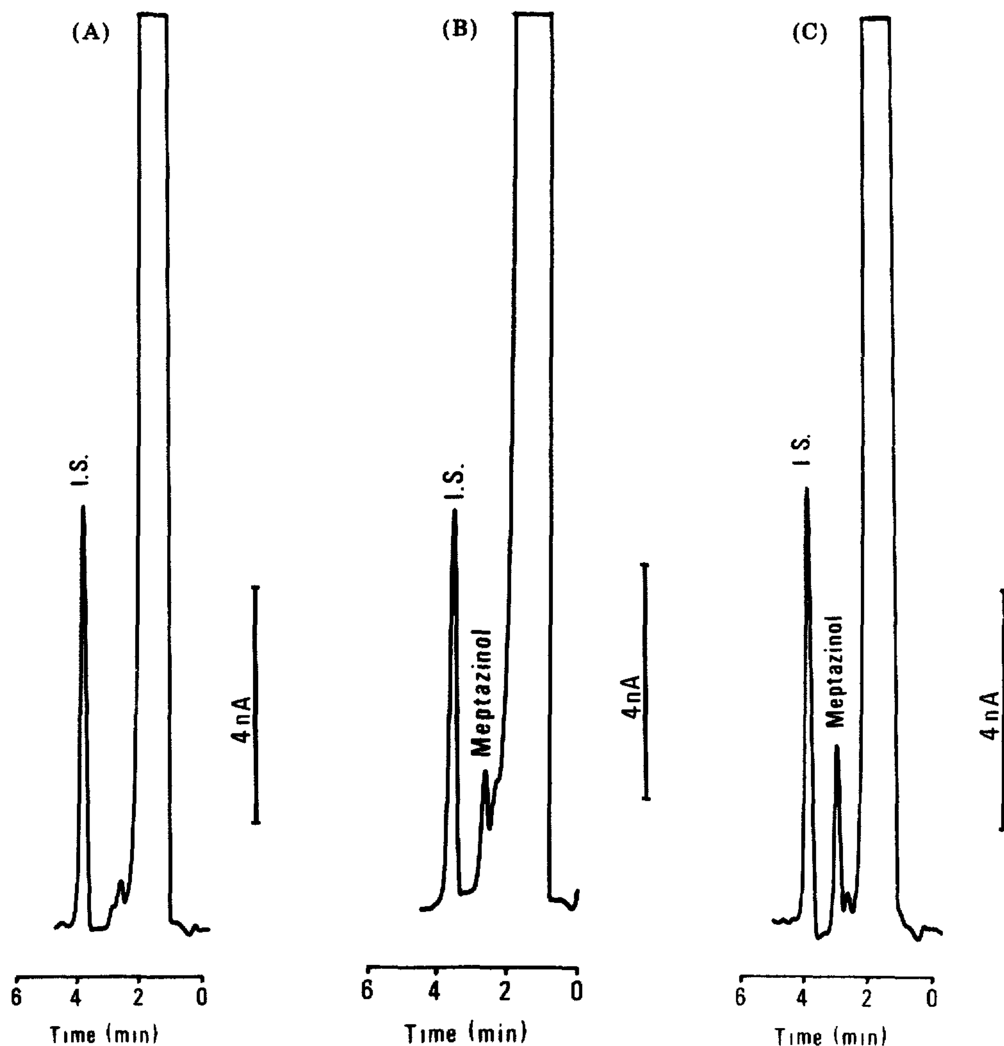

Fig. 2. Chromatograms obtained on analysis of an extract of (A) drug-free human plasma (200 μl); (B) drug-free human plasma (1 ml) containing meptazinol (1 μg/l); and (C) a plasma sample (200 μl) obtained from a patient 6 h after a 25-mg intramuscular dose of meptazinol. The concentration of meptazinol was 7 μg/l. I.S. = internal standard. For chromatographic conditions, see text.

TABLE I

INTRA-ASSAY REPRODUCIBILITY OF THE ASSAY

n = 10 at each concentration.

Meptazinol concentration (μg/l)	C.V. (%)
50	1.9
5	6.8
0.5	11.1

using three solutions prepared in bovine plasma are shown in Table I. The inter-assay C.V. measured from replicate analysis (n = 10) of a spiked solution of meptazinol (23 μg/l) prepared in bovine plasma was 8.0%. The lower limit of detection was 0.5 μg/l for meptazinol using a 1-ml sample.

No endogenous sources of interference have been found. The strong electrochemical response to the drug at a potential of +1.2 V in the organic mobile phase used in this method gives a significant improvement in sensitivity over previous methods. The use of straight-phase HPLC with non-aqueous ionic elements on silica columns for the analysis of basic drugs has been discussed in a previous publication [3]. Similarly, the technique of using highly efficient rapid micro-extraction of small sample volumes developed for HPLC [4] has been adapted and incorporated into this method.

This method has been used to measure the plasma concentration of meptazinol in man after intravenous and intramuscular doses for up to 24 h. Pharmacokinetic studies on meptazinol have shown a short elimination half-life

TABLE II

PLASMA CONCENTRATIONS OF MEPTAZINOL OBTAINED AFTER BOTH INTRAVENOUS (i.v.) AND INTRAMUSCULAR (i.m.) ADMINISTRATION OF 25 mg IN A VOLUNTEER SUBJECT

Time (h)	Concentration (μg/l)	
	25 mg i.v.	25 mg i.m.
0	ND*	ND
0.25	93	71
0.50	56	89
0.75	57	86
1	40	79
2	29	43
3	25	39
4	18	26
5	13	20
6	8	17
8	5	13
12	3	6
24	0.3	3

*ND = Not detected.

(of the order of 2 h) and a low oral bioavailability [5]. The present method has sufficient sensitivity to follow the elimination of meptazinol from plasma over a period of 24 h, as can be seen in Table II.

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REFERENCES

1 M.T. Rosseel, M.G. Bogaert, F.M. Belpaire and W. Oosterlinck, Curr. Med. Res. Opin., 3 (1975) 181.
2 T. Frost, Analyst, 106 (1981) 999.
3 R.J. Flanagan, G.C.A. Storey, R.K. Bhamra and I. Jane, J. Chromatogr., 247 (1982) 15.
4 R.J. Flanagan, G.C.A. Storey and D.W. Holt, J. Chromatogr., 187 (1980) 391.
5 R.A. Franklin, A. Aldridge and C.B. White, Brit. J. Clin. Pharmacol., 3 (1976) 497.