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DETERMINATION OF ZOMEPIRAC IN PLASMA BY HIGH-PRESSURE LIQUID CHROMATOGRAPHY

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SUMMARY

A sensitive, specific and precise high-pressure liquid-chromatographic method for the determination of the analgesic agent, zomepirac, in plasma samples is described. The lowest concentration of zomepirac that can be measured accurately and precisely (coefficient of variation $<20\%$) in a 2-ml plasma sample is 10 ng/ml. The standard curve is linear in the concentration range of 10 to 5000 ng/ml. To date, this procedure has been employed successfully in analysing over 10,000 clinical plasma samples.

INTRODUCTION

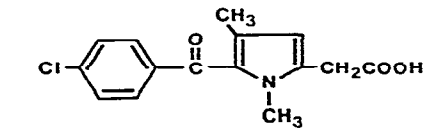
Zomepirac [5-(4-chlorobenzoyl)-1,4-dimethyl-1H-pyrrole-2-acetic acid] (I) is a new non-narcotic analgesic drug[1–4]. The pharmacokinetics and the disposition of zomepirac in man have been reported[5–6]. This paper describes a high-pressure liquid-chromatographic (HPLC) method that has been employed successfully for determining I in plasma samples from subjects in clinical studies. The procedure is sensitive, specific and simple to perform; an average of 36 plasma samples can be analysed routinely during an 8-h working day.

EXPERIMENTAL

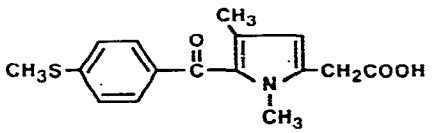
Reagents

Sulphuric acid, isoamyl alcohol and glacial acetic acid were of analytical grade (Mallinckrodt, St. Louis, Mo., U.S.A.). Hexane and isopropanol were of glass-distilled grade (Burdick and Jackson, Muskegon, Mich., U.S.A.). Heptane was of glass-distilled grade (Pollard and Company, Wilmington, Del., U.S.A.). Diethyl ether was of reagent grade (anhydrous, Mallinckrodt). Monobasic potassium phosphate and dibasic sodium phosphate used in the preparation of the pH 7.4 buffer were of certified ACS grade (Fisher Scientific, Pittsburgh, Pa. U.S.A.).

Two analogues of zomepirac (II and III) have been used successfully as the internal standard; compound III has a longer retention time, but has better stability in plasma extracts than II. I was obtained as the sodium salt dihydrate. II and III were obtained as free acids (McNeil Laboratories, Fort Washington, Pa., U.S.A.).



(I)



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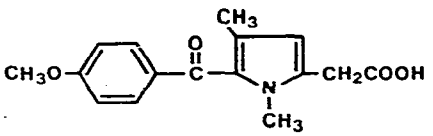

(III)

Plasma standard solutions

Plasma standards (volume: 10.0 ml) containing 10–5000 ng of I per ml of plasma were prepared as follows: 0.5 ml of an aqueous solution of zomepirac sodium, containing the appropriate amount (0.2–100.0 μg equiv.) of I was added to 9.5 ml of drug-free plasma.

Glass equipment

Disposable screw-top bottles (volume: 14.2 ml) with polyethylene-lined caps and 12-ml centrifuge tubes (conical bottom) were used for extractions. Before use, all glassware was soaked in detergent for 2 h, rinsed thoroughly with distilled water and heat-treated for 3 h at 270°. Polyethylene-lined screw caps were soaked in *n*-heptane for 1 h and dried at 60° before use.

Extraction procedure

To each sample of plasma (2.0 ml), containing I as standard or unknown in a disposable screw-top bottle were added 1.0 ml of pH 7.4 phosphate buffer and 10.0 ml of ether containing 1.0 μg of internal standard. The capped bottle was then shaken for 15 min on a table-top shaker (Eberbach Corp.) at 120 oscillations per minute and centrifuged at 681 *g* for 10 min. The supernatant ether layer was aspirated and discarded. A 0.5-ml aliquot of 6 *N* sulphuric acid and 0.9 ml of 1.5% isoamyl alcohol in heptane were added to the aqueous layer, and the bottle was capped, shaken and centrifuged as before. Then 8.0 ml of the supernatant organic layer were transferred to a 12-ml centrifuge tube and evaporated to dryness under a stream of dry nitrogen at room temperature. The dried plasma extract was kept refrigerated until a few minutes before chromatographic analysis to minimize decomposition of both I and the internal standard. A 50-μl portion of 2% isopropanol in hexane was added to the residue and mixed on a Vortex mixer for 5 sec; 20 μl of the resulting solution was injected into the liquid chromatograph.

Liquid chromatography

The HPLC system used was a Waters Model ALC/GPC 204 liquid chromato-

graph equipped with a U6K injector. A Waters Model 440 dual-channel UV detector with a 313-nm wavelength filter was employed. The column (25 cm × 2.0 mm I.D.) was of stainless steel and was packed with 10-μm LiChrosorb® Si 60 (E. Merck, Darmstadt, G.F.R.). The mobile phase [hexane–isopropanol–glacial acetic acid (93:2:5, v/v/v)] was prepared fresh daily and was filtered through a 0.45-μm Millipore® filter (Millipore Corp., Bedford, Mass., U.S.A.), and the column was conditioned with 30 ml of mobile phase before use. After conditioning the column, the flow-rate was held at 2.5 ml/min. The retention times for I, II and III were 2.4, 3.4 and 4.2 min, respectively (see Fig. 1).

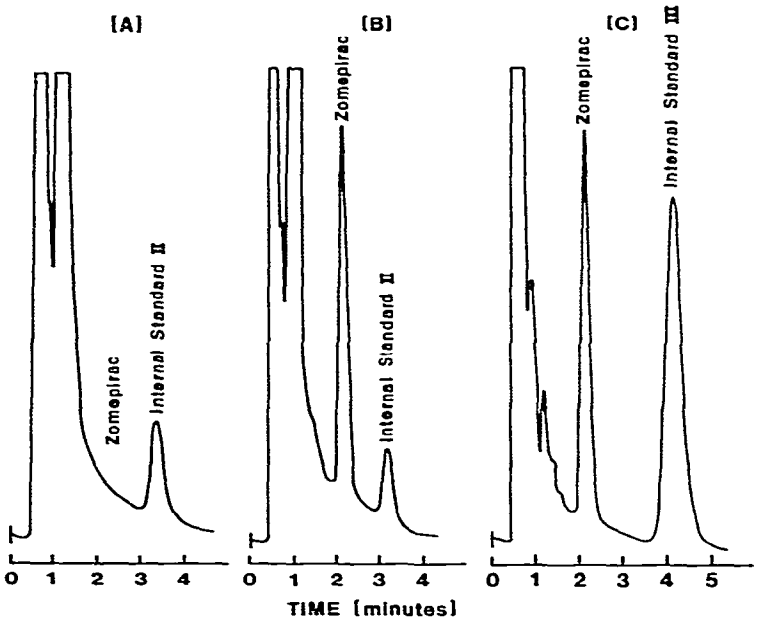

Fig. 1. Liquid chromatograms from: (A), 2 ml of blank plasma containing 200 ng of internal standard II; (B), 2 ml of plasma containing 400 ng of zomepirac and 200 ng of internal standard II; and (C), 1 ml of plasma containing 400 ng of zomepirac and 1000 ng of internal standard III.

Quantitation

Standard curves for I in plasma have been prepared by analysing standard plasma solutions according to the procedure described above. Ratios of the peak heights (I to internal standards) were plotted against concentrations of I (Figs. 2 and 3). Linear regression analysis was performed on the data. The standard curve was found to be linear in the concentration range of 10–5000 ng of I per ml of plasma with either II or III as internal standard. Therefore, peak-height ratios were used in determining zomepirac concentrations in plasma samples.

RESULTS AND DISCUSSION

Sensitivity

Compound I and internal standards II and III all absorb strongly at 313 nm

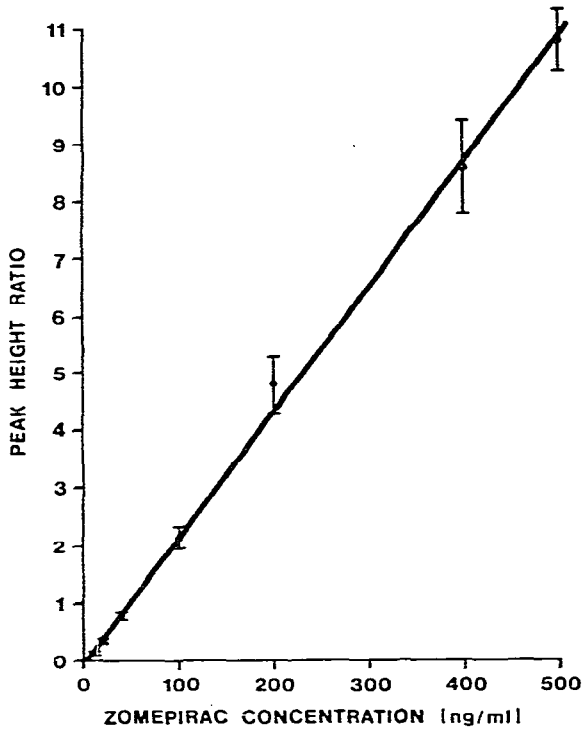

Fig. 2. Standard curve for zomepirac in plasma with II (200 ng per sample) as internal standard. Points and vertical bars represent the mean ± standard deviation values of six separate determinations at each concentration. The straight line was obtained by least squares analysis for best fit (correlation coefficient is 0.994).

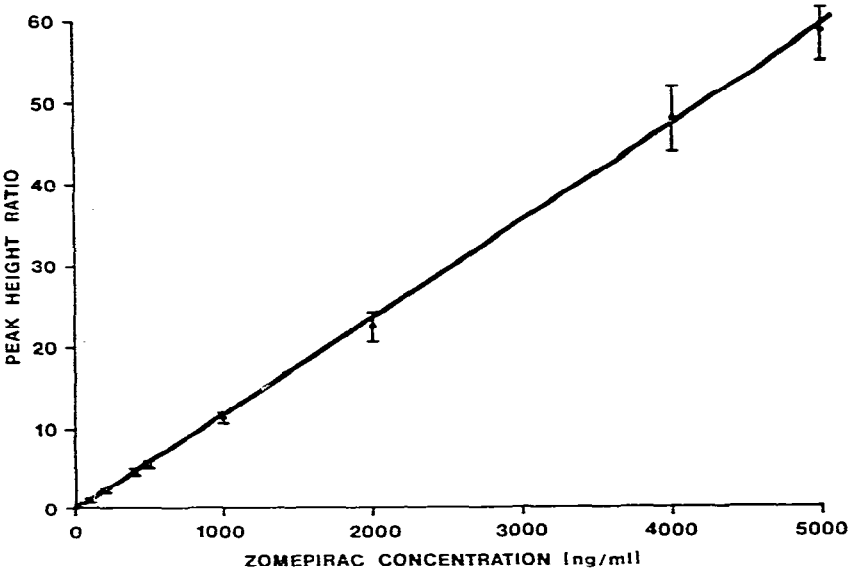

Fig. 3. Standard curve for zomepirac in plasma with III (1000 ng per sample) as internal standard. Points and vertical bars represent the mean ± standard deviation of six separate determinations at each concentration. The straight line was obtained by least squares analysis for best fit (correlation coefficient is 0.997).

(*e.g.*, for zomepirac sodium in methanol, $\varepsilon = 11{,}700$). When 2 ng of I was injected into the liquid chromatograph under the stated conditions, a peak with a signal-to-noise ratio of 12 was obtained.

The lowest concentration of I that has been determined quantitatively in 1–2 ml of plasma is 10 ng/ml. This is adequate when it is considered that the average peak plasma concentration of I following a therapeutic dose (100 mg) is *ca.* 4000 ng/ml.

Stability

Freshly prepared plasma standard solutions were compared with plasma standard solutions frozen at −10° for 2 months. The variations in peak-height ratio at each drug level between 10 and 5000 ng/ml were insignificant. However, compound I and internalstandards II and III were found to decompose slowly in dried plasma extract even when stored at −10° overnight; thus, extraction and injection of samples should be performed on the same day. Also, sample extracts should be kept in a refrigerator before injection.

Internal standards

Compound II was originally chosen as the internal standard because of its shorter retention time. However, compared with II, compound III offers better stability in plasma extracts and improved reproducibility in plasma samples containing high levels of I (2000–5000 ng/ml). Therefore, III was employed as the internal standard for analysing plasma samples in recent clinical studies.

Recovery

For 200 ng of I, 200 ng of II and 1000 ng of III seeded in 2 ml of plasma, the total recoveries after extraction were 83.0 ± 2.9%, 78.3 ± 3.7% and 80.5 ± 3.4%, respectively (mean ± S.D. for eight determinations).

Standard curves

A standard curve prepared by analysing plasma standard solutions with II as the internal standard is shown in Fig. 2. Excellent correlation was observed between the peak-height ratios and the zomepirac plasma concentrations. Linear regression analysis gave a correlation coefficient of 0.994, with a student's t of 121. A similar standard curve with III as internal standard is shown in Fig. 3. Again, excellent correlation was observed between peak-height ratios and the zomepirac plasma concentrations. Linear regression analysis gave a correlation coefficient of 0.997, with a Student's t value of 95.

For 2.0-ml plasma samples, the standard curve was found to be non-linear above 5000 ng/ml. Therefore, samples with concentrations above this level should be diluted with blank plasma before analysis.

Reproducibility

The reproducibility of the assay was very good, as is shown in Table I. For six independent determinations at each concentration over the course of 2 weeks, the coefficient of variation was less than 10% for 100–5000 ng/ml, and no more than 20% for the range of 10–100 ng/ml.

Application of the procedure to plasma samples

To date, this procedure has been employed successfully in analysing over 10,000

TABLE I

MEAN PEAK-HEIGHT RATIO OF ZOMEPIRAC TO INTERNAL STANDARD III, STANDARD DEVIATION AND COEFFICIENT OF VARIATION OF WORKING STANDARD CURVES PREPARED BY ANALYSING SEEDED PLASMA SAMPLES OVER THE COURSE OF TWO WEEKS

Each sample contained 1.0 μg of internal standard. Each result is the mean of six determinations.

Zomepirac plasma concn. (ng/ml)	*Mean peak-height ratio*	*Standard deviation*	*Coefficient of variation (%)*
10	0.10	0.01	9.7
20	0.17	0.03	20.0
40	0.40	0.04	9.8
100	1.05	0.11	10.8
200	2.01	0.15	7.6
400	4.45	0.42	9.4
500	5.23	0.49	9.3
1000	11.18	0.69	6.2
2000	22.35	1.88	8.4
4000	47.57	4.21	8.9
5000	57.96	3.25	5.6

clinical samples for I. No interference peaks due to endogenous materials have ever been observed with the clinical samples. A typical plasma concentration profile of I in man is shown in Fig. 4.

The present procedure has also been utilized to assay for I in plasma samples from rats, mice, monkeys and dogs; the results, so far, have been comparable in precision and accuracy to those from human samples.

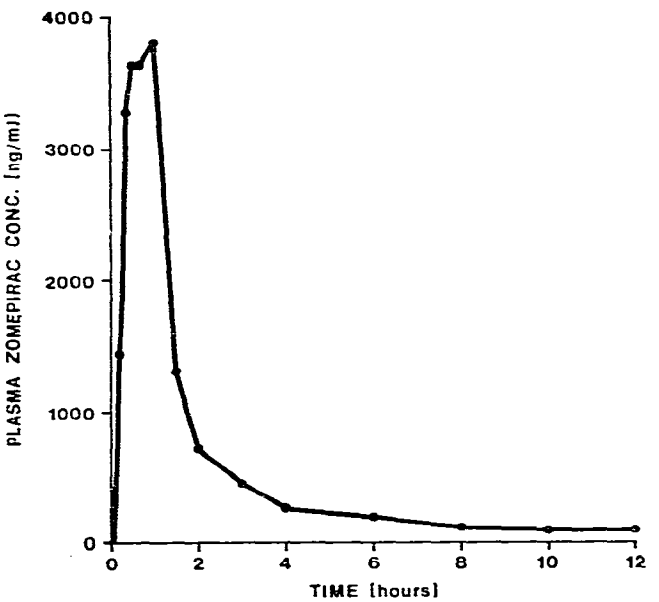

Fig. 4. Plasma-concentration profile of zomepirac in a human subject following oral administration of 100 mg of zomepirac as two 50-mg tablets.

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