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SENSITIVE GAS CHROMATOGRAPHIC QUANTITATION OF ZOMEPIRAC IN PLASMA USING AN ELECTRON-CAPTURE DETECTOR

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SUMMARY

A highly sensitive, specific and precise gas chromatographic method for the determination of the non-narcotic analgesic agent, zomepirac, in plasma is described. The pentafluorobenzyl ester derivative of zomepirac has been prepared. This enables the detection of zomepirac down to picogram levels using electron-capture detection. The lowest concentration of zomepirac which can be measured accurately and precisely (coefficient of variation <15%) is 5 ng/ml in a 2-ml plasma sample or 25 ng/ml in a 0.1-ml plasma sample. Two previously reported high-performance liquid chromatographic (HPLC) assays have detection limits of 50 ng/ml for 1-ml samples and 10 ng/ml for 2-ml samples, respectively. The present method is very useful when small sample size or interference is causing problems with the HPLC assay. This assay has been employed successfully in analyzing plasma samples from humans and monkeys as well as samples from rat milk.

INTRODUCTION

Zomepirac sodium [sodium 5-(4-chlorobenzoyl)-1,4-dimethyl-1H-pyrrole-2-acetate dihydrate] is a new non-narcotic analgesic drug [1—3]. The pharmacokinetics and the disposition of zomepirac in man have been reported [4—6].

Two high-performance liquid chromatographic (HPLC) assays for the determination of zomepirac in plasma have been published [7, 8]. These HPLC methods offer sufficient sensitivity (detection limits [7, 8]: 10 ng/ml for 2-ml samples and 50 ng/ml for 1-ml samples) for analyzing clinical plasma samples. However, when analyzing plasma samples from different animal species or from patients with concomitant medication, small sample size and interference may cause problems with the HPLC methods. The availability of another highly specific and sensitive assay would be very helpful in these situations.

This paper describes a gas chromatographic (GC) assay for the determination

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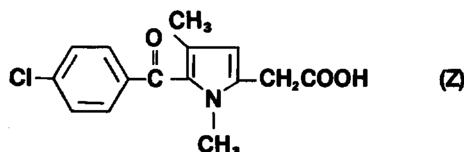
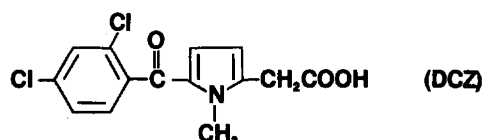
of zomepirac in plasma. The assay can readily be adapted for micro-samples. This paper also describes the pentafluorobenzylation of zomepirac by extractive alkylation. Extractive alkylation affords a method of isolating polar compounds with simultaneous derivatization. The derivatization step is carried out by adding the organic phase containing the derivatizing agent (pentafluorobenzyl bromide) to the aqueous phase containing the compound. The compound is derivatized and extracted into the organic phase in a single step.

EXPERIMENTAL

Reagents

Hydrochloric acid, potassium carbonate, toluene and isoamyl alcohol were analytical grade (Mallinckrodt, St. Louis, MO, U.S.A.). Ethyl acetate was nanograde (Mallinckrodt). Heptane was glass-distilled grade (Pollard and Company, Wilmington, DE, U.S.A.). Diethyl ether was reagent grade (anhydrous diethyl ether, Mallinckrodt). Pentafluorobenzyl bromide (PFBB) was from Pierce (Rockford, IL, U.S.A.).





A dichloro analogue of zomepirac (DCZ) was used as the internal standard. Zomepirac (Z) was obtained as the sodium salt dihydrate. DCZ was obtained as the free acid (McNeil Pharmaceutical, Spring House, PA, U.S.A.).

Plasma standard solutions

Plasma standards (volume: 10.0 ml) with zomepirac free acid concentrations ranging from 5 to 500 ng/ml were prepared as follows: 0.5 ml of an aqueous solution of zomepirac sodium, containing the appropriate amount of the zomepirac free acid, was added to 9.5 ml of drug-free plasma to give a total volume of 10.0 ml.

The internal standard solution was prepared by first dissolving 3.0 mg of DCZ in 1.0 ml of methanol. This was followed by two successive dilutions (1:100 and 1:1000) with diethyl ether to arrive at a final concentration of 30 ng/ml.

Glass equipment

Disposable screw-top bottles (volume: 28.3 ml) with polyethylene-lined caps and 15-ml centrifuge tubes with PTFE-lined screw caps were used for extraction and derivatization, respectively. Prior to use, all glassware was soaked in

chromic acid for 1 h, rinsed thoroughly with distilled water and treated for 3 h at 270°C. The PTFE-lined screw caps were soaked in *n*-heptane for 1 h and dried at 60°C.

Extraction and derivatization procedure

To each sample of plasma (0.1—2.0 ml), containing zomepirac as standard or unknown in a 28.3-ml disposable screw-top bottle, were added 1.0 ml of 1 *N* hydrochloric acid and 10.0 ml of diethyl ether containing 300 ng of internal standard. The capped bottle was then shaken for 15 min on a table-top shaker (Eberbach) at 120 oscillations per minute and centrifuged at 681 *g* for 10 min. An 8.0-ml aliquot of the supernatant diethyl ether layer was transferred to another 28.3-ml bottle containing 9.0 ml of 0.1 *N* sodium hydroxide. The mixture was shaken for 15 min and centrifufed for 10 min. The supernatant ether layer was aspirated and discarded. Two ml of 1 *N* hydrochloric acid and 10.0 ml of 1.5% isoamyl alcohol in heptane were added to the aqueous layer. The mixture was shaken for 15 min and centrifuged for 10 min. An 8.0-ml aliquot of the supernatant organic layer was transferred to a 15-ml centrifuge tube and evaporated to dryness under a stream of dry nitrogen at room temperature. To the dried plasma extract, 1.0 ml of 1 *M* K_2CO_3 and 1.0 ml of ethyl acetate solution containing 0.5% v/v PFBB were added. The centrifuge tube was capped and heated in an oven at 40°C overnight. After cooling to room temperature, 0.5 ml of supernatant organic phase was pipetted into another centrifuge tube and evaporated to dryness under nitrogen. The residue was reconstituted with 500 μl (50 μl for 0.1-ml plasma samples) of toluene and mixed on a Vortex mixer for 5 sec. A 3-μl aliquot of the resulting solution was then injected directly into the gas chromatograph.

Mass spectrometry

For the positive identification of the pentafluorobenzyl (PFB) ester derivatives of zomepirac (PFB-Z) and the internal standard (PFB-DCZ), a Finnigan 3300 quadrupole mass spectrometer was employed in conjunction with the manufacturer's Model 9500 gas chromatograph and a Finnigan Model 6100 data system. The GC—mass spectrometric (MS) system was operated in the chemical ionization (CI) mode using methane as the reagent gas. A 61.0 × 0.2 cm I.D. silanized glass column packed with 3% OV-17 on Gas-Chrom Q (60—80 mesh) was used with a methane flow-rate of 20 ml/min. During analysis the interface and the transfer line were maintained at 250°C. The column temperature was 235°C and the injection port temperature was at 280°C. The CI source was operated without external heating. The source pressure was maintained at 1 torr. The electron energy was 100 eV and electron beam emission was adjusted to 0.5 mA.

Gas chromatography

A Perkin-Elmer Model 900 gas chromatograph equipped with a ^{63}Ni electron-capture detector (ECD) was used. The column was a 122.0 × 0.4 cm I.D. silanized glass column packed with 3% OV-17 on Gas-Chrom Q (60—80 mesh). Prior to use, the column was conditioned at 280°C overnight with an argon—methane (95:5, v/v) carrier at a flow-rate of 30 ml/min.

The chromatographic conditions for the analysis were: column oven 230° C, injection port 260° C, detector 295° C. The carrier gas (argon—methane, 95:5, v/v) flow-rate was 70 ml/min. The retention times for the PFB ester derivatives of zomepirac (PFB-Z) and of the internal standard (PFB-DCZ) were 6.4 min and 9.0 min, respectively (Fig. 1).

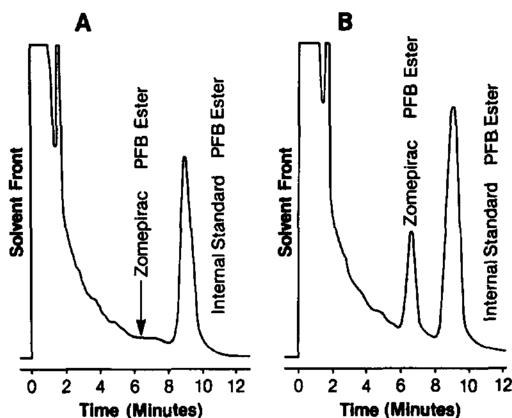


Fig. 1. Gas chromatograms from (A) predose monkey plasma sample (samples without internal standard showed no peak at around 9.0 min) and (B) monkey plasma sample 12 h after oral administration of 10 mg/kg of zomepirac sodium showing 53 ng/ml of zomepirac and 100 ng/ml of internal standard.

Quantitation

Standard curves for zomepirac in plasma were prepared by analyzing standard plasma solutions according to the procedure described above. Ratios of the peak areas (zomepirac/internal standard) were plotted against concentrations of zomepirac. Linear regression analysis was performed on the data.

RESULTS AND DISCUSSION

Reaction conditions

Pentafluorobenzylation of organic acids and phenols has previously been investigated [9, 10]. Derivatization occurs when the acid or phenol is heated with PFBB in strongly basic acetone or alcohol for some hours. More recently, an extractive alkylation technique has successfully been employed [11, 12] for derivatization. In extractive alkylation, organic acids in a basic aqueous medium are extracted by means of a positively charged counter ion (e.g. tetrabutylammonium ion) into an organic phase containing PFBB. In a poorly solvating organic phase, the anion of the organic acid becomes a highly reactive nucleophile in the displacement of bromide ion from the derivatizing reagent. However, if the partition ratio between the organic and aqueous phases is very low, as in the case of short chain fatty acids, the extraction of the organic acids into the organic phase will be inefficient and the reaction will be very slow and incomplete [13]. Since the partition coefficient of an organic acid is a function of the organic phase (e.g., lipophilicity or polarity properties) and of the pH of

the aqueous phase, reaction conditions for an individual organic acid can be optimized by manipulation of these two parameters.

With zomepirac (Z) and the internal standard (DCZ), pentafluorobenzylation in the presence of tetrabutylammonium ion was rapid and complete when using ethyl acetate as the organic phase and 1 *M* K_2CO_3 as the aqueous phase. At pH values greater than 12, however, an additional derivative of zomepirac was obtained. The exact structure of the second derivative has not been identified. Preliminary results from MS (CI) indicated that this derivative contained two PFB groups. It was also found that in the absence of the counterion, the derivatization was completed for both Z and DCZ within 4 h at 40°C. By leaving out the counter ion, the derivatization reaction became selective. This resulted in much cleaner chromatograms. Because of the relatively long (4 h) reaction time, the reaction mixture was kept overnight in the oven.

Reaction mixtures were subjected to GC—MS analysis. Only one derivative was found for both zomepirac (Z) and the internal standard (DCZ). The chemical ionization spectrum for the PFB derivative of zomepirac (PFB-Z) is shown in Fig. 2. Major *m/e* peaks observed were: 472 $(M+1)^+$, 500 $(M+29)^+$, 512 $(M+41)^+$, 139 $(ClC_6H_4CO)^+$, 436 $(M+1-HCl)^+$, 246 $(M+1-\text{PFB formate})^+$ and 360 $(M+1-\text{chlorobenzene})^+$. These ions are consistent with the addition of one PFB group to the carboxy group in zomepirac (MW = 471).

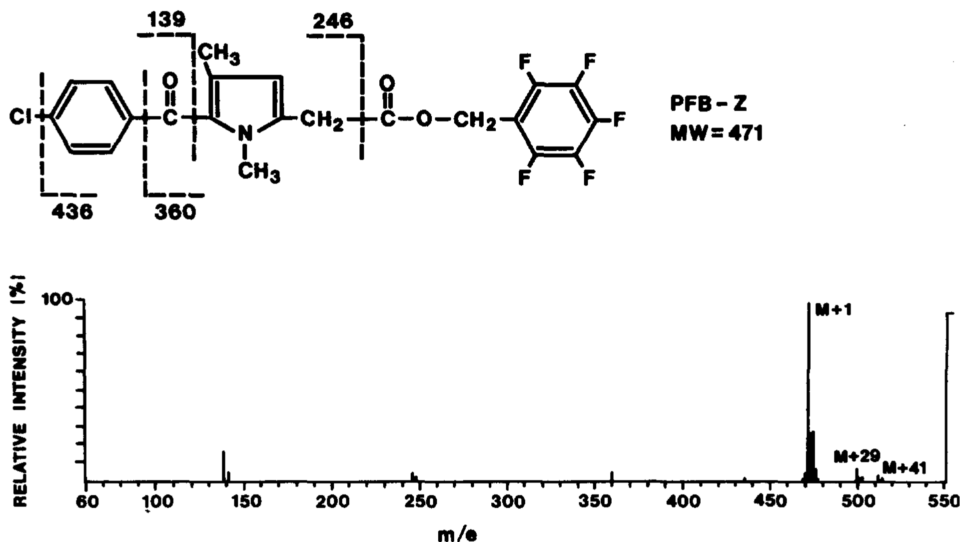


Fig. 2. Chemical ionization mass spectrum and proposed fragmentation pattern of the pentafluorobenzyl derivative of zomepirac (PFB-Z).

Sensitivity

The PFB ester derivative of zomepirac (PFB-Z) is highly electron-capture sensitive. Ten pg of this derivative when injected into the gas chromatograph under the stated conditions gave a peak with a signal-to-noise ratio of eight.

The detection limit of zomepirac that has been determined accurately and precisely (C.V. $< 15\%$) in a 2-ml plasma sample was 5 ng/ml, which is more

than adequate for all clinical samples. Furthermore, the detection limit in a 0.1-ml plasma sample was found to be 25 ng/ml, which is still adequate for most purposes. The reasons behind the low detection limit for smaller samples is quite obvious. Since only 0.3% of the derivatives in each sample was injected into the gas chromatograph in the present procedure, the percentage of sample utilized can be readily increased by using a smaller amount of toluene for reconstitution and/or by injecting a larger amount of the toluene solution. The background noise or interference in the chromatogram was found to be directly proportional to plasma sample size.

Interference

No interference peaks due to endogenous materials have ever been observed using the present procedure (Fig. 1). This procedure, to date, has been employed successfully to analyze over 500 zomepirac plasma and milk samples. The major circulating metabolite of Z (glucuronide conjugate) will not interfere in this procedure because it is eliminated in the extraction step.

Stability

Freshly prepared plasma standard solutions were compared with plasma standard solutions at the same drug concentrations which had been kept frozen at −10°C for two months. The variations in the peak area ratios at each drug level between 5 ng/ml and 500 ng/ml were insignificant ($p < 0.05$).

Toluene solutions of derivatized plasma extract containing PFB-Z and PFB-DCZ were examined by repeated injections of aliquots into the gas chromatograph. It was found that both PFB ester derivatives were stable in the presence of plasma extract for at least two days at room temperature. The stability of PFB compounds has previously been demonstrated [9—14].

Response curve

The linearity of the ECD response was demonstrated by injecting samples of different concentrations of the PFB ester derivatives of both Z and DCZ into the gas chromatograph. Peak area values were plotted against the absolute amounts of the derivatives injected to obtain response curves. The response curve for the zomepirac derivative was linear between 0.01 ng and 5.00 ng (as zomepirac) and the response for the internal standard derivative was linear between 0.05 ng and 5.00 ng. The detector response for both derivatives was found to be non-linear above the 5.00-ng level.

Recovery

GC response curves which correlate peak area with the amount of PFB-Z and PFB-DCZ per sample offer the possibility of determining total yields after extraction and derivatization for zomepirac (Z) and internal standard (DCZ) in this procedure. For 300 ng of Z and 300 ng of DCZ seeded in 2 ml of plasma, the total recoveries after extraction and derivatization were 76% (C.V. = 4.2%) for Z and 70% (C.V. = 4.8%) for DCZ (eight determinations).

Standard curve

Standard curves have been prepared by analyzing 0.1—2.0 ml of the plasma

standard solutions. Excellent correlation was observed between the peak area ratios and the zomepirac plasma concentrations. Linear regression analysis gave correlation coefficients of greater than 0.99 in all cases.

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 two weeks, the coefficients of variation were less than 5% in the concentration range of 50—500 ng/ml and were less than 15% in the concentration range of 5--50 ng/ml.

TABLE I

MEAN PEAK AREA RATIO OF PFB-Z TO PFB-DCZ, STANDARD DEVIATION AND COEFFICIENT OF VARIATION OF WORKING STANDARD CURVES PREPARED BY ANALYZING SEEDED PLASMA SAMPLES OVER THE COURSE OF TWO WEEKS (300 ng OF DCZ PER SAMPLE)

Zomepirac plasma concn. (ng/ml)	No. of determinations	Mean peak area ratio	Standard deviation	Coefficient of variation
0	6	0	0	0
5	6	0.026	0.004	0.15
50	6	0.252	0.009	0.04
200	6	1.121	0.029	0.03
350	6	2.023	0.063	0.03
500	6	2.952	0.057	0.02

For 2-ml plasma samples, the standard curve was non-linear above 500 ng/ml. Therefore, samples with concentrations above 500 ng/ml should be diluted with blank plasma before analysis.

Applications

The present method has been employed successfully in analyzing human and monkey plasma samples as well as rat milk samples. The long reaction time for derivatization does not affect sample throughput. An average of 28 plasma samples can be analyzed routinely during an 8-h working day. For each set of samples, extractions were performed in the afternoon with the derivatization step performed overnight, and injections were done the following morning with analysis of results completed before noon.

REFERENCES

1 S.A. Cooper, J. Clin. Pharmacol., 20 (1980) 230.
2 W.M. Baird and D. Turek, J. Clin. Pharmacol., 20 (1980) 243.
3 S.L. Wallenstein, A. Rogers, P.F. Kaiko, G. Heidrich, III and R.W. Houde, J. Clin. Pharmacol., 20 (1980) 250.
4 L.D. Muschek and J.M. Grindel, J. Clin. Pharmacol., 20 (1980) 223.
5 R.K. Nayak, K.T. Ng, S. Gottlieb and J. Plostnieks, Clin. Pharmacol. Ther., 95 (1980) 395.

6 J.M. Grindel, P.J. O'Neill, K.A. Yorgey, M.H. Schwartz, L.A. McKown, B.H. Migdalof and W.N. Wu, Drug Metab. Disp., 8 (1980) 343.
7 K.-T. Ng and T. Snyderman, J. Chromatogr., 178 (1979) 241.
8 C.L. Welch, T.M. Annesley, H.S. Luthra and T.P. Moyer, Clin. Chem., 28 (1982) 481.
9 F.K. Kawahara, Anal. Chem., 40 (1968) 1009.
10 F.K. Kawahara, Anal. Chem., 40 (1968) 2073.
11 H. Ehrsson, Acta Pharm. Suecica, 8 (1971) 113.
12 O. Gyllenhaal, H. Brötell and P. Hartvig, J. Chromatogr., 129 (1976) 295.
13 B. Davis, Anal. Chem., 49 (1977) 832.
14 W.J. Cole, J. Parkhouse and Y.Y. Yousef, J. Chromatogr., 136 (1977) 409.