Journal of Chromatography, 114 (1975) 159–165

CHROM. 8419

GAS CHROMATOGRAPHIC ANALYSIS OF 4-PRENYL-1,2-DIPHENYL-3,5-PYRAZOLIDINEDIONE (FEPRAZONE) AT THERAPEUTIC LEVELS IN HUMAN PLASMA

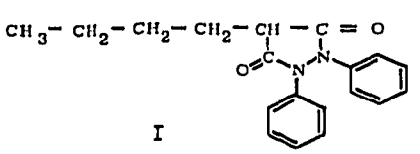
D. J. BERRY
Poisons Unit, New Cross Hospital, Avonley Road, London SE14 5ER (Great Britain)
(Received May 1st, 1975)

SUMMARY

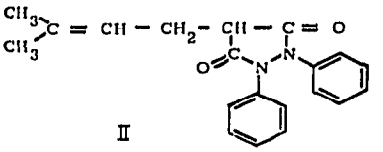
A specific and sensitive procedure for the determination of prenazone* at therapeutic levels in human plasma has been developed. The method involves extraction of the drug from acidified plasma into chloroform. After further purification by back extraction the residue is examined on a gas–liquid chromatograph fitted with a flame ionisation detector. Tetraphenylethylene is used as an internal standard for quantitation by the relative peak height technique. Plasma levels encountered after oral ingestion of therapeutic doses are reported.

INTRODUCTION

Prenazone* (II) is a new non-steroidal anti-inflammatory agent which is very similar to phenylbutazone (I) in its chemical structure. The pharmacological and toxicological properties of prenazone have been reported previously[1–5] and extended clinical trials have recently been undertaken in Great Britain. A frequently ignored aspect of such trials is the non-compliance of patients in taking their medication. In our opinion, blood level monitoring of trial drugs is highly desirable to ensure that patients, first are taking the drug and secondly, that they are doing so in the appropriate dosage.



I



II

Burns *et al.* determined phenylbutazone in plasma by a spectrophotometric method[6] and this technique can also be applied to prenazone. Phenylbutazone has also been determined in body fluids using a gas chromatographic technique[7], which is specific and more sensitive than spectrophotometry. The gas chromatographic proper-

* The approved name for prenazone has now been changed to feprazone.

ties of prenazone having been described, a method based on this procedure has now been developed for measuring prenazone in plasma[10].

An extensive study of columns and methodology for the gas–liquid chromatographic (GLC) analysis of barbiturates was recently undertaken by us[8]. Because of its acidic character and chromatographic properties, phenylbutazone was one of the few drugs that might interfere with this method, so the same procedures were investigated for prenazone analysis.

Having evolved a satisfactory method this was used to determine plasma prenazone levels following single oral therapeutic doses in six volunteers. Steady-state drug levels were also determined in patients on maintenance therapy.

EXPERIMENTAL

Reagents

The following reagents were used: Analar chloroform, redistilled before use. 0.5 *N* Sodium hydroxide and 1 *N* hydrochloric acid, prepared from concentrated solutions (Hopkin and Williams, Chadwell Heath, Great Britain) and washed three times with chloroform before use. The internal standard consisted of a solution of tetraphenylethylene (TPE) (Koch-Light, Colnbrook, Great Britain) 1.0 mg/100 ml in chloroform. Analytically pure prenazone was supplied by Istituto De Angeli (Milan, Italy).

Glass wool

The quality of the glass wool used was variable. If it was found to be so badly contaminated that it gave rise in itself to confusing peaks in the gas chromatograph, it was first washed extensively with solvents. This was conveniently achieved by treating large batches in a Soxhlet extraction apparatus.

Instrumentation

A Pye 104 Model 24 dual-column gas chromatograph, equipped with flame ionisation detectors, was used throughout in conjunction with a Hitachi 159 recorder that had been converted to 1 mV f.s.d. The column was a 5 ft. × $\frac{1}{4}$ in. I.D. coiled glass tube, which had been silanised with a 5% solution of dimethyldichlorosilane in toluene for 24 h. Glass wool was silanised in the same solution. After drying at 100°, this column was packed with 4% cyclohexanedimethanol succinate (CDMS) (Perkin-Elmer, Beaconsfield, Great Britain) on 70–80 mesh Aeropak 30 (Varian Aerograph (U.K.) Ltd., Walton-on-Thames, Great Britain). This packing was prepared as follows: 1 g of CDMS was dissolved in 100 ml of dichloromethane in a flask to which 24 g support were then added and which was left to stand, with occasional swirling, for 2 h. The solvent was removed under vacuum in a rotary evaporator, the final stages of evaporation being completed in a water-bath at 100° for 30 min. The prepared column was then packed with coated support under vacuum. The packing was then conditioned for 48 h at 250° with nitrogen carrier flowing at 55 ml/min.

The instrument settings were as follows: column temperature, 240°; hydrogen flow-rate, 45 ml/min; air flow-rate, 500 ml/min; nitrogen (carrier gas) flow-rate, 55 ml/min; sensitivity, 2×10^{-10} A.

Extraction procedure

1.0 or 3.0 ml of plasma were acidified with 2.0 ml of 1 *N* hydrochloric acid

and extracted with 15 ml of chloroform by gently shaking for 10 min in a 30-ml centrifuge tube. After spinning at 3000 rpm the organic layer was withdrawn by means of a Pasteur pipette and transferred into a conical tube via a glass wool plug fixed into a small funnel. This prevented the transfer of any precipitate present at the liquid interface. The sample was extracted by shaking with a further 10 ml of chloroform and the second organic layer pooled with the first. 5.0 ml of 0.5 *N* sodium hydroxide were added to the chloroform and shaken gently for 10 min. After centrifuging the solvent was discarded. The sodium hydroxide solution was washed with successive 10-ml aliquots of chloroform until no interface precipitate remained. To the washed sodium hydroxide extract 3.0 ml of 1 *N* hydrochloric acid were added and the aqueous phase was shaken with 10 ml of chloroform for 10 min. The chloroform was transferred to a 10.0-ml conical tube containing 0.5 ml of the internal standard solution and, with the tube immersed in a water-bath at 60°, the solvent was evaporated to dryness under a stream of air. The remaining aqueous phase was re-extracted with a further 5.0 ml of chloroform which was evaporated to dryness with the rest. This residue was carefully dissolved in 0.1 ml of chloroform and a 5-μl aliquot injected onto the gas chromatograph.

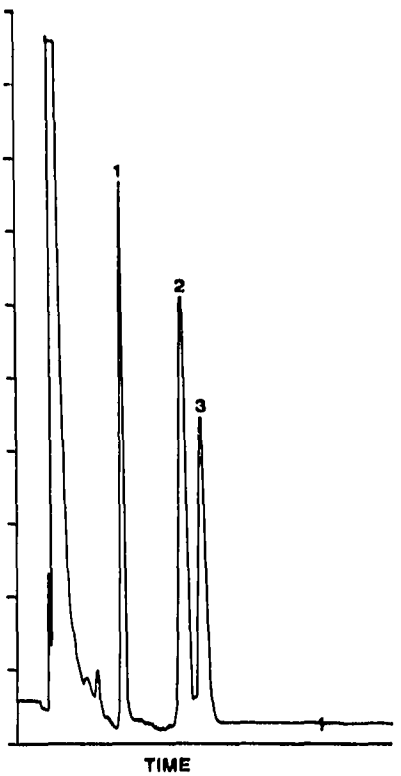


Fig. 1. Separation of TPE, phenylbutazone and prenazone on a 5 ft. × ¼ in. I.D. column of 4% CDMS operating at 240°. 1 = TPE; 2 = phenylbutazone; 3 = prenazone.

TABLE I

COMPOSITION OF STANDARD SOLUTIONS OBTAINED BY DILUTING STOCK SOLUTIONS OF TETRAPHENYLETHYLENE (100 mg/100 ml) AND PRENAZONE (100 mg/100 ml) TO 50 ml WITH CHLOROFORM

Prenazone stock solution (ml)	*TPE stock solution (ml)*	*Prenazone (μg equivalent in 0.1 ml)*
1	2.5	2
2	2.5	4
3	2.5	6
4	2.5	8
5	2.5	10
6	2.5	12
7	2.5	14
8	2.5	16
9	2.5	18
10	2.5	20

RESULTS

Qualitative recognition

The CDMS packing had been shown to be an excellent one for separating the weakly acidic barbiturate drugs[8]. Fig. 1 shows a separation of TPE, phenylbutazone and prenazone. Although barbiturates would also come through the procedure if they were present, none of those on the British market have the same retention time as prenazone on the CDMS column, whereas cyclobarbitone runs with phenylbutazone. We have found no other drugs of acidic character that chromatograph in an interfering position.

Measurement

A range of standard solutions each containing 50 μg/ml of TPE as internal standard and from 20–200 μg/ml of prenazone was made up in chloroform. Stock solutions of TPE and prenazone were made and aliquots of each mixed and diluted to 50 ml with chloroform as indicated in Table I.

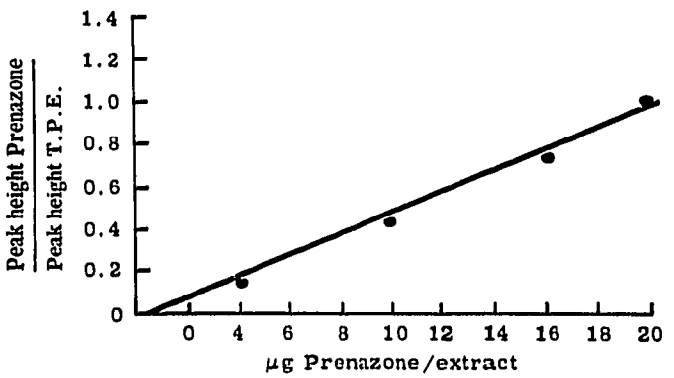


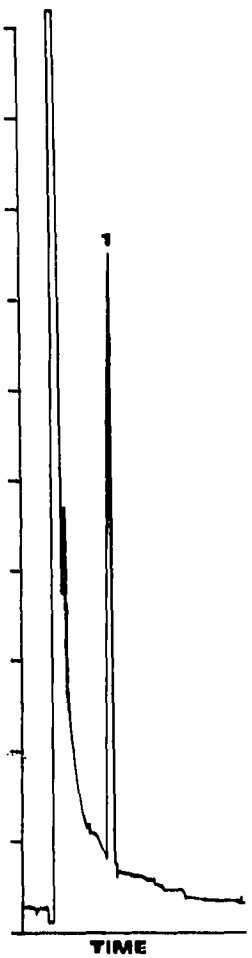
Fig. 2. Standard calibration graph relating the ratio of the peak heights of prenazone and tetraphenylethylene to the number of micrograms of prenazone in the extract.

These standard solutions were stored in a dark cupboard at room temperature and were stable under these conditions. A calibration curve was prepared daily by injecting 3- to 5-μl aliquots of the appropriate standards and plotting a graph of peak height ratios against microgram of prenazone per extract. The calibration curve was linear over this range (Fig. 2). Knowing the sample volume extracted, the drug concentration could easily be calculated.

TPE was chosen as internal standard since we had considerable experience with it on the CDMS column and it ran close to, but separated well from, the two antirheumatoid drugs of interest.

Recovery studies

Recovery experiments were performed by adding from 2 to 20 μg of prenazone to bovine plasma. This was done by evaporating to dryness in a 30-ml glass centrifuge tube suitable aliquots of a chloroform solution of prenazone, and then adding bovine



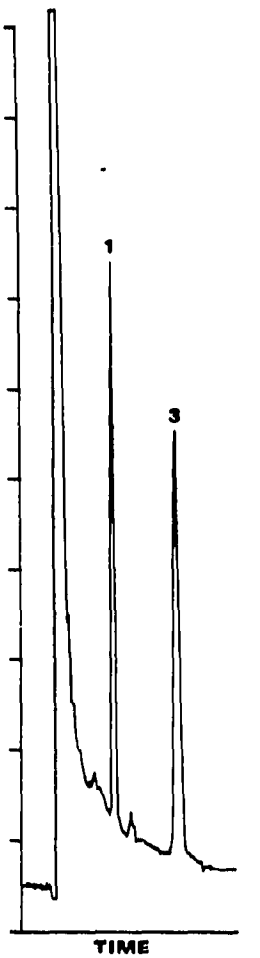


Fig. 3. Gas chromatogram of a normal plasma extract with addition of internal standard.
Fig. 4. Gas chromatogram of a plasma extract from an individual treated with prenazone.

plasma to dissolve the residue. The adequacy of the extraction procedure was then verified by subjecting these samples to the extraction procedure. In seven experiments, the mean recovery achieved was 84% with a standard deviation of ± 4%.

Specificity

The procedure has been shown to be specific for prenazone. No interfering peaks in the same region as either prenazone or TPE have been encountered from constituents of normal plasma (Fig. 3), or from exogenous components in samples derived from patients receiving a variety of other drugs. Fig. 4 shows a typical trace from a plasma extract after a single 200-mg dose of prenazone.

Application of the GLC assay to biological specimens

The procedure has been used to measure plasma concentrations of prenazone in human subjects following the administration of a single oral dose of 200 mg in capsule form (Zepelin) and the results from five experiments are tabulated (Table II). Plasma levels have also been measured in patients undergoing trials of long-term prenazone therapy for rheumatoid arthritis (Table III). All subjects should have taken 600 mg daily, but the results suggest that in two of the trials there was a high incidence of non-compliance not only to prenazone, but also to the comparison drug:

TABLE II

PRENAZONE PLASMA LEVELS IN FIVE SUBJECTS FOLLOWING A SINGLE ORAL DOSE (200 mg)

Time (h)	*Prenazone plasma level (μg/ml)*				
	D.J.B. (male, 29 years old, 145 lb.)	*L.A.C. (female, 25 years old, 118 lb.)*	*P.R. (male, 37 years old, 156 lb.)*	*J.D. (male, 19 years old, 122 lb.)*	*Y.V.P. (male, 20 years old, 160 lb.)*
1	2.2	N.D.	2.2	11.6	12.6
2	—	—	8.5	—	—
3	7.7	10.4	—	29.7	21.2
4	—	—	16.7	—	—
6	16.1	11.4	11.7	17.5	11.1
9	16.5	15.8	—	15.6	—
11	—	—	—	—	5.5
25	8.8	8.2	6.5	6.2	5.4

TABLE III

PLASMA LEVELS AND COMPLIANCE IN PATIENTS ON LONG-TERMS PRENAZONE THERAPY TRIALS

Trial No.	*Number of patients*	*Average prenazone plasma levels (μg/ml) in compliers*	*Number of prenazone non-compliers*	*Number of comparison drug non-compliers*
1	20	12.6	2	2
2	14	5.6	4	0
3	20	28.2	0	0
4	6	31.7	0	0

however, at the two centres where low prenazone levels were recorded, long periods occurred when the blood samples were not stored at 4° as specified in the trial protocol, which may be an alternative explanation. These findings indicate the value of undertaking blood level studies on drugs undergoing clinical trial, since a proper evaluation cannot be made unless one knows that patients are reliable in their drug taking. The wide variation in average plasma levels reported in Table III suggests not only that some subjects failed to take their medication, but also that in trials 1 and 2 those patients who took their medication did so irregularly. In trials 3 and 4 compliance was good, and the average prenazone plasma levels were similar.

Previous studies have shown that, for phenylbutazone, the blood level should be maintained in the region 50–90 μg/ml for maximum efficacy consistent with minimum side-effects[9]. However, the analysis for this work was done by a UV spectrophotometric method that also measures metabolites. Because prenazone is very similar, one may expect a blood level–effect relationship with this drug. In general, on equal doses, levels are about half those for phenylbutazone, but we are continuing this work to see whether a blood level–effect relationship can be established. If it can, then every rheumatology department would benefit by having access to analytical facilities when using these drugs.

ACKNOWLEDGEMENTS

I wish to thank Dr. R. Goulding, Director of the Poisons Unit and Dr. B. Donovan of De Angeli for their help and encouragement with this work. I am also grateful to Mrs. P. C. French for her expert technical assistance.

REFERENCES

1 C. Bianchi, B. Lumachi and E. Marazzi-Uberti, *Arzneim.-Forsch.*, 22 (1972) 183.
2 E. Marazzi-Uberti, C. Bianchi, M. Gaetani and L. Pozzi, *Arzneim.-Forsch.*, 22 (1972) 191.
3 C. Bianchi and G. Bonardi, *Arzneim.-Forsch.*, 22 (1972) 196.
4 E. Marazzi-Uberti, B. Lumachi, M. Gaetani and G. Coppi, *Arzneim.-Forsch.*, 22 (1972) 213.
5 M. Gaetani, R. Debens, G. Bonardi and G. Coppi, *Arzneim.-Forsch.*, 22 (1972) 216.
6 J. J. Burns, R. K. Rose, T. Chankin, A. Goldman, A. Schubert and B. B. Brodie, *J. Pharmacol. Exp. Ther.*, 109 (1953) 346.
7 I. J. McGilveray, K. K. Midha, R. Brien and L. Wilson, *J. Chromatogr.*, 89 (1974) 17.
8 D. J. Berry, *J. Chromatogr.*, 86 (1973) 89.
9 E. Bruck, M. E. Fearnley, I. Meanock, H. Patley, *Lancet*, Jan. 30 (1954) 225.
10 G. Coppi, G. Bonardi and R. Perego, *Arzneim.-Forsch.*, 22 (1972) 234.