Journal of Pharmaceutical & Biomedical Analysis
Vol. 6, No. 5, pp. 493–501, 1988
Printed in Great Britain

0731–7085/88 $3.00 + 0.00
Pergamon Press plc

The micro gas-liquid chromatographic analysis of 4-(3′,3′-dimethylallyl)-1,2-diphenylpyrazolidine-3,5-dione (feprazone) in human biosamples

D. J. BERRY*‡ and D. V. PARKE†

** Poisons Unit, New Cross Hospital, Avonley Road, London SE14 5ER, UK*
† Department of Biochemistry, University of Surrey, Guildford, Surrey GU2 5XH, UK

Abstract: A gas-liquid chromatographic method for the determination of feprazone in various biological matrixes, employing a choice of detector options, is described. After rapid, micro-scale extraction of the sample with *n*-butyl acetate at physiological pH, the solution was injected directly onto the chromatograph. Separation was with either an OV7 column and flame ionisation or electron capture detection, or with a carbowax high polymer column and nitrogen specific detection. When 100 μl of plasma was extracted the limit of accurate measurement was 2 mg l^{-1} for F.I.D. and N.P.D. and 0.5 mg l^{-1} with E.C. detection. Quantification was by comparison with a range of plasma calibrators carried throughout the procedure, and determination of peak height ratios against an internal standard incorporated into the extracting solvent. The CV of the assay throughout the concentration range normally encountered in patients undergoing feprazone treatment, ranged between 2.4 and 7.8% for the various detector options. The analytical method has been applied to samples collected both from patients and normal volunteers undergoing treatment with a range of feprazone maintenance doses.

Keywords: *Gas-liquid chromatography; flame ionisation detection; electron capture detection; nitrogen specific detection; feprazone; pyrazolidinedione.*

Introduction

Feprazone is a non-steroidal anti-inflammatory drug with a similar chemical structure and range of therapeutic activity to phenylbutazone. It is reported to have lower toxicity than phenylbutazone, its *n*-butyl analogue [1, 2], and several clinical trials have demonstrated its therapeutic efficacy [3–7]. In man, the drug is metabolised and excreted in urine mainly as the unusual C(4)-β-glucuronide [8, 9]. However, oxidative metabolism of feprazone also occurs to produce 4′-hydroxyfeprazone [4-(3′-hydroxymethyl-but-2′-enyl)-1,2-diphenylpyrazolidine-3,5-dione]. This is the only hydroxylated metabolite to be formed in man, and has been detected in the plasma of patients ingesting the parent drug. The lower toxicity of feprazone, compared with phenylbutazone in humans may be accounted for by the fact that aromatic hydroxylation occurs only in the latter.

‡ Address for correspondence.

To determine the single dose pharmacokinetics of feprazone a GLC method was developed [10]. This required the extraction of a relatively large volume of plasma, back-extraction to purify the sample, and concentration of the residue prior to chromatography on a CDMS column with flame ionisation detection. An internal standard, tetraphenylethylene, was incorporated prior to solvent concentration to compensate for any losses at this stage. To monitor clinical trials and study maintenance dose pharmacokinetics in volunteers, where the plasma concentrations would be higher than in the single dose experiments, and the number of samples would be far greater, a more rapid GLC assay was required. The present paper reports the development of, and describes in detail, three micro GLC assays for feprazone which are based upon similar principles to those outlined before [11–14]. These micro methods differ from the original procedure [10], not only in the sample preparation technique, but also in the chromatographic conditions employed; each using a different system of detection. The methods have been used successfully to study the disposition and pharmacokinetics of feprazone in human subjects [15].

Experimental

Reagents

The following reagents were used: *n*-butyl acetate (Rathburn Chemicals, Walkerburn, Scotland); phosphate buffer, pH 5.5, consisting of 4 M sodium dihydrogen orthophosphate (Analar, B.D.H., Poole, UK). Prazepam was kindly donated by William R. Warner Medical (Southampton, UK) for use as internal standard, and was prepared in two concentrations in *n*-butyl acetate, namely, solution A, 25 mg l^{-1}; and solution B, 1.0 mg l^{-1}.

Gas chromatography

Three different instrument configurations were used:

I. *Flame ionisation detector system (F.I.D.).* A Pye 104 model 24 dual column gas chromatograph equipped with flame ionisation detectors was used throughout in conjunction with a Hitachi 56 chart recorder (1 mV f.s.d.). The column was a 2 M × 2 mm i.d. coiled glass tube which had been silanised with a 5% solution of dimethyldichlorosilane in toluene; glass wool was treated with the same solution. After rinsing with methanol and drying at 100°C the column was packed with 3% O.V.7 on Varaport 30 (80–100 mesh) (Field Instruments, Richmond, UK), which had been prepared by a standard evaporation technique. The column was conditioned before use for 24 h at 300°C with nitrogen carrier gas flowing at 60 ml min^{-1}.

The instrument settings were as follows: column temperature 280°C, carrier gas (nitrogen) flow rate 60 ml min^{-1}, hydrogen flow rate 45 ml min^{-1}, air flow rate 500 ml min^{-1}, sensitivity 5×10^{-10} A, injection port setting 5.

II. *Electron capture detector system (E.C.D.).* A Pye 104 model 64 gas chromatograph was used. This was fitted with a 7 mCi ^{63}Ni electron capture detector and Pye GCD linear amplifier in conjunction with a Hitachi 56 chart recorder (1 mV f.s.d.). The O.V.7 column was prepared in an identical manner to that used with the F.I.D. system. Instrument settings were as follows: column oven temperature 270°C, detector temperature 290°C, carrier gas (nitrogen) 80 ml min^{-1}, detector current setting 6.

III. *Nitrogen Specific Detector System (N.P.D.).* A Perkin-Elmer F33 gas chromatograph, fitted with an electrically heated nitrogen-phosphorous detector head, was used in conjunction with a Hitachi 56 recorder (1 mV f.s.d.). The column consisted of a 1 m × 2 mm i.d. coiled glass tube which was silanised, as described previously, before packing with 1% Carbowax high polymer coated onto 80–100 mesh Supelcoport by the standard evaporation technique. The column was conditioned at 250°C with carrier gas (nitrogen) flowing at 40 ml min^{-1} for 24 h before use. Instrument settings were: column temperature 250°C, injector/detector temperature 250°C, nitrogen (carrier) flow 40 ml min^{-1}, hydrogen flow 3 ml min^{-1}, air flow 100 ml min^{-1}, sensitivity 32 × 10 A.

Extraction Procedures

Sample preparation was carried out in Dreyer tubes (Scientific Supplies, Vine Hill, London, UK). Eppendorff pipettes were used to dispense the plasma, and repeating Hamilton syringes used to add the buffer and internal standard solutions. All samples were analysed in duplicate and the mean results reported. If the differences between duplicates were greater than 10% the extractions were repeated.

(I) *F.I.D./N.P.D. methods*

To a clean Dreyer tube were added 10 μl 4 M phosphate buffer, 100 μl of the biological sample (calibration standard, plasma sample or faecal homogenate) and 100 μl of internal standard solution A. The tube was vortex mixed for 1 min and then centrifuged at 3000 rpm for 3 min in an Eppendorff Centrifuge 5412; 1–5 μl of the butyl acetate phase was then injected directly onto the GLC column.

(II) *E.C.D. method*

To a clean Dreyer tube were added 100 μl biological sample (or calibration standard), 10 μl 4 M phosphate buffer and 100 μl internal standard solution B. The tube was vortex mixed and centrifuged as before; 2 μl of the butyl acetate phase was injected onto the GLC column.

Quantitative Measurement

(I) *F.I.D./N.P.D. methods*

Quantitative measurement was achieved by measuring the peak heights of feprazone and prazepam to an extrapolated baseline and then calculating the ratio of peak height feprazone/peak height prazepam for each sample injection. Calibration was performed from plasma standards containing feprazone over a concentration range of 10–80 mg l^{-1}. This was achieved by adding 50, 100, 200, 400 μl of a stock solution (2 g l^{-1}) of feprazone in methanol to four separate vials containing freeze dried plasma which had been reconstituted with 10 ml distilled water (Biotrol). These standards were extracted in a similar way to the samples, with each analysis batch, and a plot of drug concentration vs peak height ratio prepared. The concentration of feprazone contained in unknown samples was determined from the peak height ratio for the sample and interpolation of the calibration graph.

(II) *E.C.D. method*

Quantification was again performed by measuring the peak height ratio of feprazone/

internal standard and relating to a set of plasma calibrators which had been prepared as above, but covering the concentration range of 2–20 mg feprazone per litre.

Quality Control

Feprazone, from an independent methanolic stock solution, was added to fresh heparinised human plasma to give concentrations of 10 and 30 mg l^{-1}. A plasma standard at each concentration was carried through the procedure with each batch of samples. All plasma calibrators and quality control material were stored deep-frozen (−20°C) when not in use.

Results and Discussion

Column selection

The macro GLC method [10], employing flame ionisation detection, was developed at a time when even the best solid supports adsorbed significant quantities of certain drugs injected onto the column; the problem was overcome by extracting a relatively large volume of plasma and injecting relatively large amounts of drug onto the column. The CDMS liquid phase provided an excellent separation of feprazone from phenylbutazone, an essential requirement, as the latter was often present in patient samples. Sample preparation included a back-extraction step to remove cholesterol from the final residue,

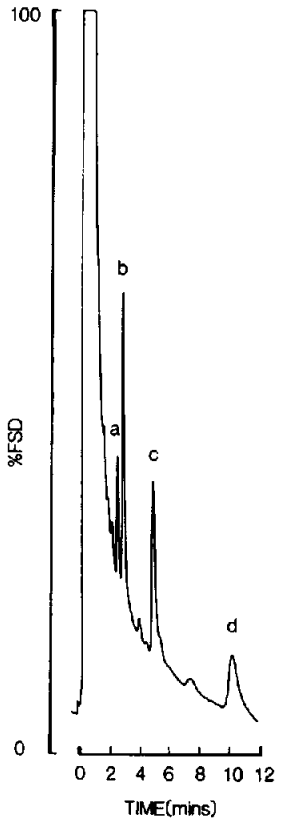

Figure 1
Gas chromatographic separation of phenylbutazone, feprazone, prazepam and cholesterol using the flame ionisation detector system. Chromatogram after micro-extraction of the 80 mg l^{-1} feprazone plasma calibration standard to which phenylbutazone had been added. a = phenylbutazone; b = feprazone; c = prazepam; d = cholesterol.

as this was detectable by F.I.D., and would have been retained for a long time on the CDMS column.

The micro GLC methods reported in the present paper were developed once less absorptive solid supports became available, thus obviating the need for sample concentration and allowing the use of more rapid preparation procedures. The OV7 liquid phase used also gave excellent separation of feprazone from its analogue phenylbutazone (Fig. 1). Furthermore, cholesterol, which was now present in the directly-extracted plasma samples and detected by F.I.D., was well separated from the drugs of interest, and eluted more rapidly than from the CDMS column, thus allowing a shorter run time. Despite the use of direct solvent extraction, the life of the OV7 column was excellent and several thousand injections could be made before its performance deteriorated.

Internal standard

Prazepam was chosen as the internal standard because it gave a response with all detector systems used and chromatographed at a convenient retention time, clear of feprazone, but before cholesterol. In addition, prazepam was known to be recovered adequately under the extraction conditions used [14].

Linearity and sensitivity

The extraction of feprazone into organic solvents at acid pH was known to be efficient, but since quantification in the present work was by reference to similarly treated plasma standards it was not necessary to investigate recovery in detail or to apply a recovery factor. However, the recovery at a concentration of 40 mg l^{-1} was approximately 35%, determined by comparing the mean peak height of 20 accurate injections of a 1:1 extract of a plasma standard with that obtained from a 40 mg l^{-1} solution of feprazone which had been prepared by direct weighing into butyl acetate. Using a plasma to solvent ratio of 1:1 the limit of accurate measurement (defined as 10 times the standard deviation of the lowest standard) was 2 mg l^{-1} for F.I.D. and N.P.D. and 0.5 mg l^{-1} for E.C. In addition, the sensitivity of the technique could be enhanced if necessary by increasing the plasma to solvent ratio up to 3:1 without increasing the frequency of emulsion formation. Calibration of this modified method requires a lower range of plasma standards which must be treated in an identical manner. The graphs of peak height of drug vs peak height of internal standard were rectilinear throughout all the ranges of calibration used with the various detector options. The slopes were F.I.D. = 0.029, N.P.D. = 0.022, E.C.D. = 0.300.

Electron capture method

The micro GLC method using electron capture detection was investigated initially as an attempt to speed up the assay by employing direct extraction with a specific detector which was not sensitive to cholesterol, thus avoiding the time delay caused by waiting for this endogenous material to elute between injections. Because the E.C.D. detector is extremely sensitive to feprazone, it was possible to decrease the quantity of plasma extracted to 10 μl. However, this resulted in poor reproducibility not only because it was more difficult to sample 10 μl than 100 μl, but also because smaller quantities of drugs were being injected onto the column and irreversible adsorption again became significant even with the best deactivated supports. Despite these difficulties, it was possible with this method to measure plasma concentrations of feprazone using very small volumes of

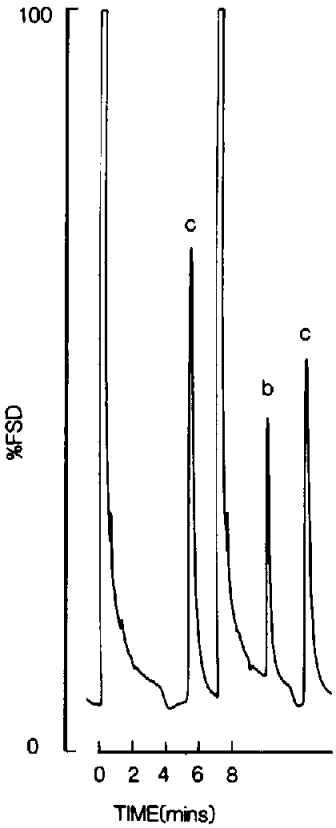

Figure 2
Gas chromatograms, using electron capture detection, of plasma extracts from an individual before and after treatment with feprazone. b = Feprazone; c = prazepam. 1 = Chromatogram after micro-extraction of plasma from an individual prior to administration of feprazone. 2 = Chromatogram from an individual 3 h after rectal administration of 300 mg feprazone (plasma concentration = 3.0 mg l^{-1}).

plasma. In addition, by extracting larger volumes, it was possible to measure the much lower concentrations of feprazone which occur in saliva, plasma ultrafiltrate and urine. The method was very specific and Fig. 2 shows some typical electron capture chromatograms.

Nitrogen detector method

The micro method with N.P.D. was a further attempt to speed up the assay by employing direct extraction together with a specific detector to avoid interference from endogenous compounds, in particular those which are present in plasma and faecal homogenate. The carbowax high polymer column produced symmetrical peaks from both feprazone and prazepam, and clean, uncluttered chromatograms in the areas of interest (Fig. 3). The method has been applied mainly to faecal homogenates, and the problems associated with heterogeneous drug distribution in this type of sample were overcome by determining several (10) replicate samples. The coefficient of variation on this number of replicates was found to be <5%. As the N.P.D. was only as sensitive as the F.I.D. to either feprazone or prazepam, sample preparation was similar for both detectors.

Precision and accuracy

Between batch reproducibilities of the F.I.D.–N.P.D. methods were assessed by repeated analysis of two genuine patient plasma samples, containing approximately 14

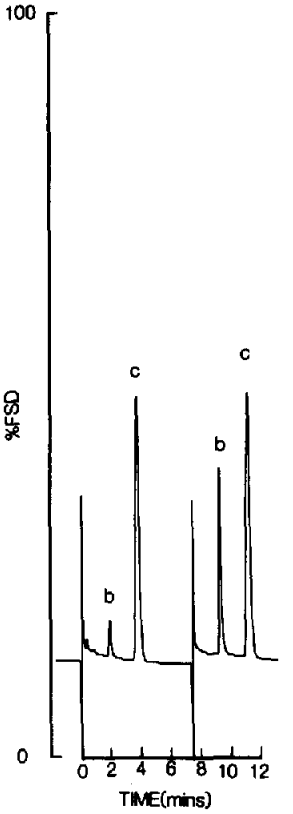

Figure 3
Gas chromatograms, using nitrogen specific detection, of extracts of faecal homogenates from an individual treated with feprazone. b = feprazone; c = prazepam. 1 = Chromatogram of an extract of a faecal homogenate, containing 14 mg feprazone per litre, from an individual treated with feprazone. 2 = Chromatogram of a faecal homogenate containing 46 mg l^{-1} of feprazone.

Table 1
Between batch reproducibility of each method for determination of feprazone

Method	*N*	Mean concentration (mg l^{-1})	S.D.	C.V. (%)
E.C.D.	12	3.0	0.07	2.3
	12	9.0	0.33	3.7
N.P.D.	12	15.4	1.00	6.5
	12	49.3	2.35	4.8
F.I.D.	12	14.3	1.12	7.8
	12	40.7	0.89	2.2

and 45 mg l^{-1} feprazone, on 12 different days. The reproducibility of the E.C.D. method was similarly assessed at 3 and 9 mg l^{-1} and Table 1 summarises the results.

It was not possible to compare the results obtained by present methods with a non-chromatographic assay, but the feprazone concentration in 50 plasma samples was measured by both N.P.D. and F.I.D. with good agreement (r = 0.98). As columns with different separating characteristics were used with these detector options, the accuracy of each method was concluded to be satisfactory.

The procedures have been shown to be specific and no interfering peaks in the same region as either feprazone or prazepam have been encountered from constituents of

normal plasma, urine, faeces or saliva. 4-Hydroxyfeprazone elutes from the OV7 column at a retention time approximately four times that of feprazone. However, this polar metabolite produces a broad tailing peak which results in poor sensitivity, and since plasma concentrations are invariably much lower than those of the parent drug it is not detected in sample extracts. In addition, no interfering exogenous compounds were found in samples derived from patients receiving a variety of other drugs.

Feprazone is stable when stored deep-frozen in various biological samples, and this was validated, not only by analysing the stored calibration standards at various times throughout a three year period against freshly prepared standards, but also by re-analysing clinical trial samples which had been deep-frozen throughout the same interval.

The value of monitoring experimental drugs in long term multicentre trials is well established and previous monitoring of feprazone has demonstrated poor compliance in some studies [10]. The micro GLC methods described here have been used to monitor more recent clinical trials, and in one of these there was evidence that plasma trough concentrations of feprazone of around 25 mg l^{-1}, achievable on a 200 mg b.d. dosage, were indicative of therapeutic efficacy in osteoarthritis [7]. In the past, a blood level-effect relationship has also been suggested for phenylbutazone [17], an analogue of feprazone.

The methods described have also been applied to a range of biological samples from healthy volunteer studies designed to assess the relationship between steady state plasma feprazone concentration and a range of daily doses [16]. The results of one such experiment are summarised in Fig. 4, indicating that the relationship between plasma concentration of feprazone and the maintenance dose is non-linear. A similar relationship has been reported for phenylbutazone although the plasma plateau concentrations are higher with this drug [18].

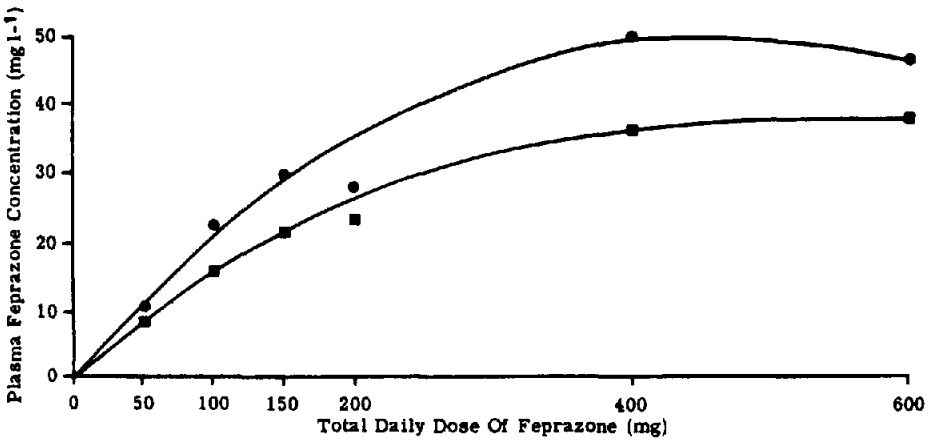

Figure 4
Mean plasma concentrations of feprazone in six normal volunteers who have been stabilised on a range of maintenance doses. After eleven days dosage (at 08.00 and 20.00 h each day), blood was drawn twice daily (at 07.30 and 13.30 h) for three successive days. The data points represent the average feprazone concentration for these three days. ● = 5.5 h post dose at 13.30 h, ■ = pre-morning dose at 07.30 h.

Conclusion

In summary, direct extraction techniques have been successfully applied to the analysis of a variety of drugs in plasma [11–14]. The present method for feprazone is similarly of considerable value. It is rapid, since it employs a short extraction time, and does not require a solvent concentration step; it is also economical in respect of reagents and apparatus, and the chromatographic run time is short, thus allowing the analysis of a large number of samples daily. The choice of detector options is a useful feature of the method and most laboratories would have access to at least one of these. The specific detectors (E.C. and N.P.D.) allow rapid throughput of direct solvent extracts of plasma, as they are insensitive to cholesterol which has a long retention time and thus delays the rate of injection with F.I.D. Specific detectors also allow one to examine a wider range of biomatrixes, e.g. urine, faecal homogenates and tissue extracts which tend to have complex endogenous backgrounds when assayed by F.I.D. The electron capture detector is more sensitive to feprazone than the other detectors and has the potential to determine drug concentrations which are an order of magnitude lower, although the absorptivity of the packed column is a limiting factor. The method has been of great value not only in determination of the steady state kinetics and disposition of feprazone in volunteers, but also for monitoring clinical trials of the drug.

References

[1] C. Bianchi and G. Bonardi, *Arzneim.-Forsch.* **22**, 196–201 (1972).
[2] M. Gaetani, R. Debus, G. Bonardi and G. Coppi, *Arzneim.-Forsch.* **22**, 216–223 (1972).
[3] R. Billings, H. C. Burry, R. Grahame, D. J. Berry and B. Donovan, *Rheumatol. Rehabil.* **14**, 61–66 (1975).
[4] I. Haslock, L. Burkinshaw, D. J. Berry, B. Donovan and V. Wright, *Rheumatol. Rehabil.* **15**, 81–86 (1976).
[5] P. M. G. Reynolds, R. D. Sturrock, A. Issacs and F. D. Hart, *Rheumatol. Rehabil.* **14**, 67–70 (1975).
[6] L. S. Bain, M. P. Lynch and G. M. Bruce, *Curr. Med. Res. Opin.* **4**, 665–669 (1977).
[7] M. R. Laurent, D. J. Berry and R. Grahame, *Pharmacotherapeutica* **3**, 393–397 (1983).
[8] H. Yamaguchi, J. Kubo, K. Sekine, T. Naruchi, Y. Hasimoto and R. Kato, *Drug. Metab. Disp.* **7**, 340–344 (1979).
[9] A. Donetti, M. G. Quintero, E. Cereda and H. Yamaguchi, in *Recent Developments in Mass Spectrometry in Biochemistry and Medicine*, Vol. 6 (A. Frigerio and M. McCarnish, Eds), pp. 203–215. Elsevier-Amsterdam (1980).
[10] D. J. Bery, *J. Chromatogr.* **114**, 159–165 (1975).
[11] D. J. Berry and L. A. Clarke, *J. Chromatogr.* **156**, 301–307 (1978).
[12] R. J. Flanagan and D. J. Berry, *J. Chromatogr.* **131**, 131–146 (1977).
[13] D. J. Berry and L. A. Clarke, *J. Chromatogr.* **150**, 537–541 (1978).
[14] D. M. Rutherford, *J. Chromatogr.* **137**, 439–448 (1977).
[15] D. J. Berry, Ph.D. thesis: University of Surrey (1982).
[16] D. J. Berry, *2nd Int. Symp. on Drug Analysis* (Brussels). Abst. No. 425 (1986).
[17] E. Bruck, M. E. Fearnley, I. Meanock and H. Patley, *Lancet* **1**, 225–228 (1954).
[18] M. Orme, B. L. J. Holt, D. V. R. Hughes, C. J. Bulpitt, G. H. Draffen, S. S. Thorgeirsson, F. Williams and D. S. Davies, *Br. J. Clin. Pharmacol.* **3**, 185–191 (1976).

[*Received for review 14 September 1987; revised manuscript received 28 January 1988*]