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GAS—LIQUID CHROMATOGRAPHIC EVALUATION OF FENQUIZONE IN BIOLOGICAL SAMPLES FOR PHARMACOKINETIC INVESTIGATIONS

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SUMMARY

Extractive alkylation was carried out on fenquizone, a sulphonamide diuretic, in order to devise a suitable method for its determination in pharmacokinetic and bioavailability studies. After extraction as a tetramethyl derivative, fenquizone was evaluated by gas—liquid chromatography with a ^{63}Ni electron-capture detector, which enables a limit of detection of 2 ng/ml of plasma or urine to be achieved. Linearity was verified in a range of 50— 10,000 pg for each injection with a fenquizone/internal standard ratio ranging from 4:1 to 1:4. Determination is very rapid, as one analysis only takes 5 min.

The preliminary results of the pharmacokinetic study performed in a volunteer human subject after a single oral administration of the drug are presented in this paper in terms of the plasma levels and the cumulative urinary excretion.

INTRODUCTION

Fenquizone (2-phenyl-6-sulphonamido-7-chloro-1,2,3,4-tetrahydro-4-quinazolinone) is a diuretic with a quinazoline structure like quinethazone and metolazone [1,2]. From the point of view of the structure—activity relationship, position 1 may be occupied by a CO- or an SO_2-group, shifting from quinazolinone to benzothiazine derivatives, such as chlorothiazide, hydrochlorothiazide, etc. [2].

The main interest of fenquizone lies in its activity at a single daily dose of 10—20 mg, which is quite low compared to other diuretics, and in its very good tolerability [3,4].

The pharmacokinetics of fenquizone were previously investigated in several animal species by detecting the ^{14}C-labelled drug in body fluids [5,6].

This paper reports an analytical method performed to achieve a sensitivity

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of 2 ng/ml which is required for human pharmacokinetic and bioavailability studies.

EXPERIMENTAL

Drugs and chemicals

Samples of fenquizone were supplied by Maggioni (Milan, Italy), and penfluridol by Studio Chimica (Milan, Italy).

All reagents were of analytical grade and were supplied by Merck (Darmstadt, G.F.R.).

Extractive alkylation procedure

Extractive alkylation was performed in a stoppered tube on 1 ml of human plasma or urine (2 ml may be used to increase sensitivity) to which 0.2 g of $NaHCO_3$ and 5 ml of methyl isobutyl ketone were successively added. The mixture was vigorously stirred and then centrifuged at 2400 *g* for 10 min. An aliquot of the organic layer was transferred to a second tube and alkalinized with 3 ml of 0.1 *N* sodium hydroxide and then shaken and centrifuged.

The organic layer was discarded and an aliquot of the aqueous layer was transferred to another test tube together with 5 ml of 0.5 *M* iodomethane in dichloromethane and 50 μl of 0.1 *M* tetrahexylammonium acid sulfate in dichloromethane. The test tube was stoppered and kept at 50°C under stirring for 20 min, after which it was cooled to room temperature and centrifuged. The organic layer, containing the tetramethyl derivative of fenquizone (Fig. 1), was dried over 500 mg of anhydrous Na_2SO_4, evaporated to dryness and finally dissolved in 100 μl of acetone. Penfluiridol was added as the internal standard (Fig. 2). This solution was ready for gas—liquid chromatographic (GLC) analysis and 1—3 μl were injected.

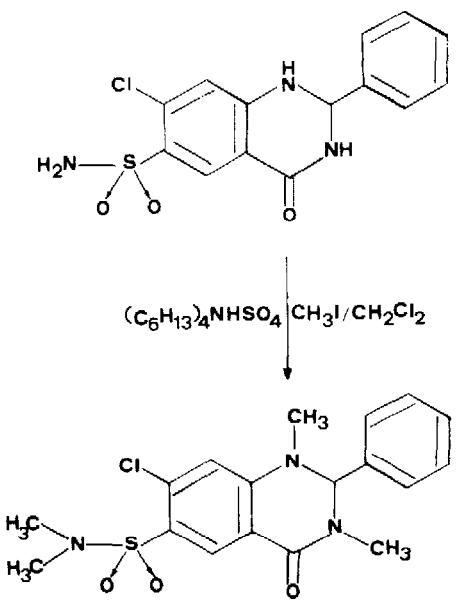
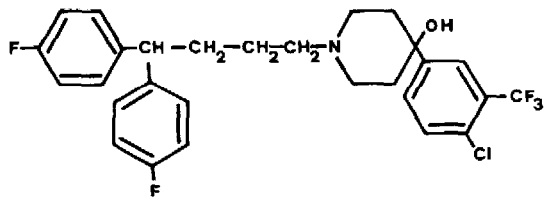


Fig. 1. N-Methylation of fenquizone.



Penfluridol

Fig. 2. Structure of penfluridol.

GLC analysis

A Perkin-Elmer Sigma 3B gas chromatograph equipped with an electron-capture detector (^{63}Ni) was employed. A silanized glass column (35 cm × 6 mm O.D. × 2 mm I.D.) was packed with 30% OV-101 on 80—100 mesh Chromosorb W AW. Oven, injection port and detector temperatures were maintained at 300°C, 320°C and 350°C, respectively. Nitrogen was used as carrier gas at a flow-rate of 60 ml/min.

The following retention times were measured: 3 min 40 sec for the fenquizone tetramethyl derivative, and 2 min 48 sec for the internal standard (I.S.) (Fig. 3). The relative retention volume of fenquizone was 1.31, assuming that of the I.S. to be 1.00.

The chemical identity of fenquizone was confirmed by GLC—mass spectrometry (Fig. 4) and the same was done to demonstrate that penfluridol was eluted unchanged.

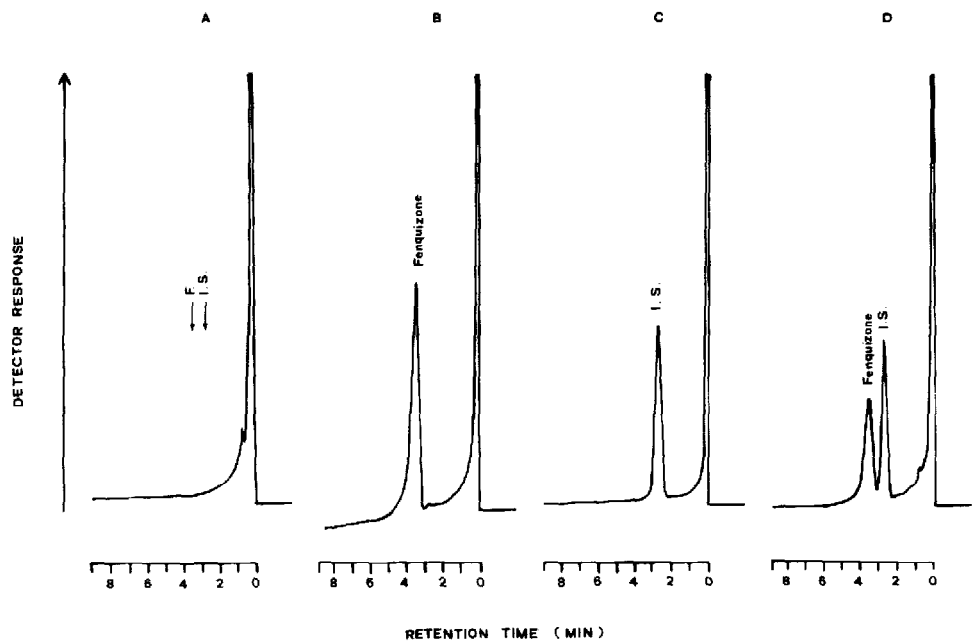


Fig. 3. Gas chromatograms of (A) a blank plasma sample in the absence of either fenquizone derivative or I.S., (B) fenquizone tetramethyl derivative, (C) penfluridol, and (D) fenquizone and I.S. after the described extraction procedure from plasma.

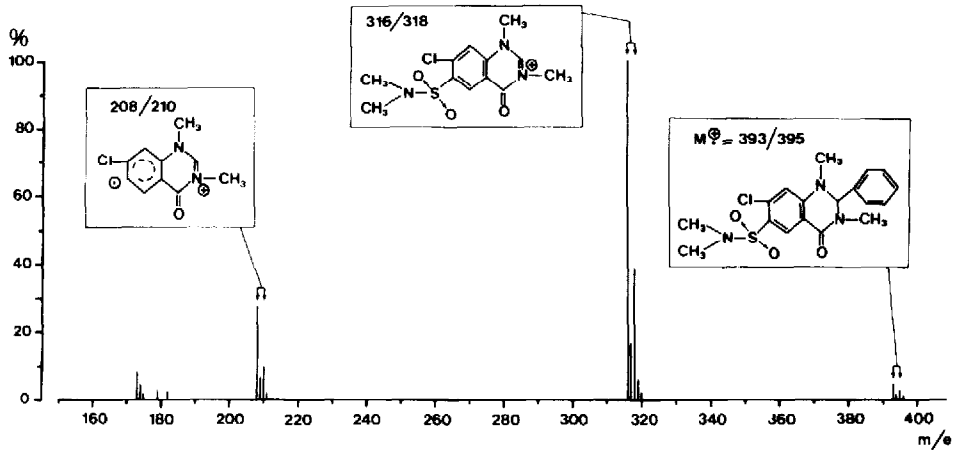


Fig. 4. Mass spectrum of the tetramethyl derivative of fenquizone, which confirms the chemical identity of the derivative used in GLC analysis.

Calibration curves, reproducibility and linearity of detector response

In order to evaluate the analytical reproducibility and the linearity of the detector response the following tests were performed:

(A) Nine different amounts of fenquizone (range 10–5000 pg) were injected into the gas chromatograph together with the I.S. at a fixed drug/I.S. ratio of 1:2. Each injection was performed twice.

(B) Fenquizone was mixed with the I.S. so as to obtain the five following drug/I.S. ratios: 1:0.25; 1:0.5; 1:1; 1:2; 1:4. Each injection was performed twice.

(C) A solution containing 250 pg of fenquizone and 500 pg of I.S. per μl was injected ten times into the gas chromatograph; the volume of each injection was 1 μl.

The recovery of the extraction was evaluated by adding nine different amounts of fenquizone, ranging from 2 to 1000 ng, to 1 ml of plasma and performing four tests at each concentration. A similar investigation was carried out on the urine in a range of concentration of the drug between 100 and 5000 ng/ml.

The concentration of fenquizone in plasma was evaluated from the fenquizone/I.S. area ratio of the peaks, which was corrected by the detector response factor and the recovery.

RESULTS AND DISCUSSION

Choice of the internal standard

Fig. 3 shows the gas chromatograms of a blank plasma sample without fenquizone, of fenquizone and of the internal standard. A series of diuretics with structures similar to that of fenquizone (furosemide, chlorothiazide, hydrochlorothiazide, chlorthalidone) were first tested as internal standards, but then discarded because their retention times were too short and there was interference between their peaks and the solvent tail. To improve resolution the

choice of a longer column and a lower temperature would have been necessary with a decrease in sensitivity and a loss of time as a consequence.

Penfluridol {1-[4,4-Bis(4-fluorophenyl)butyl]-4-bis(*p*-fluorophenyl)butyl-4-(4-chloro-α,α,α-trifluoro-*m*-tolyl)-4-piperiolinol} ($C_{28}H_{27}ClF_5NO$, M.W. = 523.99) is not structurally correlated with the thiazides (Fig. 2), but it is well evaluated in GLC by the electron-capture detector because of its six halogen atoms. Also it is quite stable because it eluted unchanged during GLC analysis, which was demonstrated by GLC—mass spectrometry, and it has a suitable retention time which allows clear separation from the fenquizone derivative and from the solvent peak (Fig. 3) to be achieved.

Overall recovery

The recovery of fenquizone extraction from plasma is depicted in Table I. By varying the amount of fenquizone added to 1 ml of plasma, the percentage recovery did not change. The mean value of the recovery, evaluated from the 36 tests, was 89.92 ± 7.10% (S.D.).

The linear relationship between fenquizone added to 1 ml of plasma (X) and fenquizone recovered (Y) was established by the least-squares method and was characterized by a linear regression coefficient of 0.9998. This linear correlation may be expressed by the function $Y = a + bX$, where $a = 0.019$ and $b = 0.9029$. The slope b, in percentage 90.29%, is very close to the mean recovery value directly calculated from the 36 tests performed.

The recovery of fenquizone from urine also gave good results with an average of 90%.

TABLE I

RECOVERY OF FENQUIZONE ADDED TO 1 ml OF PLASMA IN DIFFERENT AMOUNTS RANGING FROM 2 TO 1000 ng

Mean values, each obtained from four assays, are reported with their S.D.

Fenquizone added (ng)	Fenquizone found (ng)	Recovery* (%)
2	1.77 ± 0.24	88.75 ± 11.96
5	4.21 ± 0.44	84.25 ± 8.80
10	8.60 ± 0.65	86.00 ± 6.48
20	18.75 ± 1.63	93.75 ± 8.14
50	43.87 ± 2.72	87.75 ± 5.44
100	89.75 ± 6.60	89.75 ± 6.60
200	174.50 ± 9.98	87.25 ± 4.99
500	465.00 ± 27.39	93.00 ± 5.48
1000	897.50 ± 55.60	89.75 ± 5.56

*Mean recovery calculated from the 36 assays and covering the whole range is 88.92 ± 7.10%.

Reproducibility and linearity

Reproducibility was expressed as percentage S.D. in relation to the analytical data found in different assays. When the same solution containing 250 pg of fenquizone and 500 pg of I.S. per μl was injected ten times into the gas chromatograph, an S.D. of 2.2% could be determined. The S.D. reached 5.5%

by varying the fenquizone/I.S. ratio between 1:0.25 and 1:4 and rose to 6.3 when increasing fenquizone amounts from 10 to 5000 pg were injected at a fixed fenquizone/I.S. ratio. In both the latter cases the linearity of the response was well verified with an r value (correlation coefficient) very close to 1 (Table II).

Reproducibility in a complete analysis which includes all analytical manipulations can be evaluated from the recovery test (Table I).

An S.D. of 8.0% could be calculated as mean value from the 36 assays.

TABLE II

REPRODUCIBILITY AND LINEARITY OF DETECTOR RESPONSE IN FENQUIZONE ANALYSIS CARRIED OUT UNDER THREE DIFFERENT CONDITIONS

Condition A: varying the fenquizone injected at a fixed drug/I.S. ratio. Condition B: injecting a fixed amount of fenquizone and varying the drug/I.S. ratio. Condition C: fixing both parameters (ten assays).

Condition	Fenquizone injected (pg)	Drug/I.S. ratio	Detector response factor ± S.D.	S.D. (%)	Linear regression coefficient
A	From 10 to 5000, nine assays, each performed twice	1:2	0.441 ± 0.028	6.3	0.9994*
B	250	From 4 to 0.25, five different ratios, each performed twice	0.435 ± 0.024	5.5	0.9972**
C	250	1:2	0.446 ± 0.0097	2.2	

*Fenquizone injected versus fenquizone detected.
**Fenquizone/I.S. weight ratio versus area ratio.

Sensitivity

When tetramethyl fenquizone was injected into the gas chromatograph without any endogenous interference, small amounts of 5 or 10 pg gave a detectable response. But when fenquizone was added to a biological fluid and then extracted and analysed the lowest detectable concentration was 2 ng/ml with a reproducibility of 13.5% (S.D.). Even a concentration of 1 ng/ml could be determined, but in this case the S.D. rose to 20%.

Preliminary pharmacokinetic investigations

The extractive alkylation method just described, which had been already performed with other diuretics [7], also gave a satisfactory response with fenquizone. Indeed its high sensitivity (2 ng/ml) allows pharmacokinetic and bioavailability studies to be performed on human subjects. Fig. 5 shows plasma levels of fenquizone and Fig. 6 its cumulative urinary excretion in one healthy volunteer orally treated with 10 mg of the drug as its poassium salt, which corresponds to 8.937 mg of fenquizone as sulphonamide. Extractive alkylation may be carried out simultaneously on a series of samples and subsequent GLC analysis of each sample only takes about 5 min. Analysis is thus fairly rapid and inexpensive.

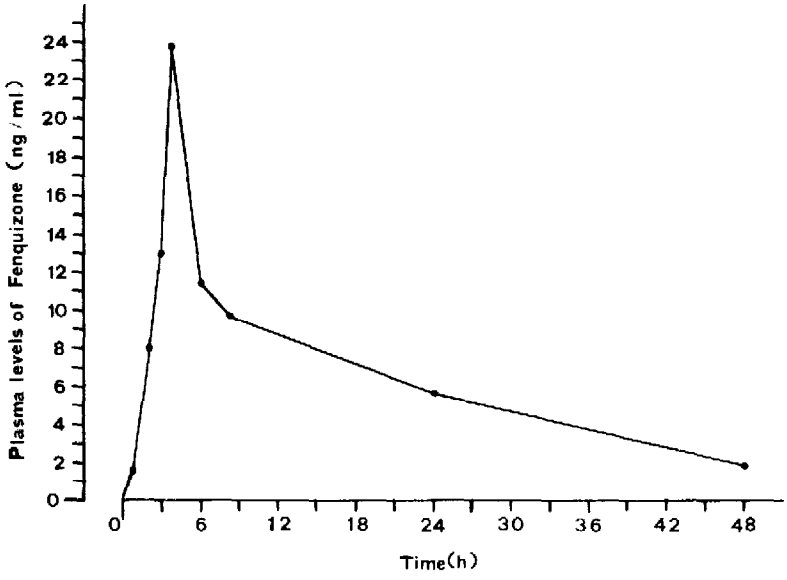


Fig. 5. Plasma levels of fenquizone determined in a human volunteer orally treated with 10 mg of the drug as its potassium salt.

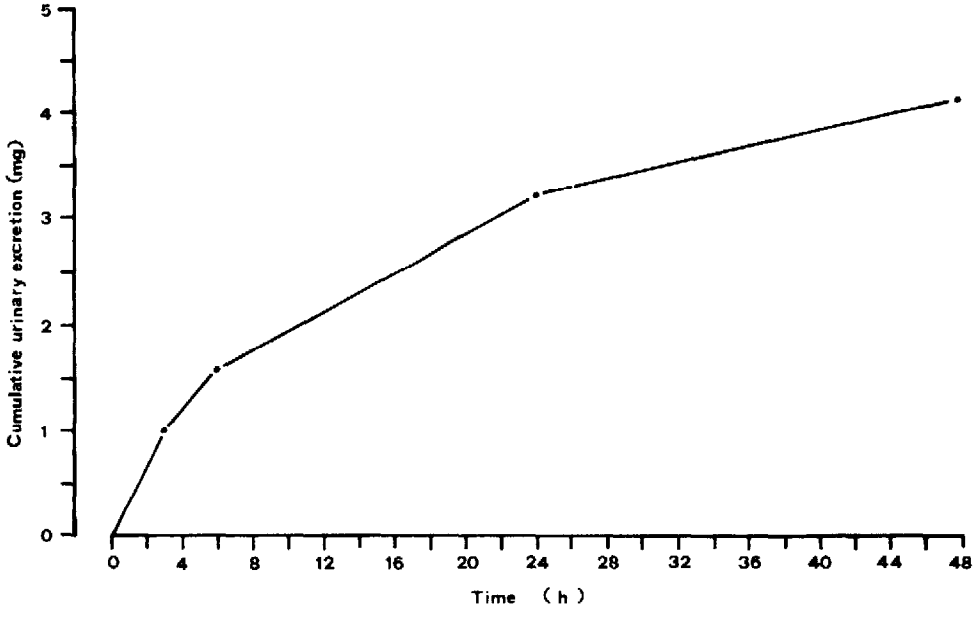


Fig. 6. Cumulative urinary excretion of fenquizone in the volunteer orally treated with 10 mg of the drug as its potassium salt.

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