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PLASMA QUANTIFICATION OF QUAZEPAM AND ITS 2-OXO AND N-DESMETHYL METABOLITES BY CAPILLARY GAS CHROMATOGRAPHY

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SUMMARY

The authors have developed a gas chromatographic method for the simultaneous quantification of quazepam in plasma and its two main metabolites, 2-oxoquazepam and N-desmethylquazepam. This method involves an extraction from plasma using butyl acetate, and an analysis by electron-capture detection on a CP-Sil 5 WSCOT capillary column. Intra- and inter-day precision and accuracy were better than 10% for each of these three compounds, even near their detection limit estimated at 0.2 ng/ml. Linearity proved satisfactory between 0.2 and 60—70 ng/ml. For endogenous plasma components, adequate specificity was achieved. Despite some inconveniences, a long analysis time, a progressive saturation of the column owing to a low oven temperature, and a relatively short life-span of the CP-Sil 5 columns, this method was the only one available in the literature for the quantification of quazepam and its metabolites from the same plasma sample. It was successfully applied to phase I studies in healthy volunteers.

INTRODUCTION

Quazepam [compound I; 7-chloro-5-(2-fluorophenyl)-1,3-dihydro-1-(2,2,2-trifluoroethyl)-2H-1,4-benzodiazepine-2-thione] is a new benzodiazepinic compound with very interesting sedative and hypnotic properties [1—3]. It has two major active metabolites (Fig. 1), 2-oxoquazepam (compound II) and N-desmethylquazepam (compound III), which have been identified in man [4]. The N-desmethyl metabolite represents the final form of several benzodiazepines, namely flurazepam and ethylloflazepate.

From the methodological point of view, only a gas chromatographic (GC)

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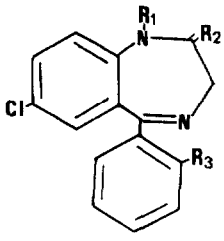

COMPOUND	N°	R_1	R_2	R_3
QUAZEPAM	I	CH_2CF_3	S	F
2-OXOQUAZEPAM	II	CH_2CF_3	O	F
N-DESMETHYL-QUAZEPAM	III	H	O	F
DELORAZEPAM (I.S.)	IV	H	O	Cl

Fig. 1. Chemical structures of quazepam, its metabolites and the internal standard used.

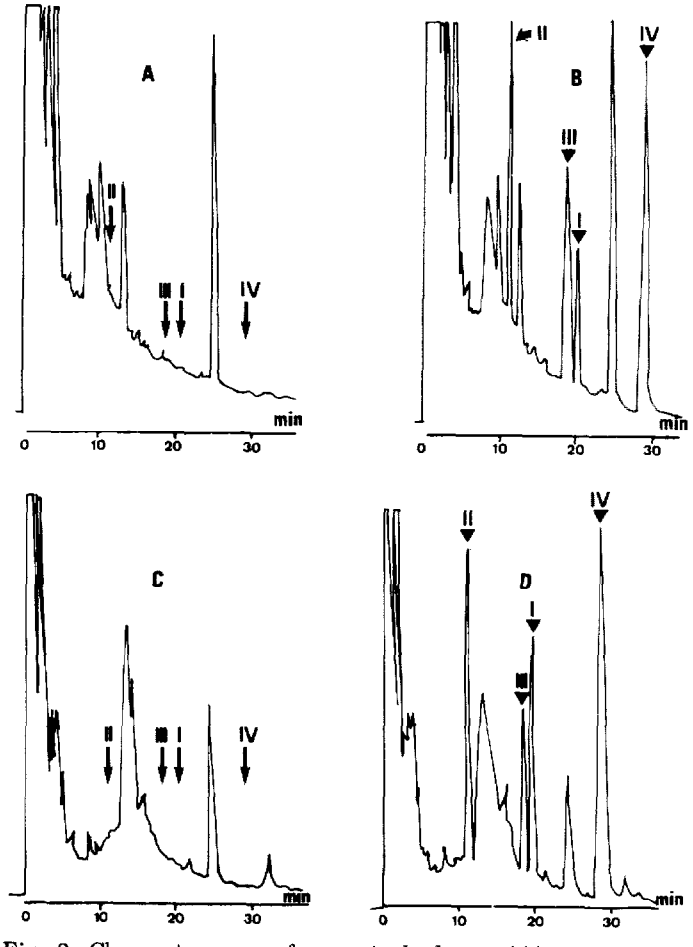

Fig. 2. Chromatograms of a control plasma (A), a control plasma spiked with 1.2 ng of compound I, 1.2 ng of compound II and 1.8 ng of compound III (B), and a subject plasma before administration (C) and 4 h following oral administration of 15 mg of quazepam (D).

method was available for the quantification in plasma of quazepam and its two main metabolites [5]. This method involves a separate quantification of compounds I and II using an OV-25 packed column, and of compound III with a SP-2250 packed column. For each of these compounds, the limit of detection is ca. 0.7 ng/ml. The authors have described here a capillary GC method allowing simultaneous quantification of compounds I, II and III from the same plasma sample, with sensitivity and specificity significantly improved when compared to the data in the literature.

EXPERIMENTAL

Chromatographic analysis

The analysis was performed on a GC system HP 5890 (Hewlett-Packard, Les Ulis, France) equipped with a glass moving-needle injector and an electron-capture detector. The carrier gas was helium N55 with a head column pressure of 0.4 bar; the auxiliary gas was argon—methane (95:5) with a flow-rate of ca. 10 ml/min. The temperature settings for the injection port and the detector were 260 and 300°C, respectively. The retention times for compounds I, II, III and their internal standard delorazepam were 20.5, 11.8, 18.8 and 28.6 min, respectively, at an oven temperature of 220°C (Fig. 2).

Capillary columns

The columns used were glass wall-coated superior capacity open tubular (WSCOT) capillary columns (25 m × 0.5 mm I.D.) with an apolar polydimethylsiloxane stationary phase (CP-Sil 5) (film thickness 1 μm), supplied by Chrompack (Orsay, France). New capillary columns were conditioned before use by several slow temperature programmings (2°C/min) from 150 to 300°C, and by heating at 300°C for 12-h periods.

Standard solutions and reagents

The standard solutions of compounds I, II, III and the internal standard delorazepam (compound IV) (Fig. 1) were prepared by carefully weighing ca. 10 mg into a 10-ml volumetric flask, then by dissolving in methanol. Serial dilutions were also prepared in methanol. Butyl acetate and toluene, both Purex brand and supplied by SDS (Peypin, France), were used, respectively, for the extraction from plasma and for the dissolution of the residue prior to GC analysis. Methanol, Uvasol brand, was supplied by Merck (Darmstadt, F.R.G.).

Extraction procedure

A 0.5-ml volume of plasma was added to a suitable volume of the internal standard solution (concentrated to ca. 20—30 μl in tapered 10-ml tubes under a stream of nitrogen), and then extracted for 5 min with 0.5 ml of butyl acetate. The tubes were gently shaken on a Vortex system to avoid serious emulsion formation. After centrifugation for 15 min at 4500 *g*, the organic phase was transferred to a 1.0-ml minivial (Pierce, Rockford, IL, U.S.A.), and then evaporated to dryness under a nitrogen stream. The residue was dissolved in 50—100 μl of toluene prior to GC analysis.

Quantification of unknown samples

The plasma concentration data were obtained from least-squares linear regression curves, established daily from three or four calibration points. Peak-height ratios were computed by means of an HP 3388A system (Hewlett Packard). Quality-control samples were analysed together with the unknowns to confirm the assay accuracy (ca. 10% of the analysed samples were quality controls).

RESULTS

Chromatograms

Fig. 2 shows the typical chromatograms obtained in such analytical conditions for a control plasma (A), a control plasma spiked with compounds I, II and III (B), a subject plasma before administration (C) and 4 h following oral administration of 15 mg of quazepam (D).

Reproducibility

The intra-assay precision (given by the relative standard deviation) and the accuracy (defined by the difference between obtained and expected concentrations) were checked for plasma concentrations of compound I ranging from 1.2 to 100.0 ng/ml, of compound II ranging from 1.2 to 60.0 ng/ml and of

TABLE I

INTRA-ASSAY REPRODUCIBILITY AND ACCURACY FOR PLASMA DETERMINATION OF QUAZEPAM AND ITS METABOLITES (n = 7)

Concentration expected (ng/ml)	Concentration obtained (ng/ml)	C.I.M.* (%)	Difference between expected and obtained concentration (%)
Quazepam			
1.2	1.18	9.8	−1.7
3.0	2.92	6.1	−2.7
15.0	14.6	3.9	−2.6
40.0	40.4	4.0	+1.0
100.0	96.2	1.8	−3.8
2-Oxoquazepam			
1.2	1.16	3.9	−3.3
3.0	3.04	3.2	+1.3
18.0	17.8	2.3	−1.1
40.0	39.6	2.9	−1.0
60.0	59.2	1.9	−1.3
N-Desmethylquazepam			
0.80	0.78	1.7	−2.5
1.98	2.06	2.8	+4.0
13.4	13.8	0.7	+3.0
40.0	38.0	4.2	−5.0
67.4	68.0	1.1	+0.9

*C.I.M. = Confidence interval of mean (significance level = 0.05).

TABLE II

INTER-ASSAY REPRODUCIBILITY AND ACCURACY FOR PLASMA DETERMINATION OF QUAZEPAM AND ITS METABOLITES (n = 7)

Concentration expected (ng/ml)	Concentration obtained (ng/ml)	C.I.M.* (%)	Difference between expected and obtained concentration (%)
Quazepam			
2.96	2.94	5.6	−0.7
4.88	5.06	3.8	+3.7
11.28	11.34	3.8	+0.5
19.88	19.46	4.6	−2.1
29.74	27.94	6.3	−6.0
2-Oxoquazepam			
2.36	2.28	3.0	−3.4
2.92	2.96	3.1	+1.4
8.46	8.56	3.3	+1.2
23.86	22.98	3.7	−3.7
35.68	35.14	2.7	−1.5
N-Desmethylquazepam			
2.22	2.14	3.2	−3.6
4.38	4.48	3.4	+2.3
10.16	10.0	1.9	−1.6
17.9	17.02	2.5	−4.9
26.76	25.54	3.6	−4.5

*C.I.M. = Confidence interval of mean (significance level = 0.05).

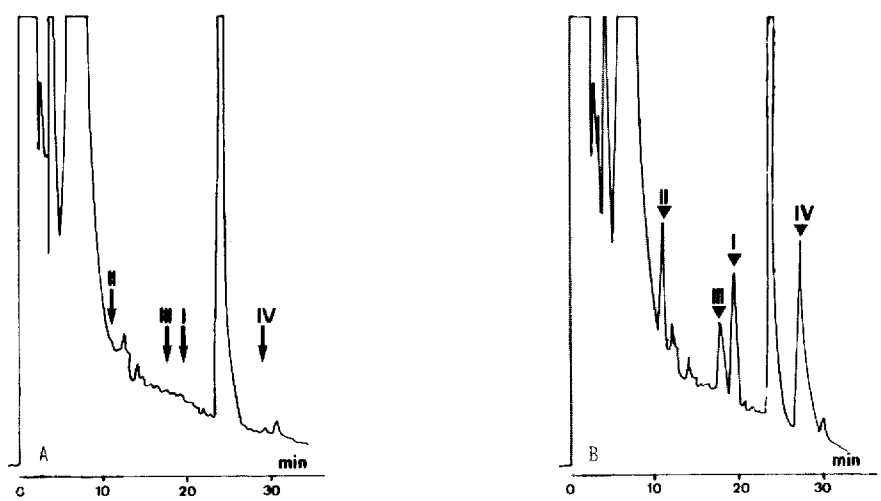

Fig. 3. (A) Chromatogram of a control plasma; (B) chromatogram illustrating the detection limit for plasma quantification of compounds I (0.4 ng/ml plasma), II and III (0.2 ng/ml plasma).

compound III ranging from 0.8 to 67.4 ng/ml. The results were acceptable within these concentration ranges (Table I).

In the same manner, the inter-day precision and accuracy, estimated from quality-control samples analysed during routine quantification of the unknowns, were satisfactory for plasma concentrations of compounds I, II and III ranging from ca. 2 to 30 ng/ml (Table II).

TABLE III

INTRA-ASSAY REPRODUCIBILITY AND ACCURACY NEAR THE DETECTION LIMIT (n = 7)

Concentration expected (ng/ml)	Concentration obtained (ng/ml)	C.I.M.* (%)	Difference between expected and obtained concentration (%)
Quazepam			
0.40	0.40	10.0	0
0.60	0.56	7.2	−6.6
2-Oxoquazepam			
0.40	0.42	7.2	+5.0
0.60	0.58	4.9	−3.3
N-Desmethylquazepam			
0.26	0.26	9.3	0
0.60	0.56	7.2	−6.6

*C.I.M. = Confidence interval of mean (significance level = 0.05).

TABLE IV

LINEARITY TESTS FOR PLASMA DETERMINATION OF QUAZEPAM AND ITS METABOLITES

Concentration added (ng/ml)	Equation of the non-weighted linear regression curve	Correlation coefficient
Quazepam		
0.4, 0.8, 1.6, 2.0	$1.3186x + 0.0290$	0.9912
1, 2, 3, 4	$0.3498x - 0.0032$	0.9995
10, 15, 25, 40	$0.0568x + 0.0109$	0.9997
37.5, 50, 100, 150	$0.0193x + 0.0068$	0.9999
2-Oxoquazepam		
0.2, 0.4, 0.6, 1	$0.5728x - 0.0008$	0.9986
1, 2, 3, 4	$0.3228x + 0.0002$	0.9990
12, 18, 30, 48	$0.0382x + 0.0575$	0.9951
20, 40, 80, 120	$0.0579x - 0.0073$	0.9998
N-Desmethylquazepam		
0.132, 0.266, 0.398, 0.664	$2.6131x - 0.0002$	0.9993
0.664, 1.328, 1.992, 2.656	$0.5843x + 0.0126$	0.9991
10, 15, 25, 40	$0.0568x - 0.0109$	0.9997
20, 40, 80, 120	$0.0463x - 0.0159$	0.9997

Limit of detection

The limit of detection, defined by a signal-to-noise ratio of 4—5, was ca. 0.2 ng/ml for compounds I, II and III (Fig. 3). Near this detection limit, the intra-assay reproducibility and accuracy were better than 10% (Table III).

Linearity

The linearity of the method was checked for concentrations of compounds I, II and III in the range of ca. 0.2 ng to ca. 120—150 ng/ml of plasma. Table IV presents the linearity test results: the correlation coefficients were in the range 0.9912—0.9999 and the intercepts of the calibration curves did not differ significantly from zero.

Extraction efficiency

The recovery of compounds I, II and III from human plasma was in the range 69—88%, regardless of the type of compound and its concentration (Table V).

Specificity

For endogenous plasma components, the specificity was satisfactory (Fig. 2). Interferences which might be due to other compounds, such as benzodiazepines, antiepileptics, etc. have not been determined because, so far, we have applied this method only to healthy volunteers who have not received other concurrent co-medication.

TABLE V

EXTRACTION RECOVERY OF QUAZEPAM AND ITS METABOLITES FROM PLASMA

Concentration (ng/ml)	Extraction recovery (%)	C.I.M.* (%)
Quazepam		
1.2	87.7	8.7
2.4	85.5	4.3
6.0	77.5	6.6
25.0	86.8	4.1
50.0	79.2	3.0
2-Oxoquazepam		
1.2	69.2	2.4
2.4	73.0	4.3
6.0	69.8	6.4
30.0	69.0	3.7
36.0	75.6	5.0
N-Desmethylquazepam		
0.80	83.3	3.4
2.38	87.1	2.2
3.96	82.7	3.7
22.50	80.8	3.1
45.00	88.0	3.5

*C.I.M. = Confidence interval of mean (significance level = 0.05).

DISCUSSION

The method described above allows simultaneous quantification of compounds I, II and III from the same plasma sample. All tests of reproducibility, linearity and specificity concerning endogenous compounds were satisfactory. Nevertheless, such a method presents some inconveniences when applied to routine plasma quantifications.

Firstly, the analysis time was very long, ca. 40 min for each injection, and, because of the use of the glass moving-needle injector, automation of the injection step could not be considered. The particular column conditions, namely CP-Sil 5 (25 m × 0.5 mm I.D.) at 220°C with a head column pressure of 0.4 bar, were required in order to achieve a complete resolution between quazepam (I) and its N-desalkyl metabolite (III). Such requirements are justified even more since peak-tailing for the desalkylated compound was sometimes observed.

Secondly, owing to the relatively low oven temperature, a saturation of the baseline level after a varying number of injections of extracted plasma samples was sometimes observed. In this case, a slow temperature programming of between 220 and 300°C at 2°C/min is needed. Moreover, after three or four weeks, the CP-Sil 5 column lost, in a non-reversible manner, a great deal of its efficiency, with, as a consequence, poor chromatographic separation of compounds I and III.

Despite these inconveniences, this method was the only one in the literature that allowed simultaneous quantification from the same plasma sample of quazepam and its two metabolites. The method fits phase I kinetic studies of quazepam, during which the metabolic behaviour of this benzodiazepine is very interesting because of the pharmacological activity of its metabolites as well as the relatively long half-life of the desalkylated derivative.

Applications

This method was used to analyse human plasma samples collected after oral administration of 15 mg of quazepam. The plasma levels of compounds I, II

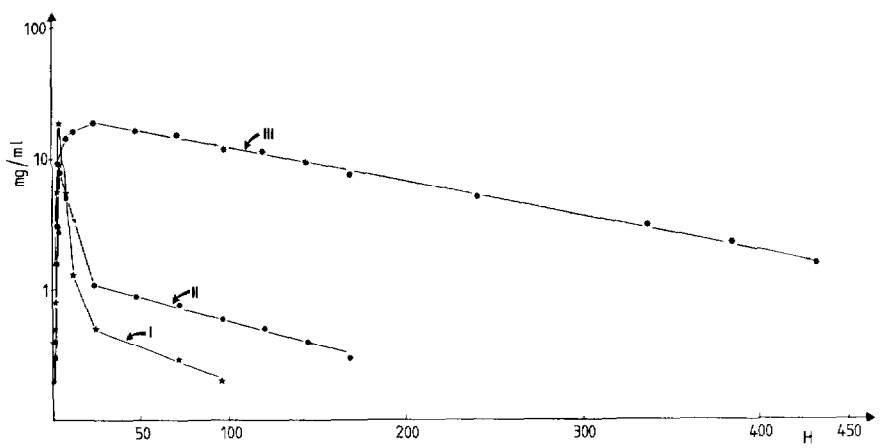

Fig. 4. Time course of plasma levels of quazepam (I), 2-oxoquazepam (II) and N-desmethylquazepam (III) following oral administration of 15 mg of quazepam to a healthy volunteer.

and III were determined at the following times: 0, 0.5, 1, 2, 3, 4, 8, 12, 24, 48, 72, 96, 120, 144, 168, 240, 336, 384 and 432 h after drug intake. Fig. 4 is the plasma level versus time curve obtained after a 15-mg administration of quazepam in one of the subjects studied. An extensive study of these experimental data will be the subject of a further publication.

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