Pharmaceutical Research, Vol. 5, No. 6, 1988

Report

Liquid Chromatographic Assay and Pharmacokinetics of Quazepam and Its Metabolites Following Sublingual Administration of Quazepam

Samir K. Gupta[1,2] and Everett H. Ellinwood, Jr.[1]

Received September 29, 1987; accepted January 6, 1988

A reverse-phase liquid chromatographic method is described for simultaneous quantification of quazepam, and two of its metabolites, 2-oxoquazepam and *N*-desalkyl-2-oxoquazepam. The method uses a solid-phase extraction procedure to prepare plasma samples. After extraction, the methanolic extract is evaporated; the residue is then reconstituted in a small volume of mobile phase and chromatographed. The total chromatography time for a single sample is about 20 min. A sensitivity of 1 ng/ml for quazepam and its metabolites is attained when 1 ml of plasma is extracted. Analytical recovery of quazepam and its metabolites added to plasma ranged from 87 to 96%. The maximum within-day and day-to-day coefficients of variation for each compound at concentrations of 20 and 60 ng/ml were 7.6 and 11.2%, respectively. The method was applied to sublingual pharmacokinetic studies of quazepam in healthy volunteers.

KEY WORDS: quazepam; metabolites; reversed phase; high-performance liquid chromatography; solid-phase extraction; plasma; sublingual; pharmacokinetics.

INTRODUCTION

Quazepam (Q) is a long-acting benzodiazepine with sedative and hypnotic properties (1). Oral studies in humans have shown that Q is rapidly and extensively metabolized to 2-oxoquazepam (OQ) and *N*-desalkyl 2-oxoquazepam (DOQ), quazepam's major active metabolites identified in plasma (2,3). These metabolites also have long elimination half-lives. At present, only two gas chromatographic methods, and no liquid chromatographic methods, are available for determination of quazepam and its two metabolites in plasma (4,5). Both gas chromatographic methods are time-consuming because of the extraction procedures, the chromatographic retention time (5), and a two-part assay procedure requiring two different columns (4).

We describe here a simple and time-efficient solid-phase extraction procedure for plasma sample preparation and a rapid and sensitive high-performance liquid chromatographic method (HPLC) for simultaneous determination of Q, OQ, and DOQ in plasma. This method was used to determine the pharmacokinetics of these compounds in healthy volunteers following sublingual administration of Q. Since an intravenous formulation of Q is unavailable and orally administered Q has a poor systemic availability, the sublingual administration can be a possible alternative to improve systemic availability.

[1] Department of Psychiatry, Duke University Medical Center, Box 3870, Durham, North Carolina 27710.

[2] To whom correspondence should be addressed.

MATERIALS AND METHODS

Reagents and Standards

Q, OQ, and DOQ were provided by Schering Corporation (Bloomfield, N.J.), and diazepam (DZ) by Hoffman–La Roche (Nutley, N.J.). HPLC-grade methanol and potassium phosphate (both monobasic and dibasic) were obtained from Fisher Scientific (Pittsburgh, Pa.). Glycine was obtained from Sigma Chemical Co. (St. Louis, Mo.).

Extraction Apparatus

C_8 Bond-Elut columns and a Vac-Elut SPS24 apparatus were obtained from Analytichem International (Harbor City, Calif.).

Instrumentation and Chromatographic Conditions

The HPLC system was equipped with a Waters Associates (Milford, Mass.) dual-piston, positive-displacement solvent delivery system (Model 501), an automatic injection module (Model 712 WISP), a programmable multiwavelength multichannel detector (Model 490), and a dual-channel electronic integrator (Model 730). Chromatographic separations were made on a Waters Associates NOVA-PAK C_{18} column (7.5 cm × 3.9-mm i.d.).

The mobile phase was 0.002 *M* phosphate buffer (pH 7.2)–methanol (40:60) filtered through a nylon 0.45-μm membrane (Schleicher and Schuell, Keene, N.H.). The chromatograph was operated at ambient temperature using a flow rate of 1 ml/min (1100 psi). Effluents were monitored at 265 nm.

0724-8741/88/0600-0365$06.00/0

In order to determine the unknown plasma Q, OQ, and DOQ concentrations, standard curves were constructed from relative peak heights (Q, OQ, or DOQ to DZ) obtained from an integrator.

Standard Solutions

Working standard solutions were prepared by dissolving 10 mg of Q, OQ, DOQ, or DZ (internal standard) in 100 ml of methanol. Sequential dilutions to 1 μg/ml were then made in 1 *M* glycine buffer (pH 10.5). Calibration standards were prepared by adding Q, OQ, and DOQ to drug-free plasma to obtain concentrations ranging from 2 to 100 ng/ml.

Preparation of Samples

Bond-Elut columns were placed on top of the Vac-Elut vacuum manifold. With the vacuum on, each column was washed with 2 ml methanol and deionized distilled water. To prevent the column from drying out, the vacuum was then shut off as soon as the water had run through each column. A 100-μl volume of DZ solution containing an appropriate concentration of DZ was added (Table I) to each column, followed by 0.5 ml of standard (2–100 ng/ml) or sample plasma onto each column. Th vacuum was then applied to draw the standards or samples through the column. The column matrix was then washed with 2 ml of deionized water followed by 50 μl of methanol. The vacuum was then disconnected, and the eluent was discarded. A 10 × 75-mm silanized glass tube (appropriately labeled) was placed under each column to collect the eluent. A 200-μl volume of methanol was added to each column, and the vacuum was then applied to draw the methanol into the collection tubes. The process was repeated with two 200-μl volumes of methanol. The combined methanol eluent was evaporated to dryness at 37°C under a gentle stream of nitrogen. The residue was reconstituted with 100 μl of mobile phase and then transferred to automatic sampling vials. Aliquots of 50–80 μl were then injected into the chromatographic system by the automatic sampler.

Analytical Recovery

Drug-free plasma spiked with 5, 20, and 50, ng/ml of Q, OQ, or DOQ was analyzed according to the above-described method without any added internal standard. Carefully measured aliquots of the reconstituted extract were injected and peak heights of each compound were measured. Absolute recovery was calculated by comparing these peak heights with those obtained by direct injection of drug standards.

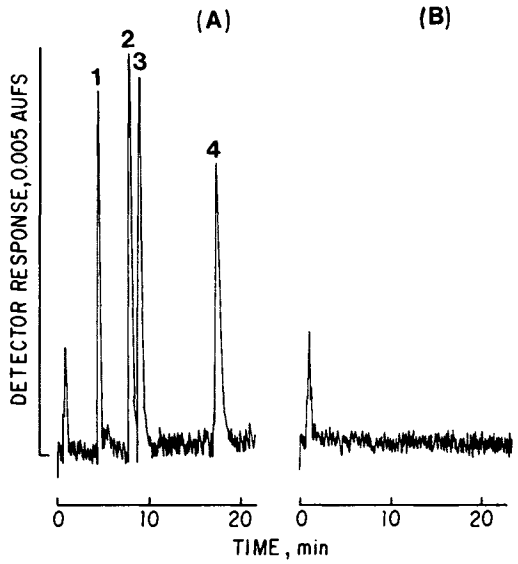

Fig. 1. (A) Chromatogram of extracted plasma containing 50 ng/ml of (1) *N*-desalkyl-2-oxoquazepam diazepam, (2) diazepam, (3) 2-oxoquazepam, and (4) quazepam. (B) Chromatogram of extracted drug-free plasma.

Pharmacokinetic Study

After giving written informed consent, two healthy male volunteers, aged 29 and 31 years and weighing 73 and 77 kg, respectively, received 15 mg of Q sublingually. The subjects fasted for at least 8 hr predose and for 3 hr postdose. The tablet of Q (Dormalin, Schering Corp., Kenilworth, N.J.) was placed under the subject's tongue and was held there for 20 min. Blood samples were drawn in heparinized tubes at 0, 15, and 30 min and 1, 1.5, 2, 4, 6, 8, 24, 30, 48, and 72 hr following drug administration. The blood samples were centrifuged, and the plasma was separated then stored at −80°C until analyzed.

Table I. Regression Analysis of the Calibration Curves of DOQ, OQ, and Q with DZ

Drug	Drug conc. (ng/ml)	DZ[a] conc. (ng/ml)	Equation[b]	r^2	N[c]
DOQ[d]	2–10	10	$Y = 0.09X + 0.05$	0.996	4
	10–100	50	$Y = 0.02X - 0.04$	0.997	5
OQ[e]	2–10	10	$Y = 0.08X + 0.06$	0.994	4
	10–100	50	$Y = 0.02X + 0.03$	0.998	5
Q[f]	2–10	10	$Y = 0.06X + 0.05$	0.998	4
	10–100	50	$Y = 0.01X - 0.06$	0.995	5

[a] Diazepam.
[b] X = drug concentration. Y = peak height ratio of drug to diazepam (internal standard).
[c] Number of points on the curve.
[d] *N*-Desalkyl-2-oxoquazepam.
[e] 2-Oxoquazepam.
[f] Quazepam.

Table II. Analytical Recovery

Spiked conc. (ng/ml)	Percentage recovery (±SD)[a]			
	DOQ	DZ	OQ	Q
5	94.2 (3.4)	90.8 (3.2)	89.6 (4.3)	91.5 (5.6)
20	96.5 (5.1)	89.2 (3.6)	92.1 (7.2)	87.4 (4.8)
50	93.6 (2.8)	92.3 (5.8)	93.2 (6.6)	89.4 (6.9)

[a] $N = 5$.

Data Analysis

Plasma Q, OQ, and DOQ time data were analyzed using both compartmental and noncompartmental methods. For the compartmental analysis, compartmental configuration and the initial estimates of the parameters were determined by ESTRIP (6). Parameter values were further refined using SAS/NLIN (7). The volume of distribution at steady state (V_{dss}/F), mean residence time (MRT), total plasma clearance (CL_p/F), absorption, formation, and elimination half-lives were determined.

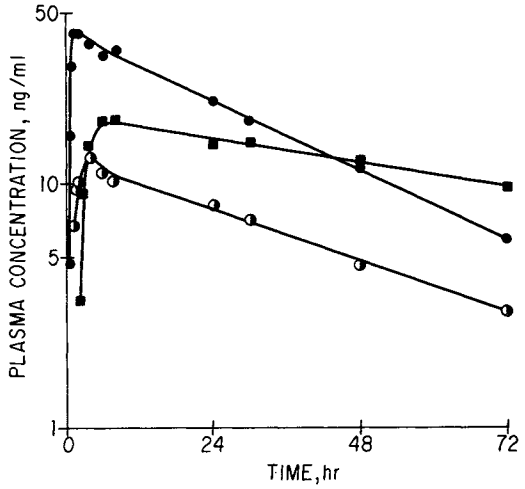

Fig. 2. Mean plasma concentrations of quazepam (●), 2-oxoquazepam (◑), and *N*-desalkyl-2-oxoquazepam (■) following sublingual administration of quazepam in healthy volunteers.

RESULTS AND DISCUSSION

Resolution and Recovery

Under the described chromatographic conditions, Q, OQ, DOQ, and DZ (internal standard) gave symmetric well-resolved peaks (Fig. 1A) with retention times of 4.74, 8.12, 9.21, and 17.56 min for DOQ, DZ, OQ, and Q, respectively. Extracts of pooled human plasma yielded no interference from endogenous plasma components, as shown in Fig. 1B.

The extraction of quazepam, its metabolites, and internal standard (DZ) from plasma by the solid-phase extraction (C_8 column) method was good. DOQ, OQ, Q, and DZ were added to drug-free pooled plasma to achieve concentrations of 5, 20, and 50 ng/ml. Average recovery ranged from 93 to 96 for DOQ, 89 to 92% for DZ, 89 to 93% for OQ, and 87 to 91% for Q (see Table II). There was no perceivable dependence on drug concentration over the range studied.

Linearity, Sensitivity, and Precision

Linearity of the detector response was evaluated by injecting known volumes of various methanolic standard solutions containing DOQ, OQ, DZ, and Q in amounts ranging from 1 to 100 ng. The detector response (peak height) was linear over this range for each compound. Relative peak height ratios of DOQ, OQ, and Q to DZ from extracted plasma samples were also linearly related to concentration over the range of 2–100 ng/ml. The calibration curves obtained for both the low (2–10 ng/ml) and the high (10–100 ng/ml) concentration ranges for DOQ, OQ, and Q were straight lines. The constants of the respective linear regression are listed in Table I.

The limits of detection, allowing a signal-to-noise ratio of 3, are 1.0 ng of DOQ, OQ, and Q, respectively. The sensitivity of the method allows for quantitation of at least 2 ng/ml of each drug extracted from only 0.5 ml of plasma; however, sensitivity is improved by using 1-ml plasma samples.

The precision of the method was assessed by repeated analyses of spiked plasma samples containing two known concentrations of DOQ, OQ, and Q (Table III). The coefficient of variation (CV) ranged from 1.6 to 7.6 for within-day

Table III. Precision of Assay for Quazepam and Its Metabolites

Within day ($N = 10$)		Day to day ($N = 10$)	
Conc. (ng/ml)	CV (%)	Conc. (ng/ml)	CV (%)
N-Desalkyl-2-oxoquazepam			
22	5.1	15.6	8.3
60.4	1.6	54.5	2.4
2-Oxoquazepam			
20.7	6.8	20.8	9.7
61.5	2.8	52.1	4.9
Quazepam			
21.3	7.6	18.2	11.2
60.8	3.4	51.7	5.7

Table IV. Pharmacokinetic Parameters of Quazepam and Its Metabolites Following Sublingual Administration of 15 mg Quazepam: Subject 1 (Age 29 Years and Body Weight 73 kg)

Parameters	Q[a]	OQ[b]	DOQ[c]
$t_{1/2}$ K_a, or $t_{1/2}$ K_m (hr)	0.35	0.67	1.13
$t_{1/2}$, λ_1 (hr)	1.40	1.83	—
$t_{1/2}$, λ_2 (hr)	26.84	36.29	71.94
Lag time (hr)	0.16	0.52	1.84
C_{max} (ng/ml)	44.11	13.70	16.49
t_{max} (hr)	1.27	3.44	5.28
MRT (hr)	39.72	50.69	107.06
V_{dss}/F (liters)	362.30	—	—
CL_p/F (liters/hr)	8.49	—	—

[a] Quazepam.
[b] 2-Oxoquazepam.
[c] *N*-Desalkyl-2-oxoquazepam.

Table V. Pharmacokinetic Parameters of Quazepam and Its Metabolites Following Sublingual Administration of 15 mg Quazepam: Subject 2 (Age 31 Years and Body Weight 77 kg)

Parameters	Q[a]	OQ[b]	DOQ[c]
$t_{1/2}$ K_a, or $t_{1/2}$ K_m (hr)	0.51	0.79	0.83
$t_{1/2}$, λ_1 (hr)	2.24	2.37	—
$t_{1/2}$, λ_2 (hr)	23.76	32.47	69.42
Lag time (hr)	0.24	0.96	2.60
C_{max} (ng/ml)	38.95	10.94	20.17
t_{max} (hr)	1.73	4.76	6.72
MRT (hr)	32.84	48.17	102.24
V_{dss}/F (liters)	326.52	—	—
CL_p/F (liters/hr)	10.81	—	—

[a] Quazepam.
[b] 2-Oxoquazepam.
[c] *N*-Desalkyl-2-oxoquazepam.

determinations and from 2.4 to 11.2% for day-to-day determinations.

Pharmacokinetic Study

The present method provided the desired sensitivity for the pharmacokinetic study of Q and its major metabolites in humans. Previously published gas chromatographic techniques are time-consuming because of the lengthy liquid–liquid extraction procedure (4,5), the long chromatographic retention time (5), and a two-part assay procedure requiring two different columns.

Average plasma Q, OQ, and DOQ concentrations were plotted as a function of time following sublingual administration of 15 mg Q (Fig. 2). Individual plasma Q, OQ, and DOQ time profiles were best characterized by a two-compartment model for Q and OQ and by a one-compartment model for DOQ with first-order absorption (or formation) with a lag time as indicated by F test in ESTRIP. The pharmacokinetic parameters of Q, OQ, and DOQ of two subjects are summarized in Tables IV and V.

Q was rapidly absorbed as indicated by its $t_{1/2}$ K_a and lag time. The large volume of distribution (V_{dss}/F) of Q is indicative of significant tissue uptake. After Q administration, OQ and DOQ appeared slowly as indicated by their lag times and formation half-lives ($t_{1/2}$ K_m). The terminal half-lives of Q, OQ, and DOQ (25.3, 34.38, and 70.68 hr) are in the range of the reported values of 25–41 hr for Q, 28–43 hr for OQ, and 69–79 hr for DOQ (2,3). Further studies are in progress to evaluate individual variability in the pharmacokinetic properties of Q.

REFERENCES

1. C. P. Robinson. *Drugs Today* 22:149–157 (1986).
2. J. M. Hilbert, M. Chung, G. Maier, R. Gural, S. Symchowicz, and N. Zampaglione. *Clin. Pharmacol. Ther.* 36:99–104 (1984).
3. N. Zampaglione, J. M. Hilbert, J. Ning. M. Chung, R. Gural, and S. Symchowicz. *Drug Metab. Dispos.* 13:25–29 (1985).
4. J. M. Hilbert, J. M. Ning, G. Murphy, A. Jimenez, and N. Zampaglione. *J. Pharm. Sci.* 73:516–519 (1984).
5. H. Bun, P. Coassolo, B. Ba, C. Aubert, and J. Cano. *J. Chromatogr.* 378:137–145 (1986).
6. R. D. Brown and J. E. Manno. *J. Pharm. Sci.* 67:1687–1691 (1978).
7. SAS Institute. *SAS User's Guide: Statistics,* SAS Institute, Cary, N.C., 1982.