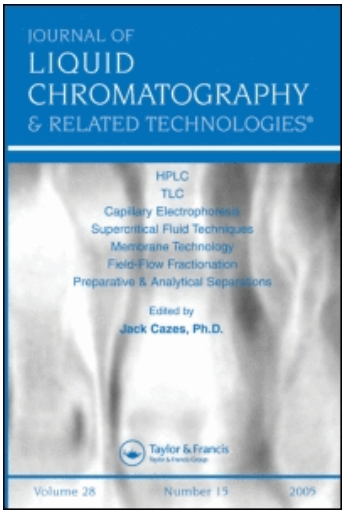

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Liquid Chromatographic Determination of Quazepam in Commercial Tablets

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LIQUID CHROMATOGRAPHIC DETERMINATION OF QUAZEPAM IN COMMERCIAL TABLETS

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ABSTRACT

A simple and rapid reverse-phase liquid chromatography method is described for quantification of quazepam in tablets. Quazepam is extracted with methanol. An aliquot (10 ul) of the diluted methanolic extract was injected into the chromatograph. Chromatographic separations were made on a Adsorbosphere C_8 column (10 cm x 4.6 mm I.D.). Chromatograph was operated at ambient temperature with a mobile phase of 0.002 M phophate buffer (pH 4.0)-methanol (40:60) using a flow rate of 1.5 ml/min. Effluents were monitored at 265 nm. Retention times for diazepam (internal standard) and quazepam were 5.41 min and 8.67 min, respectively. Excellent day-to-day reproducibility of the slope of the standard curve and recovery data were obtained.

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INTRODUCTION

Quazepam is a 1,4 benzodiazepine derivative released for clinical use in the United States as a sedative and hypnotic agent (1,2). At present tablets are the only dosage form available (Dormalin^{R}, 15 mg).

We describe here a simple and rapid high-performance liquid chromatographic procedure (HPLC) for quazepam. At present, gas chromatographic (GLC) method (3,4) is the only other analytical procedure reported in the literature. For routine analysis in quality control, GLC method is time consuming compared to the HPLC method because of long extraction procedure.

EXPERIMENTAL

Materials

Quazepam was provided by Schering Corporation (Bloomfield, NJ) and diazepam by Hoffman-La Roche (Nutley, NJ). HPLC-grade methanol and potassium phosphate (both mono- and dibasic) were obtained from Fisher Scientific (Pittsburgh, PA).

Instrumentation

The HPLC system was equipped with a Waters Associates (Milford, MA) dual-piston, positive displacement solvent delivery system (Model 501),

automatic injection module (Model 712 WISP), programable multiwavelength multichannel detector (Model 490), an electronic integrator (Model 745B). Chromatographic separations were made on a Alltech Associates Adsorbosphere C_8 column (10 cm x 4.6 mm I.D.).

Chromatographic Conditions

The mobile phase was 0.002 M phosphate buffer (pH 4.0)-methanol (40:60) filtered through a nylon 0.45 um membrane (Schleicher and Schuell, Keene, NH). The chromatograph was operated at ambient temperature using a flow rate of 1.5 ml/min (1800 psi). Effluents were monitored at 265 nm.

In order to determine the amount of quazepam in commercially available tablet, standard curves were constructed from relative peak heights (quazepam to diazepam) obtained from the integrator.

Standard Solutions

Working standard solutions were prepared by dissolving 10 mg of quazepam or diazepam (internal standard) in 100 ml of methanol. Calibration standards were prepared by adding quazepam in methanol to obtain the concentration ranging from 1-40 ug/ml. All standard solution contained 10 ug of diazepam per ml as internal standard. Calibration curves were constructed by

plotting ratios of quazepam to diazepam peak heights against known concentrations of quazepam.

Quazepam Extraction Procedure from Tablets

A representative sample, consisting of 20 tablets, was weighed to determine the average weight. Two 15 mg tablet were crushed in a glass mortar to a fine powder. An accurately weighed portion of the powder, equivalent to 20 mg of quazepam, plus 10 mg of diazepam were transferred to a 50-ml volumetric flask. About 40 ml of metahnol was added to the flask. The sample was stirred for 30 min using a small stir bar and a magnetic stirrer. The stir bar was then removed and the sample flask was brought to volume with methanol. After through mixing, an aliquot of the sample solution was transferred to a glass centrifuge tube and was centrifuged at 2000 rpm for 30 min.

A 500 ul portion of the supernatant was diluted to 10 ml with methanol prior to the injection of 10 ul into the chromatograph.

RESULTS AND DISCUSSION

Under the describe chromatographic conditions quazepam and diazepam (internal standard) gave symmetric well-resolved peaks (Figure 1) with retention times of 5.41 min and 8.67 min for diazepam and quazepam, respectively.

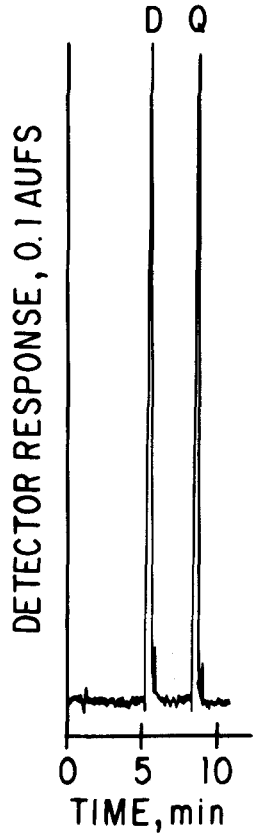

Figure 1: Chromatogram showing quazepam (peak Q) and internal standard, diazepam (peak D).

A typical standard curve is shown in Figure 2. Linearity of detector response was evaluted by injecting various methanolic standard containing quazepam over the concentration range of 1-40 ug/ml with a constant amount of internal standard (10 ug/ml). Reproducibility of the standard curve determined for

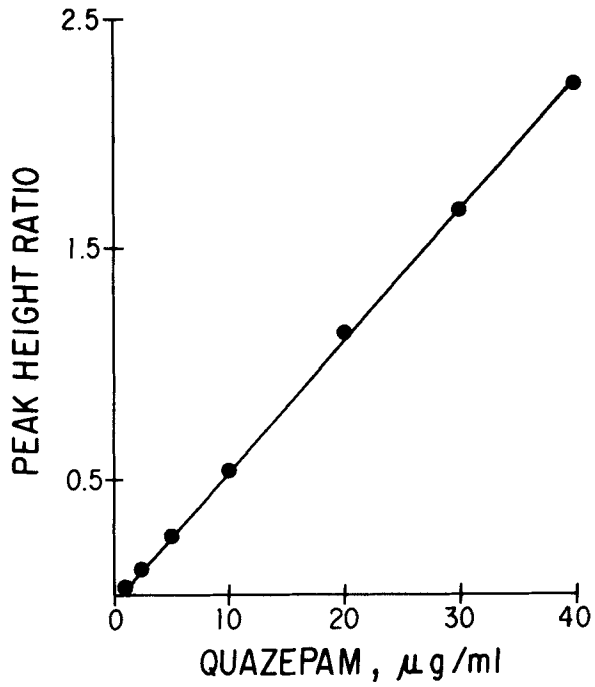

Figure 2: Typical standard curve for quazepam.

five days is indicated in Table I. Excellent day-to-day reproducibility of the slope of the curve was obtained (coefficient of variation = 3.04 %).

Analysis of Commercial Tablets

The retention times of the suspected quazepam from the tablet and pure quazepam were identical. The quazepam extraction procedure from commercial tablet was excellent with no unidentified peaks in the chromatogram after 10 ul injection of samples. Five

TABLE I

DAY-TO-DAY REPRODUCIBILITY OF THE SLOPE

Day	Slope	Coefficient of Determination
1	0.054	0.998
2	0.053	0.994
3	0.050	0.996
4	0.051	0.999
5	0.052	0.997
Mean	0.052	
S.D.	0.00158	
C.V. (%)	3.04	

TABLE II

ANALYSIS OF COMMERICAL TABLETS

Tablet Number	Claimed Amount (mg/tablet)	Obtained Amount (mg/tablet)	Percent Recovery
1	15	15.21	101.40
2	15	15.38	102.53
3	15	15.12	100.80
4	15	14.89	99.27
5	15	14.95	99.67
Mean			100.73
S.D.			1.32
C.V. (%)			1.31

commercial tablets were analyzed and the average percent recovery was 100.73 standard deviation (S.D.) 1.32 and coefficient of variation (C.V.) 1.31 % (Table II).

In conclusion, the present HPLC assay method has been found successful and will be useful for routine analysis of quazepam tablets. In addition, this method provides the basis for rapid, specific and precise quantitative method for the simultaneous determination of quazepam and its metabolites (2-oxoquazepam and N-desalkyl-2-oxoquazepam) in biological samples (work in progress).

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