Note

Liquid chromatographic determination of halazepam in commercial tablets

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Halazepam, an 1,4-benzodiazepine derivative, is used clinically as an anxiolytic agent[1,2]. At present tablets are the only dosage form available in the U.S.A. (Paxipam®, 20 and 40 mg tablet).

We describe here a simple and rapid high-performance liquid chromatographic (HPLC) procedure for halazepam. At present, a gas chromatographic (GC) method[3,44] is the analytical procedure reported in the literature. For routine analysis in quality control, the GC method is time consuming compared to the HPLC method because of the long extraction procedure.

EXPERIMENTAL

Materials

Halazepam was provided by Schering Corporation (Bloomfield, NJ, U.S.A.) and diazepam by Hoffman-LaRoche (Nutley, NJ, U.S.A.). HPLC-grade methanol and potassium phosphate (both mono- and dibasic) were obtained from Fisher Scientific (Pittsburgh, PA, U.S.A.).

Instrumentation and chromatographic conditions

The HPLC system was equipped with a Waters Assoc. (Milford, MA, U.S.A.) dual-piston, positive displacement solvent delivery system (Model 501), automatic injection module (Model 712 WISP), programable multi-wavelength multi-channel detector (Model 490), and an electronic integrator (Model 745B). Chromatographic separations were made on an Alltech Adsorbosphere C_8 column (10 cm × 4.6 mm I.D.).

The mobile phase was 0.002 *M* phosphate buffer (pH 4.0)–methanol (40:60) filtered through a nylon 0.45-µm membrane (Schleicher and Schuell, Keene, NH, U.S.A.). The chromatograph was operated at ambient temperature using a flow-rate of 1 ml/min (1100 p.s.i.). Effluents were monitored at 240 nm.

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

Standard solutions

Working standard solutions were prepared by dissolving 10 mg of halazepam or diazepam (internal standard) in 100 ml of methanol. Calibration standards were prepared by adding halazepam in methanol to obtain the concentration ranging from 1–40 μg/ml. All standard solutions contained 10 μg of diazepam per ml as internal standard. Calibration curves were constructed by plotting ratios of halazepam peak heigt against known concentrations of halazepam.

Halazepam extraction procedure from tablets

A representative sample, consisting of 20 tablets, was weighed to determine the average tablet weight. One 40-mg tablet was crushed in a glass mortar to a fine powder. An accurately weighed portion of the powder, equivalent to 20 mg of halazepam, plus 10 mg of diazepam were transferred to a 50-ml volumetric flask. About 40 ml of methanol 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 thorough mixing, an aliquot of the sample solution was transferred to a glass centrifuge tube and was centrifuged at 1034 g for 30 min.

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

RESULTS AND DISCUSSION

Under the described chromatographic conditions halazepam and diazepam (internal standard) gave symmetric well-resolved peaks (Fig. 1) with retention times of 6.88 min and 9.20 min for diazepam and halazepam, respectively.
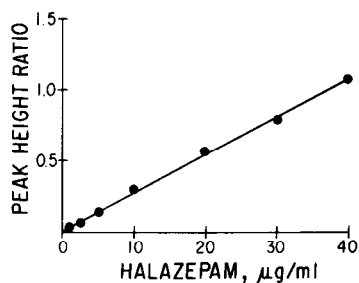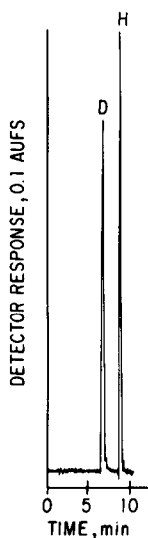

Fig. 1. Chromatogram showing halazepam (peak H, retention time = 9.20 min) and internal standard, diazepam (peak D, retention time = 6.88 min).

Fig. 2. Typical standard curve for halazepam.

TABLE I

DAY-TO-DAY REPRODUCIBILITY OF THE STANDARD CURVE FOR HALAZEPAM

S.D. = Standard deviation; C.V. = coefficient of variation.

Day	Slope	Coefficient of determination
1	0.0262	0.9994
2	0.0258	0.9986
3	0.0256	0.9991
4	0.0261	0.9989
5	0.0266	0.9990
Mean	0.02606	
S.D.	0.000385	
C.V. (%)	1.48	

   A typical standard curve is shown in Fig. 2. Linearity of detector response was evaluated by injecting various methanolic standard solutions containing halazepam over the concentration range 1–40 μg/ml with a constant amount of internal standard (10 μg/ml). Reproducibility of the standard curve determined for five days is indicated in Table I. Excellent day-to-day reproducibility of the slope of the curve was obtained (C.V. = 1.47%).

Analysis of commercial tablets

   The retention times of the suspected halazepam from the tablet and pure halazepam were identical. The halazepam extraction procedure from commercial tablet was excellent with no unidentified peaks in the chromatogram after 10 μl injection of samples. Five commercial tablet were analyzed and the average percent recovery was 101.23 with S.D. 1.18 and C.V. 1.17% (Table II).

   In conclusion, the present HPLC assay method has been found successful and will be useful for routine analysis as in quality assurance of halazepam tablets. In addition, this method provides the basis for a rapid, specific and precise quantitative method for the simultaneous determination of halazepam and its major metabolites in biological samples.

TABLE II

ANALYSIS OF COMMERCIAL TABLETS

Tablet number	Claimed amount (mg/tablet)	Obtained amount (mg/tablet)	Recovery (%)
1	40	40.67	101.68
2	40	40.48	101.20
3	40	39.88	99.70
4	40	41.15	102.88
5	40	40.27	100.68
Mean			101.23
S.D.			1.18
C.V.(%)			1.17

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