HPLC Determination of Lorazepam and Lorazepam-Related Compounds in Pharmaceutical Formulations

D. Orlovic¹ / D. Radulovic² / D. Ivanovic² / Z. Vujic²*

¹ Zorka Pharma, Narodnih heroja bb, 15000 Sabac, Yugoslavia
² Department of Pharmaceutical Chemistry and Drug Analysis, Faculty of Pharmacy, Vojvode Stepe 450, PO. Box 146, 11000 Belgrade, Yugoslavia, E-Mail: zaiv@unet.yu

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Summary
A sensitive, accurate and reproducible high performance liquid chromatographic (HPLC) procedure has been developed for the separation and analysis of lorazepam and related compounds. A 20-µL sample was separated by reversed-phase HPLC on a 150 mm x 4.6 mm, 5-µm particle, Wakosil column with 0.1 M ammonium acetate (pH adjusted to 6.0 with acetic acid)-acetonitrile-methanol, 1:1:1 (v/v) as mobile phase.

Introduction
Lorazepam is a short-acting benzodiazepine used to treat severe anxiety disorders and insomnia, in convulsions, as a premedicament and sedative for surgical and other procedures, and in anti-emetic regimens for the control of antineoplastic-induced nausea and vomiting. Quality control of Lorazepam tablets requires the separation and determination of the active substance and related compounds. There are many reports of the determination of lorazepam in human biological material and dosage forms by HPLC [1–4], UV densitometry [5], selective fluorimetry [6], difference spectrophotometry [7], and catalytic thermometric methods [8], and of the analysis of the lorazepam-related compounds 2-amino-2',5-dichlorobenzophenone (compound B), 6-chloro-4-(o-chlorophenyl)-2-quinazolinecarboxaldehyde (compound C), and 6-chloro-4-(o-chlorophenyl)-2-quinazolinecarboxylic acid (compound D) by TLC [1, 9].

We have developed a new method for quality control of lorazepam tablets, especially levels of related compounds, at Zorka Pharma; the method is more rapid than other methods reported in the literature (less than 10 min is required for complete separation of lorazepam and its related compounds), highly sensitive (traces of the related compounds can be detected), and suitable for routine quality control.

Experimental
Solvents and Chemicals

Standards of lorazepam and lorazepam-related compounds B (2-amino-2',5-dichlorobenzophenone), C (6-chloro-4-(o-chlorophenyl)-2-quinazolinecarboxaldehyde), and D (6-chloro-4-(o-chlorophenyl)-2-quinazolinecarboxylic acid) were obtained from the USA (USP; Rockville, MD, USA). Solvents used to prepare mobile phase were HPLC grade and mixtures were filtered and degassed before use.

Stock standard solutions of lorazepam-related compounds B, C, and D were prepared by dissolving 5 mg of each substance in methanol in volumetric flasks (100, 10, and 10 mL, respectively). A mixed stock standard solution was prepared by transferring accurately weighed lorazepam (50 mg) and 1, 2, and 2 mL, respectively of lorazepam-related compounds B, C, and D into a 50-mL volumetric flask, mixing, and diluting to volume with methanol.

Working standard solution was prepared by diluting the mixed stock standard solution (1 mL) to 10 mL with methanol.

Solutions for construction of the calibration curve were prepared by accurately transferring mixed stock standard solution (1, 2, 3, 4, 5, 6, 7, 8, and 9 mL) into nine 50-mL volumetric flasks and diluting to volume with methanol.

Sample solutions were prepared by accurately transferring a finally powdered tablet into a 10-mL volumetric flask, adding methanol (5 mL), sonicating for 10 min at room temperature, and diluting to volume with methanol. The solution was filtered through a 0.2 µm Millipore filter. The concentration of the solution obtained was 0.1 mg mL⁻¹ lorazepam (Figures 1 and 2).
**Chromatography**

Isocratic HPLC was performed with a GBC (Dandenong, Victoria, Australia) liquid chromatograph equipped with a Rheodyne model 7125 injector valve (20 µL sample loop), automatic sample injection, and a GBC LC 1210 UV detector. Compounds were separated on a 150 mm × 4.6 mm, 5-µm particle, Wakosil C<sub>18</sub> column (SGE, Victoria, Australia) which was maintained at ambient temperature. The mobile phase was 0.1 M ammonium acetate (pH adjusted to 6.0 with acetic acid)-acetonitrile-methanol, 1:1:1 (v/v), at a flow rate of 1 mL min<sup>-1</sup>. The injection volume was 20 µL and absorbance was monitored at 254 nm with 0.05 a.u.f.s sensitivity.

**Results**

RP-HPLC with UV detection was used for analysis of lorazepam and related compounds B, C, and D from tablets. The selectivity was determined by injecting blank samples, placebos, and standard solutions. No interfering peak was detected at the retention times of the drugs and related compounds.

The linearity of the method was determined by injecting nine solutions of concentration between 20 and 180% of the expected concentration. Over this concentration range linear regression analysis of lorazepam peak area (y) against lorazepam concentration (x) yielded the equation \( y = 2 \times 10^{-3}x + 12180 \) \( (R^2 = 0.9999; n = 9) \).

The precision of the procedure was checked by analysis of ten working standard solutions and ten sample solutions (0.1 mg mL<sup>-1</sup> lorazepam). The RSD (0.298–0.954%; Table I) was indicative of the satisfactory repeatability of the system.

The accuracy of the methods was determined by analysis of three different concentrations of lorazepam tablet (80, 100, and 120%). The low values obtained for the SD (0.89) show the accuracy and reproducibility of the method.

The high recoveries from solutions oflorazepam tablets (100.77–103.61%; Table II) were indicative of the suitability of the method for the determination of lorazepam in tablets.

The limits of detection (LOD) and quantification (LOQ) for lorazepam were determined experimentally by applying the described method to dilute solutions. The results are shown in Table III. The precision of the method was checked for within-day and between-day variation (Table IV).