

Second-derivative spectrophotometric assay of bromazepam in pharmaceutical formulations

Pablo Richter

Department of Chemistry, Faculty of Sciences, University of Chile, Las Palmeras 3425, P.O. Box 653, Santiago (Chile)

(Received 25 July 1990)

(Modified version received 8 January 1991)

Key words: Bromazepam determination; Second-derivative absorption spectrophotometry; Pharmaceutical formulation

Summary

A simple, rapid and accurate direct method has been developed for the determination of bromazepam in pharmaceutical formulations using second-derivative absorption spectrophotometry. Methanol was used as the solvent for extracting the drug from the formulations. The calibration graph was a straight line ($r = 0.9989$), passing through the origin. The proposed method was successfully applied to determine bromazepam in pharmaceutical formulations and the ingredients commonly found in commercial tablets do not interfere.

Introduction

Since the introduction of chlorodiazepoxide in 1960, a large group of the 1,4-benzodiazepine class of compounds has been investigated as anticonvulsants in addition to their anti-anxiety and muscle relaxant properties. These compounds are widely used and prescribed for therapeutic purposes. The metabolic pathway for the biological degradation of these compounds has been the subject of many analytical methods (Riedler and Wendt, 1973; Kaplan et al., 1974; Groves et al., 1981; Richter et al., 1990).

Bromazepam (7-bromo-1,3-dihydro-5-(2-pyridyl)-2H-1,4-benzodiazepin-2-one) is of clinical interest as an anti-anxiety agent, and it has been determined by atomic absorption spectrophotometry (Gonzalez et al., 1986), high-performance liquid chromatography (Hooper et al., 1985), voltammetry (Smyth et al., 1978; Lopez et al., 1987), flow injection analysis (Prada et al., 1988; Ruiz et al., 1988) and spectrophotometry (Lopez et al., 1987). All these methods are, however, relatively complicated in terms of assay procedure or equipment required for single-component tablet preparation.

The purpose of this work was to develop a direct and simple ultraviolet derivative spectrophotometric method for the determination of bromazepam in pharmaceuticals and applicable in the routine analysis of this drug as well as other benzodiazepines in the pharmaceutical industry.

Correspondence: P. Richter, Dept of Chemistry, Faculty of Sciences, University of Chile, Las Palmeras 3425, P.O. Box 653, Santiago, Chile.

Materials and Methods

Reagents

All chemicals used were of analytical-reagent grade. Bromazepam was kindly provided by Laboratorio Chile, Santiago, Chile.

Stock solutions ($50\text{--}500\ \mu\text{g ml}^{-1}$) of bromazepam were prepared by dissolving the appropriate amount of the drug in methanol. Tablets containing bromazepam were also, dissolved in methanol and assayed.

Apparatus and conditions

Derivative spectrophotometric analysis was performed using a Shimadzu UV-160 instrument with 10 mm quartz cells. Suitable settings were: second derivative mode, range from 200 to 300 nm, $\Delta\lambda = 5\ \text{nm}$, scan speed slow.

Calibration procedure

Aliquots of the stock solution of bromazepam were diluted in distilled demineralized water. The second-derivative spectra against water were then recorded over the concentration range $1\text{--}30\ \mu\text{g ml}^{-1}$. The corresponding absolute values of the derivative were obtained measuring the peak-to-peak amplitude between the wavelengths 237 and 247 nm and then were plotted against bromazepam concentration.

Sample analysis

Pharmaceutical formulations Tablet formulations containing a nominal 3 mg of bromazepam in a total mass of approx. 200 mg were analysed. According to the manufacturer the contents of the excipients in the tablets were approx.: magnesium stearate + gelatine 3–5% and lactose + starch 90–95%. Twenty tablets were thoroughly ground and mixed. Samples of approx. 1.5 mg of bromazepam were accurately weighed, dissolved in methanol, transferred into separate 50-ml calibrated flasks and diluted to the mark with methanol. The contents of the flasks were shaken for 20 min and then allowed to settle. A 1 ml aliquot of the clear supernatant was diluted to 10 ml with water. Then the assay was carried out as described above. By reference to the calibration graph the concentra-

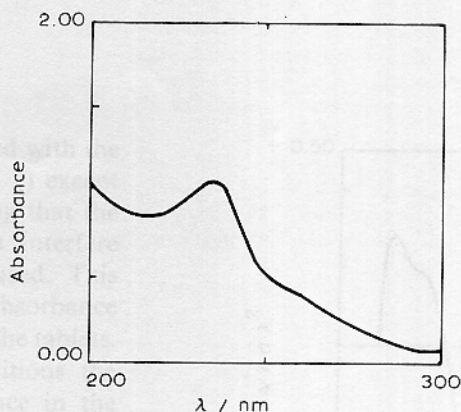


Fig. 1. UV absorption spectrum of $10\ \mu\text{g ml}^{-1}$ bromazepam vs water. Absorbance (235 nm) = 1.08.

tion of bromazepam in each sample was calculated.

In order to establish the reliability of the proposed method, known amounts of the pure drug were added to various pre-analysed bromazepam formulations and the mixtures were analysed.

The effects of the tablet excipients were also studied in order to determine their possible interference in the proposed method.

Results and Discussion

The zero-order spectrum of bromazepam dissolved in methanol shows a maximum at 235 nm

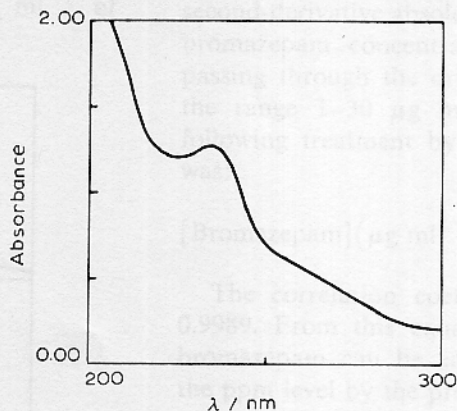


Fig. 2. UV absorption spectrum of methanolic extract of $10\ \mu\text{g ml}^{-1}$ bromazepam in the presence of excipients. Absorbance (235 nm) = 1.27.

(Fig. 1) and similar spectra were obtained with the methanolic extracts of the tablets (Fig. 2) except that the baseline was elevated, indicating that the excipients present in the formulations interfere when ordinary spectrophotometry is used. This effect results in higher values of the absorbance relative to the actual contents present in the tablets. On the other hand, under these conditions the excipients caused both slight opalescence in the samples and less reproducible results. Therefore, to make ordinary spectrophotometry applicable to the determination of bromazepam in tablets, the excipients must be removed (Lopez et al., 1987) which implies time-consuming separations.

It is known that derivative spectrophotometry is highly effective for enhancing both selectivity and reproducibility (Levillain and Fompeydie, 1986). Further, in the second-derivative spectrum, the signal from the detector is amplified and the sensitivity of the method increases markedly. On the other hand, it is widely appreciated that the differentiation degrades the signal-to-noise ratio and that some type of smoothing or low-pass filtering is required in conjunction with differentiation. However, when $\Delta\lambda$ values of five or more were used the noise was too low compared to the signal of bromazepam at the ppm levels.

Figs 3 and 4 show, respectively, typical second-derivative spectra of $10 \mu\text{g ml}^{-1}$ of

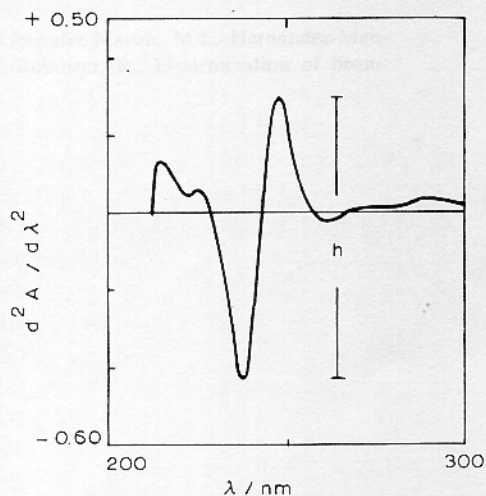


Fig. 3. Second-derivative spectrum of $10 \mu\text{g ml}^{-1}$ bromazepam vs water. $\Delta\lambda = 5$; $h = 0.723$.

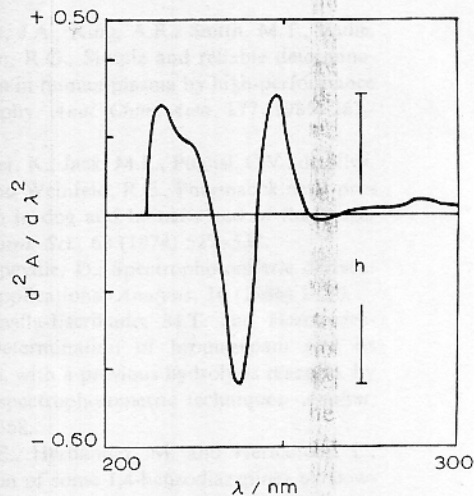


Fig. 4. Second-derivative spectrum of $10 \mu\text{g ml}^{-1}$ of methanolic extract of $10 \mu\text{g ml}^{-1}$ in the presence of excipients. $\Delta\lambda = 5$; $h = 0.729$.

bromazepam in the absence and presence of common excipients. As can be seen, the peaks in the region over 230–270 nm are of similar shape and symmetry, and the peak to peak amplitude at 237 and 247 nm (h), which remains unaltered, could be used for determination.

The calibration graph obtained by plotting the second-derivative absolute value (h) (Fig. 3) vs the bromazepam concentration was a straight line passing through the origin (intercept 0.002) over the range 1–30 $\mu\text{g ml}^{-1}$. The linear equation following treatment by the least-squares method was:

$$[\text{Bromazepam}] (\mu\text{g ml}^{-1}) = 13.79h$$

The correlation coefficient was found to be 0.9989. From this equation it can be seen that bromazepam can be easily determined down to the ppm level by the proposed method.

Identical results were obtained when the measurements were carried out against water, methanol or mixtures of these solvents. Hence, all the assays were performed by measuring the signal against water.

The precision of the method was tested with solutions of similar concentrations. The relative

standard deviation for ten solutions of $1 \mu\text{g ml}^{-1}$ of bromazepam was found to be 0.6%.

On the basis of these results, bromazepam was determined in pharmaceutical formulations. Ten assays on tablets containing a nominal 3 mg of the drug per tablet gave a mean value of 2.97 mg per tablet with a relative standard deviation of 0.5%, indicating that the method appeared to be free from interference from the background absorption due to the tablet excipients.

The method was tested by the standard addition technique and the results were found to be satisfactory. Further, ten determinations were carried out on a synthetic mixture containing bromazepam in common tablet excipients keeping the amounts of excipients relative to bromazepam as recommended by the manufacturer. The recovery was found to be $100.3 \pm 0.4\%$.

Acknowledgements

The author thanks Dr Alfonso Morales for valuable aid in the preparation of the manuscript and Sr Fernando Muñoz for carrying out the analyses of the samples. Thanks are also extended to the Department of Investigation, DTI, of the University of Chile (Project Q2443) and to the National Fund for Development of Sciences and Technology FONDECYT (Projects 90-0028 and 88/0190) for financial support.

References

Gonzalez-Perez, C., Gonzalez-Martin, M.I., Hernandez-Mendez, J. and Recio-Robuster, R., Determination of brom-

- azepam and flurazepam by atomic absorption spectrophotometry. *Quim. Anal. (Barcelona)*, 5 (1986) 420-427.
- Groves, J.A. and Smyth, W.F., Polarographic study of flurazepam and its major metabolites. *Analyst*, 106 (1981) 890-897.
- Hooper, W.D., Roome, J.A., King, A.R., Smith, M.T., Eadie, M.J. and Dickinson, R.G., Simple and reliable determination of bromazepam in human plasma by high-performance liquid chromatography. *Anal. Chim. Acta*, 177 (1985) 267-271.
- Kaplan, S.A., Alexander, K., Jack, M.L., Puglisi, C.V., de Silva, J.A.F., Lee, T.L. and Weinfeld, R.E., Pharmacokinetic profiles of clonazepam in dog and humans and of flunitrazepam in dog. *J. Pharm. Sci.*, 63 (1974) 527-532.
- Levillain, P. and Fompeydie, D., Spectrophotométrie dérivée: intérêt, limites et applications. *Analysis*, 14 (1986) 1-20.
- Lopez-Valdeon, J., Sevilla-Escribano, M.T. and Hernandez-Hernandez, L., Determination of bromazepam and its urinary metabolites, with a previous hydrolysis reaction, by voltammetric and spectrophotometric techniques. *Analyst*, 112 (1987) 1365-1368.
- Prada, D., Lorenzo, E., Hernandez, M. and Hernandez, L., Rapid determination of some 1,4-benzodiazepines by flow-injection analysis. *Microchem. J.*, 37 (1988) 257-262.
- Richter, P., Morales, A. and Lahsen, J., Voltammetric study of 7-nitro-1,4-benzodiazepin-2-ones and their acid hydrolysis products, 2-amino-5-nitrobenzophenones. *Analyst*, 115 (1990) 409-411.
- Riedler, J. and Wendt, G., In Gerattini, S., Mussini, E. and Randall, L.O. (Eds), *The Benzodiazepines*, Raven, New York, 1973, pp. 99-127.
- Ruiz-Zarobe, E., Hernandez-Blanco, M., Lorenzo-Abad, E. and Hernandez, L., Determination of bromazepam by flow injection analysis with amperometric detection. *An. Quim., Ser. B.*, 84 (1988) 120-122.
- Smyth, W.F., Smyth, M.R., Groves, J.A. and Tan, S.B., Polarographic method for the identification of 1,4-benzodiazepines. *Analyst*, 103 (1978) 497-508.