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Determination of nitrazepam and dipotassium clorazepate in the presence of their degradation products using second derivative spectrophotometry

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Summary

A method is presented for the determination of nitrazepam and dipotassium clorazepate in the presence of their acid-induced degradation products using second derivative spectrophotometry. By measuring the absolute value of the second derivative curves at the zero contribution of the corresponding degradation product, the concentration of the intact drug can be calculated directly without interference of the degradation product. The method was proved using synthetic mixtures of the intact drugs with their degradation products and its suitability to monitor the stability of the drugs was demonstrated.

Introduction

Nitrazepam and dipotassium clorazepate are members of the 1,4-benzodiazepines used as tranquillizers and hypnotics. Being 1,4-benzodiazepines, these drugs are susceptible to hydrolysis in acid medium with subsequent ring opening (Schutz, 1984). The products of hydrolysis are a substituted benzophenone and a glycine derivative.

The wide use of this group of drugs has prompted extensive literature on the analysis and study of the decomposition of these compounds. These methods include a TLC detection of the degradation products (Ebel et al., 1977), kinetic method for the study and mechanism of hydrolysis of these compounds (Wesley et al., 1976;

Wesley et al., 1977) and liquid-chromatographic determination of the decomposition rates of clorazepate (Clark et al., 1982).

The present work deals with developing a simple, rapid and accurate method for the determination of nitrazepam and dipotassium clorazepate in the presence of their acid-induced degradation products. The method is based on the use of second derivative UV spectrophotometry with a zero-crossing technique (Shibata et al., 1972; O'Haver et al., 1976; Korany et al., 1984).

Materials and Methods

All reagents used were analytical grade. Mogadon tablets (Roche), labelled to contain 5 mg nitrazepam per tablet. Tranxene capsules (Clin-Midy-Paris), labelled to contain 5 mg dipotassium clorazepate per capsule.

Apparatus

A Perkin-Elmer Model 550S UV-VIS spectrophotometer and a Hitachi Model 560 recorder were used. The second derivative curves of test and reference solutions were recorded in a 1 cm quartz cell over the range 340–200 nm. Suitable settings are: scan speed 120 nm/min; chart speed 60 mm/min; mode D_2 (second derivative = $d^2A/d\lambda^2$); ordinate maximum and minimum $\pm$ 0.009 and 0.02 for nitrazepam and dipotassium chlorazepate, respectively; response time 10 s.

Calibration graphs

Standard solutions were prepared by dissolving 50 mg of nitrazepam in 100 ml ethanol and 50 mg of dipotassium clorazepate in 100 ml distilled water; 2.0-ml portions were diluted to 100 ml with 0.1 N HCl solution. The second derivative (D_2) of the UV spectra was measured against 0.1 N HCl solution as blank. The D_2 values at 250 and 310 nm were measured for nitrazepam and clorazepate, respectively (Figs. 1 and 2).

Preparation of tablet and capsule sample

An accurately weighed amount of the powdered tablets or capsule contents equivalent to about 50 mg of the drug was extracted with ethanol (distilled water was used for clorazepate). This was filtered and made up to the mark in a 100 ml volumetric flask. The procedure was then continued as described above.

Preparation of acid-induced degradation products

About 50 mg of the drug was weighed accurately and transferred into a 100 ml beaker with 50 ml 6 N HCl solution and heated on a boiling waterbath for 1 h. It was cooled to room temperature and transferred to a 100 ml volumetric flask with 6 N HCl solution. 2.0-ml portions of this solution were neutralized with 6 N NaOH solution and diluted with 0.1 N HCl to 100 ml. The D_2 values were recorded and measured as mentioned under calibration graphs.

Results and Discussion

Figs. 1 and 2 show that the absorption curves of nitrazepam and dipotassium clorazepate in 0.1 N HCl overlapped one another considerably with those of their corresponding degradation products. The figures show also that nitrazepam pos-

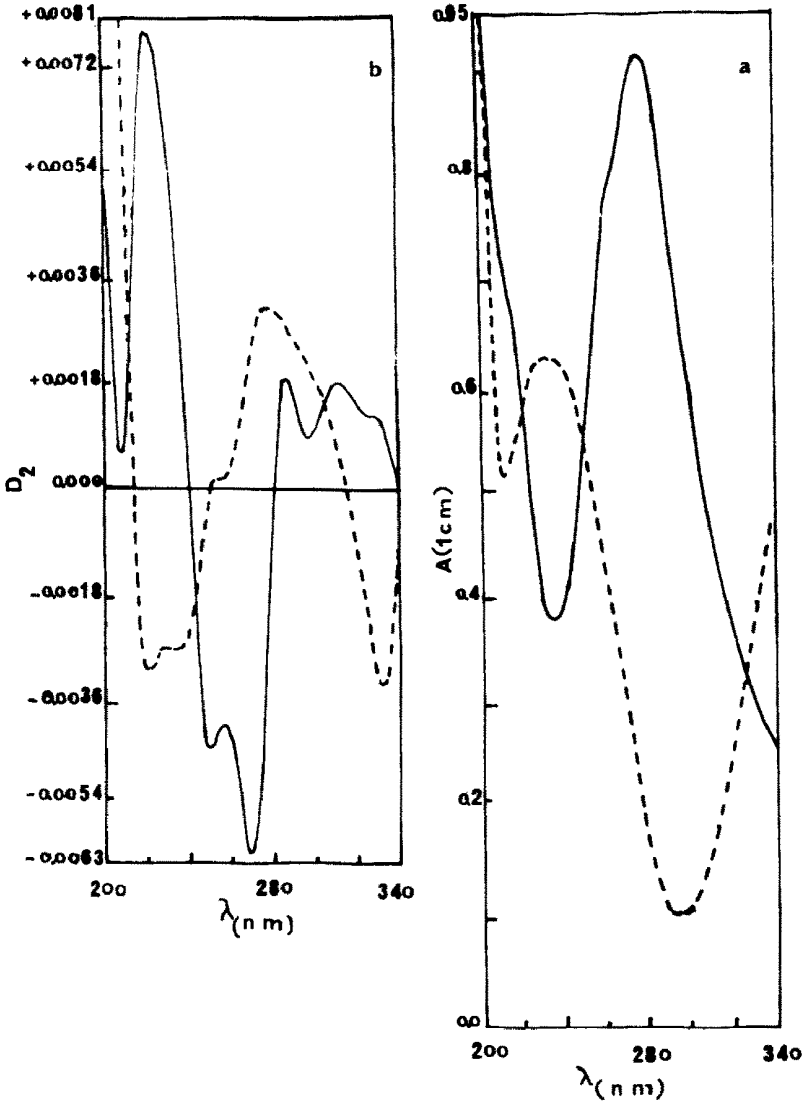

Fig. 1. Zero-order (a) and second derivative (b) spectra of 1.0 mg% nitrazepam (———) and of 1.0 mg% its degradation products (— — —) in 0.1 N HCl.

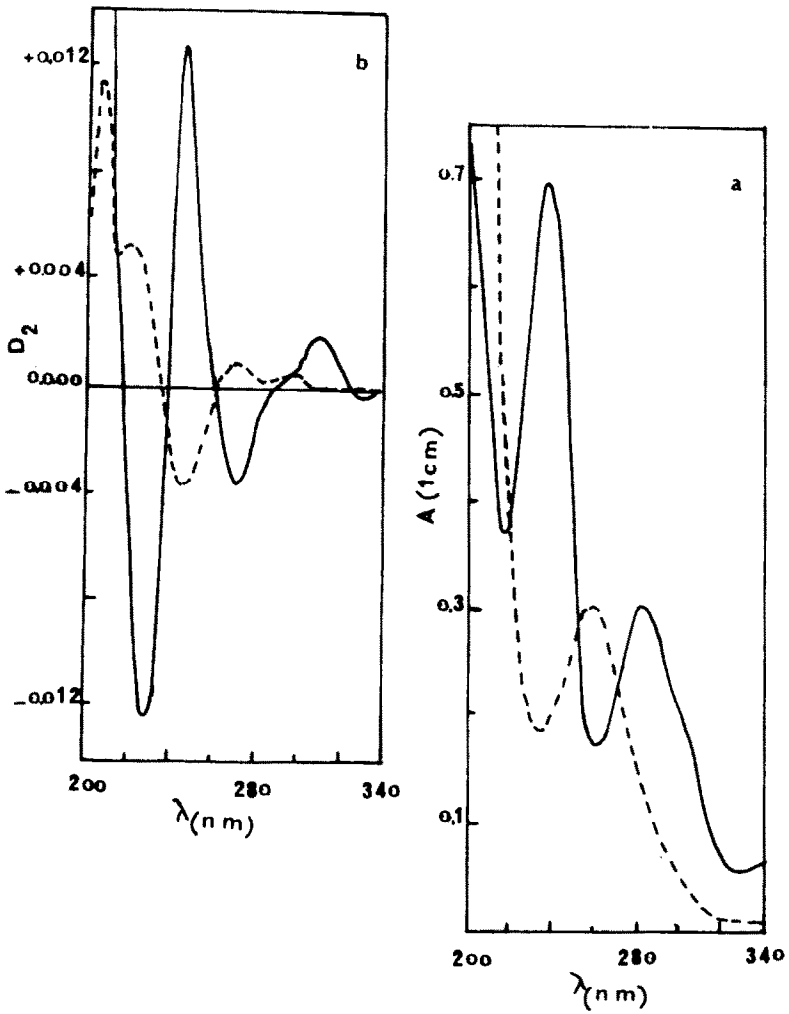

Fig. 2. Zero-order (a) and second derivative (b) spectra of 1.0 mg% dipotassium chlorazepate (———) and of 1.0 mg% its degradation products (— — —) in 0.1 N HCl.

sesses a maximum D_2 value at 250 nm while that of its degradation product at the same wavelength is equal approximately to zero. On the other hand, dipotassium clorazepate appears to have a maximum D_2 value at 310 nm while its degradation products show a D_2 value equal to zero at the same wavelength. Therefore, the absolute value of the second derivative of the zero-order sum curves of the intact drug and its degradation product at the previously mentioned wavelengths can be used to quantitate the intact drugs.

The plots of the D_2 values at 250 nm for nitrazepam and at 310 nm for dipotassium clorazepate against concentration. C, showed linear relationships within

TABLE 1

DETERMINATION OF NITRAZEPAM AND DIPOTASSIUM CLORAZEPATE BY DIFFERENT METHODS

Sample	Recovery (%) [a]		
	Proposed method	Vierordt's method	A_{max} method [c]
Nitrazepam			
Powder	100.0 ± 1.2	–	–
Mixtures [b]	100.3 ± 1.3	100.2 ± 1.3	110.6 ± 6.5
Mogadon tablets	100.1 ± 0.82	99.4 ± 0.92	99.6 ± 1.4
Dipotassium clorazepate			
Powder	100.1 ± 0.45	–	–
Mixtures [b]	99.9 ± 0.87	100.8 ± 0.68	116.1 ± 9.5
Tranxene capsules	99.6 ± 0.51	100.2 ± 0.73	100.5 ± 1.2

[a] Average of 6 determinations ± standard deviations.

[b] The concentration of the intact drug was within the range 0.3–1.0 mg% and each mixture contained 0.25 mg% of the corresponding degradation product.

[c] Wavelengths were 278 nm and 240 nm for nitrazepam and dipotassium clorazepate, respectively.

the range 0.3 –1.0 mg%. The two linear equations were found to be:

$D_2 = 0.0002 + 0.0004\ C$ ($r = 0.9986$) for nitrazepam.
$D_2 = 0.0001 + 0.0020\ C$ ($r = 0.9999$) for clorazepate.

In order to prove the validity and the applicability of the proposed method, six synthetic mixtures of nitrazepam and dipotassium clorazepate each with its degradation product were prepared and analyzed using the proposed method. The concentration of the intact drugs in these mixtures were in the range 0.3–1.0 mg% and each mixture contained 0.25 mg% of its corresponding degradation product. The mean percentage recoveries were found to be 100.3 ± 1.3 and 99.9 ± 0.87 for nitrazepam and clorazepate, respectively (Table 1).

For comparison, the A_{max} method (Dibbern and Wirbitzki, 1978) was applied for the determination of the intact drugs in the above mixtures and the results were unacceptably high due to the contribution of the degraded drug (Table 1). The error in each result decreases with the increase of the concentration of the intact drug relative to that of the degradation product.

The modified Vierordt's method (Glenn, 1960) was applied to the determination of nitrazepam ($\lambda_1 = 240$ nm, $\lambda_2 = 278$ nm) and dipotassium clorazepate ($\lambda_1 = 238$ nm, $\lambda_2 = 258$ nm) in the same mixtures and the mean recoveries were found to be 100.2 ± 1.3 and 100.8 ± 0.68, respectively (Table 1). However, the presence of a constant or linear-irrelevant absorption, as may originate from differences between batches of the sample and the reference, will certainly lead to erroneous results in the modified Vierordt's method (Glenn, 1960). On the other hand, the results

obtained using the derivative technique will not be affected by the presence of irrelevant absorption.

The proposed method was applied to the analysis of nitrazepam and dipotassium clorazepate in their pharmaceutical formulations and the results obtained were concordant with those obtained using the A_{max} method and the modified Vierordt's method (Table 1).

Degradation of nitrazepam and dipotassium clorazepate

Solutions containing 0.5 mg/ml of each drug in 6 N HCl were prepared and kept in the dark at room temperature. Aliquots equivalent to 1 mg were neutralized with 6 N NaOH and diluted with 0.1 N HCl to 100 ml at zero time and every day over a period of 6 days. The D_2 values for each aliquot was measured at the specified wavelength and the concentration of the undegraded drugs were calculated using the linear regression equations previously mentioned. The plots of log C % against time gave straight lines, indicating that, the proposed method based on measuring the D_2 values at the above mentioned wavelength is specific for the intact molecule, independent of degradation products.

With the wide availability of spectrophotometers equipped with electronic differentiator, the application of the proposed method as a stability-indicating assay could be attractive.

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