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Simultaneous determination of antazoline and its degradation product by UV spectrophotometry

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Summary

A procedure for the simultaneous determination of antazoline and its degradation product by ultraviolet spectrophotometry is described. The structure of the degradation product has been elucidated by infrared and mass spectrometry methods. The analytical method proposed is simple and selective.

Antazoline (2-[(*N*-phenyl)benzylaminomethyl]-2-imidazoline) (ANT) is an H_1 receptor antagonist of histamine which has been used as a topical agent in the treatment of ocular disorders (Goodman et al., 1980).

Several procedures for the analytical quantification of ANT, singly or in combination, have been developed. These include colorimetric (Kamalapurkar and Kamat, 1984), fluorimetric (Ayad and El-Hay, 1984), titrimetric (Massaccesi, 1985), gas-chromatographic (GC) (Perrigo et al., 1985) and high-performance liquid chromatographic (Puglisi et al., 1986; De Schutter et al., 1987) methods.

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Further, spectrophotometric methods including determinations by formation of complexes (El-Shabouri et al., 1984; Kasture et al., 1984) or by direct measurement of absorbance (Othman, 1987) have been reported. In the literature, only one report has been published regarding the decomposition of ANT (Pawelczyk et al., 1969).

In this paper, we describe the identification and assay of a degradation product, *N*-(2-aminoethyl)-2-(*N*-benzylanilino) acetamide (ABA), obtained by forced degradation heating at 50 °C of ANT in phosphate buffer solution, pH 7.4.

The main purpose of the present investigation is to develop a sensitive ultraviolet (UV) spectrophotometric procedure which allows the simultaneous assay of the relative concentrations of two species in solution.

The UV spectrophotometric method is based on the analysis of the integrated spectrals areas of the samples. A best fit of numerical data has been

found to be suitable for separating the contributions of the two components in the mixture.

UV spectra were recorded using a Perkin Elmer 330 instrument on line with a 3600 data station. The optimized operating conditions for recording the spectra, using a 1.0 cm quartz cell, were: wavelength range, 220–270 nm; scan speed, 60 nm/min; abscissa format, 10 nm/cm.

The purity of products was checked by thin-layer chromatography (TLC) on precoated silica gel plates (F_{254}, Merck). The mobil phase consisted of butanol/acetic acid/water (65 : 25 : 10, v/v). The mass spectra (MS) were recorded using a KRATOS MS25RF at 70 eV. The IR spectra were recorded in $CHCl_3$ on a Perkin Elmer FT-IR 1700X spectrometer. Antazoline phosphate was obtained from Sigma and used without further purification. Other reagents, including water, were of analytical reagent grade. Standard solutions of ANT and ABA in the range 1–12 μg/ml were prepared by dissolving separately in buffer solutions of sodium phosphate at pH 7.4. UV spectra of working standard solutions were recorded over the 220–270 nm range against a blank.

The resulting degradation product (ABA) was characterized on the basis of its IR spectrum which, in fact, showed a typical C = O absorption band at 1669 cm^{-1} and two bands at 3393–3408 cm^{-1} due to the NH_2 function, both of which were absent in the spectrum of the initial com-

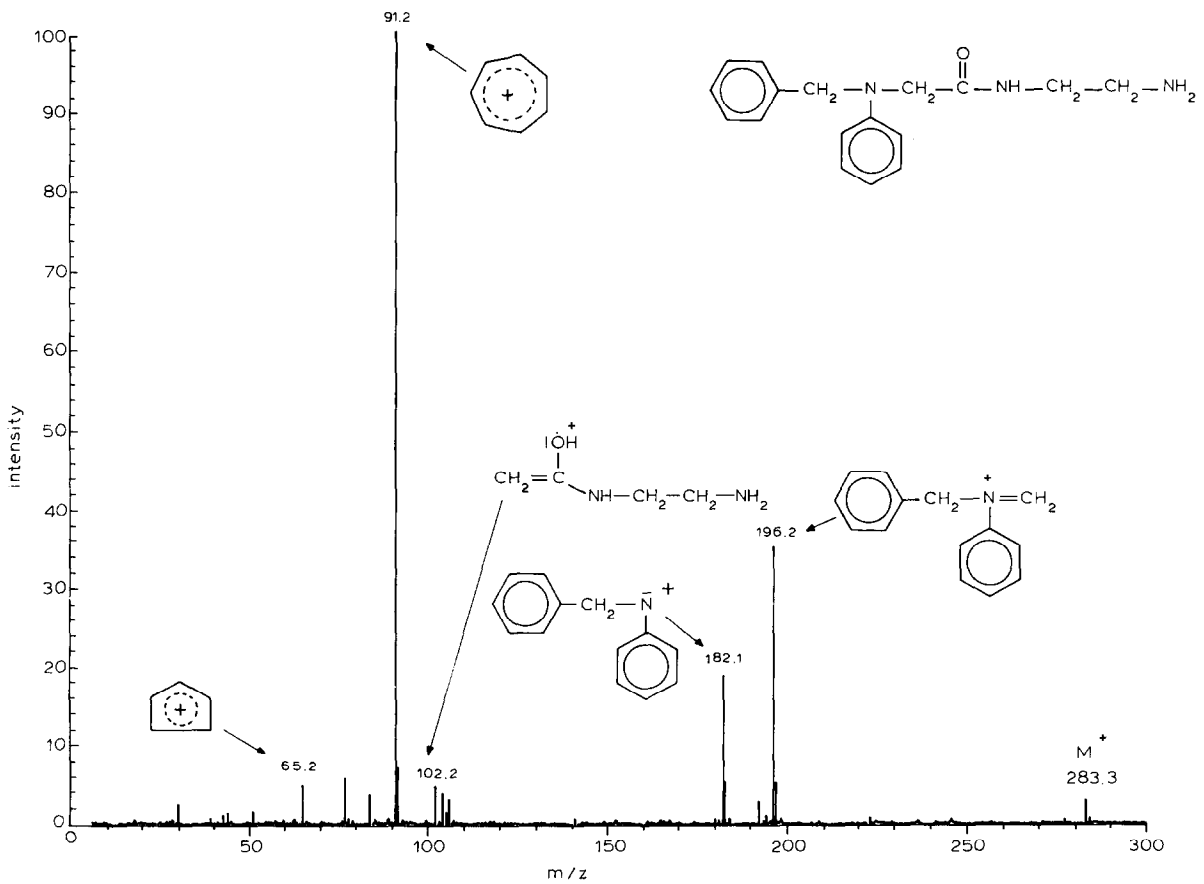

Fig. 1. MS spectrum of ABA at 70 eV.

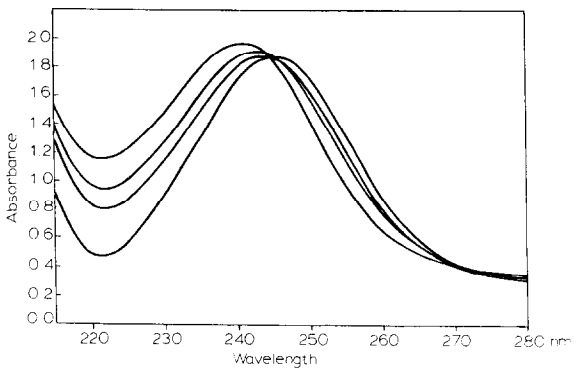

Fig. 2. Typical spectral changes induced by degradation of ANT in phosphate buffer solution at pH 7.4 at 50 °C.

pound (ANT). Moreover, the structure of ABA was elucidated by the MS spectrum as shown in Fig. 1.

The degradation behavior of ANT solution at 50 ± 0.2 °C was observed for up to 912 h. The course of degradation and the formation of a single degradation product were visualized by TLC and GC methods. The first observation, after 48 h, shows that little decomposition of ANT had occurred, while at the end of the degradation reaction the drug had been completely transformed with a stoichiometric yield.

Solutions of ANT subjected to degradation were followed by monitoring the shape of the absorption maximum. Fig. 2 represents the spectral changes due to the degradation reaction of samples.

The initial absorption maximum at 240.9 nm (ANT) was found to be shifted until an asymptotic value of 245.6 nm (ABA) was reached.

In order to develop a sensitive procedure which allows the simultaneous determination of two species in solution, a computerized UV spectrophotometric method was performed. The spectral contributions of two species were determined using spectral deconvolution by a best fit of the numerical data. Data analysis was performed on an IBM 4341 model 11 computer, using a non-linear least-squares program (MINUIT, CERN-library; Geneva, Switzerland).

The precision of the assay was determined by independent analyses on six aliquots of the same sample; the method was found to be reproducible

TABLE 1

Precision of the assay of ANT and ABA in phosphate buffer solution at pH 7.4

Sample [a]	Assay value [b] (μg/ml)	
	ANT	ABA
1	5.021	4.908
2	5.012	4.951
3	4.994	5.015
4	5.038	5.012
5	5.019	4.899
6	4.990	5.098
Mean	5.012	4.980
RSD	0.34%	1.50%

[a] Aliquots of standard solutions formulated at 5.000 μg/ml.
[b] Each value is the mean of five determinations.

with relative standard deviations (RSD) of 0.34 and 1.50% for ANT and ABA, respectively (Table 1).

The linearity of the method was also determined; five standards containing separate concentrations of ANT and ABA in the range 1–12 μg/ml were analysed according to this method. The calibration curve obtained for ANT showed a correlation coefficient of 0.9999 and the equation for the linear regression line was $y = 6.8438x - 0.0353$, where y is the area and x the concentration (μg/ml) of drug. The equation for the linear regression line of ABA was $y = 6.5800x - 0.3782$

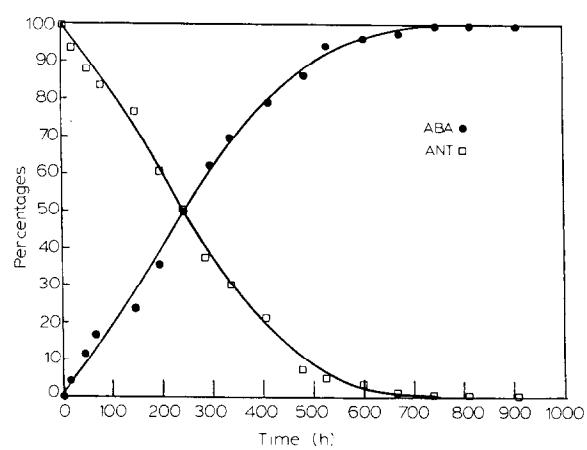

Fig. 3. Time course for the relative percentages of the two components during the degradation process.

with a correlation coefficient of 0.9998. The data obtained were expressed as relative percentages of the two components in solution vs time of degradation (Fig. 3).

The equation interpolated from the curve is a polynomial of order 5 for both compounds.

In conclusion, the method outlined in the present paper is simple and combines both specificity and sensitivity for the simultaneous estimation of ANT and ABA. At the same time, the above analytical procedure offers a rapid and efficient potential method of stability control of antazoline in pharmaceutical preparations, as well as in assessing the degradation product of the drug.

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