Journal of Pharmaceutical & Biomedical Analysis
Vol. 6, Nos 6–8, pp. 1013–1018, 1988
Printed in Great Britain

0731–7085/88 $3.00 + 0.00

Deconvolution of UV absorption spectra of 1,4 benzodiazepines in aqueous solution*

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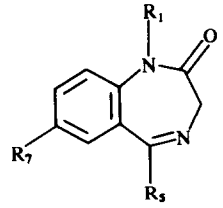
Keywords: *Bromazepam, nitrazepam, flunitrazepam, UV absorption spectra, deconvolution.*

Introduction

The application of deconvolution procedures to UV-visible absorption spectra of chemical compounds in solution can reveal characteristics of the electronic bands hidden under the spectral envelope [1]. Investigation of these bands may provide more detailed or precise information on the structural and analytical properties of the substances under study.

In this communication, a deconvolution method for the determination of some 1,4 benzodiazepines, such as bromazepam, nitrazepam and flunitrazepam in aqueous acidic solutions is described.

The results obtained allow the quantitative expression of the concentration-independent absorption intensity of the drugs as a function of wavelength, in a closed



Name	R_1	R_5	R_7
Bromazepam	H	2-Pyridyl	Br
Flunitrazepam	CH_3	2-Fluorophenyl	NO_2
Nitrazepam	H	Phenyl	NO_2

*Presented at the "International Symposium of Pharmaceutical and Biomedical Analysis", September 1987, Barcelona, Spain.

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mathematical form. This expression seems to provide a more concise way for describing important qualitative and quantitative analytical and spectrophotometric properties of the drugs.

Experimental

Reagents

The following drugs were used without further purification: bromazepam (mp 273°C) (Roche); nitrazepam (mp 226°C) (Prodes) and flunitrazepam (mp 165°C) (Roche).

Apparatus

Absorbance values were determined in 10.00 mm silica quartz cells, at 25°C, using a Hitachi 150–20 double beam recording spectrophotometer in the 200–400 nm range. The spectral bandwidth was 2 nm and scan speed was set at 50 nm min^{-1}. For orientative deconvolution purposes, first and second derivatives spectra of samples were also recorded. The digitized spectra were collected at 1 nm intervals and subjected to numerical analysis. A program in basic was written for use with an Olivetti M20 computer.

Procedures

All solutions were prepared with deionized and distilled water. The standard solutions that were used to determine molar absorptivities from absorbance measurements were prepared by serial dilution of stock solutions of the reference compounds. The pH was adjusted by adding sufficient 1.0 M hydrochloric acid to ensure that only the ionized drug species was present. Concentrations of the standard solutions ranged from 0.01 to 0.05 mM for bromazepam and flunitrazepam and from 0.001 to 0.006 mM for nitrazepam. In all cases, six standard solutions of different concentration were subjected to absorbance measurement.

Results and Discussion

The absorbance values of the aqueous solutions of the benzodiazepine drugs complied with the Lambert–Beer law. Common linear least-squares fits allowed the calculation of the molar absorptivities at 1 nm intervals. These results are represented graphically as dots in Fig. 1.

The band shapes most frequently encountered in absorption profile analysis are the symmetric Gaussian and Lorentzian or Cauchy functions. However, Gaussian shapes are the most suitable for representing the irregular electronic absorption spectral contour of polyatomic molecules in solution [2], which, generally, is composed of a large number of overlapping vibrational subfeatures. One mathematical form to express them is:

$$E(\nu) = \sum_{i=1}^{N} E_i \exp\{-\ln 2[(\nu - \nu_i)/\delta_i]^2\}$$

where frequency, ν, rather than wavelength, is used. E_i is the maximum molar absorptivity at each band central frequency ν_i, δ_i, is the halfwidth, which refers to the

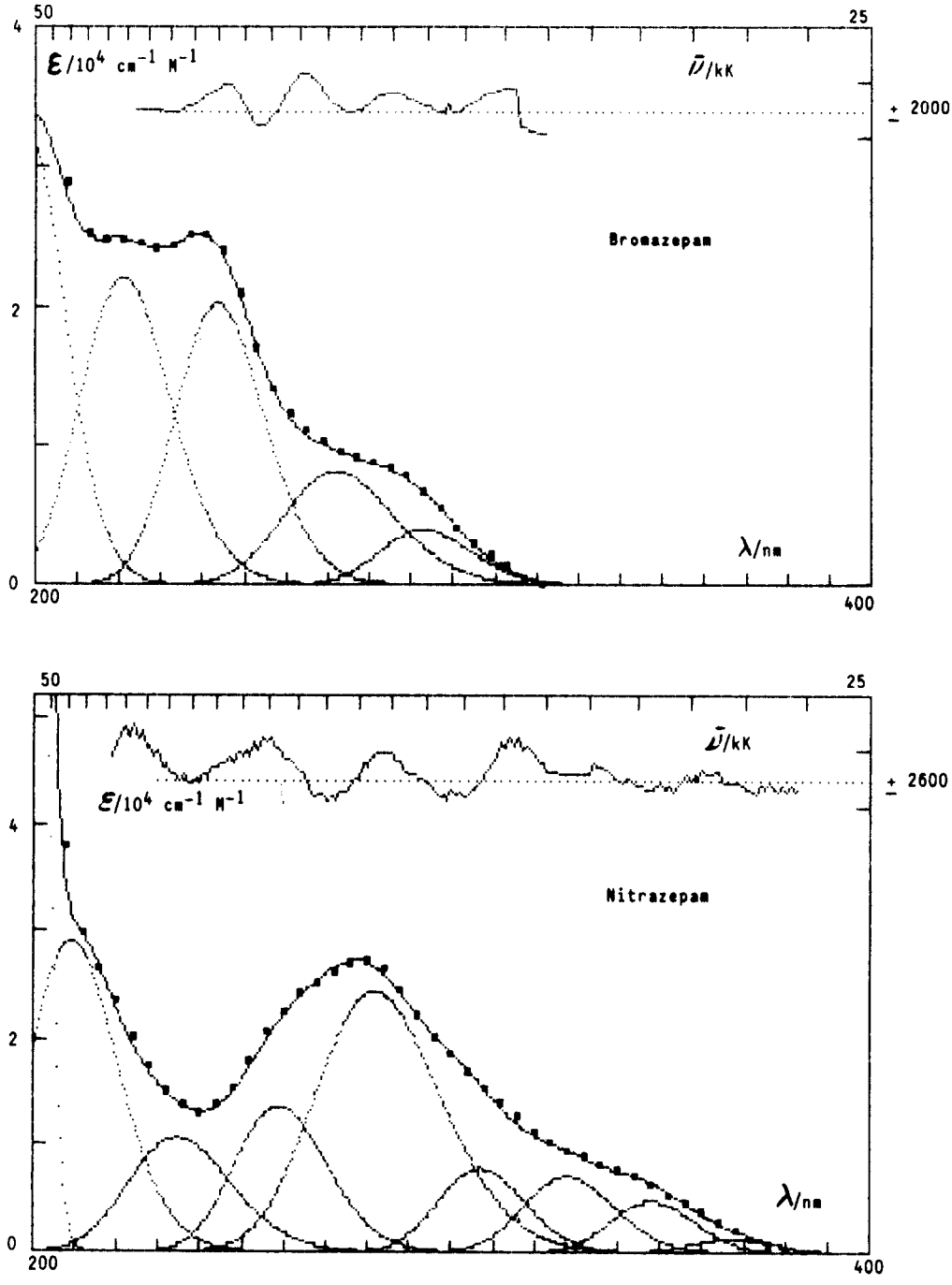

Figure 1
Deconvolution of UV absorption spectra of 1,4 benzodiazepines in aqueous acidic solution. Experimental molar absorptivities are plotted at 4 nm intervals for clarity. Dotted lines are the Gaussian component bands and solid line denotes the calculated spectral contour. The upper line is the absolute deviation from experimental values.

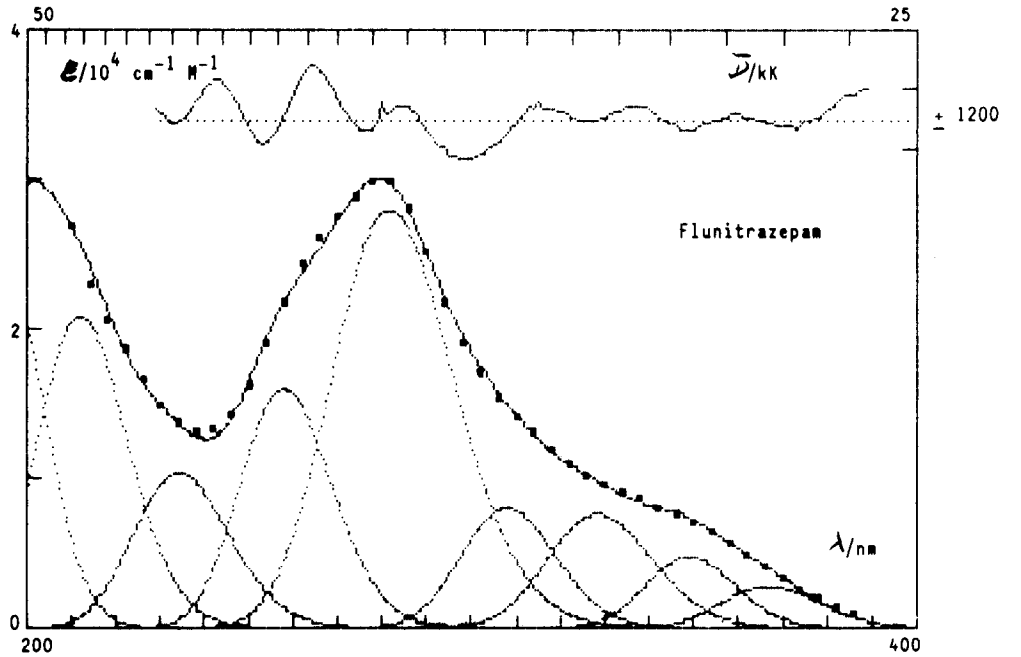

Figure 1 (continued)

frequency width of the band at a point where absorption is half the maximum, and N is the minimum number of Gaussian bands that can reproduce the spectrum.

Computer methods for the resolution of electronic band profiles fall into several related strategic levels of analysis; the so-called differential methods are used most frequently [3]. These methods employ spectral derivatives to estimate the minimum number of Gaussian bands that can reproduce the spectral shape [4]. In the present study, the computer program is based on an algorithm that takes the band central frequencies as initial parameters, as indicated by the first and second derivatives of the spectra [4]. The curve-resolving operation relies on the following procedure: (a) the computer operates in a conversational mode, so the user has complete control of the fitting process. Absorbance values are used as input absorption intensities. A first approximation can be obtained by sequential modification of the band parameters to give the best possible fit. Goodness of fit is followed using the root mean square deviation as indicator. The experimental vs calculated spectra, as well as the absolute deviation vs wavelength, are also displayed; (b) the process is accomplished by the use of a simple iterative least-squares grid search routine to optimize the parameters previously obtained [5].

Application of the method to the absorbance-wavelength experimental data led to the deconvolution of the spectral profiles in terms of a minimum number of Gaussian bands. The band halfwidth and central frequencies were seen to be independent of drug concentration. Accordingly, deconvolved spectra were obtained in terms of molar absorptivities. These results for the resolved spectra are shown together with the experimental data in Fig. 1. Table 1 shows the deconvolution parameters for the drugs

Table 1
Deconvolution parameters

$\bar{\nu}$/kK (λ/nm)	$10^4\,\epsilon/cm^{-1}\,M^{-1}$	δ/kK
Bromazepam		
50.25 (199)	3.17 ± 0.14	2.40
45.25 (221)	2.20 ± 0.09	2.60
40.98 (244)	2.01 ± 0.06	2.10
36.76 (272)	0.81 ± 0.02	2.10
34.18 (293)	0.40 ± 0.02	1.50
Flunitrazepam		
50.76 (197)	2.20 ± 0.09	2.30
47.17 (212)	2.08 ± 0.09	2.60
42.74 (234)	1.04 ± 0.04	2.30
38.76 (258)	1.60 ± 0.05	1.80
35.46 (282)	2.76 ± 0.08	2.00
32.47 (308)	0.80 ± 0.03	1.30
30.40 (329)	0.76 ± 0.03	1.30
28.65 (349)	0.48 ± 0.02	0.95
27.25 (367)	0.28 ± 0.01	0.95
Nitrazepam		
50.25 (199)	14.50 ± 0.70	1.00
47.85 (209)	2.91 ± 0.14	2.80
42.55 (235)	1.06 ± 0.04	2.60
38.61 (259)	1.36 ± 0.04	1.90
35.46 (282)	2.43 ± 0.08	2.20
32.57 (307)	0.79 ± 0.03	1.30
30.49 (328)	0.71 ± 0.03	1.20
28.74 (348)	0.48 ± 0.02	0.95
27.25 (367)	0.13 ± 0.01	0.82

studied. It should be noted that the short wavelength Gaussian tails around 197 nm have no significance and were only introduced to give a suitable fit to the absorbance data below 220 nm.

In all cases, band maxima and halfwidths are sufficiently different to allow the structural characterization of these drugs. Rigorous study of the spectroscopic transitions occurring in these systems cannot be undertaken without detailed chemical description of the molecules. However, the analytical utility of the deconvolution method becomes more apparent if the results are considered in mathematical form as a linear combination of the component Gaussian bands. According to the parameter values shown in Table 1, these expressions for the molar absorptivities of the aqueous acidic solutions of bromazepam, nitrazepam and flunitrazepam, seem to offer a more concise and convenient way to describe important qualitative and quantitative spectrophotometric properties of these drugs. A knowledge of molar absorptivities throughout the 200–400 nm wavelength range, provides a simple procedure for the quantitative spectrophotometric determination of aqueous mixtures of these drugs, and the effects of pH and temperature on complex equilibria, etc. Furthermore, spectral deconvolution studies combined with traditional kinetic research may contribute to the establishment of reaction mechanisms. In particular, in a kinetic study of the acid hydrolysis reactions of 1,4-benzodiazepines, the application of the deconvolution method eliminated the interferences produced by reaction of the reagents and products of the reaction [6].

Acknowledgements — Thanks are due to Roche and Prodes for generous gifts of samples.

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[*Received for review 23 September 1987; revised manuscript received 3 November 1987*]