International Journal of Pharmaceutics, 50 (1989) 75–78
Elsevier

IJP 01693

Simultaneous determination of naphazoline and diphenhydramine hydrochlorides in nasal drops by second-order derivative UV spectroscopy

G. Santoni [1], P. Mura [2], S. Pinzauti [2], P. Gratteri [2] and E. La Porta [2]

[1] *Stabilimento Chimico Farmaceutico Militare, Florence, (Italy)*
and [2] *Dipartimento di Scienze Farmaceutiche, Università di Firenze, Florence (Italy)*

(Received 5 July 1988)
(Accepted 10 August 1988)

Key words: Naphazoline hydrochloride; Diphenhydramine hydrochloride; Simultaneous determination; Second-derivative UV spectroscopy; Nasal drops

Summary

A second-derivative spectroscopic method for the simultaneous determination of naphazoline hydrochloride and diphenhydramine hydrochloride in nose drops was developed. Solutions of this drug combination in 0.1 M hydrochloric acid were analyzed by measurement of the amplitudes of, respectively, the positive peak at 288 nm with respect to the negative peak at 282 nm, and the negative peak at 249 nm with respect to the base line. The method allows the specific, rapid and accurate determination of the binary mixture in the tested concentration range of 1–5 μg/ml for naphazoline, and 10–50 μg/ml for diphenhydramine.

Naphazoline hydrochloride (NPZ), a potent vasoconstrictor, and diphenhydramine hydrochloride (DPH), a well-known antihistamine, can be combined in topical decongestants (nasal drops or sprays) for the treatment of allergic rhinitis (Bryant and Cormier, 1983). The chemical structures of these two drugs are shown in Fig. 1.

While several spectrophotometric and chromatographic methods are described for assaying either naphazoline (Abdel Salam et al., 1986; Jane et al., 1985; Hoogewijs and Massart, 1983; Bauer and Krogh, 1983) or diphenhydramine (Bambagiotti-Alberti et al., 1987; Hill and Langner, 1987; Korani et al., 1986; Sakai and Ohno, 1986), no method has been described for their simultaneous quantitation in two-components mixtures. The zero-order UV spectra of NPZ and DPH overlap in the 275–240 region and the corresponding absorption maxima differ only by approximately 20 nm (Clarke, 1986), making difficult their simultaneous determination by conventional UV spectroscopy.

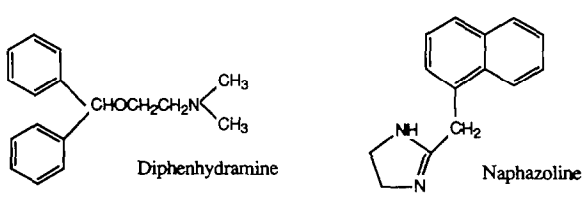

Fig. 1. Chemical structures of NPZ and DPH.

Correspondence: S. Pinzauti, Università di Firenze, Dipartimento di Scienze Farmaceutiche, Via G. Capponi 9, 50121 Firenze, Italy.

In recent years the derivative transformation of spectral data has been shown to offer a powerful tool for both the qualitative and quantitative analysis of drug mixtures (Morelli, 1988; Traveset et al., 1980; Tobias, 1983; Fasanmade and Fell, 1985) due to the ability of the method both to eliminate matrix interferences (Fell, 1978; Levine and Federici, 1982) and enhance resolution (Fell, 1978; Gill et al., 1982; Tobias, 1983).

This paper describes the novel application of a second-order derivative method for the simultaneous determination of NPZ and DPH in nose drops, which overcomes the problems due to overlapping spectral bands and eliminates the need for separation procedures.

Drugs and reagents. NPZ and DPH (Sigma Chemical) were used without further purification. Samples of 4 batches of an isotonic nose drop application form, manufactured for the Italian army (labelled to contain (m/v): 0.025% NPZ, 0.25% DPH, 0.5% sodium chloride, 0.5% chlorbutol and water q.s.), were obtained locally from the Stabilimento Chimico Farmaceutico Militare. Other reagents used, including water, were of analytical reagent grade.

Apparatus. A Perkin-Elmer Mod. 200 UV-visible spectrophotometer equipped with a Hitachi model 200-6629 derivative module and 1-cm quartz cells was used. The second derivative spectra were recorded in triplicate and optimised with the following instrumental parameters: wavelength range, 320–230 nm; scan speed, 240 nm/min; slit width, 2 nm; response time, 6 s.

Solutions. Stock solutions of NPZ (0.1 mg/ml) and DPH (1 mg/ml) in 0.1 M hydrochloric acid were prepared. A series of working standards (1–5 μg/ml NPZ and 10–50 μg/ml DPH) were obtained by appropriate dilution with 0.1 M HCl. NPZ-DPH binary mixtures were also prepared so that the concentration ratio between the analyte and the potentially interfering drug could span the range from 50 to 200% of their ratio in the assayed pharmaceutical preparation.

Analysis of nose drops. 1 ml of the NPZ-DPH solution was mixed in a 100-ml volumetric flask and diluted to volume with 0.1 M HCl. The second-order derivative spectrum was recorded against 0.1 M HCl and the peak amplitudes between the negative peak at 282 nm with respect to the positive peak at 288 nm ($^2D_{282,288}$), and the negative peak at 249 nm with respect to the derivative zero ($^2D_{249}$) were measured for NPZ and DPH, respectively. Calculations were made from the calibration curves plotting the peak amplitude (mm) against concentration (μg/ml).

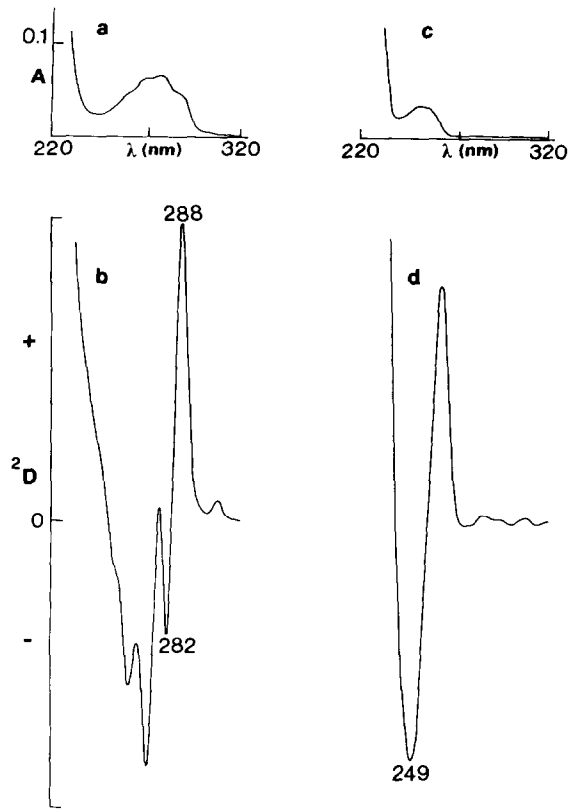

Fig. 2. Zero-order (a, c) and second-order (b, d) derivative UV spectra of NPZ HCl (a, b; 2.7 μg/ml) and DPH HCl (c, d; 33.1 μg/ml) in 0.1 M HCl. The amplitudes of the positive peak at 288 nm with respect to the negative peak at 282 nm, and the negative peak at 249 nm with respect to the base line, were used for quantitation.

The transformation of zero-order data resolved the broad absorption bands of NPZ into their component bands (Fig. 2a, b) and the new profiles clearly showed peaks where previously shoulders and inflections has been seen. Similarly (Fig. 2c, d), the spectrum of DPH was resolved into two sharp peaks. On observing the superimposed second-order derivative spectra of NPZ and DPH (Fig. 3c), it is evident that not all peaks recorded

would be useful in the quantitation of drug mixtures owing to some interferences. The spectrum analysis revealed that the derivative signal $^2D_{282,288}$ was specific for NPZ and $^2D_{249}$ for DPH; these amplitudes were selected because the respective signal magnitude of the interfering component was negligible at the chosen wavelengths.

The zero-order and the second derivative spectra of a NPZ–DPH mixture (at the same respective concentrations as in Fig. 3c) are presented in Fig. 3a, b; in particular $^2D_{282,288}$ for NPZ and $^2D_{249}$ for DPH are unchanged.

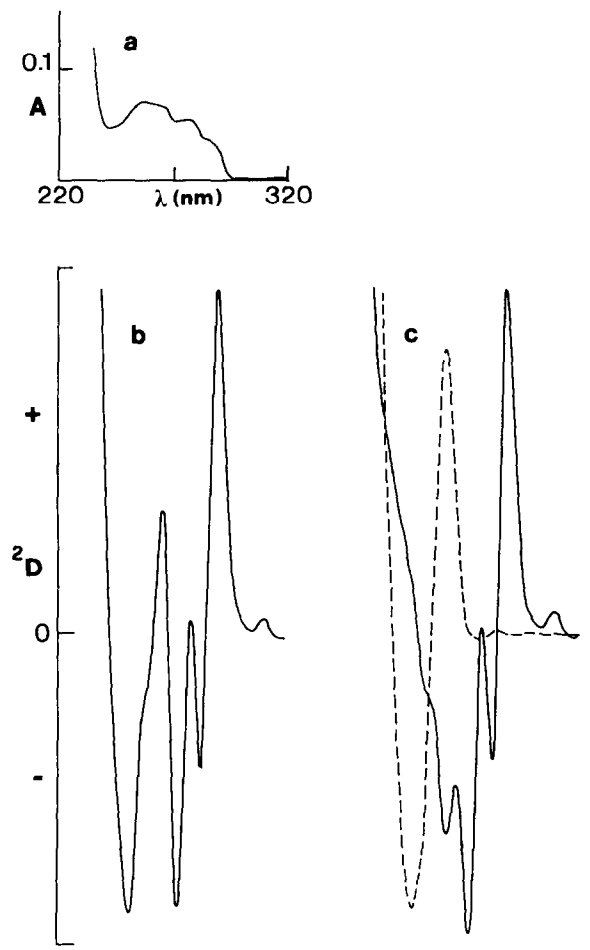

Fig. 3. Zero-order (a) and second-order (b) derivative UV spectra of a binary mixture of NPZ (2.7 μg/ml) and DPH (33.1 μg/ml) in 0.1 M HCl. c: second-order derivative of NPZ (solid line) and DPH (broken line) overlaid to show areas of spectral overlap.

TABLE 1

Analyses on 4 separate batches of a nasal drop preparation labelled to contain 0.025% NPZ and 0.25% DPH

Batch	Average recovery, % (C.V., $n = 5$)	
	Naphazoline	Diphenhydramine
1	99.5(1.2)	103.1(1.5)
2	97.6(2.0)	101.2(0.8)
3	98.7(1.9)	97.1(1.0)
4	102.4(0.7)	98.2(1.9)

Linear correlations were obtained between the respective derivative amplitude and the corresponding drug concentration over the range of 1–5 μg/ml for NPZ and 10–50 μg/ml for DPH. The least-square regression equations (average of 4 determinations) were $y = 40.96x + 2.5$, $r = 0.9996$, for NPZ, and $y = 1.720x + 0.5$, $r = 0.9997$, for DPH; where y is ^2D in mm and x is the concentration in μg/ml. The 95% confidence limits for the calibration graphs were typically ±0.04 μg/ml for NPZ and ±0.35 μg/ml for DPH at the central calibration concentration of 2.5 μg/ml and 25 μg/ml, respectively.

Interaction studies for constant NPZ or DPH levels, but varying DPH or NPZ concentrations, showed that the selected derivative amplitude was independent of the presence of the other drug; in fact, the recovery was in every instance close to quantitative.

The results of the analyses on a topically applied rhinological dosage form are presented in Table 1. The relative standard deviations for both drugs were less than 2%.

We conclude that the described second-derivative spectroscopic method does have the potential for application to stability studies since it permits rapid, precise, accurate and low-cost analyses of NPZ-DPH mixtures in nasal application forms without extraction procedures, and is easily applied to routine usage, thus confirming its possibilities as an analytical tool for simultaneous quantitation of drugs in multicomponent pharmaceutical preparations.

Acknowledgements

This study was financed by the Italian Ministry of Education.

References

Abdel Salam, M., Issa A.S. and Mahrous, M.S., A sensitive spectrophotometric method for the determination of some imidazoline derivatives by 2,6-dichlorophenol-indophenol. *Anal. Lett.*, 19 (1986) 2207–2220.

Bambagiotti-Alberti, M., Pinzauti, S. and Vincieri, F.F., Simultaneous capillary gas chromatographic determination of guaifenesin, dextromethorphan and diphenhydramine in cough-cold syrup. *Pharm. Acta Helv.*, 62 (1987) 175–176.

Bauer, J. and Krogh, S., High-performance liquid chromatographic stability-indicating assay for naphazoline and tetrahydrazoline in ophthalmic preparations. *J. Pharm. Sci.*, 72 (1983) 1347–1349.

Bryant, B.G. and Cormier, J.F., Cold and allergy products. In *Handbook of Nonprescription Drugs*, 7th edn., Am. Pharm. Ass., Washington DC, 1983, pp. 130–148.

Clarke, *Isolation and Identification of Drugs*, 2nd edn., Pharmaceutical, London, 1986, pp. 557, 799.

Fasanmade, A.A. and Fell, A.F., Determination of chlorpromazine and its sulphoxide in pharmaceutical dosage forms by third order derivative ultraviolet spectroscopy, *Analyst*, 110 (1985) 1117–1124.

Fell, A.F., Analysis of pharmaceutical dosage forms by second derivative ultraviolet-visible spectroscopy. *Proc. Anal. Div. Chem. Soc.*, 15 (1978) 260–267.

Gill, R., Bal, T.S. and Moffat, A.C., The application of derivative UV-visible spectroscopy in forensic toxicology. *J. Forensic Sci. Soc.*, 22 (1982) 165–171.

Hill, D.W. and Langner, K.J., HPLC photodiode array UV detection for toxicological drug analysis. *J. Liq. Chromatogr.*, 10 (1987) 377–409.

Hoogewijs, G. and Massart, D.L., Development of a standardized analysis strategy for basic drugs using ion-pair extraction and high-performance liquid chromatography. I. Philosophy and selection of extraction technique. *J. Pharm. Biomed. Anal.*, 1 (1983) 321–329.

Jane, I., McKinnon, A. and Flanagan, R.J., High-performance liquid chromatographic analysis of basic drugs on silica columns using nonaqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection. *J. Chromatogr.*, 323 (1985) 191–225.

Korani, M.A., Bedair, M. and El-Yazbi, F.A., Use of orthogonal polynomials for unequal intervals to eliminate interference in spectrophotometric analysis. Simultaneous determination of ephedrine hydrochloride and diphenhydramine hydrochloride in two-component mixtures. *Analyst*, 111 (1986) 41–44.

Levine, R.L. and Federici, M.M., Quantitation of aromatic residues in proteins: model compounds for second-derivative spectroscopy. *Biochemistry*, 21 (1982) 2600–2606.

Morelli, B., First and second derivative spectrophotometric assay of mixtures of cefuroxime and cephalexin. *J. Pharm. Biomed. Anal.*, 6 (1988) 199–209.

Sakai, T. and Ohno, N., Simultaneous two- and three-component determinations in multicomponent mixtures by extraction-spectrophotometry and thermochromism of ion-association complexes. *Talanta.*, 33 (1986) 415–419.

Tobias, D.Y., First-derivative spectroscopic determination of acetaminophen and sodium salicylate in tablets. *J. Ass. Off. Anal. Chem.*, 66 (1983) 1450–1454.

Traveset, J., Such, V., Gonzalo, R. and Gelpi, E., Derivative and graphical procedures for correction of irrelevant UV spectrophotometric absorption in changeable matrixes. *J. Pharm. Sci.*, 69 (1980) 629–633.