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Short Communication

Determination of homatropine hydrobromide in eye drops by second-order derivative spectroscopy

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Introduction

Homatropine hydrobromide is an anticholinergic agent commonly used as a mydriatic and cycloplegic, and is commonly formulated as 1.0 and 2.0% (w/v) eye drops. In the British Pharmacopoeia, homatropine hydrobromide in "Homatropine Eye Drops" is determined by a gas-chromatographic method, involving chloroform extraction and derivatization with silylating agents [1]. For "Homatropine Hydrobromide Ophthalmic Solution" monographed in the United States Pharmacopeia, the assay method is based on the spectrophotometric measurement of an isooctane extract from a solution after reaction with ceric sulphate [2]. In the British Pharmaceutical Codex (BPC) 1973, homatropine hydrobromide is determined by adding an excessive amount of sodium tetraphenylboron and titrating the excess with cetylpyridinium chloride, with different sample pretreatment steps for eye drops containing different preservatives [3]. In addition to the above official procedures, there are other methods of analysis of homatropine hydrobromide preparations, including colorimetric and chromatographic determinations [4].

Based on the successful application of derivative spectroscopy to the analysis of certain pharmaceutical dosage forms, in particular to the determination of hyoscine hydrobromide in eye drops containing chlorhexidine [5], and the usefulness of the technique in the assay of benzenoid compounds [6, 7], it was found that homatropine hydrobromide in eye drops can be rapidly determined by measuring the second-order derivative of the absorption spectrum with results closely comparable to those obtained by official procedures. The method requires very simple sample preparation, involving only one dilution step.

The proposed procedure can be applied similarly to the assay of atropine sulphate eye drops.

Experimental

Apparatus

A Shimadzu UV-265 FW Spectrophotometer was used under the following conditions: derivative mode, second; wavelength range, 240–290 nm; photometric range, +0.1 to −0.1; $\Delta\lambda$, 2 nm; scan speed, slow (50 nm min^{-1}); abscissa scale, 10 nm cm^{-1}.

Formulations and standards

Homatropine hydrobromide 1 and 2% (w/v) eye drops and atropine sulphate 0.5 and 1.0% eye drops, all BPC 1973 grade, were prepared by the Manufactory of the Central Medical Store of the Hong Kong Government.

Homatropine hydrobromide and atropine sulphate standards were supplied by the same manufactory and were assayed to be of 99.9 and 99.1% purity, respectively by the non-aqueous titration method described in the British Pharmacopoeia.

Procedure

Two millilitres of a 1% homatropine hydrobromide eye drop, or 1.0 ml of a 2% sample,

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were diluted to 100 ml with 0.01 M hydrochloric acid. The second-order derivative UV spectrum was obtained using a 1-cm silica cell and the peak-to-peak amplitude between the wavelengths 254 and 257 nm was measured.

The content of homatropine hydrobromide in the eye drops was calculated by referring to a calibration graph obtained by using standard solutions of homatropine hydrobromide at concentrations of 5–25 mg in 100 ml of 0.01 M hydrochloric acid.

The procedure was also applied to the determination of atropine sulphate in eye drops.

Results and Discussion

Homatropine hydrobromide exhibits UV absorption in the region of 240 to 290 nm, with characteristic small sharp peaks at 251, 257 and 263 nm, respectively. A typical absorption spectrum in 0.01 M hydrochloric acid is shown in Fig. 1(a).

A diluted sample solution of homatropine hydrobromide eye drop was found to show elevated absorption, as shown in Fig. 1(a), probably due to the presence of preservatives such as 0.1% (w/v) of chlorhexidine acetate or benzalkonium chloride as monographed in the British Pharmacopoeia.

The second and third-order derivative spectra of a standard solution of homatropine hydrobromide are shown in Figs 1(b) and 1(c). It was found that a diluted solution of the sample eye drop showed almost identical spectra, suggesting the possibility of elimination of interference.

The effect of the presence of preservatives

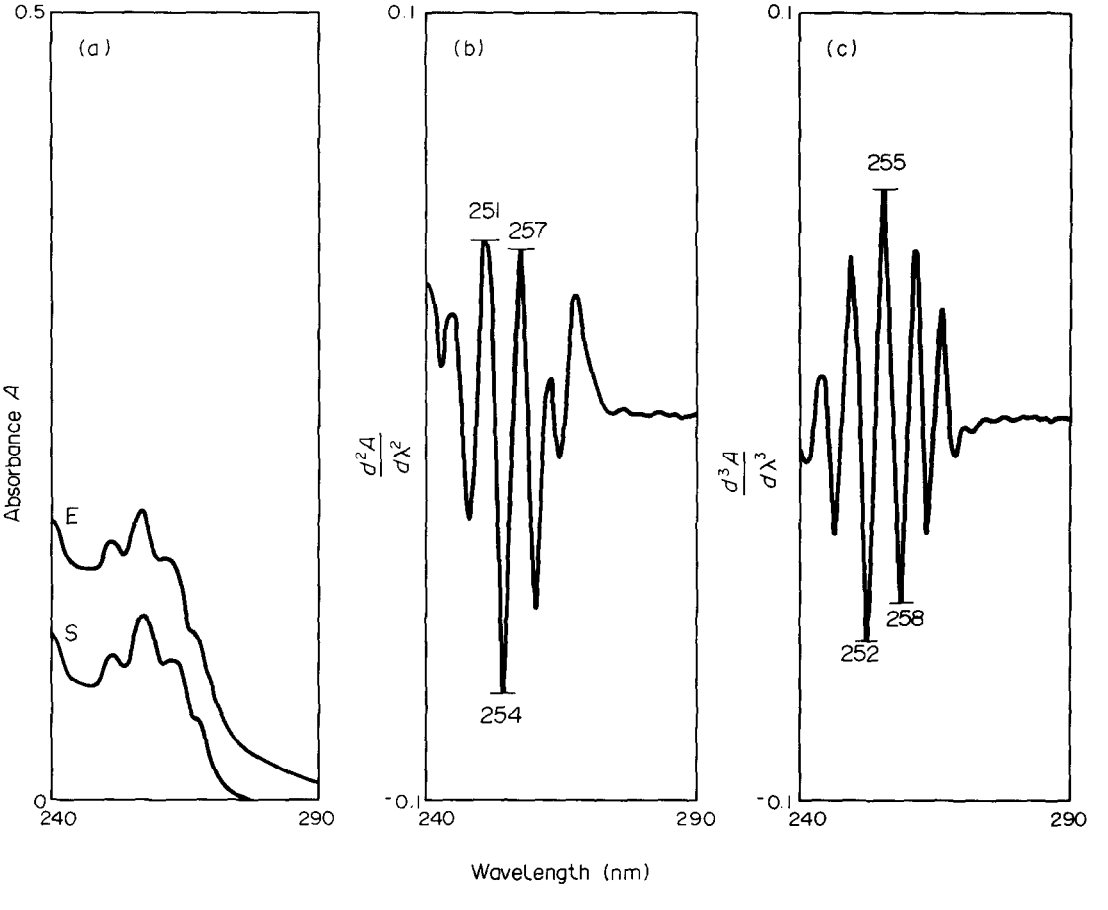

Figure 1
(a) UV absorption spectra of homatropine hydrobromide in 0.01 M hydrochloric acid: S, standard at 50 mg l^{-1}; E, eye drop sample. (b) Second-order and (c) third-order derivative spectra of homatropine hydrobromide.

was studied by preparing blank solutions containing appropriate amounts of preservatives. A typical example was 0.01% of chlorhexidine acetate. The absorption in the zero-order mode would cause interference by contribution to the background absorption. For the second and third-order derivatives, interference was shown to be negligible. Other common preservatives such as benzalkonium chloride, sodium metabisulphite, phenylmercuric acetate and phenylmercuric nitrate were found to have an absorptivity lower than chlorhexidine. They did not give interference in second or third-order derivative mode.

Quantitative study was carried out by preparing calibration graphs from standard solutions of homatropine hydrobromide in 0.01 M hydrochloric acid within a 50–250 mg l^{-1} concentration range. It was found that by measuring the peak-to-peak amplitude between wavelengths 254 and 257 nm, 251 and 254 nm of the second-order derivative spectra, and that between 255 and 258 nm, 252 and 255 nm of the third-order derivative spectra [see Figs 1(b) and 1(c)], proportional linear calibration graphs passing through the origin were obtained. Correlation coefficient, intercept and slope, with 95% confidence limits, of these graphs are shown in Table 1. Any of these four graphs could be used for calculation, but for simplicity, the measurement of the 254 and 257 nm peak amplitude in the second-order derivative spectra was used throughout the quantitative determination of homatropine hydrobromide.

Two batches of homatropine hydrobromide eye drops were analysed by the proposed procedure of derivative spectroscopy and the results were compared with those obtained by one of the official procedures, *viz* the USP spectrophotometric method. A summary of the results is shown in Table 2.

Recovery studies were performed in two synthetic solutions of 1% (w/v) of homatropine hydrobromide, containing 0.01% (w/v) chlorhexidine acetate and 0.01% (w/v) of benzalkonium chloride, respectively. Recovery was found to be 99.8 and 99.9%, respectively. The standard deviations of both results were ±0.8% based on five determinations.

The proposed procedure was found to be equally applicable to the assay of atropine

Table 1
Calibration graphs for the determination of homatropine hydrobromide and atropine sulphate

Compound	Derivative mode	Peak-to-peak amplitude between wavelengths (nm)	Slope/derivative signal $(ppm)^{-1}$*	Intercept (scale 0–0.12)†	Correlation coefficient
Homatropine hydrobromide	Second	254 and 257	4.6×10^{-4} (±2.2%)	-1.8×10^{-4} (±1.4%)	0.9999
	Second	251 and 254	4.7×10^{-4} (±2.5%)	-2.2×10^{-4} (±1.5%)	0.9998
	Third	255 and 258	4.3×10^{-4} (±2.6%)	$+1.7 \times 10^{-4}$ (±1.5%)	0.9998
	Third	252 and 255	4.8×10^{-4} (±1.5%)	$+2.8 \times 10^{-4}$ (±0.9%)	0.9999
Atropine sulphate	Second	254 and 257	5.4×10^{-4} (±2.4%)	-7.1×10^{-4} (±1.7%)	0.9998
	Second	251 and 254	5.1×10^{-4} (±1.5%)	-5.4×10^{-4} (±1.0%)	0.9999
	Third	255 and 258	5.0×10^{-4} (±1.8%)	-8.1×10^{-4} (±1.2%)	0.9999
	Third	252 and 255	5.1×10^{-4} (±3.1%)	-2.3×10^{-4} (±2.0%)	0.9997

*95% Confidence limit given in parentheses.
†95% Confidence limit, expressed as percentage of full scale, given in parentheses.

Table 2
Determination of homatropine hydrobromide and atropine sulphate in eye drops

Sample	Labelled strength	Percentage of labelled strength found	
Homatropine hydrobromide eye drops			
		Proposed method*	USP method
1	1%	100.1 (±0.9)	100.9
2	2%	101.0 (±0.6)	101.8
Atropine sulphate eye drops			
		Proposed method*	BPC method
1	0.5%	104.0 (±1.0)	104.7
2	1.0%	102.0 (±0.8)	102.5

*Mean of five determinations; standard deviation given in parentheses.

sulphate which presents a second-order derivative spectrum similar to homatropine hydrobromide. Similar results are shown in Tables 1 and 2.

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