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The assay of tropane derivatives in formulations by second derivative ultraviolet spectrophotometry

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The development of a second order derivative spectrophotometric assay of atropine, hyoscine and benztropine in formulations is described. The assay involves the extraction into 1,2-dichloroethane of the tetraphenylborate salt of the alkaloids precipitated from pH 6 solution by an excess of sodium tetraphenylboron and measurement of the amplitude near 273 nm to its shorter wavelength satellite in the second derivative spectrum. The second derivative amplitude gives a more precise determination of the alkaloids than absorbance. A two-point bracketting standardization of the amplitude is required owing to a small but significant intercept in the calibration data. The procedures are sufficiently sensitive and precise for the batch and unit-dose assay of tablets of atropine sulphate (0·6 mg) hyoscine hydrobromide (0·3 mg) and benztropine mesylate (2 mg), and for the assay of atropine sulphate injection (0·4 mg ml^{-1}) and tincture of belladonna.

Of the methods available for the assay of the tropane alkaloids hyoscyamine and hyoscine, many lack the sensitivity necessary in formulations at the low levels of drug found (typically 0·3 to 1·0 mg per unit dose).

The reaction of sodium tetraphenylboron with certain organic cations, including tropane alkaloids, to form 1 : 1 salts poorly soluble in aqueous solution has been used as the basis of several titrimetric procedures for these compounds (Johnson & King 1962; Abadia et al 1982; Gur'ev et al 1981; Uspenskaya 1975). In the present work atropine, hyoscine and benztropine are determined in formulations by second derivative uv spectrophotometry after extraction of their tetraphenylborate salts into dichloroethane.

MATERIALS AND METHODS

Spectrophotometer. Second derivative uv spectra were recorded in 1 cm silica quartz cells from 300 to 260 nm using a Perkin-Elmer 522 double-beam ultraviolet-visible recording spectrophotometer with Keyboard Expansion Accessory operating in the second derivative mode which generates the derivative spectra by electronic digital differentiation. The spectral slitwidth was 1 nm, the scan speed 60 nm min^{-1}, the response (time constant) 2 s, and the minimum and maximum ordinate settings were −0·3 and +0·3 respectively.

Drug substances. Atropine sulphate (BDH Chemicals Ltd), hyoscine hydrobromide (BDH Chemicals Ltd), benztropine mesylate (Merck Sharp and Dohme Ltd) and Hyoscyamine hemisulphate (Sigma London Chemical Co. Ltd) were used without further purification.

Reagents. Sodium tetraphenylboron (BDH Chemicals Ltd) was used at a concentration of 10 mg ml^{-1} in water; the pH adjusted to approximately 9 with a few drops of 0·1 M sodium hydroxide) and stored in an amber coloured bottle.

McIlvaine's buffer pH 6, prepared by dissolving 7·74 g citric acid and 17·93 g anhydrous disodium hydrogen phosphate in water and diluting to 1 litre.

1,2-Dichloroethane, analytical-reagent grade (BDH Chemicals Ltd).

Standard solutions. Dissolve approximately 30 mg atropine sulphate or hyoscine hydrobromide or 100 mg benztropine mesylate (accurately weighed) in water and dilute to 50 ml. Transfer aliquots (5 ml and 15 ml) of the appropriate standard solution to two volumetric flasks (100 ml) and dilute to volume with pH 6 buffer. Pipette 10 ml of each standard solution into a separating funnel (100 ml), add sodium tetraphenylboron solution (2 ml) and dichloroethane (10 ml or, in the assay of benztropine mesylate, 30 ml) and shake the contents of the funnels for 1 min. When the phases have separated, filter the lower dichloroethane layer through Whatmans no 1 paper. Record the second derivative absorption spectrum of the tetraphenylborate salt in the clarified extract using in the reference cell a dichloroethane extract of pH 6 buffer (10 ml) treated as described for the standard solutions.

Sample solutions

Tablets. Weight and powder 20 tablets. Shake a quantity of powder containing about 0·6 mg atropine

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sulphate or hyoscine hydrobromide, or 2 mg benztropine mesylate, accurately weighed in a separating funnel, with pH 6 buffer (10 ml) for 5 min. Alternatively, for unit dose assays, powder a tablet in a separating funnel with a glass rod and shake the powder with pH 6 buffer (10 ml) for 5 min. Treat the aqueous extract of the tablet powder as described above for the standard solutions from the words 'add sodium tetraphenylboron solution (2 ml) . . .'.
Injections. Transfer the injection solution (1·0 ml containing 0·4 mg or 0·6 mg atropine sulphate) to a separating funnel containing pH 6 buffer (10 ml) and continue the assay as decribed above for the standard solutions, from the words 'add sodium tetraphenylboron solution (2 ml) . . .'.

Treatment of the results
Measure in mm the largest amplitude in the derivative spectra of the dichloroethane extracts of the more concentrated standard (a_1), the less concentrated standard (a_2) and the sample solution (a_3), from the minimum near 273 nm to its shorter wavelength satellite (Fig. 1). Calculate the content of the drug in the sample solution (q_3) from the *linear*, but *non-proportional* relationship that exists between the measured amplitude and quantity of drug, as follows:

$$q_3 = \frac{(a_3 - a_1)(q_1 - q_2) + q_1(a_1 - a_2)}{a_1 - a_2}$$

where q_1 and q_2 are the quantities of drug in the 10 ml of the more concentrated and less concentrated standard solutions respectively.

RESULTS AND DISCUSSION

Derivative spectrophotometry is a well established technique for the assay of drugs in mixtures and formulations (Fell 1978; Traveset et al (1980). Its principal advantages are that it discriminates in favour of substances with narrow absorption bands against those with broad spectral bandwidth and provides better resolution of overlapping absorption bands. It is particularly suitable for the assay of benzenoid substances displaying fine structure of narrow spectral bandwidth (250–280 nm) in samples that show interference in straightforward absorption measurements from co-formulated drugs and formulation excipients (Davidson & Elsheik 1982; Jones & Marnham 1981; Fell & Smith 1982). This has already been shown with second derivative spectroscopy of extracts in 0·1 M hydrochloric acid of tablets and capsules containing 2 mg or more of a benzenoid drug (Davidson & Hassan 1983). However the very

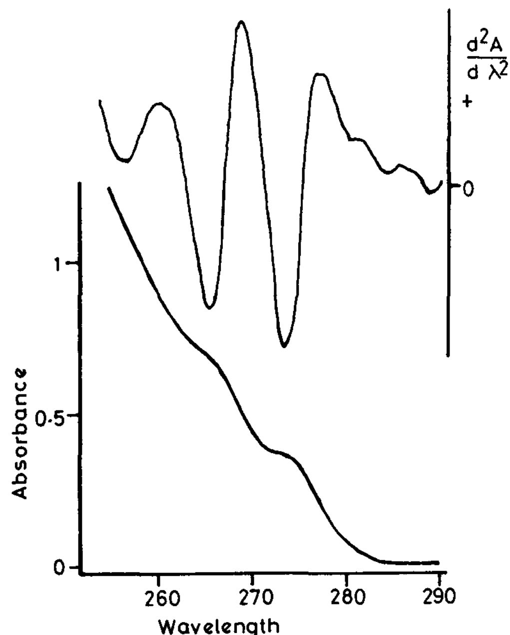

Fig. 1. The zero order (lower) and second order (upper) derivative spectra of atropine tetraphenylborate ($1{\cdot}727 \times 10^{-4}$ M) in dichloroethane.

high excipient/drug ratio in tablets of atropine sulphate (0·6 mg) and hyoscine hydrobromide (0·3 mg) distorts their second and even their fourth derivative spectra resulting in systematic errors in this simple procedure.

Sodium tetraphenylboron has been used in the present work to form salts with such alkaloids which are then extractable into organic solvents. The fine structure of the phenyl moieties of the salt gives a second order derivative spectrum in which the amplitudes are rectilinearly related to the concentration of the alkaloid in the sample.

Development of the method
To select the extracting solvent, suspensions of the precipitated tetraphenylborate salt of atropine in approximately neutral aqueous solution were extracted with various immiscible organic solvents. Non-polar and slightly polar solvents, e.g. iso-octane and carbon tetrachloride, did not completely extract the salt whereas the more polar dichloromethane, chloroform and dichloroethane completely clarified the aqueous suspension and gave the characteristic zero order and second derivative spectra of the tetraphenyl moiety. 1,2-Dichloroethane, having the highest boiling point, the highest specific gravity and the shortest uv cut-off, was selected.

Fig. 1 shows the zero order and second order derivative spectra of atropine tetraphenylborate in dichloroethane prepared as described for standard solutions. The minima in the second derivative spectrum, after correction for displacement of the spectrum in the direction of the scan (Talsky et al 1978) correspond with the wavelengths of the shoulders at 273 nm and 265 nm in the zero order spectrum. The largest amplitude in the derivative spectrum measured from the minimum at 273 nm to its shorter wavelength satellite provides the maximum sensitivity and was chosen as the measured value from which the concentration is calculated.

The pH of the aqueous solution required for the efficient precipitation of the tetraphenylborate salt and for its extraction into dichloroethane was determined by measuring the amplitudes in the second derivative spectra of dichloroethane extracts of atropine sulphate buffered at pH values in the range 3 to 8 and treated with sodium tetraphenylboron solution as described in methods. The derivative spectra were also obtained for extracts of similarly treated volumes of the buffer solutions containing no alkaloid. The results, in Fig. 2 show that free tetraphenylborate is extracted in significant quantities into dichloroethane from aqueous solution below pH 5·5: above this figure only traces are observed. The measured amplitude in the extracts of atropine using buffers in the pH range 5 to 8 is constant. A citrate-phosphate buffer (pH 6; McIlvaine 1921), was chosen to avoid base-catalysed hydrolysis of atropine and hyoscine at higher pH.

The effects of varying the sodium tetraphenylboron concentration on the efficiency of salt formation with atropine, its extraction into dichloroethane and on the linearity of the response were investigated. Although the mole ratio of the salt is known to be 1:1 (Johnson & King 1962) a large excess of sodium tetraphenylboron, given by 2 ml of a 1% m/v solution of the reagent, is required to produce a rapid and quantitative formation of the salt and to give derivative amplitudes linearly related to the concentration of the alkaloids over the required ranges (Table 1).

For volumes of dichloroethane between 5 and 50 ml, the product of the measured amplitude of the tetraphenylborate salt in the extract and the solvent volume was constant for each alkaloid, indicating high extraction efficiencies of the salts. The volume of dichloroethane (10 ml) selected for the assay of atropine or hyoscine is a compromise between high sensitivity and convenience; 30 ml was required for

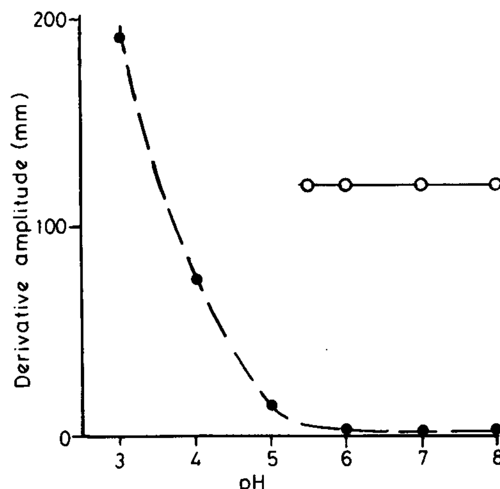

FIG. 2. The effect of pH on the second derivative amplitudes of dichloroethane extracts of atropine tetraphenylborate (open circles) and of buffer solutions containing no alkaloid (closed circles).

Table 1. Calibration and precision data for the measurement of absorbance at 273 nm (A_{273}) and second derivative amplitude (D^2) in mm.

	Atropine sulphate		Hyoscine hydrobromide		Benztropine mesylate	
Calibration	A_{273}	D^2 (mm)	A_{273}	D^2 (mm)	A_{273}	D^2 (mm)
Quantity assayed (mg)	0–1·2	0–1·2	0–1·2	0–1·2	0–3	0–3
Number of solutions	7	7	7	7	7	7
Slope*	0·610	202·0	0·546	184·1	0·135	67·1
Intercept	0·019	6·25	0·045	5·18	0·027	6·02
Correlation coefficient	0·9995	0·9999	0·995	0·9999	0·996	0·9998
Precision						
Quantity assayed (mg)	0·6	0·6	0·3	0·3	2	2
Number of measurements	6	6	6	6	5	6
Relative standard deviation	0·0475	0·0185	0·1116	0·0181	0·0757	0·0147
Variance ratio [limiting value of F]	6·59	[5·05]	38·02	[5·05]	26·52	[5·19]

* A_{273} or derivative amplitude (in mm) mg^{-1} drug.

complete extraction of benztropine tetraphenylborate in the unit dose assays of benztropine tablets (2 mg). There were no detectable quantities of the salts in second extracts of the aqueous raffinates to which a further 2 ml of sodium tetraphenylboron solution had been added.

Table 2. Assay results

Formulation	Declared strength	Found (% declared strength) B.P.	New method
Atropine sulphate tablets	0·6 mg	98·5	99·3
Atropine sulphate injection	0·4 mg ml^{-1}	98·0	100·7
Atropine sulphate injection	0·6 mg ml^{-1}	101·3	99·2
Hyoscine hydrobromide tablets	0·3 mg	100·0	101·0
Benztropine mesylate tablets	2·0 mg	96·4	98·0
Benztropine mesylate tablets	2·0 mg	98·7	100·5
Tincture of belladonna	0·3 mg ml^{-1} (as hyoscyamine)	95·7	103·0

Linearity and precision

A linear relationship, significant at the 99% probability level, obtains between the quantity of drug assayed and both the absorbance and derivative amplitude (Table 1). However, for each calibration series a small, but significant, intercept was obtained. This precludes the single-point standardization normally used in spectrophotometric procedures where Beer's Law is obeyed, and necessitates the adoption of a two-point bracketting standardization of the analytical response.

The precision of both the absorbance measurements and derivative amplitudes was determined and the relative standard deviations of absorbances at 273 nm and derivative amplitudes of the extracts (Table 1) are significantly different at the 95% probability level, as determined by the Variance Ratio test (F test), consequently the derivative amplitude, which provided the more precise result was selected. The satisfactory relative standard deviation of the derivative assays indicates their adequate sensitivity and precision for unit dose assays (uniformity of content) of solid formulations of the drugs.

As the amplitudes of second order derivative bands of substances displaying vibrational structure have been shown to decrease when the temperature of the solution increases, with temperature coefficients of the order of −0·8% per degree (Davidson 1983), the recording of the derivative spectra at room temperature was made without delay.

Assay of formulations

To test the application of the method, the concentrations of atropine sulphate, hyoscine hydrobromide or benztropine mesylate in several dosage forms were determined by the derivative procedure and also by the procedures of the British Pharmacopoeia (1980). Good agreement of the results obtained (Table 2) confirming that the proposed procedure is accurate and sufficiently sensitive for the assay of these low dose formulations.

The assay of tincture of belladonna for its principal alkaloid hyoscyamine and minor related alkaloids may be performed using hyoscyamine hemisulphate or atropine sulphate as the standard substance, since both give identical derivative amplitudes on an equimolar basis. The only sample preparation required is a preliminary extraction of the tincture with dichloroethane, to remove uv-absorbing components of the tincture, before the addition of the sodium tetraphenylboron solution. The results with the tincture were reasonably concordant with those obtained by using the British Pharmacopoeia procedure (1980).

The similarity of the derivative spectra of the dichloroethane extracts of the drugs examined in this report and of other drugs since examined, together with the many substances known to give insoluble precipitates with sodium tetraphenylboron (Cross 1965; Casy 1977) suggest that the derivative procedure may form the basis for the assay of other nitrogenous bases including non uv-absorbing substances.

REFERENCES

Abadia, V., Crovetto, G., Thomas, J. (1982) Cienc. Ind Farm., 1: 121–124: Chem. Abs. (1982) 97: 115370r

British Pharmacopoeia 1980 HM Stationery Office, London pp 40, 41, 232, 233, 234, 583, 736, 778, 833

Casy, A. F. (1977) Proc. Anal. Div. Chem. Soc. 14: 292–294

Cross, J. T. (1965) Analyst 90: 315–324

Davidson, A. G. (1983) Ibid. 108: 728–732

Davidson, A. G., Elsheik, H. (1982) Ibid. 107: 879–884

Davidson, A. G., Hassan, S. M. (1983) J. Pharm. Sci in the press

Fell, A. F. (1978) Proc. Anal. Div. Chem. Soc. 15: 260–267

Fell, A. F., Smith, G. (1982) Anal. Proc. 19: 28–33

Gur'ev, I. A., Lizunova, G. M., Korenman, I. M., Medvedeva, O. N. (1981) Zh. Anal. Khim. 36: 130–133; Chem. Abs. (1981) 94: 167146e

Johnson, C. A., King, R. E. (1962) J. Pharm. Pharmacol. Suppl. 14: 77T–82T

Jones, R., Marnham, G. J. (1981) J. Pharm. Pharmacol. 33: 458–459

McIlvaine, T. C. (1921) J. Biol. Chem. 49: 183–186

Talsky, G., Mayring, L., Kreuzer, H. (1978) Angew. Chem. Int. Ed. Engl. 17: 785–799

Traveset, J., Such, V., Gonzalo, R., Gelpi, E. (1980) J. Pharm. Sci. 69: 629–633

Uspenskaya, S. I. (1975) Tr. Tsentr. Aptech. NII: 190–197; Chem. Abs. (1976) 84: 95687a