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A spectropolarimetric assay of (−)-adrenaline in compendial formulations

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A method is described for the determination of (−)-adrenaline in certain formulations containing adrenaline hydrogen tartrate at concentrations down to 0·18 mg ml^{-1} (1 : 10 000 adrenaline). The assay is based upon a spectropolarimetric measurement at 249 nm of sample solutions, suitably treated to remove interfering substances. The rotation of the sample solutions is corrected for the rotation of the tartaric acid species which is determined by a difference rotation measurement on equimolar sample solutions at pH 1·1 and pH 5·6. The concentration of (−)-adrenaline in the sample is calculated from the net rotation at 249 nm due to the (−)-adrenaline and the total concentration of adrenaline ((+)- and (−)-isomers) determined by a published spectrofluorimetric method. The assay is specific for (−)-adrenaline in the presence of (+)-adrenaline, (+)-tartaric acid, adrenaline sulphonic acid and low levels of adrenochrome.

Although adrenaline has been used pharmaceutically for about 70 years, the assessment of the quality and potency of adrenaline formulations still presents an interesting challenge to the analyst. The adrenaline concentration may be determined by a variety of reliable methods including gas-liquid chromatography (Watson & Lawrence 1977), high-pressure liquid chromatography (Fu & Sibley 1977) and spectrofluorimetry (Prasad et al 1973). These procedures permit the specific assay of adrenaline in the presence of adrenaline sulphonic acid which may be formed in preparations containing sodium metabisulphite as the antioxidant and in the presence of adrenochrome and other oxidation products. However, they do not distinguish between the physiologically active laevorotatory (−)-isomer of adrenaline and the relatively inactive dextrorotatory (+)-isomer and are therefore unable to detect racemization which may occur in moderately acidic solution (Haddock, 1933) or during sterilization by autoclaving.

The simple polarimetric procedures which are used for the determination of the optical purity of many drugs are not sufficiently sensitive to determine the low concentrations of adrenaline in many preparations (e.g. 0·1% m/V in adrenaline injection B.P. and adrenaline solution B.P.). The sensitivity is further reduced in formulations containing adrenaline as the diastereoisomeric hydrogen tartrate due to the dextrorotatory tartrate almost cancelling the laevorotation of the adrenaline. Attempts to improve the sensitivity have included concentrating the adrenaline solution by vacuum distillation (Rosenblum et al 1949) and ion-exchange chromatography (Hellberg 1955) but very large volumes of the preparations, corresponding to 100-125 mg of adrenaline, are required for each assay.

The British Pharmacopoeia (1968 and 1973) adopted the method of Welsh (1955) for the determination of (−)-adrenaline in adrenaline injection B.P. and adrenaline solution B.P. The procedure requires 30 ml of the formulation and involves the separation of the adrenaline from the interfering tartrate species by conversion to O^3, O^4-*N*-triacetyladrenaline which is determined gravimetrically after extraction into chloroform. The concentration of (−)-adrenaline is determined by measurement of the optical rotation at the sodium D line of the derivative dissolved in a small volume of chloroform. The extraction, evaporation and drying stages of the assay are time-consuming and very careful weighing technique is required to achieve reasonable accuracy and precision since the mass of the derivative is only about 50 mg. Higuchi et al (1959) have reported that other acetylated products are formed in addition to the triacetyl derivative which lead to variable errors and have suggested a modification to Welsh's method which involves a column chromatographic isolation of the triacetyl derivative followed by a spectrophotometric determination of total adrenaline and a polarimetric assay of (−)-adrenaline.

Due to recent advances in instrumentation, modern spectropolarimeters have become capable of high sensitivity and precision. In this paper, a spectropolarimetric procedure is described for the assay of (−)-adrenaline in compendial formulations containing adrenaline hydrogen tartrate. The method

is used in conjunction with a sensitive spectrofluorimetric method (Prasad et al 1973) for the determination of total adrenaline and is applied directly to the aqueous formulations after suitable treatment to remove interfering substances.

MATERIALS AND METHODS

Materials

0·5M Hydrochloric acid. M Acetate buffer, pH 5·6. Dissolve sodium acetate trihydrate (123·85 g) and glacial acetic acid (5·4 g) in water and dilute to 1 litre.

Other reagents were of A. R. quality.

Reference compounds

Adrenaline hydrogen tartrate (BDH Ltd.). $[\alpha]_D^{20}$ of the triacetyl derivative (Welsh, 1955) = −95·3°. (c = 0·91; chloroform). Lit. −95°. (−)-Adrenaline (BDH Ltd.) $[\alpha]_D^{20} = -51{\cdot}4°$ (C = 4; M hydrochloric acid). Lit. −50° to −53°. (±)-Adrenaline (BDH Ltd.) $[\alpha]_D^{20} = 0°$ (c = 1; M hydrochloric acid). (+)-Tartaric acid A.R. (BDH Ltd.) $[\alpha]_D^{20} = +12{\cdot}00°$ (C = 20, water) Lit. +11·98°. (−)-Tartaric Acid (BDH Ltd.) $[\alpha]_D^{20} = -12{\cdot}00°$ (C = 20; water) Lit. −11·98°. Adrenochrome (BDH Ltd.). Adrenaline sulphonic acid was prepared by the method of Dibbern & Pilcher (1961). Found: C, 43·3; H, 5·3; N, 5·5; S, 13·0. $C_9H_{13}NO_5S$ requires C, 43·7; H, 5·3, N, 5·7; S, 13·0. $[\alpha]_D^{20} = 0°$ (C = 1; water).

Spectropolarimeter. Optical rotatory dispersion (ORD) spectra were measured at 27°C in a Cary 60 spectropolarimeter using 1 cm silica quartz cells. The slits were programmed to give a constant spectral bandwidth of 1·5 nm, the pen period was 3 and the scan speed was 15 nm min^{-1}. In the assay of (−)-adrenaline at 249 nm the slit was set to 1·5 mm and the Range switch was adjusted to give maximum pen response e.g. 0·2° and 0·02° full scale range for 1:1000 and 1:10 000 solutions of adrenaline.

Determination of total adrenaline concentrations

Assay the sample solutions for total adrenaline by the spectrofluorimetric procedure of Prasad et al (1973).

Spectropolarimetric assay of (−)-adrenaline concentrations

Standard solutions. Dissolve (−)-adrenaline (about 60 mg, accurately weighed) in 0·1M hydrochloric acid (20 ml) and dilute the solution to 100 ml with water. Measure the optical rotation of the solution at 249 nm (α_{249}) in a 1 cm cell and subtract the α_{249} of 0·02M hydrochloric acid measured in the same cell.

Dissolve (+)-tartaric acid (about 80 mg, accurately weighed) in water and dilute to 100 ml. Transfer aliquots (15 ml) to two volumetric flasks (25 ml). To one flask add 0·5M hydrochloric acid (5 ml) and to the other flask add pH 5·6 acetate buffer (5 ml). Dilute the contents of both flasks to 25 ml and measure the α_{249} of each solution using 0·1M hydrochloric acid and pH 5·6 acetate buffer (diluted 1 + 4 with water) as the blank reagents.

Adrenaline injection B.P.

Transfer aliquots of the injection (3 ml) to two volumetric flasks (5 ml). Add pH 5·6 acetate buffer (1 ml) to one flask and dilute the solution to 5 ml with water. Add 0·5M hydrochloric acid (1 ml) to the other flask, pass oxygen-free nitrogen through the solution till the odour of sulphur dioxide is no longer detected (about 10 min) and dilute the solution to 5 ml with water. Measure the α_{249} of the solutions as described above for the standard solutions of (+)-tartaric acid.

Adrenaline solution B.P.

Extract an aliquot of the solution (7 ml) three times with carbon tetrachloride (10 ml) to remove chlorocresol and treat two aliquots (3 ml) as described above for *adrenaline injection B.P.*

Adrenaline and atropine spray, compound, B.P.C.

Add 0·1M sodium hydroxide solution slowly from a graduated pipette (2 ml) to 5 ml of the preparation until a precipitate persists, noting the volume required (usually 1·6 to 1·8 ml). Add a volume of water to bring the total volume to 8 ml and extract the solution four times with carbon tetrachloride (10 ml) to remove papaverine. Transfer aliquots (1 ml) to two volumetric flasks (5 ml) and continue the assay as described above for *adrenaline injection B.P.* from the words 'add pH 5·6 acetate buffer (1 ml) . . . '.

Zinc sulphate and adrenaline eye-drops B.P.C.

To 3 ml of the eye-drops add pH 5·6 acetate buffer (1 ml) and 0·1M ethylenediamine tetra-acetic acid solution (1 ml). Add 0·5M hydrochloric acid (1 ml) to 3 ml of the eye-drops in a volumetric flask (5 ml) and continue the assay as described above for *adrenaline injection B.P.* from the words 'pass oxygen-free nitrogen . . . '.

Treatment of the results

Calculate the molecular rotation at 249 nm of the standard adrenaline solution ($[\Phi]_A$) and the standard tartaric acid solutions at pH 1·1 and pH 5·6 ($[\Phi]_{TA}^{1\cdot1}$ and $[\Phi]_{TA}^{5\cdot6}$ respectively) from the equation

$$[\Phi] = \frac{\alpha_{249} \times \text{mol. wt}}{C \times 1}$$

where α_{249} is the measured optical rotation at 249 nm, 1 is the pathlength of the solution in dm, mol. wt is the molecular weight of the species and C is the concentration in g/100 ml.

From the measured optical rotation at 249 nm of the sample solutions at pH 1·1 and pH 5·6 ($\alpha_u^{1\cdot1}$ and $\alpha_u^{5\cdot6}$ respectively), the difference rotation of the sample solutions ($\Delta\alpha_u = \alpha_u^{1\cdot1} - \alpha_u^{5\cdot6}$) and the difference molecular rotation of (+)-tartaric acid ($\Delta[\Phi]_{TA} = [\Phi]_{TA}^{1\cdot1} - [\Phi]_{TA}^{5\cdot6}$), calculate the concentration of (−)-adrenaline in the sample in g/100 ml using the formula

$$\left[0.5 + 0.5 \frac{\left(\alpha_u^{1\cdot1} - \frac{\Delta\alpha_u \times [\phi]_{TA}^{1\cdot1}}{\Delta[\phi]_{TA}}\right) \times \text{Mol.Wt}_A \times D}{C_u \times [\phi]_A \times 1}\right] \times C_u$$

where C_u is the concentration of the total adrenaline in g/100 ml determined by spectrofluorimetry, mol. wt_A is the molecular weight of adrenaline (183·2) and D is the dilution factor of the sample solutions.

RESULTS AND DISCUSSION

Choice of assay conditions

The ultraviolet (u.v.) absorption spectra and ORD spectra of (−)-adrenaline, (+)-tartaric acid and adrenaline hydrogen tartrate are shown in Figs 1a, 1b and 1c, respectively. Both (−)-adrenaline and adrenaline hydrogen tartrate have anomalous ORD spectra showing a weak Cotton Effect around 280 nm, corresponding to the λ_{max} in their absorption spectra, superimposed upon intense laevorotation in the u.v. region. (+)-Tartaric acid is also laevorotatory in this region and like adrenaline hydrogen tartrate shows greater rotation in 0·1M hydrochloric acid than in pH 5·6 solution while the rotation of adrenaline is unaffected by the pH change.

As optical rotation measurements may be subject to error if the solution is highly absorbing, the wavelength chosen for the assay was 249 nm corresponding to the wavelength of minimum absorption and maximum $[\Phi]/\epsilon$ of adrenaline. Although the slopes of the ORD spectra of (−)-adrenaline and (+)-tartaric acid are rather steep at 249 nm, no difficulties were experienced with 'wavelength setting errors' (Ismail & Glenn 1964) as the smallest wavelength scale division on the Cary 60 spectropolarimeter is 0·2 nm. Standard solutions of (−)-adrenaline and (+)-tartaric acid gave $[\Phi]_{249}$ values which were sufficiently precise over a four year period to permit the use of these values in the assay with only occasional checking.

The effect of pH was further investigated by measuring the $[\Phi]_{249}$ of solutions of (−)-adrenaline,

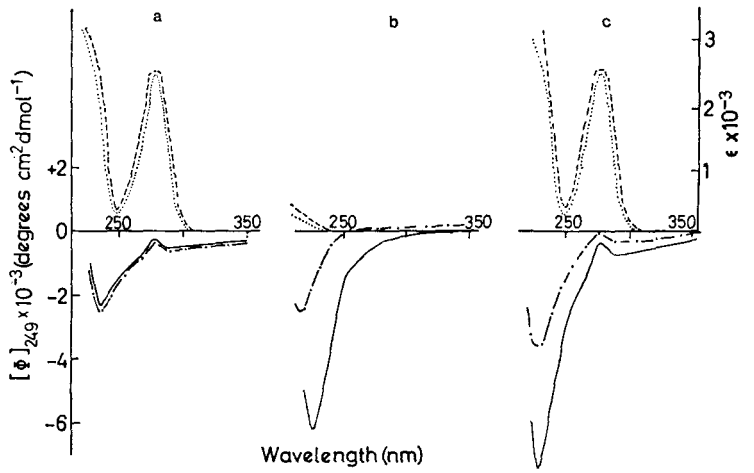

FIG. 1. The ORD spectra in pH 1·1 solution (———) and pH 5·6 solution (—·—·—) and absorption spectra in pH 1·1 solution (· · · · · · · · · · · ·) and pH 5·6 solution (- - - - - - - -) of (a) (−)-adrenaline, (b) (+)-tartaric acid and (c) adrenaline hydrogen tartrate. The ORD and absorption spectra of (−)-adrenaline in pH 1·1 solution and the absorption spectrum above 245 nm of adrenaline hydrogen tartrate in pH 1·1 solution are superimposed upon the spectra of the corresponding pH 5·6 solutions.

(+)-tartaric acid and adrenaline hydrogen tartrate at various pH values in the range 0·5–6·5. The results in Fig. 2 confirm that the $[\Phi]_{249}$ of (−)-adrenaline is constant in this pH range while (+)-tartaric acid and adrenaline hydrogen tartrate show maximum rotation below pH 1·5 and minimum rotation above pH 5·5. The difference molecular rotations ($[\Phi]_{249}^{1\cdot1}$–$[\Phi]_{249}^{5\cdot6}$) of (+)- tartaric acid and adrenaline hydrogen tartrate are identical due to the

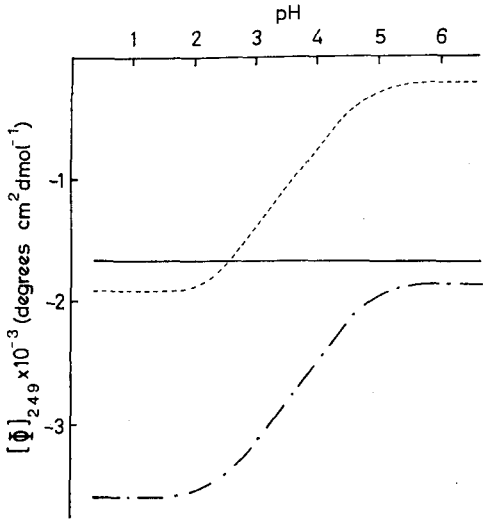

FIG. 2. The effect of pH on the molecular rotation at 249 nm ($[\Phi]_{249}$) of (−)-adrenaline (————) (+)-tartaric acid (– – – – – –) and adrenaline hydrogen tartrate (——— · ———).

equal concentration of the tartaric acid moiety. As (−)-adrenaline racemizes in strongly acidic solution and is easily oxidized in alkaline solution, acetate buffer pH 5·6 and 0·1M hydrochloric acid (pH 1·1) were chosen to provide maximum difference rotation of the tartaric acid and to minimize stability problems. (−)-Adrenaline and (+)-tartaric acid in pH 1·1 and pH 5·6 solutions give α_{249} readings which are stable for at least 60 min.

At all wavelengths (Fig. 1) and pH values in the range 0·5–6·5 (Fig. 2) the [Φ] of adrenaline hydrogen tartrate is the sum of the individual [Φ] values of (−)-adrenaline and (+)-tartaric acid and this forms the basis of the assay of (−)-adrenaline in formulations containing adrenaline hydrogen tartrate. The α_{249} of the (−)-adrenaline is obtained by correcting the α_{249} of the sample at pH 1·1 for the α_{249} of the (+)-tartaric acid which is calculated from the $\Delta\alpha_{249}$ of the sample. The concentration of (−)-adrenaline in the sample is then calculated from the net α_{249} and the total concentration of adrenaline determined by spectrofluorimetry.

Fig. 3 shows the variation of α_{249} of (−)-adrenaline, (+)-tartaric acid and adrenaline hydrogen tartrate at pH 1·1 with concentration. The α_{249} of (+)-tartaric acid is proportional to concentration over the concentration range studied. The α_{249} of (−)-adrenaline and adrenaline hydrogen tartrate is proportional to concentration up to 4·4 mM but slight deviation from proportionality above this concentration occurs. It is recognized (Chignell & Chignell 1972) that highly absorbing solutions give rise to erroneous α values by reducing the intensity of light passing to the photomultiplier which results in stray light effects and reduced signal-to-noise (s/n) ratios. The maximum A_{249} consistent with proportional α_{249} was found to be 1·1. The formulations of adrenaline were therefore diluted to give solutions whose A_{249} were around 0·86, where s/n is greatest, but did not exceed 1·1.

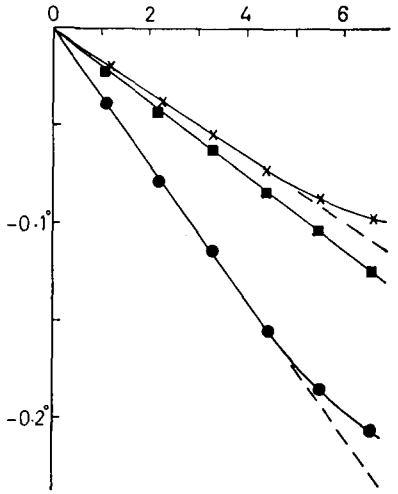

FIG. 3. The variation of optical rotation at 249 nm (α_{249}: ordinate) of (−)-adrenaline (×), (+)-tartaric acid (■) and adrenaline hydrogen tartrate (●) in pH 1·1 solution with concentration (mM: abscissa).

The effect of enantiomorphic composition on the $[\Phi]_{249}$ of adrenaline was investigated by mixing standard solutions (3·3 mM) of (±)-adrenaline and (−)-adrenaline to provide solutions of (−)-adrenaline ranging from 50–100%. Mixtures of (+)- and (−)-tartaric acid (3·3 mM) were similarly prepared to contain 0–100% (+)-tartaric acid. The results in Fig. 4 show that α_{249} of adrenaline and tartaric acid is linearly related to the isomer composition of the solutions.

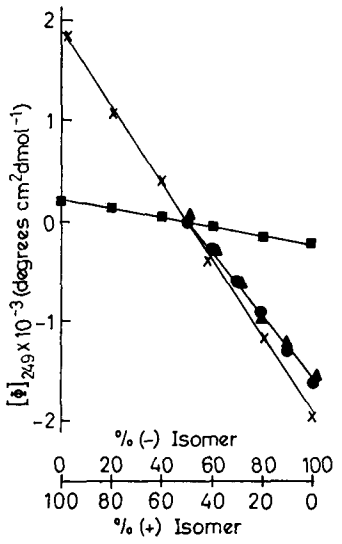

FIG. 4. The variation of molecular rotation at 249 nm ($[\Phi]_{249}$) of (−)-adrenaline at pH 1·1 (●) and pH 5·6 (▲) and of (+)-tartaric acid at pH 1·1 (×) and pH 5·6 (■) with enantiomorphic composition of the solution (abscissa).

Accuracy, precision, specificity and sensitivity

To simulate formulations which have undergone varying degrees of racemization, oxidation and adrenaline sulphonic acid formation, mixtures of (±)-adrenaline, (+)-tartaric acid, adrenaline hydrogen tartrate, adrenaline sulphonic acid and adrenochrome were analysed for (−)-adrenaline by the spectropolarimetric method after the concentrations of total adrenaline had been determined by spectrofluorimetry. As adrenaline sulphonic acid and adrenochrome have greater absorptivities at 249 nm than adrenaline, solutions containing significant concentrations of these decomposition products require greater dilution than specified in the materials and methods section to ensure that the A_{249} does not exceed 1·1. The results in Table 1 show concentrations of (−)-adrenaline in good agreement with the theoretical values and confirm that the procedure is specific for (−)-adrenaline in the presence of (+)-adrenaline, (+)-tartaric acid and the optically inactive adrenaline sulphonic acid and adrenochrome.

Table 1. The concentrations of (−)-adrenaline [(−)A] determined in mixtures containing adrenaline hydrogen tartrate [AHT], (±)-adrenaline [(±)A], (+)-tartaric acid [(+)TA], adrenaline sulphonic acid [ASA] and adrenochrome [AC].

Composition of the Mixture mM					Total (−)A Added mM	(−)A Found mM	% Recovery
AHT	(±)A	(+)TA	ASA	AC			
4·35	1·08	1·09	—	—	4·89	4·87	99·7
3·26	0·54	1·30	0·54	0·11	3·53	3·54	100·2
3·26	1·08	2·16	1·09	—	3·80	3·82	100·5
2·17	2·17	3·24	1·09	—	3·26	3·24	99·3
2·17	1·08	3·24	1·09	—	2·71	2·68	98·8
1·08	1·08	4·32	3·26	0·17	1·62	1·59	98·0

A sample of adrenaline solution B.P. assayed ten times by the fluorimetric/spectropolarimetric procedure gave a mean concentration of (−)-adrenaline of 0·966 mg ml^{-1} with a relative standard deviation of 0·93%.

Although there is no longer a compendial formulation containing 0·1 mg ml^{-1} (1 : 10 000) adrenaline, the procedure was assessed for application at this concentration by analysing an injection solution made up to the following formula (Pharmaceutical Society Laboratory Report No. 21; 1964): 18 mg adrenaline hydrogen tartrate, 100 mg sodium metabisulphite and 800 mg sodium chloride dissolved in water and made up to 100 ml of solution. The % recovery of (−)-adrenaline was 101·1% and the relative standard deviation in six assays was 2·7%. The spectropolarimetric assay is thus more sensitive than Welsh's method which has been considered to be insufficiently sensitive for 1 : 10000 formulations (Prasad et al 1973). In addition, the present method requires no more than 8 ml of 0·1% formulations for each assay (6 or 7 ml for the spectropolarimetric assay and 1 ml for the fluorimetric assay) compared with 30 ml required for Welsh's method.

Interferences

Certain excipients and co-formulated drugs present in adrenaline preparations were found to interfere in the spectropolarimetric assay of (−)-adrenaline. These interferences were easily overcome, however, by suitable treatment of the formulation before the spectropolarimetric measurement. The major source of interference was the intense absorption of light at 249 nm by other components in the formulation. Thus chlorocresol in adrenaline solution B.P. and papaverine in atropine and adrenaline compound spray B.P.C. absorb intensely at 249 nm and are removed by extraction into carbon tetrachloride. The anti-oxidant sodium metabisulphite yields the intensely absorbing sulphurous acid on acidification with 0·5M hydrochloric acid. Bubbling oxygen-free nitrogen through the solution accelerates the decomposition of the sulphurous acid to the gaseous sulphur dioxide. The interference from the strongly absorbing adrenochrome and other oxidation products is the most difficult to overcome.

Lightly coloured formulations containing up to 3% of adrenochrome can be assayed after appropriate dilution to keep the A_{249} below 1·1, but badly decomposed solutions cannot be assayed by the method.

Zinc sulphate in zinc sulphate and adrenaline eye-drops B.P.C. reduces the rotation of the tartrate species at pH 5·6 but not at pH 1·1, due to the formation of a zinc tartrate complex given by the ionized tartaric acid in pH 5·6 solution. Addition of the stronger complexing agent EDTA was found to abolish completely the interference due to the zinc ions. This is consistent with the published stability constants of (+)-tartaric acid and EDTA with zinc ($1{\cdot}26 \times 10^3$ and 5×10^{16} respectively; Perrin & Dempsey 1974).

Table 2. The concentrations of (−) adrenaline[(−)A], and total adrenaline [(±)A], determined in compendial formulations, commercially available (C) and prepared extemporaneously without sterilization (E).

	Found (% declared)			
	(±)A		(−)A	
Source	fluorimetry	B.P. gravimetry	This method	B.P. polarimetry
Adrenaline injection B.P., 98·9 mg/100 ml declared				
C	97·6	98·9	96·3	94·9
C	95·4	96·1	94·1	94·9
E	99·6	102·4	99·6	101·7
E	99·1	100·6	100·0	101·1
Adrenaline solution B.P., 98·9 mg/100 ml declared				
C	97·6	96·8	97·1	99·1
C	94·5	92·4	92·1	92·5
E	99·9	100·9	99·4	101·7
E	100·7	101·6	99·7	99·1
Atropine and adrenaline spray, compound B.P.C., 439·7 mg/100 ml declared				
C	98·1	—	95·5	—
C	99·7	—	98·1	—
E	99·7	—	99·8	—
E	100·4	—	100·1	—
Zinc sulphate and adrenaline eye-drops B.P.C., 49·5 mg/100 ml declared				
C	95·8	—	95·0	—
C	99·7	—	96·7	—
E	100·8	—	100·3	—
E	99·0	—	98·9	—

Assay results

Several compendial formulations of adrenaline either prepared extemporaneously without sterilization or purchased locally were assayed for (−)-adrenaline by the spectropolarimetric/fluorimetric assay. For comparison, samples of adrenaline injection B.P. and adrenaline solution B.P. were assayed by the gravimetric/polarimetric assay in their B.P. monographs.

Good recoveries of (−)-adrenaline were obtained by the spectropolarimetric/fluorimetric method in all the extemporaneous formulations (Table 2) including compound spray of atropine and adrenaline B.P.C. and zinc sulphate and adrenaline eye-drops B.P.C. for which no other procedures specific for the (−)-isomer have been reported. The concentrations of (−)-adrenaline and total adrenaline in the commercially available preparations were slightly lower than the stated concentrations, but were within permitted limits. The concentrations of (−)-adrenaline were also slightly lower than the concentrations of total adrenaline determined fluorimetrically, indicating that slight loss of potency on storage or during autoclaving occurs in these formulations due to racemization and adrenaline sulphonic acid formation. Comparison of the results shows that reasonably concordant concentrations are obtained by the spectropolarimetric/fluorimetric and B.P. methods for adrenaline injection B.P. and adrenaline solution B.P.

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