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Note

Determination of isoprenaline by ion-pair high-pressure liquid chromatography

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Investigations on the stability of isoprenaline in aqueous solution and in formulated products have been hampered by the lack of a method for assay of the drug in the presence of a likely impurity (isoproterenone), degradation products (N-isopropylnoradrenochrome and N-isopropylnoradrenolution) and antioxidants (sodium metabisulphite, ascorbic acid and disodium edetate).

The colorimetric method of the British Pharmacopoeia[1] for isoprenaline hydrochloride injection is not specific for isoprenaline since isoproterenone and isoprenaline sulphonic acid (formed by interaction of isoprenaline and sodium metabisulphite) will also undergo a colour reaction with ferrous sulphate–citrate solution. Similarly ultraviolet (UV) spectrophotometry is not specific for isoprenaline. The methods of the United States Pharmacopeia[2] and Kaistha[3], based upon the formation of an ether-soluble ion-pair of isoprenaline with di(2-ethylhexyl)phosphoric acid, have been reported[4] to be tedious and imprecise. Spectrofluorimetry[5] has been shown[6] to be unreliable under certain conditions in the presence of ascorbic acid and disodium edetate. The gas–liquid chromatographic method of Watson and Lawrence[7], which involves formation of a silyl derivative, is time-consuming, tedious and imprecise. Ghanekar and Das Gupta[8] have devised an ion-pair high-pressure liquid chromatographic (HPLC) technique to determine isoprenaline and other catecholamines; these workers determined isoprenaline in partly degraded solutions but did not confirm the validity of the method in the presence of added degradation products or antioxidants.

In the present work we describe an ion-pair HPLC method to determine isoprenaline hydrochloride in the presence of an impurity, degradation products and antioxidants.

MATERIALS AND METHODS

Apparatus

The modular liquid chromatograph consisted of a twin-head reciprocating pump (Milton Roy) with pulse-dampening and a column (100 × 4.5 mm I.D.) packed with ODS-Hypersil-5 μm (Shandon Southern Products). The variable-wavelength UV detector (Cecil 212, Cecil Instruments) was set at 280 nm; the detector signal was recorded on a RES11-20 potentiometric recorder (Servoscribe) and monitored by an integrator (Honeywell Instruments).

Chemicals and solutions

Isoprenaline hydrochloride (Riker Labs., Loughborough, Great Britain) and adrenaline acid tartrate (BDH, Poole, Great Britain) were used as supplied; stock solutions (0.1 %) of these were freshly prepared in purified water immediately before use.

Methanol 99% (Koch-Light, Colnbrook, Great Britain), sodium lauryl sulphate (BDH), L-ascorbic acid (BDH), disodium edetate (BDH) and sodium metabisulphite (May & Baker, Dagenham, Great Britain) were used as received.

A solution of N-isopropylnoradrenochrome was prepared by oxidation with silver oxide of isoprenaline (100 mg) in anhydrous methanol (100 ml dried over anhydrous sodium sulphate) by the method of Heacock and Scott[9]. The reaction, which was carried to completion by the addition of further quantities of silver oxide, was followed by HPLC analysis. The deep red solution contained no detectable amounts of isoprenaline.

A solution of N-isopropylnoradrenolutin was prepared by the addition of 5 ml of 10% aqueous sodium hydroxide solution to 100 ml of the solution of N-isopropylnoradrenochrome[9]. No crystals were obtained from the bright yellow solution. Solutions of N-isopropylnoradrenochrome and N-isopropylnoradrenolutin were stored at −20° until required. The UV-visible spectra agreed with those published[9].

Isoprenaline sulphonic acid and isoproterenone were prepared by the methods of Prasad *et al.*[5]; melting points, and infrared (IR) and UV spectra agreed with published data.

Procedure

A 2-ml volume of isoprenaline hydrochloride solution (0.1 %) was mixed with 2 ml of adrenaline acid tartrate solution (0.1 %) as the internal standard. An aliquot (5 to 20 μl) was injected by syringe into the chromatograph and eluted with water–methanol–acetic acid–sodium lauryl sulphate (70:30:2:0.002) at a flow-rate of 1.4 ml/min (pressure 1100 p.s.i.g.).

Standard solutions of isoprenaline hydrochloride (0.1 to 0.8 mg/ml) were chromatographed after addition of the internal standard and the peak area ratios were measured.

Solutions containing isoprenaline sulphonic acid, N-isopropylnoradrenochrome, N-isopropylnoradrenolutin and isoproterenone were each chromatographed separately. The antioxidant solutions (sodium metabisulphite, 1%; ascorbic acid, 0.1 % and disodium edetate, 0.01 %) were also tested when fresh and after autoclaving at 116° for 30 min.

RESULTS

The retention times for isoprenaline hydrochloride and adrenaline acid tartrate were 9.5 and 6.5 min, respectively; of the compounds tested, only isoproterenone had a longer retention time (10.5 min) and it was fully resolved under these conditions (Fig. 1). The retention volumes (V_R), capacity factors (k') and resolution factors (R_s) are given in Table I. N-isopropylnoradrenochrome and N-isopropylnoradrenolutin gave single peaks which were resolved (Fig. 2). Isoprenaline sulphonic acid, ascorbic acid, disodium edetate and sodium metabisulphite all gave peaks at, or close to, the solvent peak.

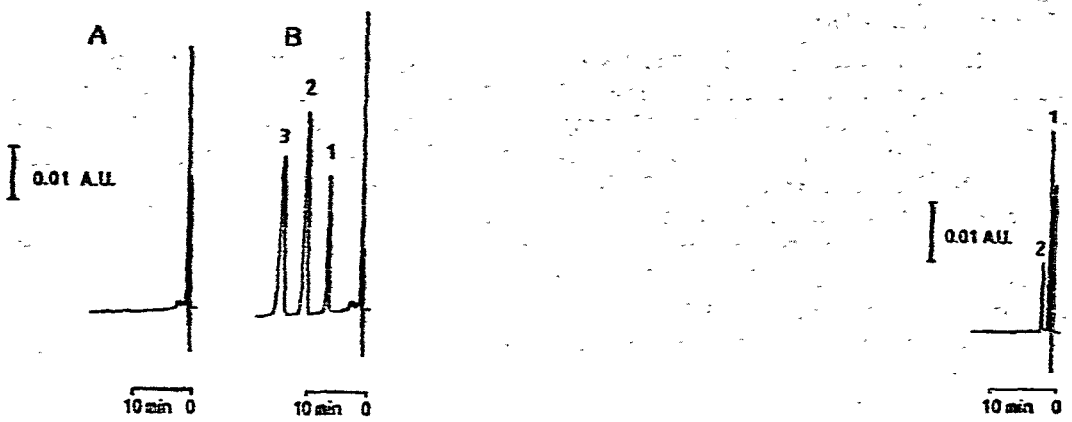

Fig. 1. Chromatography of isoprenaline, isoproterenone and adrenaline (internal standard). A, Blank; B, sample containing adrenaline acid tartrate (1), isoprenaline hydrochloride (2) and isoproterenone (3).

Fig. 2. Chromatography of oxidation products of isoprenaline: N-isopropylnoradrenochrome (1) and N-isopropylnoradrenolutin (2).

TABLE I

CHROMATOGRAPHIC VALUES FOR ADRENALINE ACID TARTRATE, ISOPRENALINE HYDROCHLORIDE AND ISOPROTERENONE

Compound	*Retention volume (ml)*	*Capacity factor*	*Resolution factor**
Adrenaline acid tartrate	9.4	7.9	3.9
Isoprenaline hydrochloride	14.6	12.9	—
Isoproterenone	20.2	18.3	2.9

* Separation from isoprenaline hydrochloride.

Autoclaved solutions of ascorbic acid gave several peaks; all had retention times less than 2.5 min and so did not interfere. No changes were observed in the chromatograms for solutions of disodium edetate or sodium metabisulphite after autoclaving.

Thus, the HPLC method of analysis of isoprenaline is not affected by any of the following substances: degradation products (N-isopropylnoradrenochrome, N-isopropylnoradrenolutin); impurities (isoproterenone); the sulphonic acid derivative formed with sodium metabisulphite; antioxidants (ascorbic acid, sodium metabisulphite, disodium edetate) in fresh and autoclaved solutions.

The relationship between peak area ratio and concentration of isoprenaline hydrochloride was rectilinear in the range 0.1 to 0.8 mg/ml and 95% confidence limits at 0.5 mg/ml were $\pm$ 2% ($n = 24$). The coefficient of variation of replicate determinations ($n = 12$) was 0.61% and the limit of detection of isoprenaline hydrochloride was 100 ng.

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