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Note

Assay for epinephrine and its impurities using reversed-phase high-performance liquid chromatography

Application to control of an eye-drop solution

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Epinephrine is a catecholamine used as a sympathomimetic in numerous pharmaceutical preparations[1], in particular in ophthalmology where it is used to treat glaucoma. It is therefore important to develop analytical methods with which to test its purity and to determine it. The fact that epinephrine is an unstable molecule renders its determination even more important.

Epinephrine is easily oxidizable in aqueous solution. This is accentuated by the presence of oxygen or heavy metals, by exposure to light and to alkaline pH, or by increase in temperature[2,3]. The final product of oxidation is adrenolutin (Fig. 1).

Several analytical procedures are used to determine epinephrine: fluorimetry[4], colorimetry[5], ultraviolet spectrophotometry[6], polarography[7], gas chromatography[8]

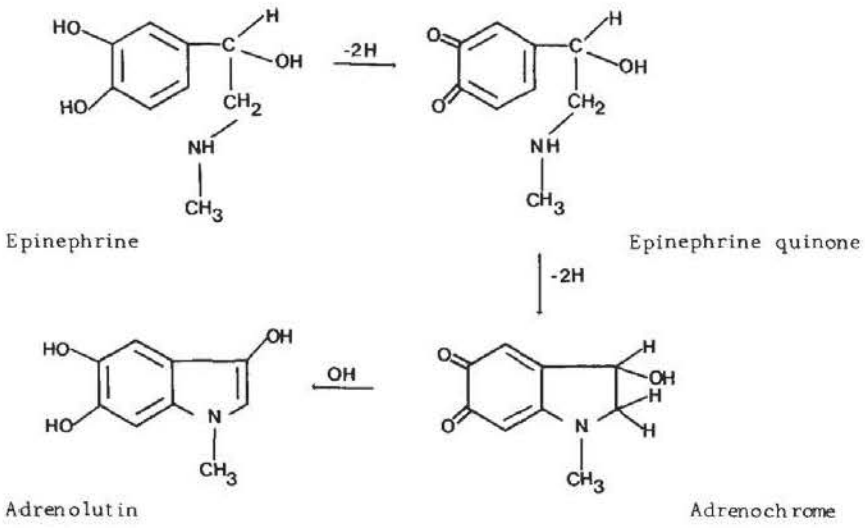

Fig. 1. Oxidation of epinephrine.

and high-performance liquid chromatography (HPLC). Reversed-phase HPLC has been used to detect epinephrine in injectable solutions[9], epinephrine and norepinephrine in intravenous solutions[10]. The separation of epinephrine and its oxidation products has been reported[11]. However, to our knowledge no method has been described for simultaneous detection of epinephrine, and of norepinephrine, adrenalone and adrenochrome which are possible impurities of epinephrine.

We thus report a reversed-phase HPLC procedure which enables not only the analysis of epinephrine present in an eye-drop solution, but also the detection of three possible impurities of epinephrine. Two of these, adrenalone and norepinephrine, arise from the methods used to obtain epinephrine. The third, adrenochrome, is produced by epinephrine oxidation.

EXPERIMENTAL

A Waters Model 6000 A pump fitted with an U6K universal injector was used in combination with an UV M480 spectrophotometer and a Model 833A integrator (Merck, Hitachi, France). A data processor was used to calculate retention times and peak areas.

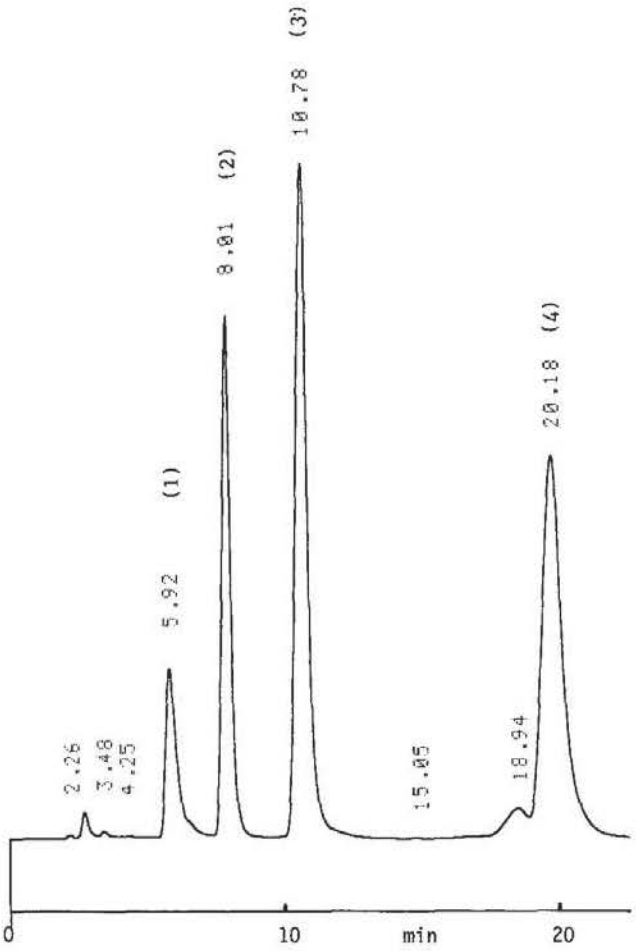

Fig. 2. Representative chromatogram of epinephrine (3) in the presence of adrenochrome (1), norepinephrine (2) and adrenalone (4). Values at peaks indicate retention times in min.

TABLE I

EPINEPHRINE CONTENT OF EYE-DROP SOLUTIONS

C.V. = Coefficient of variation.

Sample	*Epinephrine content (g/100 ml) (n = 5)*	*C.V. (%)*
Eye-drop solution containing 1% epinephrine, stored for 3 years	0.985	1.22
Eye-drop solution containing 1% epinephrine, stored for 2 years	0.999	1.22
Eye-drop solution containing 1% epinephrine, stored for 1 year	1.031	0.66

Separations were carried out under isocratic conditions using an RT 250-4 LiChrosorb RP select B 5-μm Hibar column (Merck). The mobile phase was methanol–0.05 *M* phosphate buffer pH 2 (5:95) containing 0.03 *M* sodium *n*-heptanesulphonate. The flow-rate was 1 ml/min and detection was performed at 220 nm at a sensitivity of 0.5 a.u.f.s.

The standard solutions (10 μl) employed were as follows: epinephrine, 0.5 mg/ml (Sigma); norepinephrine, 0.25 mg/ml (Sigma); adrenalone, 0.5 mg/ml (Fluka); adrenochrome, 0.5 mg/ml (Siccap-Emmop); epinephrine (0.5 mg/ml) + adrenalone (0.5 mg/ml) + adrenochrome (0.5 mg/ml) + norepinephrine (0.25 mg/ml).

Sample

A 10-μl volume of an eye-drop solution containing 1% epinephrine and stabilized with isoascorbic acid was diluted 1/20 in distilled water after storage for 1, 2 and 3 years at room temperature.

RESULTS AND DISCUSSION

A calibration graph was linear for epinephrine concentrations in the range 0.05–1 mg/ml ($n = 4$) with a correlation coefficient of 0.999. For the concentrations investigated the coefficients of variation were between 0.79 and 1.55%. The limit of detection of epinephrine was < 5 μg/ml at a signal-to-noise ratio of 2:1.

Fig. 2. shows a chromatogram of epinephrine (retention time 10.78 min) in the presence of adrenochrome, norepinephrine and adrenalone whose retention times are 5.92, 8.01 and 20.18 min respectively. It should be noted that commercial adrenochrome is an impure product; in addition to the main peak at 5.92 min there were two secondary peaks at 18.94 and 21.04 min.

Eye-drop solution

Determination of epinephrine in eye-drop solutions was carried out using external calibration (Table I).

Fig. 3 shows the chromatogram of an eye-drop solution after storage for 3 years. At this wavelength (220 nm) there is no interference from other constituents. A peak corresponding to isoascorbic acid is observed at 2.77 min.

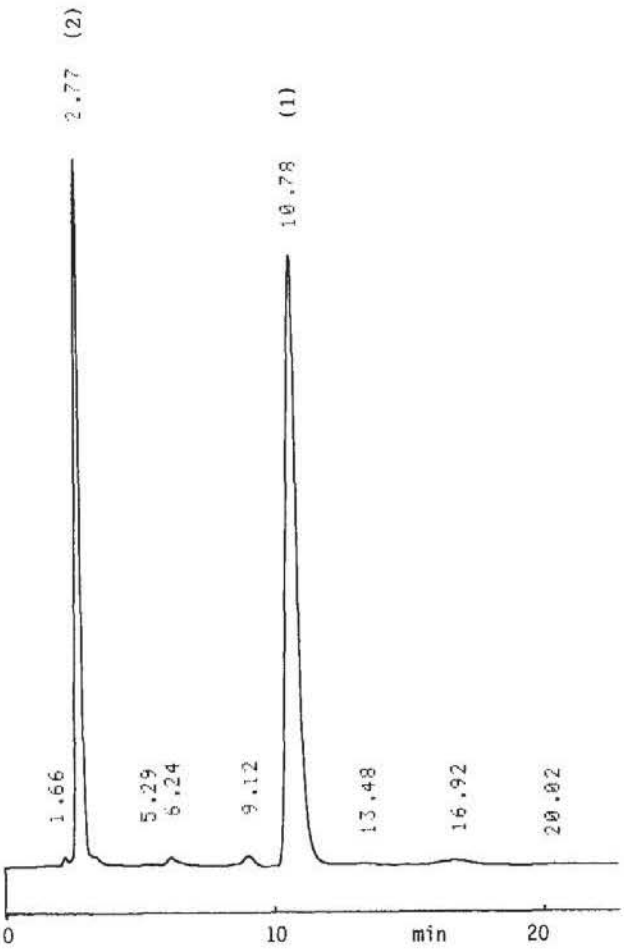

Fig. 3. Typical chromatogram of an eye-drop solution containing 1% epinephrine (1) and stabilized with isoascorbic acid(2), stored for 3 years at room temperature. Values at peaks indicate retention times in min.

This procedure is rapid, sensitive and reliable. It is thus possible to determine epinephrine in an eye-drop solution and detect possible impurities (adrenalone, norepinephrine and adrenochrome). The method seems well suited to routine control of eye-drop solutions.

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