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Note

Reversed-phase high-performance liquid chromatography of isoprenaline solutions using an acidic mobile phase

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Various methods have been utilized for the analysis of catecholamines either in biological material to recognize metabolic disorders or in pharmaceutical preparations to monitor the content of active substances. High-performance liquid chromatographic (HPLC) methods have become increasingly important in both fields because of their specificity, sensitivity and simplicity.

The most popular chromatographic mode applied to the analysis of catecholamines is reversed-phase ion-pair chromatography with anionic surfactants. In pharmaceutical analysis, this technique has been used to determine epinephrine[1–7], norepinephrine[5,7] and isoprenaline[5–8] in various formulations, and recently it has been employed in the US Pharmacopeial method[9] for the assay of these three compounds.

By this method[8], isoprenaline (IS) can be determined without any interference from degradation products, impurities or antioxidants. While the oxidative degradation products, N-isopropylnoradrenochrome and N-isopropylnoradrenolutin, give resolved peaks, isoprenalinesulphonic acid (ISA), the degradation product formed by the reaction of IS with bisulphite used as an antioxidant, is eluted with almost zero retention. Thus this specific chromatographic method of analysing IS preparations cannot furnish any information on the presence of the ineffective degradation product ISA.

The objective of the present work is to develop a reversed-phase HPLC method allowing simultaneous determination of IS and ISA. The method is intended for studying the effect of temperature on the stability of commercially available injection solutions of IS containing sodium bisulphite as an antioxidant.

The use of an aqueous mobile phase containing nitric acid or trichloroacetic acid for the separation of norepinephrine, epinephrine and dopamine and their metabolites on octadecyl-bonded silica columns was reported by Asmus and Freed[10]. An aqueous mobile phase containing sulphuric acid was used for a selective determination of epinephrine in pharmaceutical preparations[11].

The applicabilitity of such a mobile phase to the present analytical problem was examined.

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EXPERIMENTAL

Reagents and chemicals

Isoprenaline hydrochloride, epinephrine and norepinephrine were obtained from Léčiva Praha (Czechoslovakia). The degradation product ISA was synthesized from isoprenaline hydrochloride and sodium bisulphite according to the published procedure[12]. The oxidation products N-isopropylnoradrenochrome and N-isopropylnoradrenolutin were prepared by oxidation of isoprenaline hydrochloride with silver oxide[13]. Epinephrinesulphonic acid and norepinephrinesulphonic acid were prepared similarly to ISA[14,15].

4-Hydroxymandelic acid (purum grade) used as internal standard was purchased from Fluka (Buchs, Switzerland). Methanol, analytical grade (Lachema, Brno, Czechoslovakia), and distilled water used in the mobile phase were distilled before use. Sulphuric acid (95–97%) and perchloric acid (70%) were obtained from Merck (Darmstadt, F.R.G.), formic acid (99.7%), acetic acid (98%) and monochloroacetic acid from Lachema. Trichloroacetic acid was purchased from International Enzyme Limited (Windsor, U.K.). All acids were of analytical grade.

All other chemicals used were of analytical-reagent grade from Lachema.

Standard solutions for quantitation were prepared by dissolving isoprenaline hydrochloride and recrystallized dried ISA in distilled water containing sodium bisulphite (1.0 mg/ml) and disodium edetate (0.05 mg/ml). The concentrations of IS and ISA were in the ranges of 0.021–0.197 and 0.002–0.174 mg/ml, respectively.

The solution of the internal standard (0.24 mg/ml) was prepared in the same way as the solutions of IS and ISA. A 30-μl volume of the internal standard solution was added to 0.5 ml of the solution to be analyzed and an 8-μl aliquot was injected into the chromatograph.

Chromatography

All measurements were performed with HPLC apparatus consisting of a Varian Model 8500 liquid chromatograph with a stop-flow injector, a Varichrom UV 50 variable-wavelength UV detector and a Spectra-Physics Model SP 4100 computing integrator. An analytical HPLC column, 250 mm × 4.6 mm I.D. Ultrasphere ODS (5 μm), was used.

The mobile phase used for quantitations was methanol–0.01 *M* perchloric acid (5:95). The flow-rate was 1.0 ml/min and the UV detector was operated at a wavelength of 280 nm and 0.05 a.u.f.s.

The capacity factors were calculated in the usual way from the retention times of the component of interest and of an unretained compound (potassium nitrate). The quantitative measurements were carried out by peak area measurement.

RESULTS AND DISCUSSION

Chromatographic behaviour of IS and ISA

Mobile phases containing various acids were tested for the chromatography of IS and ISA. Purely aqueous acidic mobile phases, suitable for chromatography of very polar catecholamines (norepinephrine, epinephrine), gave too high a retention of IS, therefore a mobile phase comprising an aqueous solution of an acid and meth-

TABLE I

CAPACITY FACTORS, k', OF CATECHOLAMINES AND CATECHOLAMINE SULPHONIC ACIDS ON OCTADECYL-BONDED SILICA WITH 0.01 *M* AQUEOUS ACID AS THE MOBILE PHASE

Compound	*Sulphuric*	*Perchloric*	*Formic*	*Acetic*	*Monochloro-acetic*	*Trichloro-acetic*
Norepinephrine	0.2	0.5	1.2	2.5	0.7	1.8
Epinephrine	0.7	1.2	2.2	5.5	1.6	4.4
IS	6.1	–	–	–	–	–
Norepinephrine-sulphonic acid	0	0.1	0.1	0.1	0.1	0.1
Epinephrine-sulphonic acid	0.3	0.3	0.3	0.3	0.2	0.3
ISA	2.5	2.9	2.4	–	2.6	2.9

anol (9:1) was also used. The results are reported in Tables I and II. For comparison, the retentions of epinephrine and norepinephrine and the corresponding sulphonic acids were also measured and are listed.

The only catecholaminesulphonic acid retained on octadecyl-bonded silica with the aqueous acidic mobile phase was ISA. The two remaining, strongly polar sulphonic acids were eluted with almost zero retention.

While the capacity factor of ISA did not depend on the type of acid used in the mobile phase, the chromatographic behaviour of IS was strongly affected by this factor. Good peak symmetry and high efficiency were obtained with three strong acids. The sequence of these acids in order of increasing retention of IS and of the other catecholamines is sulphuric acid, perchloric acid and trichloroacetic acid, the latter giving a significantly higher capacity factor than perchloric or sulphuric acids.

An increased retention obtained with aqueous solutions of trichloroacetic acid as the mobile phase has already been reported for dopamine, norepinephrine, epinephrine and their metabolites and explained in terms of a high affinity of trichloroacetate ion, acting as an ion-pairing reagent, for the reversed-phase packing[10].

Table II shows that the highest retention of IS was found with the mobile phase containing acetic acid. However, highly asymmetric and broad peaks were obtained, again in agreement with the results of Asmus and Freed[10].

TABLE II

CAPACITY FACTORS, k', AND SEPARATION FACTORS, α, OF IS AND ISA ON OCTADECYL-BONDED SILICA WITH 0.01 *M* AQUEOUS ACID–METHANOL (9:1) AS THE MOBILE PHASE

Compound	*Sulphuric*	*Perchloric*	*Formic*	*Acetic*	*Monochloro-acetic*	*Trichloro-acetic*
Epinephrine	0.2	0.4	1.5	3.7	0.7	1.2
IS	0.7	1.5	4.1	8.3	2.1	3.9
ISA	0.4	0.5	0.6	0.6	0.6	0.6
α_{IS-ISA}	1.4	3.0	6.8	13.8	3.5	6.5

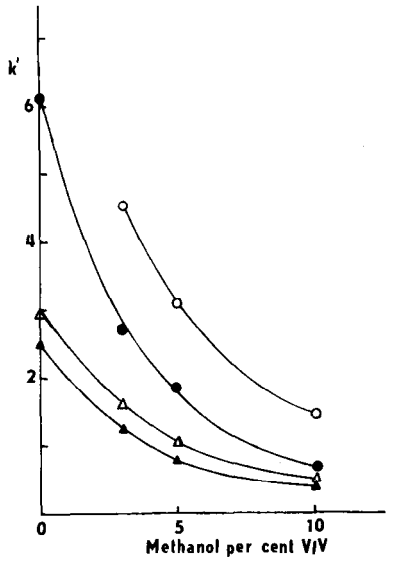

Fig. 1. Dependence of the capacity factors, k', on the composition of the mobile phase: IS (○) and ISA (△) with the mobile phase methanol–0.01 *M* perchloric acid; IS (●) and ISA (▲) with the mobile phase methanol–0.01 *M* sulphuric acid. Column: Ultrasphere ODS.

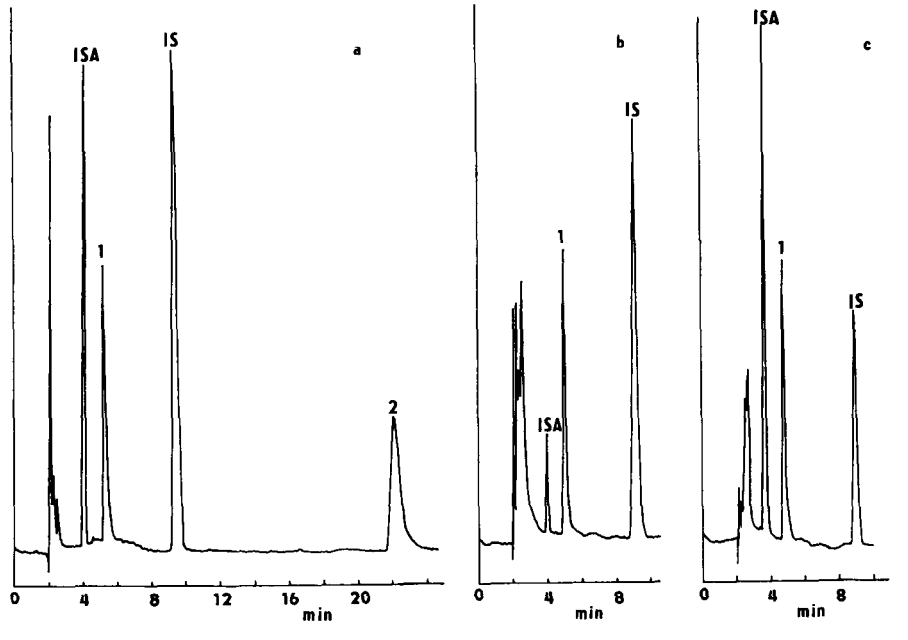

Fig. 2. Chromatograms of (a) a synthetic mixture of ISA, IS, internal standard (1) and N-isopropylnoradrenochrome (2), (b) an IS injkection solution (declared content of IS, 0.2 mg/ml) stored under normal conditions for 3 years and (c) an IS injection solution kept at 60°C for 3 months. Column: Ultrasphere ODS. Mobile phase: methanol–0.01 *M* perchloric acid (5:95). Detection: 280 nm, 0.05 a.u.f.s.

TABLE III

LINEARITY AND PRECISION OF THE HPLC METHOD

Compound	*Amount injected (μg)*	*C.V. (%)*		*Concentration range (mg/ml)*	*Correlation coefficient, r*
		n = 10 within 1 day	*n = 10 within 2 days*		
ISA	0.2	1.3	2.1	0.002–0.174	0.9995
IS	1.4	1.2	2.0	0.021–0.197	0.9997

Somewhat less unfavourable chromatographic behaviour of IS (and of the other catecholamines) was found with a mobile phase containing formic acid or monochloroacetic acid.

The values of the separation factor listed in Table II demonstrate the influence of the different acids used in the mobile phase on the separation of IS and ISA. That with trichloroacetic acid being too great, perchloric and sulphuric acids were chosen as suitable components of the mobile phase.

In order to determine the optimum composition of the mobile phase, the dependence of the capacity factors on the methanol content was measured and is shown in Fig. 1.

As the oxidative degradation products of IS were more strongly retained when the mobile phase containing sulphuric acid was used, the mobile phase containing perchloric acid was preferred. The separation efficiency achievable under the suggested conditions is illustrated in Fig. 2a, for a synthetic mixture.

Quantitation

The stability-indicating ability of the suggested HPLC method is shown in Fig. 2b,c, where chromatograms of two IS solutions are presented. N-Isopropylnoradrenochrome was not detected in any solution stored in ampoules at a temperature within the range of -20 to $+70$°C. The only degradation product found was ISA.

For the quantitation of IS and ISA, the internal standard method was used and 4-hydroxymandelic acid was selected as the internal standard. The linearity of the method was tested by analyzing standard solutions containing both compounds at five concentration levels in the range listed in Table III. The lower value of the concentration range for ISA also corresponds to the limit of quantitation. Both the dependences of the peak area ratios on the concentration were linear, as shown by the values of the correlation coefficient, r, given in Table III.

The precision of the method was evaluated on the basis of the results of ten analyses of a three-years-old IS solution. The coefficients of variation (C.V.) given in Table III indicate a satisfactory precision.

CONCLUSION

High efficiency, good retention and peak symmetry of IS, as well as of two other catecholamines, were obtained on octadecyl-bonded silica with a simple mobile phase containing strong acids (sulphuric, perchloric or trichloroacetic acid).

This simple, rapid and precise chromatographic method was successfully applied to the separation of IS and its degradation products, and to the simultaneous quantitation of IS and its ineffective degradation product ISA.

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