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Racemisation and oxidation in adrenaline injections

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Adrenaline injection fluids aged between 3 and 33 years were analyzed with respect to oxidation and racemization. The oxidation was determined by ion-pair reversed phase HPLC, and the degree of racemization was determined by derivatization of the adrenaline isomers to diastereomeric forms and subsequently separated by reversed phase HPLC. 10% adrenaline was oxidized after about 11 years, while 10% L-adrenaline was converted to D-adrenaline after only 4 years. After about 4 years, the injections contained less than 90% active adrenaline.

For military purposes, drugs may be stored for years past their ordinary expiration date. In Norway, the storage conditions vary considerably due to the shifting climate and a decentralized storage system with many small depots of varying quality.

The shelf-life of pharmaceutical preparations may be estimated by accelerated studies at high temperatures. In practice, such studies are of limited value where extremely long-term storage is concerned. Therefore, shelf-lives should be based on retrospective studies of drugs stored under realistic conditions.

The Norwegian armed forces store their drugs for emergency purposes for up to 15 years. Earlier studies have shown that several drugs may be stored for this long without a deterioration of quality [1, 2].

Adrenaline is an important drug in military medicine. In Norway, the shelf-life of ampoules with adrenaline injections is 3 years. The present study was undertaken to document the long-term stability of adrenaline injections under extreme storage conditions.

There are two optical isomers of adrenaline, of which only L-adrenaline is biologically active and used for injections.

L-adrenaline is easily racemized in acidic solutions [3]. The kinetics of the racemization have been determined, and the reaction rate was estimated to 10% racemization at pH 3–3.5 in 3 years [4]. Later, it was shown that adrenaline injections in ampoules stored for 7.5 years at a temperature less than 15°C had racemized only to a very small extent [5]. Injections of local anaesthetics containing adrenaline contained 5% or less D-adrenaline after the expiration date [6].

Adrenaline in solutions is easily oxidized and the reaction is catalyzed by bases [7–10]. The reaction is complex, and only the intermediate degradation products

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have been identified [8]. The end products are characterized as coloured melanins. 10–12% oxidation has been shown in 7.5 year old solutions [5].

The most common method of protecting adrenaline in injections against oxidation is by using antioxidants such as sodium bisulfite or sodium metabisulfite. But the bisulfite may also attack adrenaline, and the reaction product is adrenaline sulfonic acid [11–13]. The rate of bisulfite addition is normally low compared to the oxidation rate.

HPLC has been widely used for analysis of adrenaline in the recent years. Total adrenaline may be determined by reversed phase ion-pair liquid chromatography [14]. Recent developments in enantiomeric separation have made it possible to determine D- and L-adrenaline by reversed phase liquid chromatography after derivatization of the isomers to diastereomeric forms [6, 15].

Experimental

Chemicals

Methanol and citric acid monohydrate, both of analytical grade, Merck (Darmstadt, F.R.G.); potassium dihydrogenphosphate, analytical grade, Riedel-de Haën (Seelze, F. R. G.); L-adrenaline hydrogen tartrate, Boeringer Ingelheim (Ingelheim, F. R. G.); isoprenaline sulfate, NMD (Oslo, Norway); DL-adrenaline hydrochloride and hydrazine hydrate 80%, TCI (Tokyo, Japan); 2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl isothiocyanate (GITC), Polysciences (Warrington, PA, U.S.A.); dimethylformamide, analytical grade, BDH Chemicals (Poole, England).

Solutions

Buffer pH 3: 1.05 g citric acid was dissolved in 250 ml methanol and 700 ml water. The pH was adjusted to 3.0 and the solution was diluted to 100 ml.

Buffer pH 2.9: 1.36 g KH_2PO_4 was dissolved in 900 ml water. The pH was adjusted to 2.9 and the solution was diluted to 1000 ml.

Internal standard: 200 mg isoprenaline sulfate was dissolved in 100.00 ml buffer, pH 3.

Apparatus

The Shimadzu LC 4 A (Kyoto, Japan) liquid chromatograph with gradient mixer and variable UV-spectrophotometric detector was used.

Total adrenaline: The samples were chromatographed on a Hibar Lichrocart RP 18 column, 250×4 mm, 7 µm particles, Merck (Darmstadt, F. R. G.). The detector was operated at 280 nm. The mobile phase was 20 ml PIC B7 reagent, Waters (Milford, MA, U.S.A.), 250 ml methanol and water to 1000 ml. 20 ml PIC B7 reagent provides 0.005 M heptasulfonic acid and buffer pH 3 when diluted to 1000 ml. The flow rate was 1.5 ml/min at ambient temperature.

D- and L-adrenaline: The samples were chromatographed on a Spherisorb ODS 2 column, 250×4.6 mm, 5 µm particles, Phase Sep (Queensferry, U. K.). The detector was operated at 254 nm. The mobile phase was 630.00 ml aqueous buffer pH 2.9 and methanol to 1000.00 ml. After 22 min, the mobile phase was changed by a gradient mixer to 300.00 ml aqueous buffer pH 2.9 and methanol to 1000.00 ml. After 36 min, the original concentrations of the mobile phase was reconstituted. The flow rate was 1.5 ml/min at ambient temperature.

Samples

Samples of 18 batches of adrenaline injections stored in 6 different military depots for 3–30 years were kindly supplied by the regional military pharmacies in Norway. Altogether, 24 different combinations of batches and depots were analyzed. The adrenaline injections contained L-adrenaline hydrogentartrate corresponding to 1 mg/ml of L-adrenaline base.

Sample preparation

Total adrenaline: 1.00 ml adrenaline injection and 1.00 ml internal standard solution were diluted to 10.00 ml with buffer pH 3. 20 µl of the solution was injected into the chromatographic system.

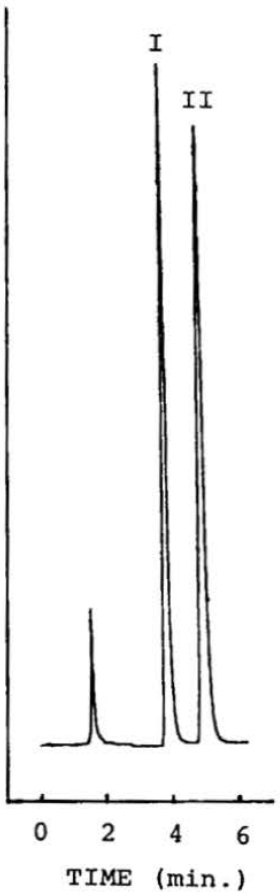

Fig. 1. *A chromatogram of total adrenaline (I) and internal standard, isoprenaline (II).*

D- and L-adrenaline: 1.00 ml adrenaline injection was diluted to 10.00 ml with 35% methanol in water. 1.00 ml of this solution was evaporated at 100°C with N_2 till dryness. 100 µl 2% GITC in dimethylformamide was added to the residue. After 10 min in a water-bath at 50°C, 20 µl of 0.5% hydrazine in dimethylformamide was added. After 10 minutes at room temperature, 1.00 ml mobile phase was added. 20 µl of the solution was injected into the chromatographic system.

Reproducibility

Total adrenaline: A solution containing L-adrenaline hydrogen tartrate corresponding to 0.1 mg/ml adrenaline base, and 0.2 mg/ml isoprenaline sulfate was injected 10 times into the chromatographic system.

D- and L-adrenaline: 10 aliquots of a solution containing a mixture of L-adrenaline hydrogen tartrate and DL-adrenalin hydrochloride corresponding to 1 mg/ml adrenaline base, 75% of

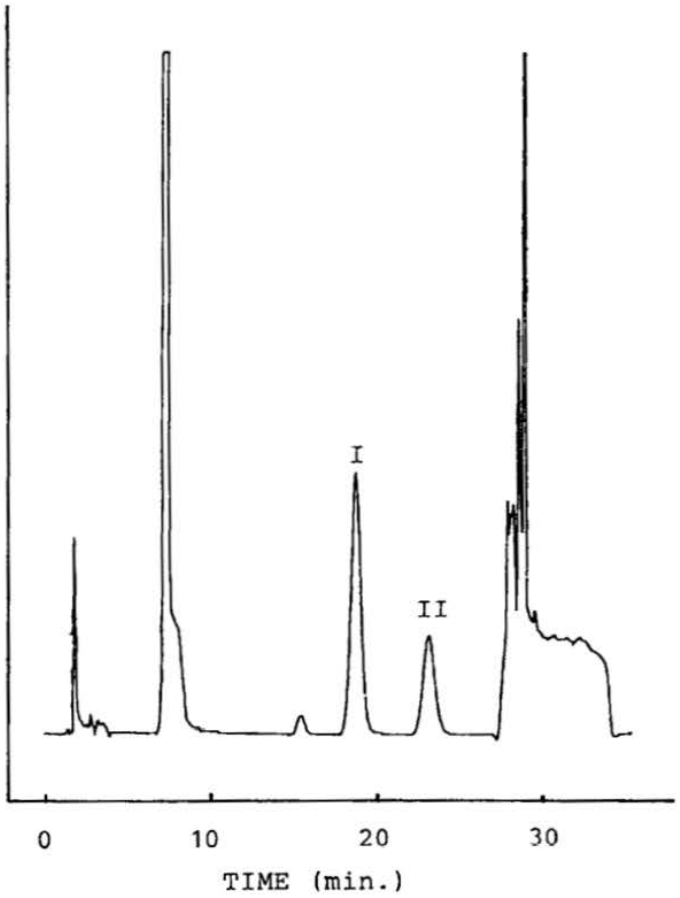

Fig. 2. *A chromatogram of L- and D-adrenaline derivatized with GITC.*
The ratio between L- and D-adrenaline concentrations is to 3:1.
I=L-adrenaline,II=D-adrenaline.

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