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## Note

# Determination of adrenaline by ion-pair high-performance liquid chromatography

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Polarimetric[1] and fluorimetric[2] determinations of adrenaline are non-specific and have poor reproducibility. Gas chromatographic determination entails a tedious preparation of the sample, including an extraction and a derivatization[3]. Finally, analysis on ion-exchange columns does not permit the simultaneous separation of other basic compounds; also, the lifetime of such columns does not in practice exceed 3 months[4]. In the present paper, it is shown that quantitative determination by ion-pair chromatography is simple, rapid and does not have the disadvantages of the above techniques.

## EXPERIMENTAL

### *Apparatus*

A home-made chromatograph was used, consisting of a Type M-6000-A piston pump, a Type U-6-K septum-less injector and a column (30 × 0.4 cm I.D.) filled with 10-$\mu$m silica beads covered with octadecylsilane ($\mu$Bondapak $C_{18}$), all supplied by Waters Ass. (Milford, Mass., U.S.A.). The column outlet was connected to a Model LC-55 UV-detector (Perkin-Elmer, Norwalk, Conn., U.S.A.). The detector signal was evaluated with a W + W 1100 recorder (Electronic Inc., Basle, Switzerland) and an integrator (Minigrator; Spectra Physics, Santa Clara, Calif., U.S.A.).

### *Chemicals*

Research grade methanol and acetic acid were obtained from E. Merck, Darmstadt, G.F.R. The sodium salt of hexanesulphonic acid was purchased from Eastman-Kodak, Rochester, N.Y., U.S.A. All of the chemicals were used without further purification. l-Adrenaline (E. Merck) satisfied the norm required by various pharmacopoeias (norm DAB 7).

### *Procedure*

An aqueous solution of 0.10% adrenaline containing 0.3% of $NaH_2PO_4 \cdot 2H_2O$ (pH 4.4) and 0.04% $Na_2S_2O_5$ was prepared as a standard. Samples to be analyzed were adjusted to about the same concentration of adrenaline. Ten microlitres of each of these solutions were injected into the chromatograph and eluted with methanol–water (1:1) containing 0.06% hexanesulphonate and 0.2% acetic acid at a flow-rate of 1.2 ml/min. The elution time for adrenaline was *ca.* 3 min (Fig. 1).

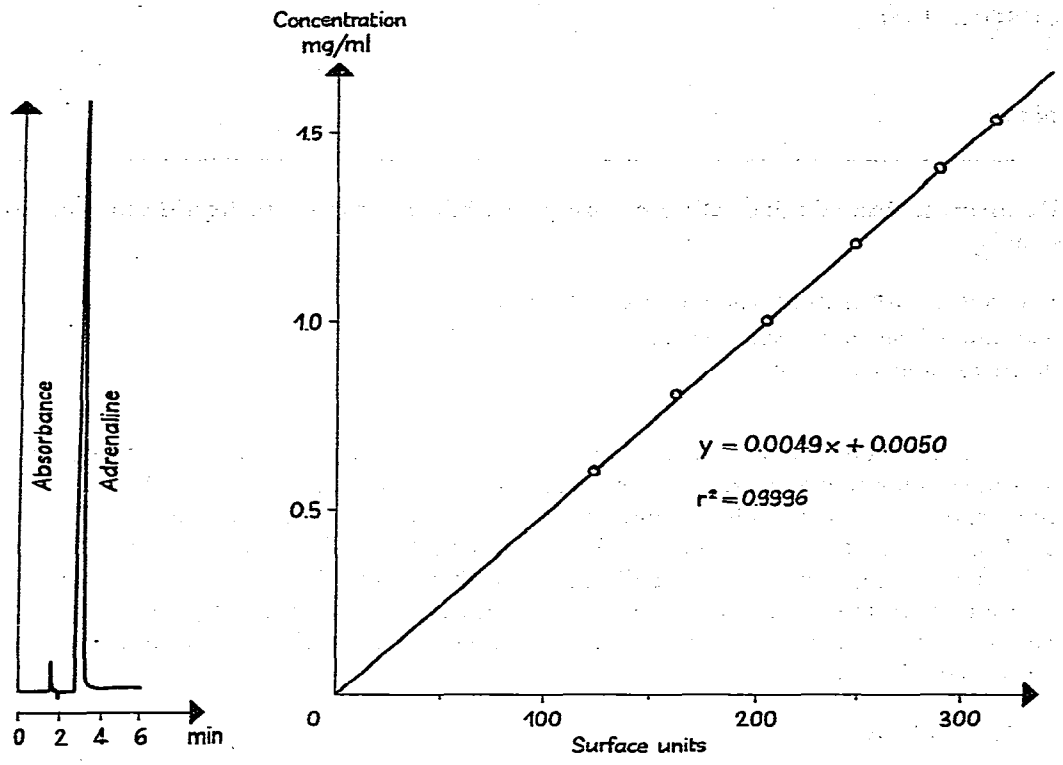


Fig. 1. Chromatogram of a solution of adrenaline. Chromatographic conditions: column, 30 × 0.4 cm I.D. filled with μBondapak $C_{18}$; mobile phase, methanol–water (1:1) containing 0.06% hexanesulphonate and 0.2% acetic acid; flow-rate, 1.2 ml/min; pressure drop, 1200 p.s.i.; injection volume, 10 μl of 0.1% solution.

Fig. 2. Analytical evaluation function.

## RESULTS

For the ion-pair chromatography of organic bases, alkanesulphonic acids have often been proposed as possible protonating agents since they do not absorb in the UV region[5]. When hexanesulphonic acid is used, the presence of acetic acid is necessary in order to prevent hydrolysis of the conjugated acid of the adrenaline. The ion-pair formed was eluted with methanol–water (1:1) after *ca.* 3 min on a chemically bonded octadecyl monolayer, and the adrenaline hexanesulphonate was well separated from impurities and degradation products. The wavelength of detection was 279 nm where the molar extinction coefficient of adrenaline, $\varepsilon_0$, is 2600 l/mol·cm.

Injections of various quantities of adrenaline showed that the specific response at the chosen wavelength was constant up to 2.0 mg/ml (Fig. 2). The reproducibility was evaluated in terms of the relative standard deviation: $\sigma_{rel.} = 1.04\%$ ($n = 7$) between $x = 0.5$ and 1.5 mg/ml. The detection limit was equal to 0.06 mg/ml.

In order to prevent the degradation of adrenaline in pharmaceutical solutions, antioxidants are added. These are either not eluted or are not detected, but are of limited efficiency and old solutions may show a decrease in the adrenaline content.

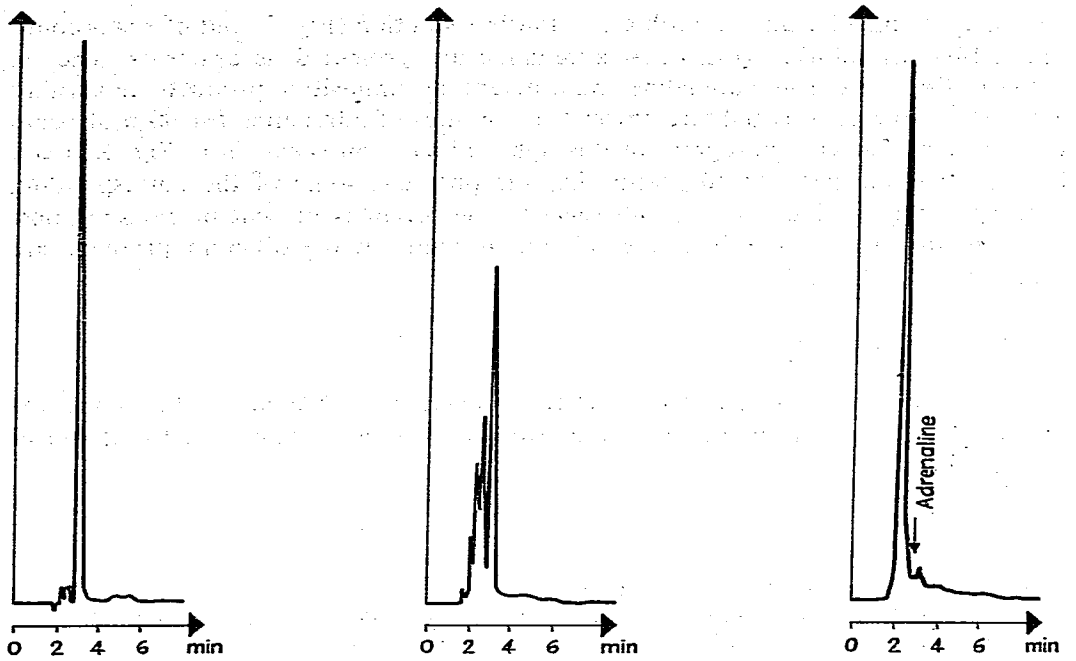


Fig. 3. Chromatogram of a three-month old solution of adrenaline. Chromatographic conditions as in Fig. 1.

Fig. 4. Chromatogram of a six-month old solution of adrenaline. Chromatographic conditions as in Fig. 1.

Fig. 5. Chromatogram of a solution of adrenaline recorded after oxygen bubbling for 60 h at room temperature. Chromatographic conditions as in Fig. 1.

TABLE I

RETENTION TIMES OF DIFFERENT ADRENALINE SOLUTIONS (SEE ALSO FIGS. 1, 3 AND 4)

| *Sample* | *Retention times (sec)* * | | | | | | | *Fig.* |
|---|---|---|---|---|---|---|---|---|
| | *Decomposition products* | | | | | | *Adrenaline* | |
| Reference soln. (0.1% adrenaline) | | | | | | | 188 (100) | 1 |
| Colourless soln. after 3 months | 115 (>1) | 133 (1.5) | 152 (4) | | 278 (1.5) | 303 (>1) | 186 (93) | 3 |
| Red-brown soln. after 3 months | 115 (1.5) | 134 (9) | 148 (6) | 165 (28.5) | 272 (>1) | | 186 (55) | 4 |
| Decomposed soln. obtained by oxygen bubbling | | | 152 (94) | | 209 251 (total: 4) | 226 | 194 (2) | 5 |

* Relative amounts are given in parentheses.

Chromatograms of a three-month old colourless solution (Fig. 3) and of a six-month old red-brown solution (Fig. 4) of adrenaline are presented as examples. The six peaks in Fig. 4 are from adrenaline and different decomposition products. In another experiment oxygen was bubbled through a solution of adrenaline for 60 h at room temperature. The chromatogram of this light red solution is shown in Fig. 5. It can be seen that the peak of adrenaline has disappeared; some of the corresponding decomposition products may be identical to the products present in old solutions. The retention times as well as the relative amounts of the different products are summarized in Table I.

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