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Note

Use of high-pressure liquid chromatography in the assay of aldosterone in urine

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It has been demonstrated that high-pressure liquid chromatography (HPLC) is suitable for the analysis of synthetic mixtures of steroids [1,2]. Up to now HPLC has not often been used in clinical steroid practice, although a few assays for cortisol and corticosterone have been described [3—5].

In our laboratory we are using HPLC with success in aldosterone assay in urine for the final separation and quantification. For several years we have estimated aldosterone in urine by means of gas—liquid chromatography (GLC), after conversion of aldosterone into a stable derivative, its corresponding γ-lactone [6]. We have now replaced GLC by HPLC, thus avoiding the oxidation of aldosterone. The two methods are compared, and HPLC is shown to be an improvement.

MATERIALS AND INSTRUMENTS

A Varian liquid chromatograph Model 8500 was used, equipped with a stop-flow injector, a 254-nm single-wavelength detector, and a 250 × 2.2 mm SI-10 MicroPak column (silica gel, particle size 10 μm). Samples were injected with a 10-μl Hamilton syringe.

Thin-layer chromatography (TLC) was performed on thin-layer plates pre-coated with silica gel 60 F_{254} (E. Merck, Darmstadt, G.F.R.). Components separated on the plates were revealed by short-wave UV irradiation, under a Camag UV lamp Type TL 900.

All solvents were of analytical reagent grade.

METHOD

(a) The acid-labile conjugate of aldosterone, its 18β-D-glucosiduronic acid [7], in 24-h urine is hydrolysed at pH 1 at room temperature for 24 h.

(b) Hydrolysed urine is split into two equal portions (duplicates), and each

portion is extracted three times with one-third volume dichloromethane. The pooled extract is washed successively with 1*M* sodiumhydroxide and 0.1*M* sodium hydroxide, both solutions being saturated with sodium chloride, and twice with water saturated with sodium chloride.

(c) The washed extract is dried over anhydrous sodium sulphate, filtered and evaporated to dryness in a rotary evaporator at 30°. The residue is transferred with acetone into a 10-ml centrifuge tube and the solution is evaporated to dryness under nitrogen.

(d) The residue is dissolved in 2 ml of 70% methanol in water and washed three times with 3 ml of toluene.

(e) The washed solution is split into two equal portions, and 10 μg of aldosterone is added to one portion.

(f) After being evaporated to dryness under nitrogen, the residue is purified by TLC. Three solvent systems are used, and after each TLC step the area corresponding to the area of the aldosterone standard is scraped off and eluted with acetone. The solvent systems are: (1) chloroform—methanol—water (90:10:0.8); (2) ethyl acetate—methanol—water (85:15:1); and (3) benzene—acetone—water (70:30:0.5) (twice in succession, on the same thin-layer plate).

(g) Quantification of aldosterone is performed by HPLC, after the addition of 5—10 μg of prednisolone to the eluate as internal standard. The amount of added prednisolone depends on the expected amount of aldosterone, as judged by viewing the last thin-layer plate under UV irradiation (254 nm). The eluate is evaporated to dryness under nitrogen, the residue is dissolved in 50 μl of dichloroethane; 1—10 μl of this solution is injected onto the column. The eluent used is 1.5% methanol in chloroform, half saturated with water, prepared by passing 2 l of 1.5% methanol in chloroform through a column 50 cm long and 1 cm I.D., filled with 20 g of silica gel, 30—120 mesh, that had been coated with 6 ml of water, and mixing this water-saturated solvent with an equal volume of water-free solvent. The flow-rate is 70 ml/h, which corresponds to a pressure drop of 1100 p.s.i.

RESULTS AND DISCUSSION

The separation of pure aldosterone and prednisolone, injected directly onto the column, is shown in Fig. 1; the shape of the aldosterone peak is symmetrical.

Fig. 2 shows the chromatogram of a urine extract, obtained according to the method described; here the aldosterone peak is not symmetrical as in Fig. 1, but shows a shoulder. The shoulder is caused by the effect of TLC on aldosterone; this is illustrated in Fig. 3, showing the chromatogram of pure aldosterone after TLC. Possibly a shift in the equilibrium between two tautomeric forms of aldosterone [8] (Fig. 3) is responsible for this effect.

To correct for the loss of material due to three elutions from the thin-layer plates, aldosterone standard was added to a part of each urine extract (see Method). The recovery was 63.1 ± 9.4% (n = 215).

The precision of both methods, GLC and HPLC, was estimated from duplicate assay by the equation [9]:

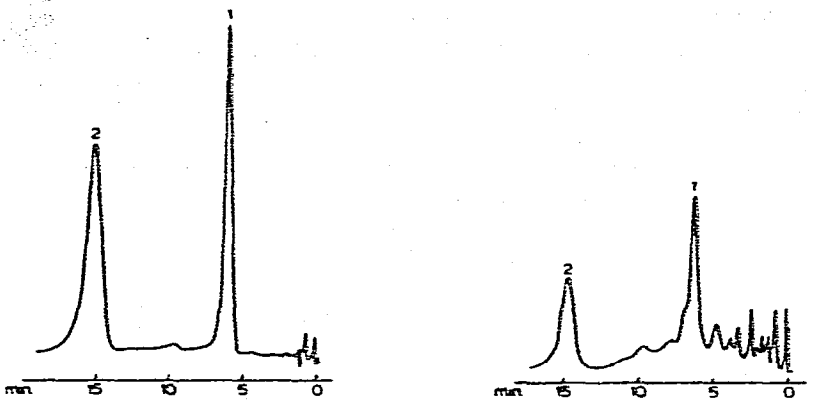

Fig. 1. Separation of pure aldosterone (1) and prednisolone (2). Column, SI-10 MikroPak; eluent. 1.5% methanol in chloroform half saturated with water; flow-rate, 70 ml/h; detection UV detector 254 nm.

Fig. 2. Chromatogram of a urine extract. 1 = Aldosterone, 2 = prednisolone (internal standard). For conditions see Fig. 1.

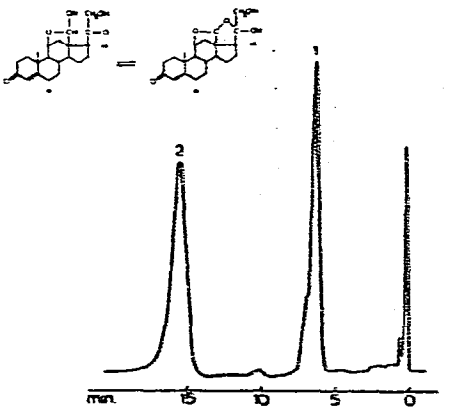

Fig. 3. Chromatogram of pure aldosterone after TLC. 1 = Peak + shoulder of aldosterone, possibly a mixture of two tautomeric forms of aldosterone a and b. 2 = prednisolone. For conditions see Fig. 1.

$$CV = \sqrt{\frac{\Sigma d^2}{2n}}$$

where CV = coefficient of variation; $d = \left(\frac{x_2}{x_1} - 1\right) . 100$,

with $x_2 \geqslant x_1$ (x_1, x_2 are duplicate values); and n = number of duplicate assays.

Results showed that for HPLC CV was 16 (n = 215), with a lower detection

limit of 5 nmoles/24 h whereas for GLC CV was 22 (n = 71), with a lower detection limit of 10 nmoles/24 hr. Normal values were 17.6 ± 12.6 nmoles/24 h (n = 15), range 5—45 nmoles/24 h.

Thus, in our hands, HPLC in aldosterone assay in urine appeared to be more precise and more sensitive than GLC. In addition, the conversion of aldosterone into a stable derivative, required for GLC, is avoided.

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