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GAS CHROMATOGRAPHIC DETERMINATION OF LEVELS OF ALDADIENE IN HUMAN PLASMA AND URINE FOLLOWING THERAPEUTIC DOSES OF SPIRONOLACTONE

J. CHAMBERLAIN
Searle Scientific Services, Lane End Road, High Wycombe, Bucks. (Great Britain)

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SUMMARY

A specific and sensitive procedure for the determination of aldadiene in human plasma and urine after therapeutic doses of spironolactone has been developed. The method involves extracting the metabolite from the plasma or urine into dichloroethane with subsequent separation and detection on a gas chromatograph equipped with an electron-capture detector. Androst-4-ene-3,6,17-trione is used as an internal standard for quantitation by the relative peak height technique.

INTRODUCTION

Spironolactone (3-(3-oxo-7α-acetylthio-17β-hydroxyandrost-4-en-17α-yl)propionic acid-γ-lactone) is a synthetic steroid which provides effective treatment for resistant oedema and ascites by blocking the action of aldosterone. One of its principal metabolites is aldadiene[1] (3-(3-oxo-17β-hydroxyandrosta-4,6-dien-17α-yl)propionic acid-γ-lactone). A fluorescence technique has been used to determine the metabolite in plasma and urine[2]. This fluorescence method lacks specificity and it was thought that gas chromatography (GC) would offer a more reliable and rapid method for monitoring levels of aldadiene in plasma and urine. Preliminary experiments showed that aldadiene was a strong absorber of thermal electrons, thereby offering a means of sensitive and specific detection after GC.

EXPERIMENTAL

Reagents

The internal standard, androst-4-ene-3,6,17-trione, was prepared by chromium trioxide oxidation[3] of dehydro-*epi*-androsterone in the presence of air. A standard solution of 1 μg/ml in ethanol was prepared. Aldadiene was obtained from our own collection of reference steroids. All other reagents and solvents were obtained from Hopkin & Williams Co. Ltd., Chadwell Heath, Essex, Great Britain, and were not further purified prior to use.

Gas chromatography

A Pye 104 chromatograph equipped with a single ^{63}Ni electron-capture detector and injection point heater was used. Silanised glass columns (1.5 m long × 4 mm I.D.) were packed with 2% OV-1 on CQ 80–100 mesh (JJ's (Chromatography) Ltd., Hardwick Trading Estate, Kings Lynn, Norfolk, Great Britain) and conditioned overnight in a slow stream of nitrogen at 250°. Chromatography was at 250° with a carrier of oxygen-free nitrogen (120 ml/min).

Extraction procedure

Plasma. To each analysis tube was added 200 μl of the internal standard solution, 1 ml plasma, 0.1 ml 0.25% sodium hydroxide and 5 ml dichloroethane with shaking after each addition. After the layers had been allowed to separate the top (aqueous) layer was removed and the organic extract washed with a further 1 ml of water, dried by filtration through Whatman No. 1 filter paper and evaporated to dryness. The residue was dissolved in 1 ml ethanol and 10–15 μl was used for GC.

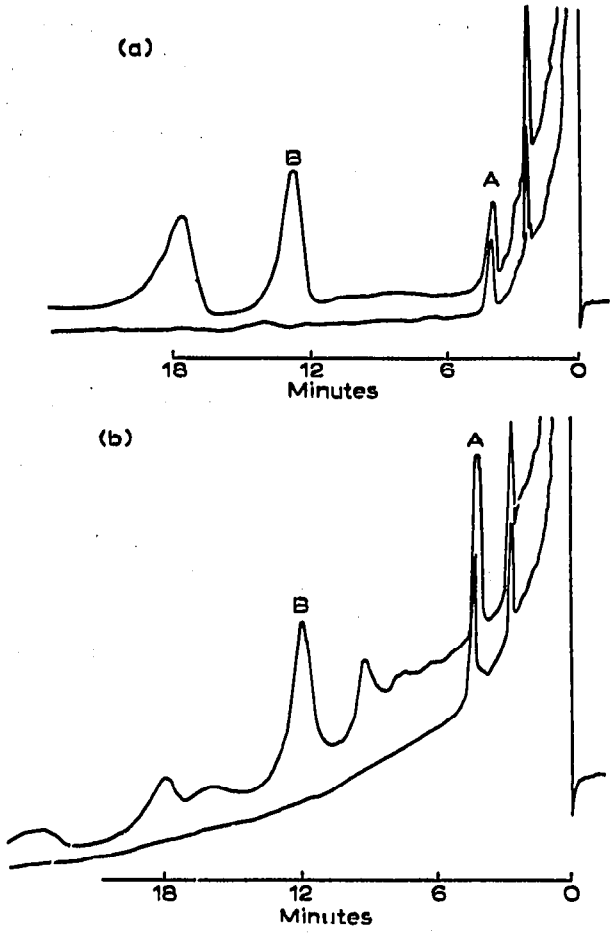

Fig. 1. (a) Gas chromatogram of a dichloroethane extract of urine from a volunteer who had ingested 100 mg spironolactone. (b) Gas chromatogram of a dichloroethane extract of plasma from the same volunteer. In each case the lower trace is from extracts of samples obtained prior to medication. A is the internal standard, androst-4-ene-3,6,17-trione. B is aldadiene corresponding to 3 mg/l in urine (a) and 14.0 μg/100 ml in plasma (b).

Urine. The procedure was as above using 5 ml urine and omitting the 0.25% sodium hydroxide.

Measurement

The retention times of androst-4-ene-3,6,17-trione and aldadiene under the conditions described were 4 and 12 min, respectively (Fig. 1). A linear response, as judged by the relative peak height technique, was observed for the range 0–15 mg/l urine and 0–150 μg/100 ml plasma. (Fig. 2).

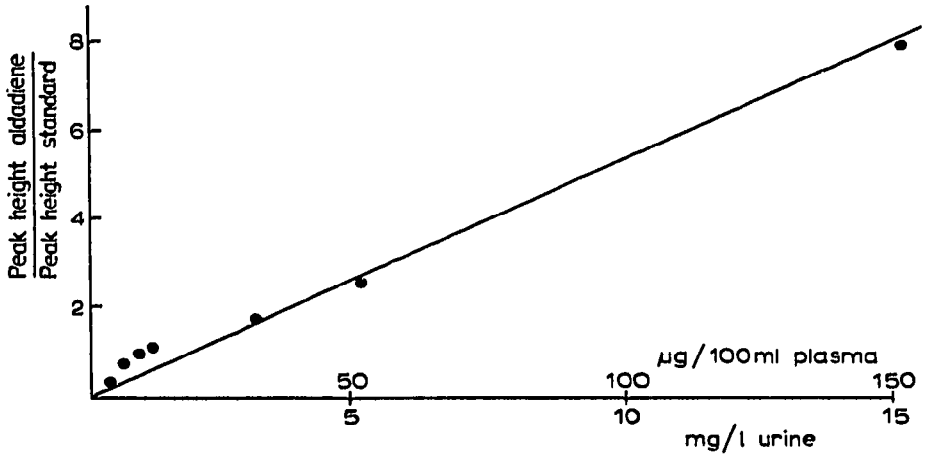

Fig. 2. Calibration curve for quantitation of aldadiene in plasma and urine.

Accuracy

Varying amounts of aldadiene were added to urine and plasma and the mixtures assayed by the present technique. The mean recovery was 96% (range 89–101%) with a standard deviation of $\pm$ 4.

Specificity

The detector used is specific for electron-absorbing molecules. Fig. 1 shows chromatograms obtained from plasma and urine extracts before and after treatment with spironolactone. Table I shows a comparison of values obtained from the analysis of urine and plasma samples assayed by the present technique and the fluorescence technique of GOCHMAN AND GANTT[2]. The lower values obtained by the present technique may be due to the higher specificity obtained by the GC separation from aldadine of other fluorescent metabolites.

Application

Plasma and urine were collected from male volunteers who ingested 100 mg spironolactone (Aldactone-A, G.D. Searle & Co. Ltd.). Venous blood samples were drawn at 0, $\frac{1}{2}$, 1, 2, 4, 6 and 24 h; urine was collected at 0, 2, 4, 6 and 24 h.

RESULTS AND DISCUSSION

Plasma levels of aldadiene reached a peak of 10–15 μg/100 ml approximately 3 h after administration of Aldactone-A (Fig. 3). GANNT *et al.*[4] reported peak plasma

TABLE I

COMPARISON OF PRESENT METHOD (A) AND FLUORESCENCE METHOD (B) FOR ESTIMATION OF ALDADIENE IN PLASMA AND URINE

Plasma (μg/100 ml)		*Urine (mg/l)*	
A	*B*	*A*	*B*
0	0	0	0
3.6	3.5	2.6	4.0
15.7	31.0	2.5	5.6
12.5	18.0	2.0	3.9
1.5	3.0	1.27	1.9
0.7	2.0	0.15	0
3.3	5.5	2.00	2.2
15.3	23.0	3.00	7.3
14.2	15.0	2.44	6.0
24.3	28.0	1.27	1.6

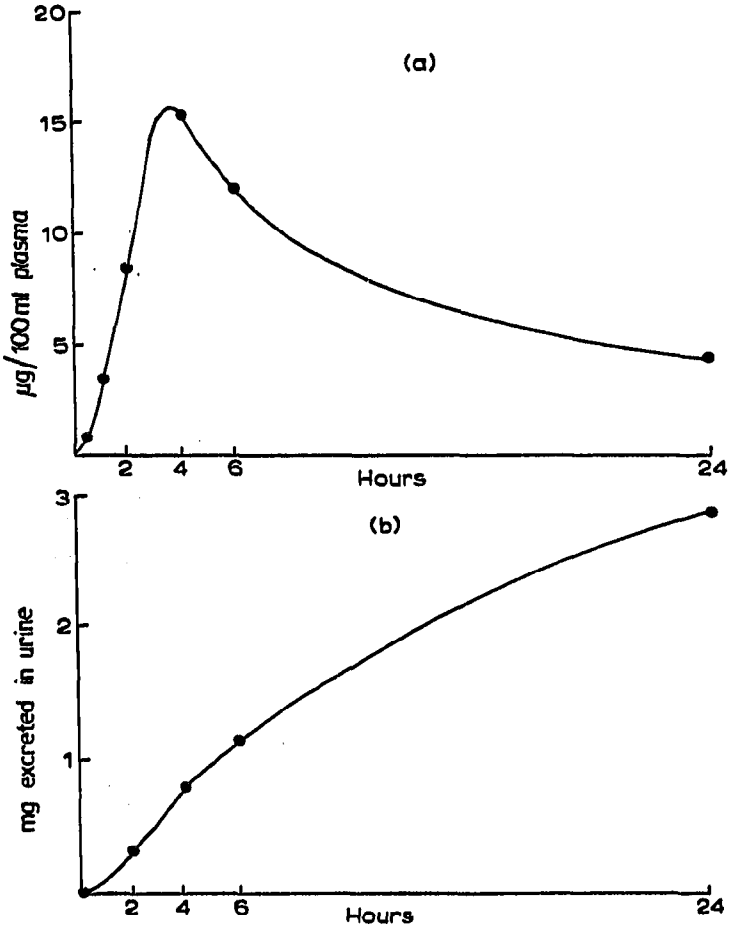

Fig. 3. (a) Plasma concentration–time curve indicating levels of aldadiene found in a male subject after an oral dose of Aldactone-A containing 100 mg spironolactone. (b) The corresponding urine levels of aldadiene in the same subject.

levels at three h of 40 μg/100 ml after a 200 mg dose of Aldactone-A, using the fluorescence technique. The lower values observed in the present study could be attributed to the greater specificity of the GC method.

Urinary excretion of aldadiene reflects the plasma levels of the metabolite (Fig. 3). About 4 mg is excreted in the 24 h after a 100 mg oral dose, and this is in agreement with figures quoted by GANTT *et al.*[4]

Inspection of the gas chromatograms obtained (Fig. 1) reveals the presence of several compounds after spironolactone treatment, in addition to the aldadiene usually measured to monitor levels of the drug. It is not clear, at present, whether these compounds are also metabolites of spironolactone or whether they represent increased excretion of natural products due to the action of spironolactone. This is being investigated.

In recent years it has become apparent that the increased specificity and potency of modern drugs requires very careful control of dose levels. For some drugs there may be considerable individual variation in absorption, metabolism and elimination and dosages are best individualised by monitoring blood levels, or in some cases urinary excretion, of the drug or its metabolites. The present technique provides a rapid, specific and sensitive procedure for correlating plasma and urine levels of aldadiene with biological activity of spironolactone.

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