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Short Communication

Thin-layer chromatography of urinary 17-oxosteroids using dansylhydrazine as a prelabelling reagent[a]

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ABSTRACT

This paper describes a modification of a high-performance liquid chromatographic method for measurement of 17-oxosteroids in biological fluids for use with thin-layer chromatography and fluorometric scanning detection. After extraction from urine samples with Separon-C_{18} microcolumns, free oxosteroids were labelled with dansylhydrazine in acetonitrile–acetic acid and chromatographed on silica gel F-254 plates with the solvent system chloroform–methanol (97:3). Linearity of fluorescence detection (Shimadzu CS-9000 densitometer) was obtained between 30 and 1000 ng.

INTRODUCTION

Kawasaki *et al.* [1] and Iwata and Suga [2] have described a method for the measurement of 17-oxosteroids in biological fluids. It involved labelling with dansylhydrazine, high-performance liquid chromatographic (HPLC) separation and fluorometric detection.

We have modified the method for use with thin-layer chromatography (TLC). Free oxosteroids are extracted after hydrolysis from urine samples with Separon SGX C_{18} cartridge (Tessek), labelled with dansylhydrazine in acetonitrile–acetic acid, chromatographed on silica gel F-254 plates in the solvent system chloroform–ethanol, and detected by fluorescence at 310 nm (excitation; the emission wavelength is eliminated by a filter) on the densitometer Shimadzu CS-9000. A reproducible separation was obtained, and lineary in the fluorescence intensity was observed in the range of 30–1000 ng.

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EXPERIMENTAL

Urine samples (4 ml) were hydrolysed with arylsulphatase and β-glucuronidase (Boehringer, Mannheim, Germany; 70 μl of the commercial solution in 4 ml of 2 *M* acetate buffer, pH 4.6) at 37°C for 48 h. Then they were extracted by Separon SGX C_{18} cartridges (Tessek, Prague, Czechoslovakia). The cartridges were washed with 3 ml of water and 3 ml of 20% methanol, and the 17-oxosteroids were eluted with 3 ml of methanol and dried under nitrogen. The internal standard (5 μg of corticosterone in 20 μl of methanol) was added, and the labelling reaction was performed by adding 200 μl of 0.2% (w/v) of dansylhydrazine (Sigma, Deisenhofen, Germany) in acetonitrile (Merck, Damstadt, Germany) and 50 μl of acetic acid to the dry sample. The mixture was incubated at 37°C for 45 min, them 200 μl of a 0.5% (w/v) suspension of sodium pyruvate (Lachema, Brno, Czechoslovakia) in acetonitrile was added. After further incubation at 37°C for 20 min, 3 ml of chloroform and 1 ml of 0.25 *M* sodium hydroxide were added. After vortex-mixing for 1 min the water layer was discarded. Another 2 ml of water were added, and after vortex-mixing the water layer was again discarded. The chloroform layer was dried with anhydrous sodium sulphate and evaporated under nitrogen. The residue was dissolved in chloroform, and 100-μl samples were used for spotting.

Each 100-μl chloroform extract was applied by a Hamilton syringe to a TLC plate (Silica Gel F-254 Merck, 20 × 20 cm). The plate was developed twice in chloroform–ethanol (97:3). The chromatography was performed in a tank not saturated with the solvent system at room temperature (22°C) at a distance of 18 cm. The fluorescence was quantatively measured at 310 nm (excitation; the emission wavelength was eliminated by a filter) on the Shimadzu CS-9000 densitometer.

RESULTS AND DISCUSSION

The R_F values after double developement are: corticosterone, 0.22; 11β-hydroxyetiocholanolone, 0.29; 11β-hydroxyandrosterone and oxoetiocholanolone, 0.32; oxoandrosterone, 0.38; etiocholanolone, 0.49; dehydroepiandrosterone, 0.56; androsterone, 0.60. Known amounts of individual oxosteroids were added to a sample of patient's urine, and the recoveries (after subtracting the result without addition of the standard from the total result with the addition of the standard, expressed as μg per 4 ml) and coefficients of variation (C.V.) were calculated. The results are shown in Table I.

This modification of the method described by Kawasaki *et al.* [1] and by Iwata and Suga [2] provides a simple possibility of group estimation of oxosteroids without HPLC; the chief advantage is the possibility of running ten urine samples in one chromatography step.

TABLE I

ANALYTICAL RECOVERY OF 17-OXOSTEROIDS FROM URINE AND PRECISION OF ASSAY

Steroid	Amount added (μg per 4 ml)	Final amount[a] (μg per 4 ml)	Recovery (%)	C.V. (%)
11-Hydroxyetiocholanolone	0	2.04 ± 0.016		0.78
(3α,11β-dihydroxy-5β-androstan-17-one)	5.0	6.83 ± 0.514	97.0	7.35
11-Hydroxyandrosterone	0	4.33 ± 0.51		11.77
(3α,11β-dihydroxy-5α-androstan-17-one) and oxoetiocholanolone (3α-hydroxy-5β-androstan-11,17-dione)	5.0 + 5.0	14.05 ± 0.644	98.4	4.58
Oxoandrosterone	0	1.26 ± 0.036		3.05
(3α-hydroxy-5α-androstan-11,17-dione)	5.0	6.16 ± 0.257	98.6	4.17
Etiocholanolone	0	7.49 ± 0.423		5.84
(3α-hydroxy-5β-androstan-17-one)	5.0	12.32 ± 0.322	98.65	2.61
Dehydroepiandrosterone	0	2.21 ± 0.101		4.57
(3β-hydroxy-5-androstan-17-one)	5.0	7.30 ± 0.174	101.3	2.38
Androsterone	0	4.25 ± 0.106		2.49
(3β-hydroxy-5α-androstan-17-one)	5.0	9.13 ± 0.18	98.86	1.97

[a] Means ± 95% confidence intervals; $n = 6$.

REFERENCES

1 T. Kawasaki, M. Maeda and A. Tsuji, *J. Chromatogr.*, 226 (1981) 1.
2 J. Iwata and T. Suga, *J. Chromatogr.*, 474 (1989) 363.