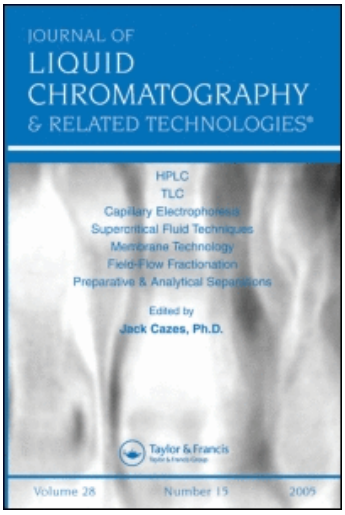

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Enzymatic Detection of Urinary Steroid-17β-Glucuronides after Gel Filtration

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ENZYMATIC DETECTION OF URINARY STEROID-17β-GLUCURONIDES AFTER GEL FILTRATION

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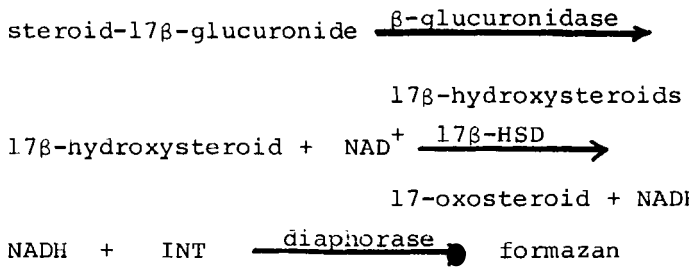
ABSTRACT

An enzymatic detection of urinary steroid-17β-glucuronides is described. The principle of the method is as follows; after gel filtration with Sephadex G-25 β-glucuronidase is added to each effluent fraction and incubated for 20 h at 37 °C. After hydrolysis, 3β,17β-hydroxysteroid dehydrogenase is added and incubated for 20 min at 37 °C. An absorbance at 500 nm is read aginst sample of first fraction effluent.

INTRODUCTION

An enzymatic detection of steroid-3α-glucuronide, steroid-3α-sulfate, and steroid-3β-sulfate in urine after gel filtration has been described previouly(1). In this paper, an enzymatic detection of steroid-17β-glucuronide after gel filtration is described. The principle of the method is as follows;



MATERIALS

All reagents were of analytical grade obtained from commercial sources and used without futher purification. 3β, 17β-hydroxysteroid dehydrogenase (3β, 17β-HSD) from P. testosteroni (EC 1.1.1.51), 2-p-iodophenyl-3-p-nitrophenyl-5-phenyltetrazolium chloride (INT), and diaphorase (EC 1.6.99.2, from C. kluyveri) were purchased from Sigma chemical Co.

Sulfatase/β-glucuronidase from H. pomatia (EC 3.1.6.1) was prepared of 200 units as sulfatase/ml in 0.05 M acetate buffer (pH 5.0). β-Glucuronidase from E. coli (EC 3.2.1.31) was prepared of 500 Fishman units/ ml of phosphate buffer (0.1 M, pH 6.8).

Reagent for color development for 17β-hydroxysteroid was described previouly (2,3): 25 U of 3β,17 β-HSD, 30 mg of NAD^+,100 U of diaphorase and 15 mg of INT were dissolved in 0.2 M K_2HPO_4 (pH 9.0) of 100 ml.

METHODS

Sephadex Gel filtration (1,4)

Sephadex G-25 was swollen by heating a suspension of the particles in acetate buffer (0.05 M, pH 5.0) for 4 h at 90 °C under constant stirring. The fines were removed by several decantations and the slurry was poured directly into the column (1 x 20 cm) which was then washed for 3 h with acetate buffer.

Urine sample, a part of 24 h urine, was centrifuged for 3 min at 3000 rpm and supernatant of 1 to 2 ml was applied. One fraction of effluent contains 1.3 ml and 30 fractions were collected.

Procedure for detection of steroid-17β-glucuronide

To 0.6 ml of effluent of each fraction is added 0.1 ml of β-glucuronidase solution and incubated for 20 h

at 37 °C. After hydrolysis of steroid-17β-glucuronide, 1 ml of color development reagent for 17β-hydroxysteroid is added and incubated for 20 min at 37 °C. Absorbance at 500 nm was read against the sample of first fraction.

RESULTS AND DISCUSSION

A chromatogram for androsterone-glucuronide, dehydroepiandrosterone-sulfate, estrone-sulfate and estriol-16-glucuronide of standard compounds was shown in FIGURE 1. Hydrolysis with β-glucuronidase from E. coli and sulfatase/β-glucuronidase from H. pomatia was compared for steroid-17β-glucuronides detection using sample from patients of adrenal tumor. As shown in FIGURE 2 , only

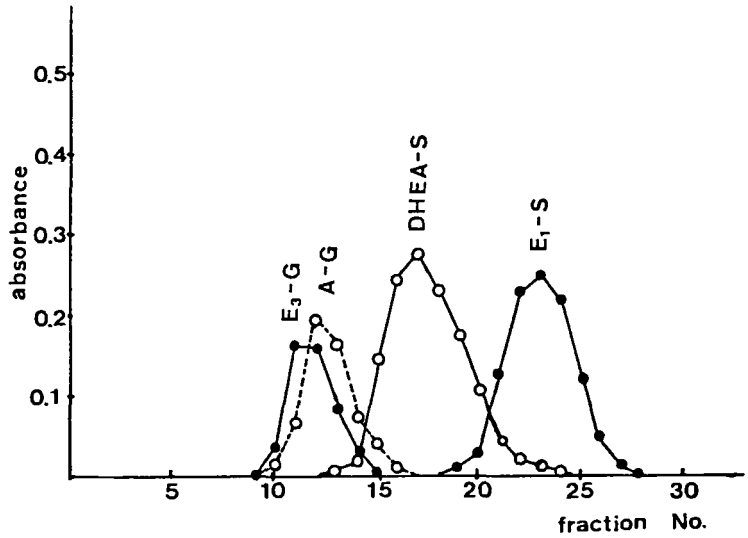


Fig. 1. Gel filtration of standard steroid conjugate.(1) Methods for each conjugated steroids were performed by enzymatic detection method described previously.

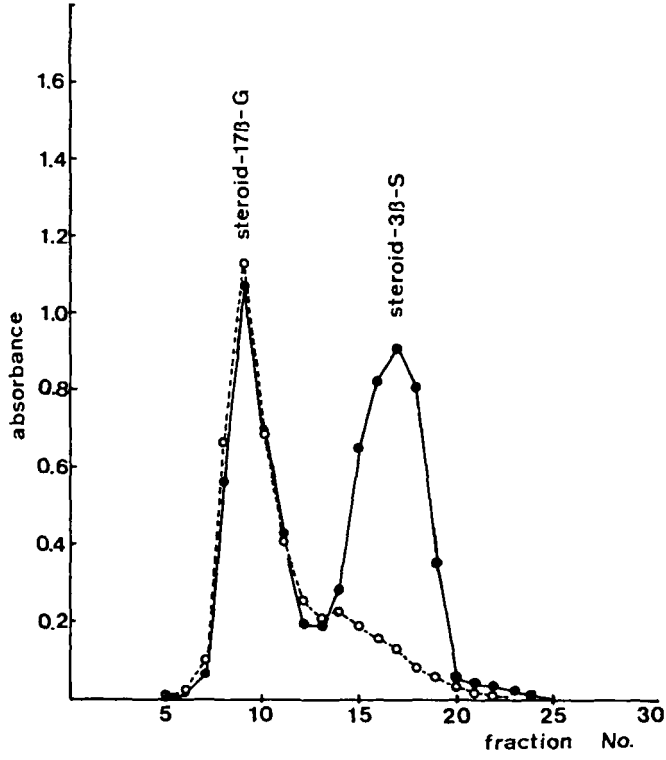


Fig. 2.Gel filtration of steroid-17β-glucuronide and steroid-3β-sulfate in urine of patient of adrenal tumor. o------o, hydrolysis with β-glucuronidase and ●——● , hydrolysis with sulfatase/β-glucuronidase.

17β-glucuronides was detected by hydrolysis with β-glucuronidase from E. coli and 3β-sulfate and 17β-glucuronide were detected by hydrolysis with sulfatase/β-glucuronidase from H. pomatia. From this chromatogram, most of the 17β-hydroxy group is conjugated with glucuronic acid. As a reference of other steroid conjugates excretion,

steroid-3α-glucuronide, steroid-3α-sulfate and steroid-3β-sulfate of the same sample which is detected by previously described method were shown in FIGURE 3. (1) The direct enzymatic detection method for steroid-17β-glucuronide can be detected for a sample of elevated excretion of 17β-hydroxysteroids, at least 5 mg/litter

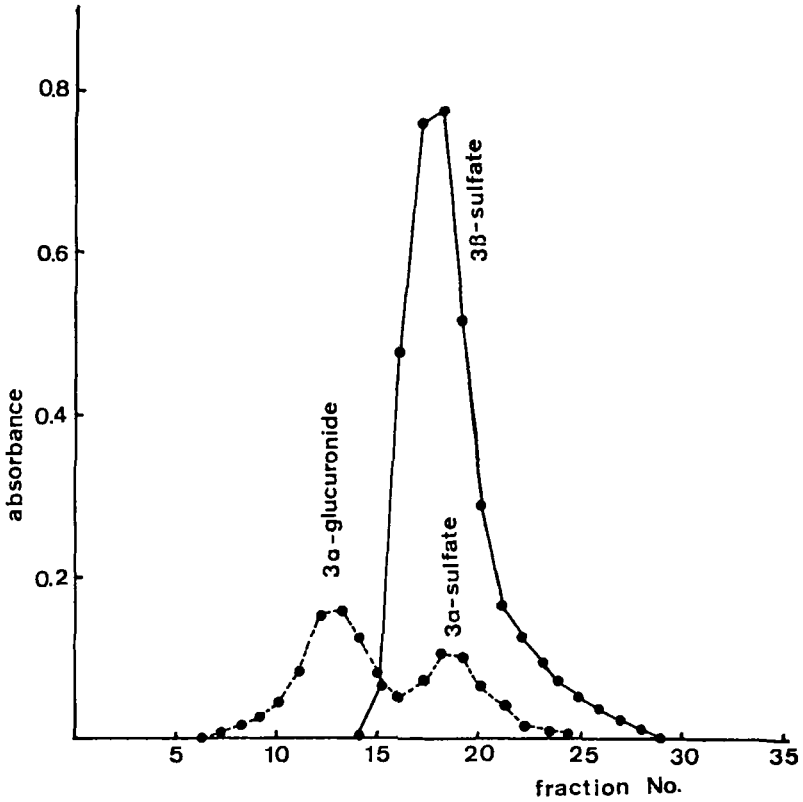


Fig. 3. Gel filtration of steroid-3α-glucuronide, steroid-3α-sulfate, and steroid-3β-sulfate in urine of patient of adrenal tumor. Each steroid conjugate was detected by previously described methods (1).

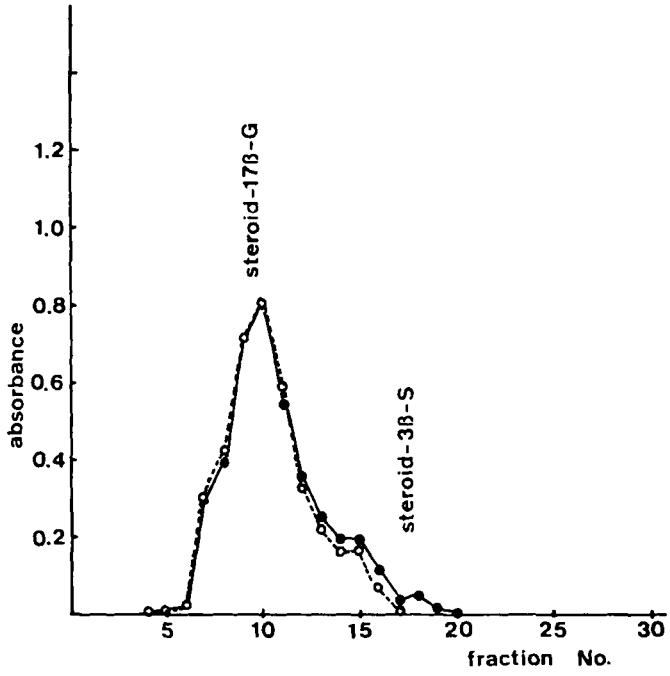


Fig. 4. Gel filtration of steroid-17β-glucuronide and steroid-3β-sulfate in urine of patient of breast tumor. o-----o, hydrolysis with β-glucuronidase and ●——●, hydrolysis with sulfatase/β-glucuronidase.

as total 17β-hydroxysteroids determined by the previously described method (2) such as breast tumor shown in FIGURE 4.

An enzymatic detection of nonconjugated steroid has been described so far (2,3,4,5,6,7,8), but extraction by organic solvent is required in a procedure so that automated analysis can not be performed. This enzymic method for determination of steroid-17β-glucuronide has a possiblity for automated analysis using

high performance liquid chromatograhy and flow reaction systems.

REFERENCES

Non-standard abreviations; DHEA-S; dehydroepiandrosterone sulfate, A-G; androsterone glucuronide, E_1-S; estrone-sulfate, E_3-G; estriol-16-glucuronide:

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