Application of Glass ODS for The HPLC Analysis of Estrone 17-β-Estradiol and Estriol*

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Reversed phase high-performance liquid chromatography (HPLC) with an ODS porous glass column (HITACHI GEL #3161) was used to assay estrone, 17-β-estradiol, and estriol. Using acetonitrile-water (30/70) as a mobile phase at a flow rate of 1 mL/min, the three steroids were analysed in 5 min. The results demonstrate that the present packing materials was much advantageous to analyse these three steroids.

INTRODUCTION

High-performance liquid chromatography (HPLC) has become a powerful tool for rapid analysis of steroids. Several HPLC systems have been described for the separation of steroids in pharmaceutical preparations and in biological fluids [1-6]. For further improvement of the separation efficiency, we have been applying the porous glass ODS (HITACHI GEL #3161) as a packing material and this time we applied the column for separating estrone, 17-β-estradiol, and estriol.

EXPERIMENTAL

Packing method of the column

HITACHI GEL #3161 was packed into a 150 x 4.6 mm I. D. stainless steel column by a slurry packing method. 1.6g of the packing material was added to the mixture consisted of tetrabromoethane 40 vol%, carbon tetrachloride 40 vol%, and dioxane 20 vol%. The slurry was placed in an ultrasonic cleaner for about 30 sec to be dispersed well and poured into the packer. Chloroform was fed by operating the pump for 30 min.

After chloroform treatment, methyl alcohol was fed for 20 min. Then methyl alcohol-water (5/5) (v/v) was flowed for 25 min.

Materials

Guaranteed reagent grade Estrone, 17-β-Estradiol, and Estriol were supplied from Tokyo Kasei Kogyo Co., Ltd. Japan. HPLC grade acetonitrile (Wako Pure Chemical Japan) and freshly ion exchanged and distilled water were used as eluent. Other chemicals were of reagent grade.

Apparatus

A Toyoda high-performance liquid chromatograph Model CCPD 8000, equipped with a Rheodyne injector Model 7125 and a Toyoda UV detector Model UV-8000, was operated at ambient temperature. Samples were dissolved in the mobile phase. The eluent was degassed by vacuum and ultrasonication for 30 min just before use.

RESULTS AND DISCUSSION

Attempts were made to find the optimal conditions for the separation of the samples at a flow rate of 1 mL/min. The capacity factors for the steroids

*Received 25 July, 1987
Revised 25 Sep. 1987
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Figure 1  Effect of acetonitrile concentration in the mobile phase on capacity factors of estriol, 17-β-estradiol, and estrone.

Figure 2  A chromatogram of estrone, 17-β-estradiol, and estriol.

Packing Material: HITACHI GEL #3161
Column size: 150 x 46 mm I.D.
Mobile phase: acetonitrile-water (30/70)
Detection: 254 nm
Flow rate: 1.0 ml/min
Temperature: ambient
Column pressure: 250 kgf/cm²
Key: 1, estriol; 2, 17-β-estradiol; 3, estrone

Figure 3  Effect of acetonitrile concentration in the mobile phase on capacity factors of cortisone (1), hydrocortisone (2), prednisolone (3), triamcinolone (4), progesterone (5), norethisterone (6), estriol (7), estrone (8), and 17-β-estradiol (9) attained with 30% acetonitrile in water. With 20% acetonitrile the retention time becomes too long for routine analytical work, though the separation was tolerable. A typical chromatogram obtained is displayed in Fig. 2.

Cortisone and hydrocortisone are glucocorticoid. Prednisolone and triamcinolone are synthetic anti-inflammatory steroid, and progesterone and norethisterone are corpus luteum hormone. Those are standardized steroids at the 11th Japanese Pharmacopoeia. Separation behavior of those compounds was also examined, and results are shown in Fig. 3.

To summarize, HITACHI GEL #3161 used in the present work is quite suitable for rapid analysis of steroids. Estrone, 17-β-estradiol, and estriol were separated successfully with 30% acetonitrile in water. Further studies are in progress in our laboratories.

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