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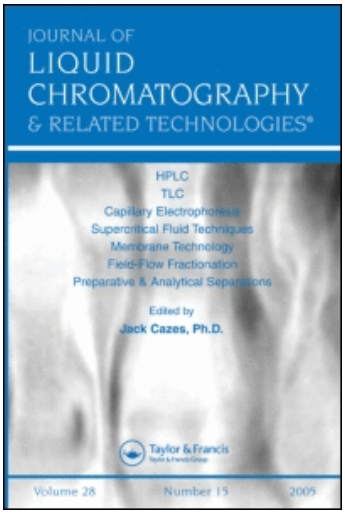

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Capillary Electrophoresis of Steroid Conjugates

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CAPILLARY ELECTROPHORESIS OF STEROID CONJUGATES

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ABSTRACT

Capillary Electrophoresis of Steroid Conjugates

Advances in medical sciences have mandated increased separation efficiency and high level sensitivity. This is especially true when sample availability is in the ultra micro range. Because of this a study of capillary electrophoresis (CE for separation of steroid conjugates was carried out. There are few methods available to separate these molecules without first hydrolysis of the conjugates. The initial focus was on the estrogen conjugates since many of these are readily available for reference material. We avoided the use of derivative formation often necessary for optimal sensitivity.

The determination of steroids and their metabolites, the sulfates and glucuronides has been hampered for considerable time due to tedious methodology. Since this laboratory has been involved in this area for a number of years it seemed appropriate to investigate the utility of capillary electrophoresis (CE) for the separation of these substances. Only recently has there been reported investigation

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on the use of CE for separation of small molecules; most of the prior work being with macromolecules and proteins. Chromatographic methods have been applied but required a high level of analytical skill. High performance liquid chromatography has been used but, has not been developed to a level of universal acceptance in this area.

Recent reports have indicated that CE can be successfully applied to smaller molecules. K.J. Lee, et al. have shown the separation of antiepileptic drugs in plasma, phenobarbital among them (1). Pleasance, et al. used CE for separation of sulfa type pharmaceuticals including sulfamethazine (2). Lukkari, et al. (3) were able to resolve ten β-blockers from urine by CE. Holland and Sepaniak (4) separated 10 mycotoxins in 45 minutes using micellar electrokinetic capillary chromatography. Wernly and Thormann (5) used this technique in analyses of drugs of abuse in human urine. Whang and Chen (6) separated riboflavin, thiamine, nicotinamide and pyridoxal using capillary zone electrophoresis. Smith and Khaledi (7) studied the effect of pH on separation of phenols.

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CE thus has been indicated to be an attractive tool for pharmaceutical analysis because of ease of operation and high sensitivity. This report describes results obtained with CE of a number of steroid conjugates.

Experimental

Steroids

The steroids were obtained as follows: estriol-3-glucuronide and estriol-16α-glucuronide were obtained from Sigma Chemical Co. (St. Louis, MO); estriol-3-sulfate, estrone-sulfate, dehydroepiandiosterone-sulfate, equilenin sulfate, and equilin sulfate were generously supplied by Wyeth-Ayerst (Rouses Point, NY). All chemicals were EM Science (Gibbstown, NJ) reagent grade.

The biological samples were aliquots of samples collected from the clinic or from animals during the course of other experiments. All samples were filtered through 0.45 μm. Millipore filters prior to injection into the system. 2-4 nl. were injected by electromigration.

The instrument used was an ISCO Model 3850 (Lincoln, NB) capillary electrophoresis system. This provided variable current and voltage capabilities. The detection system was on column variable wavelength.

The capillary was an uncoated fused silica column of 50 μm. ID and 50 cm length with the detection window at 31 cm. In the experiments reported the buffer used was a borate-sodium hydroxide solution of pH 10.2 and concentration of 0.2 mm. in borate. This was routinely degassed by filtering through a 0.45 μm. filter (Millipore). Urine samples were diluted 1:50 and the amniotic samples 1:25 with the buffer.

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Results and Discussion

Table 1 shows that the steroid conjugates listed are readily separated. The estrogen conjugates are those present in urine or blood. These are present in larger quantities in the urine of pregnant women or pregnant mares. Because estriol-3-glucuronide did not separate completely from the 16-analogue, further investigation was carried out to improve the resolution. It was found that reducing the current increased resolution along with an increased retention time that resulted in a more complete separation of these two homologues as seen in Table II. Figure 1 indicates that the resolution provided by the conditions used was excellent for separation of the standard steroid conjugates studied. It is of interest that the sulfates have longer migration times than the glucuronides.

Figure 2 shows the separation of conjugates from amniotic fluid. The peak at 6.88 (13.7 mm) coincides with that of estriol-3-glucuronide. Figure 3 shows the

Table 1

Migration Times (min.) for Steroid Conjugates

44mA, 20kV, 210nm

Estriol-16α-Glucuronide	13.4	DHA* Sulfate	15.9
Estriol-3-Glucuronide	12.0	Equilin Sulfate	16.2
Estriol-3-Sulfate	14.55	Equilenin Sulfate	17.5
Estrone-Sulfate	16.60		

*dehydroepiandiosterone

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Table II

Effect of Voltage on Separation

(Time in min.)

	44 mA 20 kV	18 mA 10 kV
Estriol-16α-Glucuronide	12.5	16.57
Estriol-3-Glucuronide	13.0	14.91

separation of estriol-16α-glucuronide from urine of term pregnancy. The peak at 17.85 min. has the migration shown by estriol-16α-glucuronide. Figure 4 shows the separation of conjugates in the urine of a rat with a bile fistula to which was administered phenobarbital. At present the separated peaks are not identified but some presumably are phenobarbital metabolites.

The results given in the foregoing discussions demonstrate that the conditions for capillary electrophoresis as described may provide means to separate steroid

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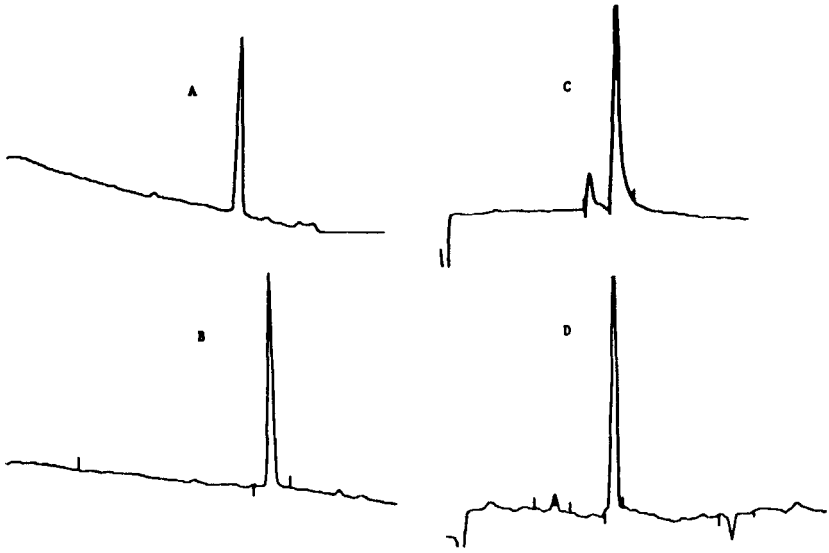

Figure 1 Resolution of Steroid Conjugates by Capillary Electrophoresis

a Estriol-3-glucuronide

b Estriol-16α-glucuronide

c Estrone-sulfate

d Estriol-3-sulfate

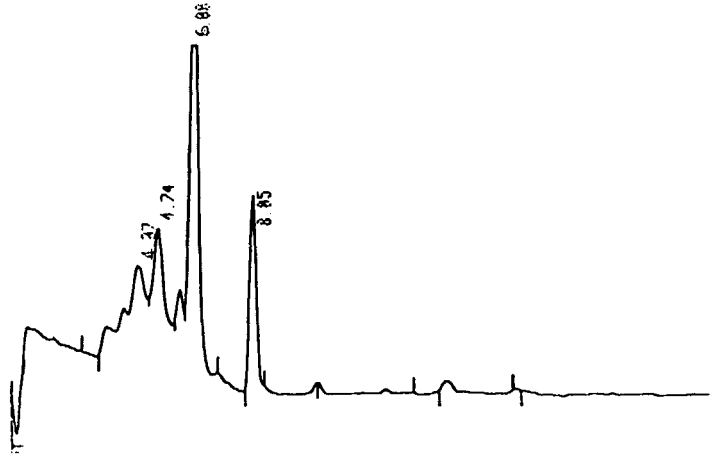

Figure 2 Separation of Components of Amniotic Fluid from Term Pregnancy. Peak at 13.0 min. coincides with E3-3-glucuronide.

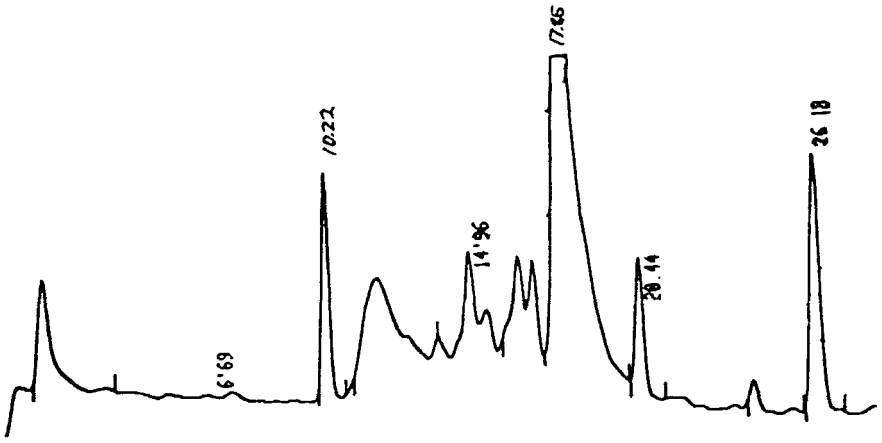

Figure 3 Separation of Components of Urine of Term Pregnancy. Peak at 17.85 is E3-16-glucuronide as noted by migration time.

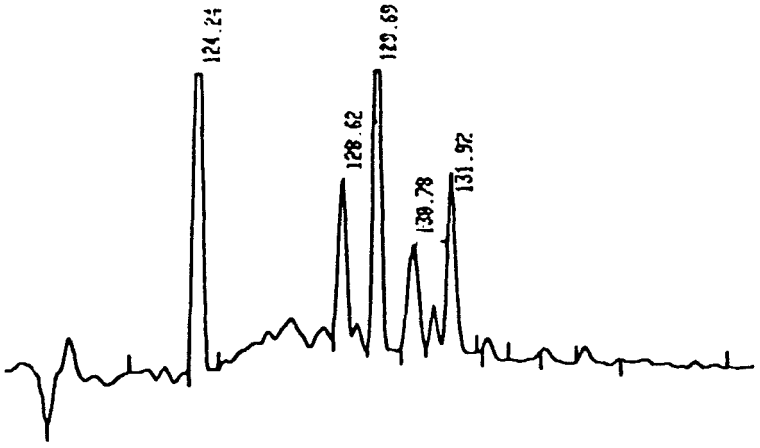

Figure 4 Separation of Components of Rat Urine. Separated Peaks as yet not Identified.

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conjugates in biological media. Further work is in progress to evaluate the utility of this tool in metabolic studies.

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