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Note

Liquid chromatographic assay of urinary estriol and electrochemical detection with a battery powered detector

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Urinary estriol represents the final metabolism of estrogens. In pregnancy, estriol levels increase rapidly especially after the 12th week of gestation. Frequent monitoring of this metabolite is utilized in diagnosis of fetal distress in threatened abortions such as toxemia and diabetes.

At the present time, the main methods for estriol assay are colorimetric and fluorimetric [1, 2]. Such methods require lengthy extraction and clean-up steps and they are subject to many interferences [3]. Recently, high-performance liquid chromatography with ultraviolet (UV) detection has been employed for estriol assay [4, 5].

Electrochemical detection has been applied for the detection of many of the urinary metabolites. It is highly sensitive and selective. In this paper we illustrate that estriol can be detected electrochemically with great sensitivity after separation by liquid chromatography. Electrochemical detection of estriol is about 20-fold more sensitive than UV detection. This greater sensitivity allows smaller sizes of sample and solvents to be used, thus speeding up the extraction steps. Column life is greatly increased because of the smaller size of the sample injected.

We used a battery-powered detector in this work. Since the electrochemical detector consumes just minute amounts of electricity, it is well suited to be powered by a battery. The advantages of such a detector are: it would operate free from line noise and would be easier to construct. The detector can be assembled for less than US$75 for parts.

MATERIALS AND METHODS

Equipment

A pump, Model 110A (Altex Scientific, Berkeley, CA, U.S.A.) was used to

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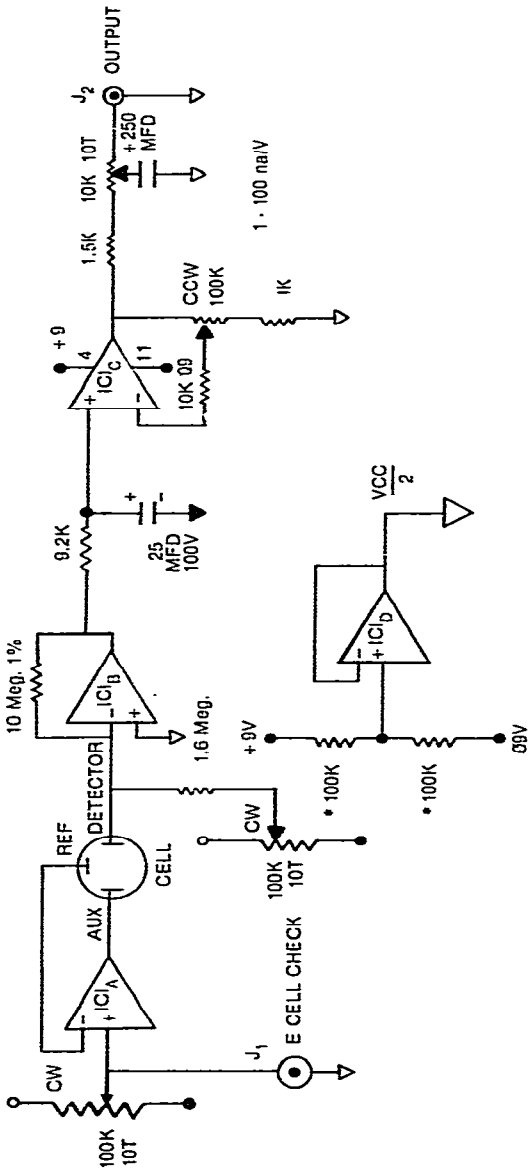


Fig. 1. Schematic diagram of the detector.

deliver the solvent through a 150 × 4.6 mm I.D. column of 5 μm average particle size of C_{18} silica gel (Altex) at a flow-rate of 1.2 ml/min. The samples were introduced through a 30-μl loop injector, Model 7120 (Rheodyne, Berkeley, CA, U.S.A.). The effluent was monitored at 275 nm using a UV detector, Model 785 (Micromeritics Instrument Corp., Norcross, GA, U.S.A.) followed by a thin-layer electrochemical cell (Bioanalytical Systems, West Lafayette, IN, U.S.A.).

Detector

The schematic diagram of the detector is illustrated in Fig. 1. The detector is powered by two 1.4-V mercury batteries and one 9-V alkaline battery. These batteries run for a few months. The detector has a linear range between 1—250 ng of 5-hydroxy-3-indoleacetic acid. The detector responds to as low levels as 0.5 ng of the latter compound.

Reagents

Extraction solvent: 15 ml ethyl acetate and 15 ml methanol were added to 70 ml chloroform.

Pump solvent: 20% acetonitrile in 15 m*M* phosphate buffer, pH 3.5.

Stock estriol standard: 100 mg estriol dissolved in 1000 ml methanol.

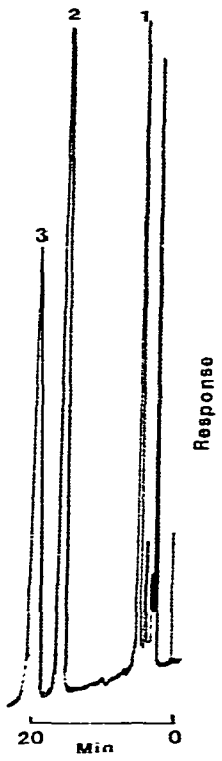
Procedure

Urine (100 μl) was buffered with 25 μl of 2 *M* phosphate buffer, pH 6.4 and hydrolyzed with 45 units of glucuronidase (Sigma, St. Louis, MO, U.S.A.) at 60°C for 30 min. After cooling the tubes 500 μl of the extracting solvent were added and the contents were vortex-mixed for 20 sec. The organic layer was separated from the aqueous layer by centrifugation at 9000 *g* for 30 sec. A 50-μl aliquot of the organic layer was removed into the evaporating vial and evaporated at 60°C without air. The contents of the vials were reconstituted with 100 μl of pump solvent and an aliquot of 25 μl was injected on the column.

RESULTS AND DISCUSSION

Estrogens contain a phenolic group, so they are expected to undergo oxidation under an applied potential. The separation and electrochemical detection of the three main estrogens is illustrated in Fig. 2. Estradiol and estrone do not elute from the column using the routine solvent of 20% acetonitrile in 15 m*M* phosphate buffer, pH 3.5 but require increasing the acetonitrile concentration up to 35%. The 16- and 17-epiestriol coelute with estriol; however, the level of epiestriol in urine is about 50-fold less than estriol. The oxidation potential of estriol is illustrated in Fig. 3. For routine use the oxidation potential was set at 0.9 V. Since the oxidation potential depends on many variables [6, 7], it needs to be determined for each instrument. The capacity factor for estriol (Fig. 4) was not affected with pH change.

Representative chromatograms of estriol assay by the present method are illustrated in Fig. 5. The assay is linear by the peak height method between 1—30 mg/l. The average recovery of 10 mg/l standard added to urine is 87%.



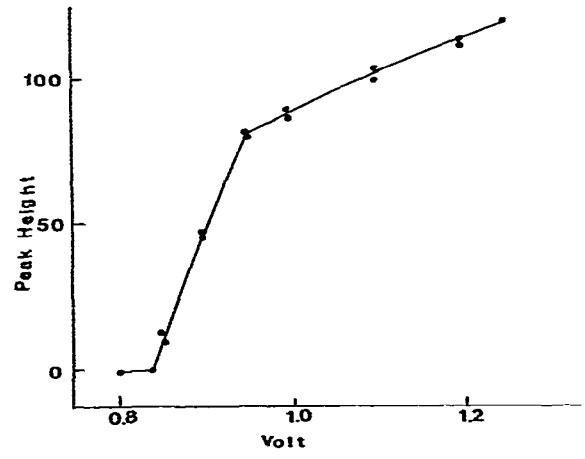


Fig. 2. Separation of estriol (1), estradiol (2) and estrone (3). Eluting solvent: 35% acetonitrile in 15 m*M* phosphate buffer, pH 3.5.

Fig. 3. Oxidation potential of estriol.

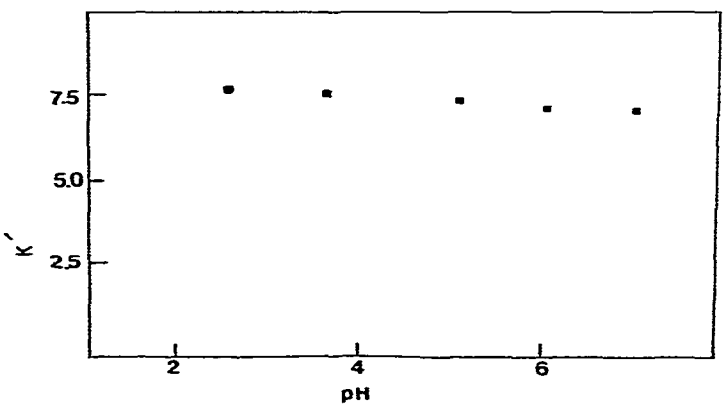


Fig. 4. Capacity factor, *k'*, of estriol against pH.

Two samples with values of 13 and 19 mg/l by gas chromatography gave 12.6 and 16.6 mg/l, respectively, by the present method. Three urine samples from pregnant females — in the trimester — had estriol levels by the present method of 13.6, 14.1 and 17.5 mg/l, while 30 urines from males and non-pregnant

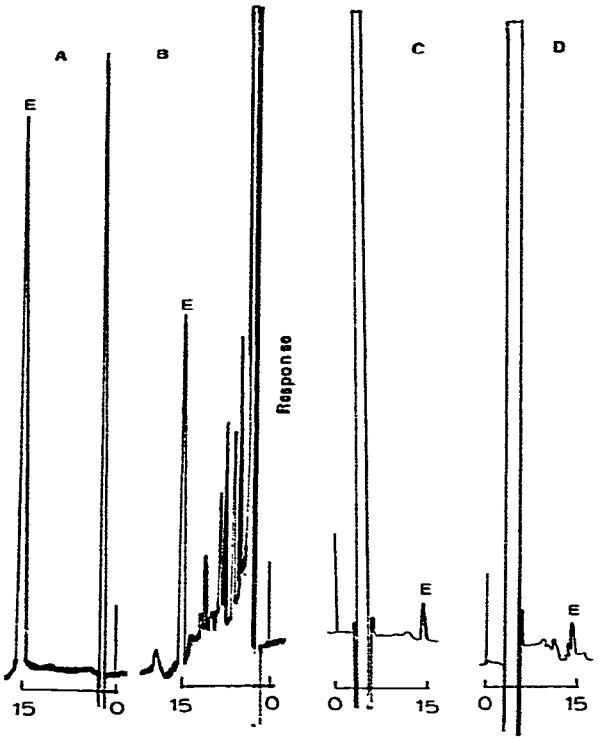


Fig. 5. Chromatograms of (A) estriol (E) standard, 10 mg/l by electrochemical detection; (B) estriol (E) extracted from the urine of pregnant female; electrochemical detection; (C) estriol (E) standard, 10 mg/l; UV detection, 275 nm, 0.010 absorbance; (D) estriol (E) extracted from urine of pregnant female same as (B); UV detection at 275 nm, 0.010 absorbance.

females had undetectable levels. Usually estriol in non-pregnant females is about 100-fold less than in pregnant females. If estriol is to be assayed from non-pregnant females larger urine samples, and more important, further additional clean-up steps are required.

Estriol assay by electrochemical detection is about 20-fold more sensitive than the UV detection at 275 nm (Fig. 5). Estriol concentrations as low as 0.2 mg/l can be assayed by this method. This high sensitivity allows smaller volumes of sample, solvent and enzyme to be used, thus greatly speeding up the extraction step. Column life is greatly extended. The column can be used for over 1000 injections provided that maintenance is carried out as previously described [8]. Although electrochemical detection is more sensitive than UV detection for estriol, the radioimmunochemical methods remain more sensitive than either of them.

The standard deviation for 15 within-run assays by this method is 0.52 with a mean of 11.4 mg/l and a coefficient of variance of 4.5%.

The battery-powered detector is easy to assemble and repair while operating free from line noise. It has similar sensitivity to the electrical one [7, 8]. When it is connected after a UV detector, it will increase the capability of the liquid chromatograph without extra expensive investment.

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