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ANALYSIS OF UNCONJUGATED ESTRIOL IN SERUM USING RADIAL COMPRESSION: ADVANTAGES OVER RADIOIMMUNOASSAY

Concentrations of unconjugated estriol in maternal serum or plasma increase throughout pregnancy, particularly during the third trimester. To manage the time of delivery of difficult pregnancies, physicians typically measure the level of this compound as an indicator of fetal well-being. Conventionally, this measurement is done by radioimmunoassay (RIA) techniques. Some significant drawbacks of the RIA methods include the large number of standards and controls needed for the assays, the limited shelf life of the expensive reagents and the burden of record keeping associated with all RIA procedures. These considerations prompted Kaplan and Hohnadel (1) to develop an LC method for the analysis of unconjugated estriol as an alternative to RIA.

Two types of reversed-phase columns were used during development of the method: a μBONDAPAK™ C_{18} steel column and a RESOLVE™ C_8 cartridge with an RCM-100® Radial Compression Module. Slightly different eluents were needed to achieve similar separations with the two column types, but both methods "separated estriol from other peaks equally well," and showed "similar precision and accuracy," according to the authors. It was also noted that the advantages of the RESOLVE™ C_8 cartridges over the stainless steel C_{18} columns were "their lower cost, increased flexibility when changing columns, and the ability to perform the analysis at higher flow rates with low back pressures."

The experimental conditions for the analysis are summarized in Table 1. Electrochemical detection was used to achieve the desired sensitivity and selectivity. Serum samples were extracted by shaking with diethyl ether for 10 minutes,followed by solvent removal under a nitrogen stream and reconstitution in methanol. Propiophenone was included in the methanol as an internal standard. 10-50 μl were injected directly into the isocratic LC system, to give typical chromatograms such as those shown in Figure 1. As little as 400 pg of injected estriol could be detected.

In order to compare the accuracy of the LC and RIA methods for estriol, the analytical recovery of known amounts of estriol from pooled serum was determined. The results were 99.6% for LC and 113% for RIA, indicating good recovery of material for both methods. The within-run and between-run precisions of the RIA and LC methods were statistically indistinguishable except at the lowest concentration of estriol analyzed. The calibration lines for the two methods were also statistically identical. For the LC method, a number of steroids known to occur in serum at levels similar to that of estriol were analyzed. No significant interferences to the quantitation of estriol were observed.

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In summary, the LC method was found to be equivalent to RIA in terms of accuracy, precision and sensitivity. Specificity was excellent for the LC method, and the previously mentioned drawbacks to RIA were avoided. The sample throughput was not quite as good as for automated RIA instruments, but could probably be improved. Nonetheless, the authors were able to conveniently analyze samples within 60 minutes of their receipt.

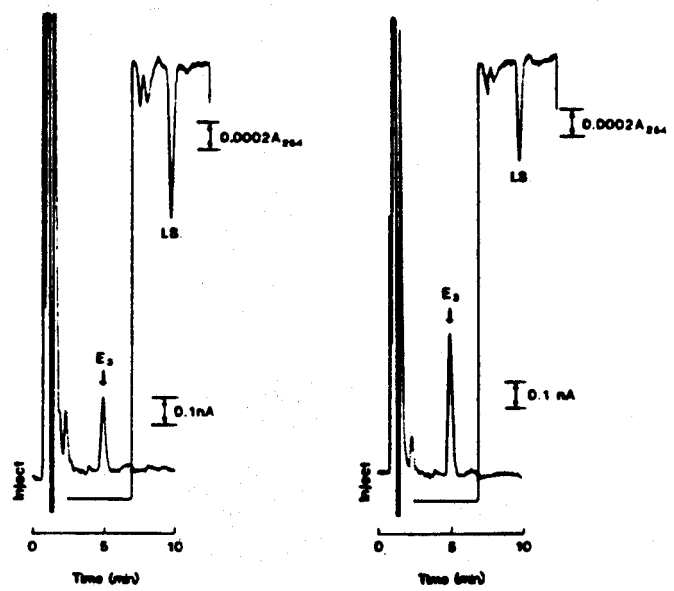
TABLE 1

CHROMATOGRAPHIC CONDITIONS FOR LC ANALYSIS OF ESTRIOL

Buffer:	180 mM disodium hydrogen phosphate 8.5 mM EDTA
Columns/Eluents:	μBONDAPAK™ C_{18} (steel), acetonitrile/buffer (24/76) Flow Rate: 2 ml/min
	RESOLVE™ C_8 cartridge, acetonitrile/buffer (27/73) Flow Rate: 3 ml/min
Detection:	UV, 254 nm (Waters M480) Electrochemical, +0.75V vs Ag/AgCl (BAS LC-4A)

FIGURE 1

CHROMATOGRAMS OF EXTRACTED SERUM FROM A PREGNANT WOMAN WITHOUT (LEFT) AND WITH ADDED ESTRIOL (E_3) STANDARD (RIGHT), AS OBTAINED WITH USE OF Radial-PAK™ C_8 CARTRIDGES.



1. Kaplan, L. A. and Hohnadel, D. C., Clin. Chem., 29, 1463 (1983).