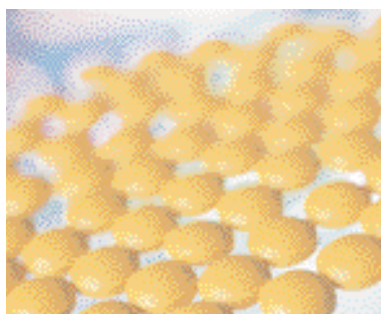


An LC Method for the Separation and Determination of Ethynodiol Diacetate and Ethinyl Estradiol in Tablets

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The author describes a method for the separation and determination of **the two active ingredients in the contraceptive pill** by liquid chromatography with UV detection.

The United States Pharmacopeia's (USP) separation method for ethynodiol diacetate (EDDA) and ethinyl estradiol (EED), two active ingredients in contraceptive pills, uses a cyano column and a mobile phase of methanol:acetonitrile:water (15:35:50) (1). However, several separation attempts using both cyano and phenyl columns did not produce satisfactory results. Thus, a study was performed to improve the chromatography.

A literature search revealed few references to liquid chromatography (LC) separation of a EDDA and EED mixture. Carignan and colleagues used an RP-2 column, a dual-wavelength detection, and an internal standard to analyze a mixture of EDDA, EED, and mestranol (2). López de Alda and Barceló used a LiChrospher 100 RP-18 column for separation with mass spectrometry detection (3).

The present work uses phenyl columns because the ring structures (Figure 1) of the two steroids should interact well with the stationary phase of the phenyl column. The work describes the determination of the content uniformity of 10 tablets, each containing 1-mg EDDA and 50- μ g EED.

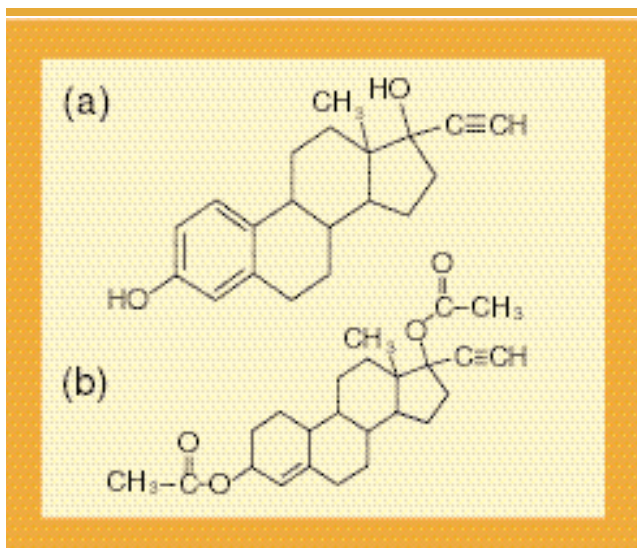


Figure 1: (a) Ethinyl estradiol. (b) Ethynodiol diacetate.

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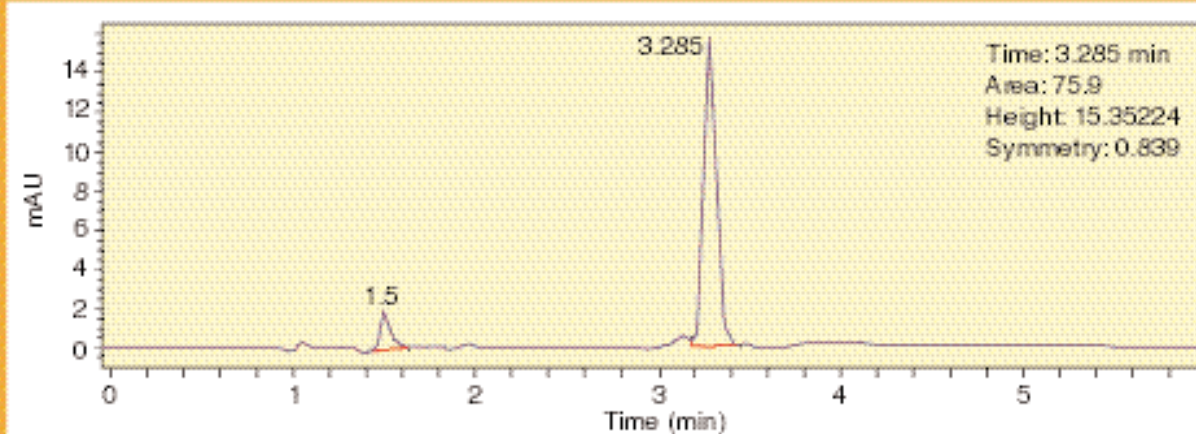


Figure 2: LC chromatogram of 2.5 µg/mL of ethinyl estradiol standard.

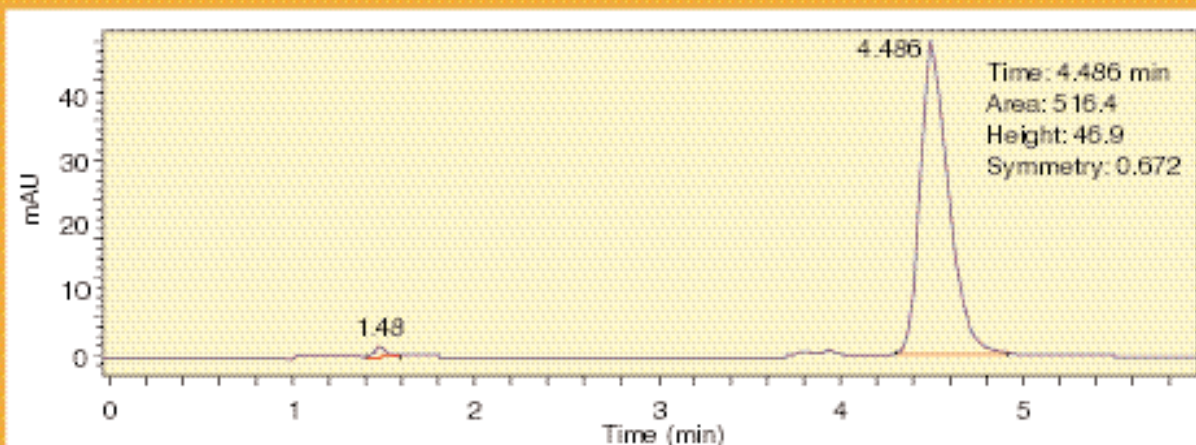


Figure 3: LC chromatogram of 50 µg/mL of ethynodiol diacetate standard.

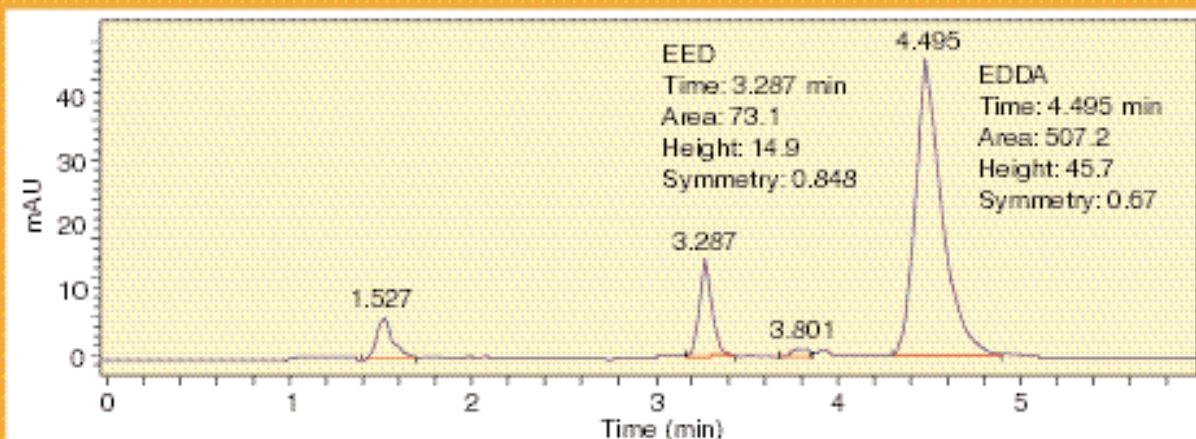


Figure 4: LC chromatogram of extract from tablet 8, showing ethinyl estradiol and ethynodiol diacetate.

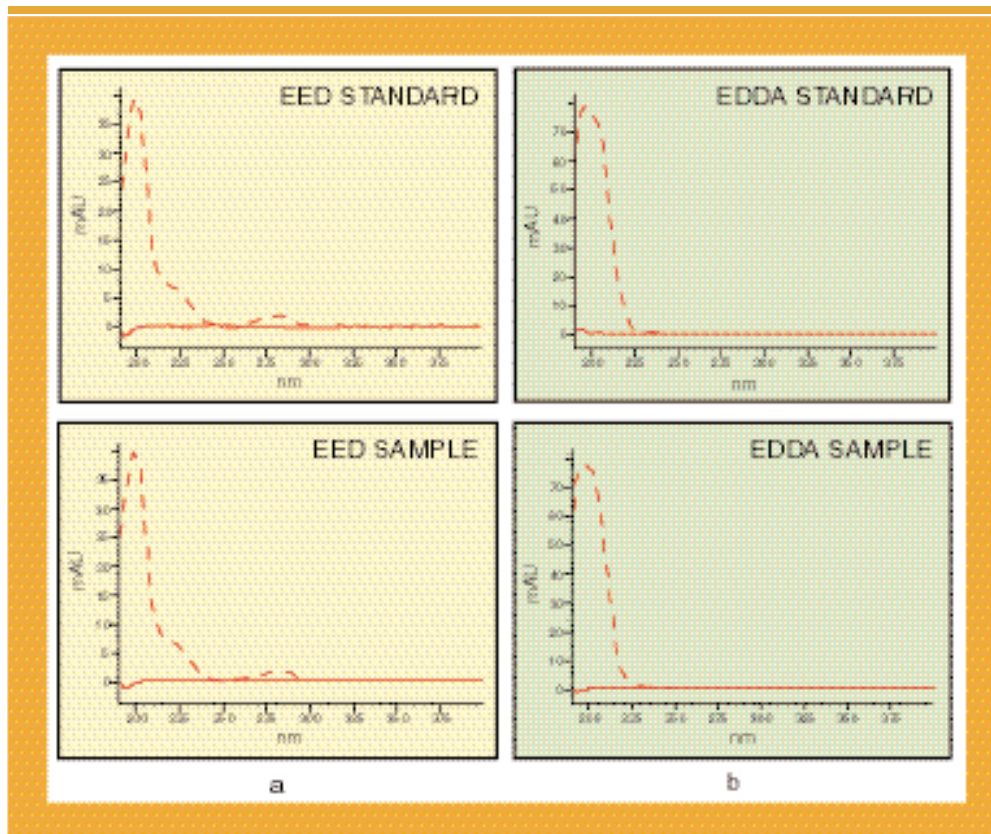


Figure 5: (a) UV spectra taken during the LC chromatograms at 3.3 min for ethinyl estradiol standard (2.5 $\mu\text{g}/\text{mL}$) and a sample; (b) UV spectra taken during the LC chromatograms at 4.5 min for ethynodiol diacetate standard (50 $\mu\text{g}/\text{mL}$) and a sample.

Methods

Materials. Reagent and chemicals.

- acetonitrile, methanol, and MilliQ water (Millipore Corp., Billerica, MA) (high-purity or high-performance liquid chromatography [HPLC] grade)
- mobile phase: 13% methanol, 30% acetonitrile, and 57% water.
- USP Reference Standards, EED (Lot P), and EDDA (Lot 10A033)
- commercial tablets in dosage form, EDDA (1 mg), and EED (50 μg).

Apparatus.

- HPLC system: HP model 1100 system equipped with a vacuum degasser, quaternary pump, auto sampler, temperature-controlled column chamber, diode array detector, LC 3D chemstation, Kayak XA computer, laser jet printer, and a 5- μm , 250 \times 4.60-mm Phenomenex Ultramex 5 phenyl column
- solvent filtration system: vacuum suction and 47-mm diameter, 0.45- μm nylon membrane filters
- sonicator
- 10-mL disposable syringes with 0.45- μm nylon syringe filters
- volumetric glassware: Class A volumetric pipettes and Class A volumetric flasks.

Sample preparation. Ten separate samples were prepared by dissolving each tablet in 20-mL mobile phase with a 10-min sonication at room temperature. The 10 solutions were filtered

with 0.45- μm nylon syringe filters and \sim 2 mL of the initial filtrate were discarded before sample collection.

Standard solutions. EDDA. All calibration standards were prepared using the mobile phase. The 60- and 50- $\mu\text{g}/\text{mL}$ standards were prepared by dissolving 6- and 5-mg EDDA, respectively, in 100-mL mobile phase. The 40- and 25- $\mu\text{g}/\text{mL}$ standards were prepared by diluting the 50- $\mu\text{g}/\text{mL}$ standard.

EED. A stock 5- $\mu\text{g}/\text{mL}$ standard was prepared by dissolving 5-mg EED in 1-L mobile phase using a volumetric flask. The 1.5-, 2.5-, and 4- $\mu\text{g}/\text{mL}$ standards were prepared by diluting the stock standard. The LC conditions were: λ was 210 nm, 1-mL/min flow rate, 10- μL injection, and 20 $^{\circ}\text{C}$ column temperature.

Results and discussion

The two steroid hormone ingredients of oral contraceptive tablets—EDDA and EED—were separated and quantified.

Among the available phenyl columns, only the two Phenomenex phenyl columns with more than 9000 theoretical plates yielded good results. The phenyl groups in the stationary phase interacted favorably with the ring structures of EED and EDDA yielding retention times of 3.3 and 4.5 min, respectively, with a resolution of 2.9. The tailing factor for EED was 1, and the EDDA peak tailed slightly. The total run time was 5 min.

To satisfy the system suitability requirements, both the 2.5- $\mu\text{g}/\text{mL}$ EED and the 50- $\mu\text{g}/\text{mL}$ EDDA standards were tested six times, and the percent relative standard deviation (RSD) using the peak areas were calculated. The percentages RSD for EED was 0.2% and 0.1% for EDDA. The USP requirement for the percent RSD is 2.0% for five trials. Typical chromatograms for EED and EDDA are shown in Figures 2 and 3.

The linearity of EED and EDDA were established from four separate standards for both EED and EDDA. The EED standards ranged from 1.5 to 5 $\mu\text{g}/\text{mL}$, and the EDDA standards ranged from 25 to 60 $\mu\text{g}/\text{mL}$. These concentration ranges bracketed the sample concentration. The plots of peak areas versus concentration were linear; the correlation coefficient (r) for EED was 0.9997 and 0.9995 for EDDA.

Tablet 8 was selected randomly from the 10 sample solutions. A 1-mL portion of the extract from tablet 8 was spiked with 1 mL of the 2.5- $\mu\text{g}/\text{mL}$ EED standard. A second 1-mL portion

was spiked with 1 mL of the 50- $\mu\text{g}/\text{mL}$ EDDA standard. The recoveries were 99.7% for EED and 100.3% for EDDA which are normal values for tablet dosage forms because the matrix effects are minimal.

Figure 4 represents tablet 8 of the 10 chromatograms obtained from the determination of content uniformity of the commercial dosage form. Because the 10 tablets were dissolved separately in 20-mL

mobile phase, the sample concentration of EDDA was 50 $\mu\text{g}/\text{mL}$ and that of EED was 2.5 $\mu\text{g}/\text{mL}$. For content uniformity, the range was 90.1–96.8% for EED and 92.1–98.5% for EDDA. Percent RSD for EED and EDDA was 3.2 % and 2.7%, respectively, which is lower than the USP requirement of 6% for 10 tablets.

The UV spectra between 200–375 nm were taken at 3.3 and 4.5 min for a tablet

dissolved in 20-mL mobile phase and standards. The spectra were quite identical for both the sample and the standards (Figure 5).

Conclusion

The phenyl column coupled with the mobile phase yielded a good, rapid separation that enabled the determination of both ethynodiol diacetate and ethinyl estradiol in the same sample. The method generated excellent analytical results for several commercial samples of the finished dosage forms.

Acknowledgements

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