Waters
Lab Highlights
An Internal Communication of Applications and Techniques
LAH 0091 2/83
Doc # M1027
AN/PA,/MD,OC/HR/SS

THF AS THE ORGANIC MODIFIER IN REVERSED-PHASE SEPARATION OF STEROIDS

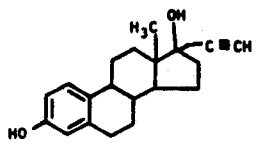
Reversed-phase separations of simple neutral compounds are usually performed with methanol or acetonitrile as the organic component of the mobile phase. Occasionally, with a particular stationary phase, methanol or acetonitrile will fail to provide sufficient selectivity for a pair of solutes in a sample. The frequent response is, "I need a different column which will give me the resolution I need." Actually, a different organic modifier may provide the necessary selectivity. Tetrahydrofuran, which is used infrequently for reversed-phase in pharmaceutical applications, may offer the needed selectivity without changing the column.

Contraceptive steroids are closely related compounds. Three different materials are used, either alone or in combinations of two, as oral contraceptives (Structures in Figure I). A single assay for all three compounds in less than ten minutes was required. The separations of ethinyl estradiol and norethindrone with methanol (Chromatogram 1) and acetonitrile (Chromatogram 2) are poor. Insufficient selectivity was obtained with either mobile phase on μBONDAPAK™ C_{18}. When the percentage of organic modifier was decreased to increase the resolution of this pair, mestranol eluted too late to meet the ten minute time limit. Changing to THF and decreasing the percentage of organic modifier from 70% to 50% to maintain retention (THF is a stronger solvent) provided the rapid separation shown in Chromatogram 3. Notice that the elution order of norethindrone and ethinyl estradiol is reversed in THF relative to both methanol and acetonitrile.

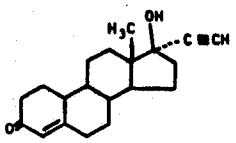
FIGURE I

COMPONENTS OF FORMULATIONS

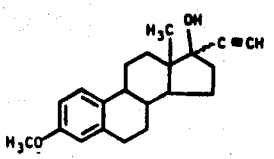
ETHINYL ESTRADIOL
MW = 296.39



NORETHINDRONE
MW = 298.41



MESTRANOL-
MW = 310.42



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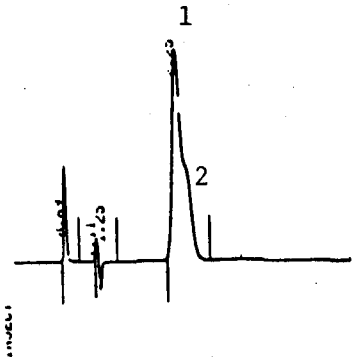
SEPARATION OF ETHINYL ESTRADIOL AND NORETHINDRONE

1)

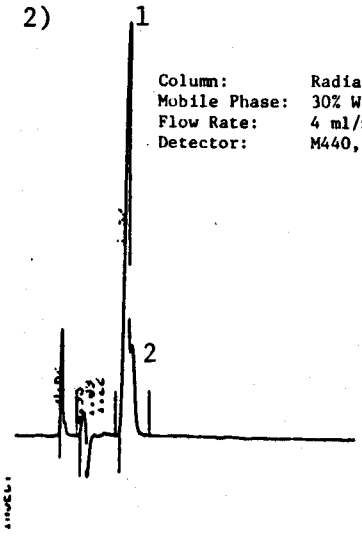
Column: Radial-PAK™ μBONDAPAK™ C_{18}
Mobile Phase: 30% Water/70% Methanol
Flow Rate: 4 ml/min
Detector: M440, 280 nm, 0.1 AUFS



SEPARATION OF ETHINYL ESTRADIOL AND NORETHINDRONE

2)

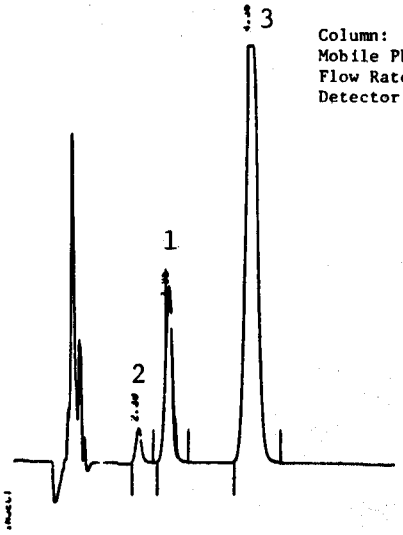
Column: Radial-PAK™ μBONDAPAK™ C_{18}
Mobile Phase: 30% Water/70% Acetonitrile
Flow Rate: 4 ml/min
Detector: M440, 280 nm, 0.1 AUFS



OPTIMUM SEPARATION OF NORETHINDRONE, ETHINYL ESTRADIOL AND MESTRANOL

3)

Column: Radial-PAK™ μBONDAPAK™ C_{18}
Mobile Phase: 50% Water/50% Tetrahydrofuran
Flow Rate: 4 ml/min
Detector M440, 280 nm, 0.1 AUFS



1. Ethinyl Estradiol
2. Norethindrone
3. Mestranol

Hal Richardson