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Rapid Profiling of Human Sex Hormones

Rapid separation of human sex hormones in Biological Samples using the JASCO X-LC system

Introduction:

Hypertension is a serious problem among African American population, especially women. According to the American Heart Association (AHA), around 42.6% of African American men above the age 20 and 46.6% of African American women have hypertension[1]. According to another study conducted by AHA, hypertension was the cause of death of 5,762 African American men and 6,664 African American women in 2004.[1]Men and postmenopausal women are known to have greater incidences of hypertension compared to premenopausal women. This suggests that the female sex hormones have an effect on blood pressure[2]. Estrone (E1), estradiol (E2), estriol (E3) and progesterone are the main female sex hormones in the body. Out of all these, estrone is assumed to be the only one present in variable quantities in postmenopausal women. 2-hydroxyesterone (2-OHE1), 16_-hydroxyestrone (16_ -OHE1) and 4- steroid hormone from the androgen group which is present at much higher concentration in men than in women.
Determining the link between sex hormones with the pathogenesis of hypertension is complex.

This application note describes a 6.1 min method for identifying a mixture of 8 sex hormones using Jasco X-LC. Separation of these compounds using conventional HPLC typically takes around 16 minutes. hydroxyestrone (4-OHE1) are the major metabolites of estrone. The ratio of 2-hydroxyesterone (2-OHE1) to 16_-hydroxyestrone (16_ -OHE1) is considered to be an index of breast cancer risk [3]. HPLC typically takes around 16 minutes. hydroxyestrone (4-OHE1) are the major metabolites of estrone. The ratio of 2-hydroxyesterone (2-OHE1) to 16_-hydroxyestrone (16_ -OHE1) is considered to be an index of breast cancer risk [3]. Testosterone is a steroid hormone from the androgen group which is present at much higher concentration in men than in women.

Figure 1: Conversion of Progesterone to Estrogenic Compounds

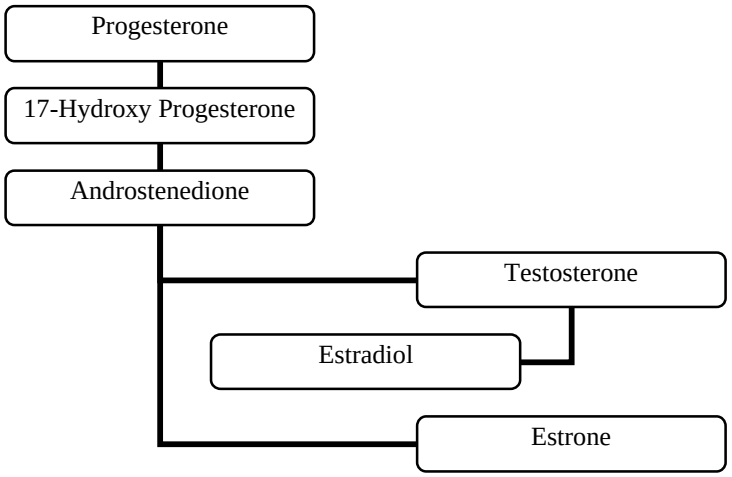
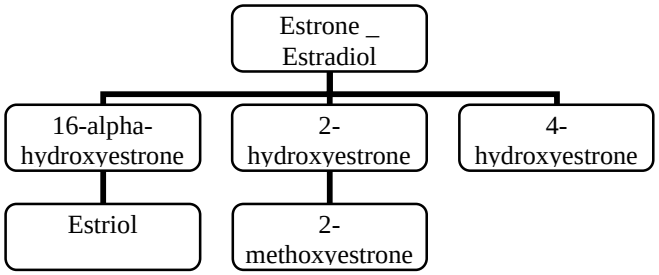


Figure 2: Metabolism of Estrogenic Compounds



Experimental:

Sample Preparation:
All the hormones were dissolved in 1 ml methanol such that the stock mass concentration for all was 1mg/ml.Working standards for the analytes were prepared by serial dilution using ACN. 2-methoxy Estrone, 4-hydroxy estrone were obtained from Sigma Aldrich, 2-hydroxy estrone, 16-α-hydroxyestrone were obtained from Steraloids, estrone, E1; estradiol,E2; estriol, E3; were obtained from Fluka.

XLC system and operating conditions:
System: Jasco X-LC
Mobile Phase A: Water
Mobile Phase B: ACN
Flow Rate: 0.165 ml/min

Gradient:

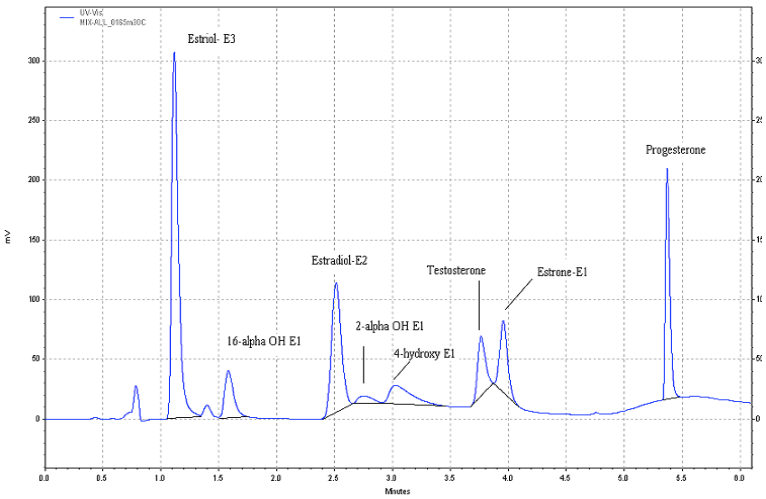
Time (min)	*%B*
0.0	*40*
2.0	*40*
3.8	*60*
4.5	*80*
6.0	*80*
6.10	*40*

Stationary Phase:	Restek Pinnacle DB-Biphenyl 1.9 _m (50 x 2.1 mm)
Column Temperature:	30°C
UV Detection:	220 nm
Injection Volume:	1_l

Results and Discussion:

Figure 2 is an X-LC chromatogram of a standard mixture of hormones (50 ng each). The compounds are separated by a gradient elution of water and acetonitrile on a Restek Pinnacle DB Biphenyl 1.9_m (50 x 2.1 mm) at a flow rate of 0.165 ml/min. Wavelength 220 nm was used for the detection of these compounds. Resolution of testosterone from estrone, is 1.25 in this method, acceptable for quantitative determinations of both species.

Figure 2. X-LC chromatogram of a standard mixture of hormones.(critical pair resolution, 1.25)



References:

1. American Heart Association- Statistical Fact S Sheet- Populations 2008 update.

2 2. Khalil AR, Hypertension, 2005, 46, 249-254.

3 3. Ursin G et al, Environ Health Perspect, 1997, 105, 6 601-605.

4

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