

## **Direct analysis of estrogen hormone in plasma using online SPE coupled with HPLC with between column derivatization**

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Estrogens are a group of steroid compounds, named for their importance in the estrous cycle, and functioning as the primary female sex hormones. The three major naturally occurring estrogens in women are estrone (E1), estradiol (E2), and estriol (E3). Their analysis plays an important role in disease diagnosis.

The analysis of estrogens are performed using HPLC or LC –MS. Since the concentration of the hormones can be very low and the sensitivity with UV or MSD detection is not satisfactory, derivatization is normally used to enhance the sensitivity. Derivatization can also differentiate these hydrophobic hormones from lipids of similar polarity.

In manual derivatization, variation in reagent amount and heating condition can affect the reproducibility of the analysis. Besides, most derivatizations involve adding derivatizing reagents (mainly dansyl chloride and basic buffer) to plasma before SPE cleanup. The sample matrices can cause more variation to the analytical results.

This note demonstrate direct analysis of estrone in plasma using online SPE with between column derivatization. The SPE-04 three-in-one online SPE is used to perform all the necessary sample preparation and introduction of sample to an HPLC.

### **Materials and methods**

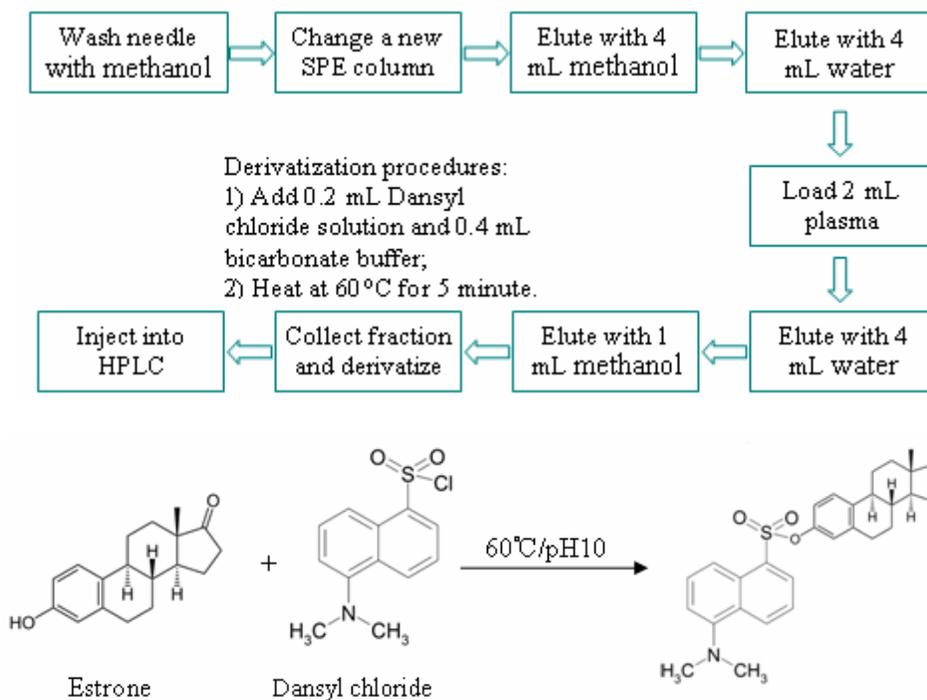
#### **Instruments**

The instruments consist of a SPE-04 three-in-one online SPE and an Agilent 1100 HPLC with a quaternary pump and a variable wavelength detector. The SPE and HPLC are synchronized using a remote cable.



## Methods

The following diagrams describe the automatic working procedures of the online SPE and the relevant derivatization reactions:



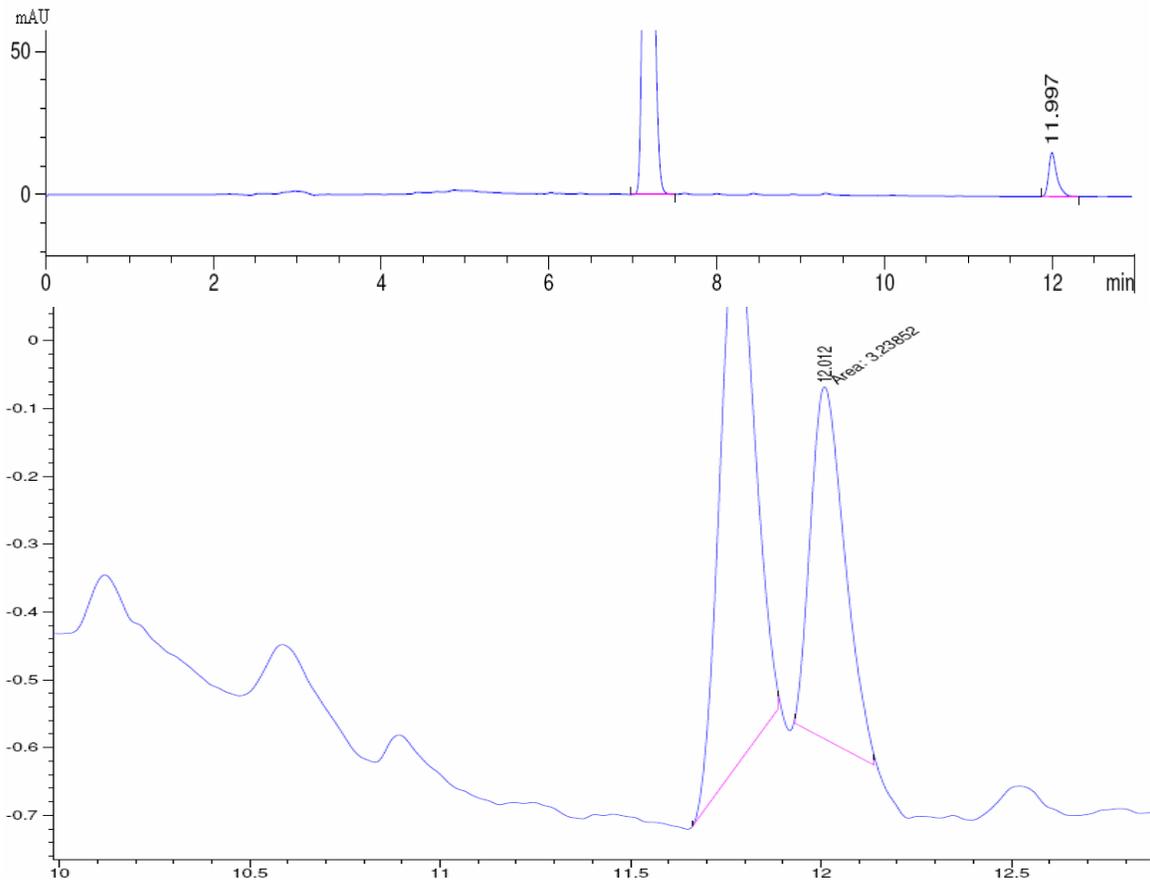
The SPE column is PromSil C18, 500-mg/3-mL from PromoChrom. Since the plunger for SPE column can seal the column well, normal SPE columns from most suppliers can be used. There is no need for a special cap or adapter.

The HPLC analysis uses a PCTsil-C18 column (4.6x250 mm) for separation. The flowrate is 1.2 mL/min. The elution start with 30% acetonitrile+ 0.1%TFA and increases to 90% acetonitrile over a period of 8 minutes. The acetonitril concentration is reduced to 30% after holding at 90% for 4 minutes. Detection wavelength is 360 nm. A 20- $\mu$ L derivatized sample is injected for HPLC analysis. The retention time of derivatized estrone is 12.0 minutes.

## Results

### 1. Sensitivity

Plasma sample is spiked with estrone stanard at 2 and 20 ppm level and diluted with water at 1:1 ratio. The diluted sample is analysed using the approaches described in the above diagram.



Upper figure: plasma sample spiked with 20 ppm estrone

Lower figure: plasma sample spiked with 2 ppm estrone

The interference from plasma sample is effectively removed using C18 SPE column. The derivatization allows use of a long detection wavelength, which also significantly reduce the background noise. The detection limit is below 0.5 ppm. More improvement in sensitivity can be achieved if a fluorescence detector or LC-MS is used.

## 2. Repeatability

Water spiked with estrone at 20 ppm level was analysed to estimate the reproducibility of the instrument. Below is the result:

Repeat	Peak Area(s.mAU)
1	131.6
2	135.3
3	138.0
4	133.9
Average	134.7
CV%	2.0

In spite of many cleanup steps and derivatization reactions, good repeatability is achieved. This is because the online SPE can well control each elution step and the derivatization conditions and considerably reduce chance of error.

### More informatin on SPE-04 three-in-one online SPE

**SPE-04 3-in-1** model is a flexible and versatile platform for automatic sample preparation. It can perform multiple tasks: offline SPE, online SPE, normal sample injection, and online derivatization with controlled temperature.

The control software for online SPE is user friendly and is compatible with most HPLC software.

The software uses methods and sequences for the automation. It has similar structure as Agilent Chemstation. Users of HPLC can easily understand the SPE-04 software.

The software can perform overlapped injection. When HPLC is performing an HPLC run, SPE-04 can start processing the next sample.

In online mode, SPE-04 can perform derivatization under controlled temperature. This function is very useful for analysis of amino acids, hormones, and some pesticides.

