A Sensitive LC-MS/MS method for the Quantification of Ethinyl Estradiol and Drospirenone in Human Plasma

INTRODUCTION
Estrogens and progestogens play an important role in fertility and sexual development, as well as in cancer risk. In modern world estrogen and progestosterone combination based drugs are widely used as most effective contraceptive agents. One of such combination is Ethinyl estradiol (EE), Figure 2a and Drospirenone. The mean bioavailability of EE is reported to be 45%. EE is known to undergo extensive metabolism and also it highly protein bound makes its bio availability very low in human system. Drospirenone (Figure 2b) is a novel synthetic progestogen with a pharmacological profile similar to its natural progestogen. It will be metabolized completely but the metabolites may occur any biological activities and undergo hepatic and renal elimination. Drospirenone and ethinyl estradiol combination in formulation have similar efficiency and safety profile to other low dose oral contraceptive. It has also been reported that it has less side effect with regards to weight gain, mood change etc. Highly sensitive and accurate low level quantification becomes essential for the bioequivalence studies for such molecules. The main objective of this work is to develop and validate a high sensitive and reproducible method for EE (1 pg/ml) in human plasma using AB SCIEX Triple Quad™ 5500 LC/MS/MS system (Fig. 1).

Key Features of the Method using AB SCIEX Triple Quad™ 5500 System
A sensitive, reproducible and cost effective LC/MS/MS method was developed for a GLP bioanalytical laboratory using simple liquid liquid extraction sample preparation method

The LLOQ for Ethinyl Estradiol in plasma was 0.993 pg/ml (10 ng on column) 3N ratio of 68 with good precision and accuracy for n = 12 in. human plasma

Accuracy and precision parameters for analyte EE, Drospirenone were not shown. Organic phase were collected and evaporated to dryness under nitrogen stream. The residue in each tube were dissolved in the 200μL of Ammonium bicarbonate (pH 11) followed by 3mL vortex. 

Sample Preparation: Plasma (50μl) samples were spiked (2%) with Ethinyl Estradiol (EE) and Drospirenone standard with 10μl, internal standard solution. The samples were then extracted in the BEB2- Hexane mixture and centrifuged at 12,000g. Organic phase were collected and evaporated to dryness under nitrogen stream. The residue in each tube were dissolved in the 200μL of Ammonium bicarbonate (pH 11) followed by 3mL vortex. 

RESULTS
Ethinyl estradiol was derivatized with dansyl chloride to obtain the maximum sensitivity in plasma sample (Fig 3). Darnyl - EE produced the Q1MS ion m/z 532.0 and major product ion 171.1 which correspond to (5-dimethylamino) naphthalene moiety. The best sensitivity was achieved in ESI positive mode. The mass spectrometric parameters for both the compounds are given in Table 1. A linear calibration curve was constructed using the 1/x2 regression. The calibration curve for EE was linear in a dynamic range of 0.993-300.48 pg/ml in plasma sample (Fig 8) with r value 0.9980 and similarly calibration curve for drospirenone shown in figure 9 has r value 0.9944

MATHEMATICAL AND MATERIALS

Sample Preparation: Plasma (50μL) samples were spiked (2%) with Ethinyl Estradiol (EE) and Drospirenone standard with 10μl, internal standard solution. The samples were then extracted in the BEB2- Hexane mixture and centrifuged at 12,000g. Organic phase were collected and evaporated to dryness under nitrogen stream. The residue in each tube were dissolved in the 200μL of Ammonium bicarbonate (pH 11) followed by 3mL vortex. Darnyl estradiol (0.5 mg/ml, solution in acetone) was added to derivatized the Ethinyl Estradiol. Reaction mixture tubes were kept for 10 min at 60°C in water bath. LLE was again performed with TMS - Hexane (2mL) followed by vortex and centrifugation. Organic phase were collected and evaporated and finally reconstituted in acetonitrile and water (200μL) for quantification in AB SCIEX Triple Quad™ 5500 LC/MS/MS system.

NPLC Conditions: A Shimadzu Nexera with 30AC auto sampler system was used with a C18 (50 x 2.1 mm, 5μm) analytical column maintained at 38°C. A gradient elution was employed, consisting of 5% Ammonium Formate buffer mobile phase A and acetonitrile/methanol (mobile phase B) at a flow rate of 300μL.min. The total run-time for the method was 8.00 minutes. The injection volume was set to 10μL. The rinsing solution was Methanol: Water (50:50) mixture.

REFERENCES
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