

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



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## INTRODUCTION

Estrogens and progesterone play an important role in fertility and sexual development, as well as in cancer risk. In modern world estrogen and progesterone 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 can be 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 progesterone. It will be metabolized completely but the metabolites are not shown 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 been also reported that it has less side effect with regards to weight gain, mood change etc. Highly sensitive and accurate low level quantitation becomes essential for the bioequivalence studies for such molecules. The main objective of this work is to develop and validate a highly sensitive and reproducible method for EE (1.0 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

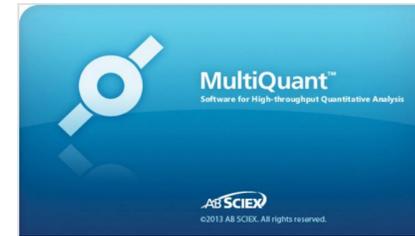
**The LLOQ for Ethinyl Estradiol in plasma was 0.993 pg/ml (10 fg on column)** S/N ratio of 68 with good precision and accuracy for n = 12 inj. in human plasma **Accuracy and precision** for Ethinyl Estradiol and Drospirenone are between 80-120% meeting the validation requirements for regulated bio analytical labs

**New, patented QJet® 2 Ion Guide improves sensitivity and robustness:** The patented QJet® 2 Ion Guide design yields improved ion containment and operates at higher pressures providing better collisional focusing to enhance ion transmission for ultimate sensitivity

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AB SCIEX Triple Quad™ 5500 system



MultiQuant™ Software for streamlining data processing and increasing MS quantitation productivity

## MATERIALS AND METHODS

**Sample Preparation:** Plasma (500µL) samples were spiked (2%) with Ethinyl Estradiol (EE) and Drospirenone standard with 10µL internal standard solution. The samples were vortex with 2ml of TBME: n-Hexane mixture and centrifuged at 4°C. Organic phase were collected and evaporated to dryness under nitrogen stream. The residue in each tube were dissolved in the 200ul of Ammonium bicarbonate (pH:11) followed by 2min vortex. Dansyl chloride (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 TBME : n-Hexane (2mL) followed by vortex and centrifugation. Organic phase were collected and evaporated and finally reconstituted in acetonitrile and water (200µL) for quantitation in AB SCIEX Triple Quad™ 5500 LC/MS/MS system

**HPLC Conditions:** A Shimadzu Nexera with 30AC auto sampler system was used, with a C18 (50 x 2.1 mm, 5µm) analytical column maintained at 30°C. A gradient elution was employed, consisting of 5mM 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 8.0 minutes. The injection volume was set to 10µL. The rinsing solution was Methanol: Water (50/50) mixture.

## MS parameters:

Analyte	MRM	Dwell Time (ms)	DP	EP	CE	CXP
Dansyl-Ethinyl Estradiol	530.2/171.1	200	100	15	49	16
D4 - Dansyl-Ethinyl Estradiol	534.2/171.0	200	100	15	49	16
Drospirenone	367.1/97.00	200	100	121	29	14
D4-Drospirenone	371.1/97.00	200	100	121	29	14

Table 1: MRM transitions and optimised MS parameter for compounds

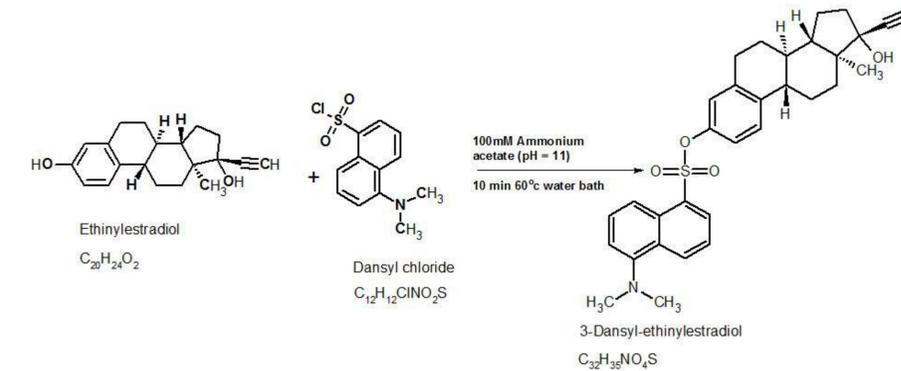


Figure 3: Chemical derivatization of Ethinyl estradiol using dansyl chloride

## RESULTS

Ethinyl estradiol was derivatised with dansyl chloride to obtain the maximum sensitivity in plasma sample (Fig 3). Dansyl - EE produced the Q1MS ion m/z 530.2 and major product ion 171.1 which correspond to 5-(dimethylamino)-naphthalene moiety. The best ionization 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/X<sup>2</sup> regression. The calibration curve for EE was linear over a dynamic range of 0.993-300.48 pg/mL in plasma sample (Fig 8) with an r value 0.9980 and similarly, calibration curve for drospirenone shown in figure 9 has r value 0.9994

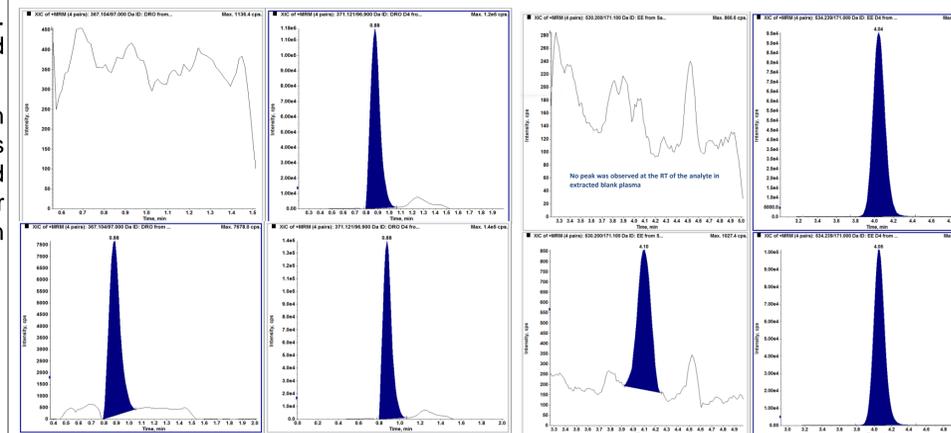


Figure 4: The chromatograms for drospirenone in zero blank plasma sample (upper pane) and 1003.63 pg/mL spiked in plasma sample (lower pane) and 0.993 pg/mL spiked in plasma sample (lower pane)

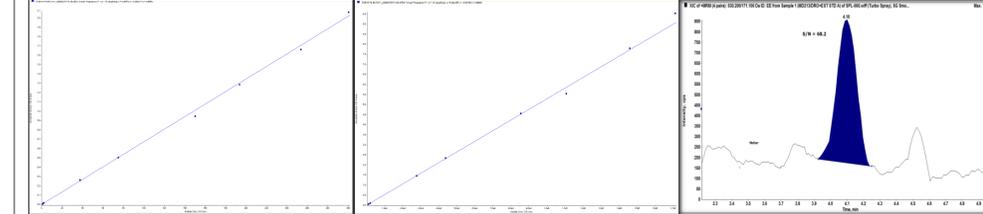


Figure 8: Calibration curve of Dansyl-Ethinyl Estradiol in plasma from 0.993 pg/ml to 300.485 pg/ml. The method has shown excellent linearity over the concentration range with r = 0.9980. Figure 8: Calibration curve of drospirenone in plasma from 1003.63 pg/ml to 171152.24 pg/ml. The method has shown excellent linearity over the concentration range with r = 0.9994. Figure 7: Signal to noise calculation for Dansyl-Ethinyl Estradiol in extracted plasma sample at LLOQ level (0.993 pg/mL) is 68.3

Analyte	Sample Name	Exp. Con (pg/ml)	Cal. Con (pg/ml)	Number of values used	%CV	Accuracy
Ethinyl Estradiol	LLOQ	0.9980	1.0406	12	4.5702	104.27
	LQC -1	2.6740	2.7786	12	1.6824	103.91
	MQC -1	150.2430	149.2491	12	1.0126	99.33
	HQC -1	246.3980	242.2303	12	2.2463	98.30
Drospirenone	LLOQ	1004.0070	1052.0814	12	2.0557	104.78
	LQC -1	2706.2190	2885.9109	12	0.8237	106.63
	MQC -1	84569.3450	84438.5864	12	1.0554	99.84
	HQC -1	140948.9090	141724.8942	12	2.464	100.55

Table 3. Statistic calculation of quality controls (n=12 inj) in human plasma sample for Dansyl-Ethinyl Estradiol and Drospirenone

## CONCLUSIONS

- A highly sensitive method for Ethinyl Estradiol and Drospirenone was developed and validated using AB SCIEX Triple Quad™ 5500 LC/MS/MS system in human plasma. The developed method is sensitive, reproducible and cost effective for bioanalytical laboratory with good precision and accuracy

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