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## Determination of Estradiol in Plasma with Negative Chemical Ionization GC-MS/MS on TSQ Quantum GC™

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Estradiol is an endogenous hormone that has been implicated in many physiological effects for both genders. The detection of estradiol in biological fluids is of clinical importance to disease diagnosis and progression. While both LC-MS/MS and GC-MS methods have been widely used for the detection of estradiol in biological fluids, the GC-MS method has the advantage of being more sensitive, capable of detecting femtogram (fg) levels on column as compared to low pictogram (pg) levels using LC-MS/MS (1).

In this note, a triple quadrupole GC-MS/MS method using the TSQ Quantum GC is reported to detect estradiol from plasma samples in Negative Chemical Ionization (NCI) mode. The estradiol was extracted from plasma and derivatized with pentafluorobenzoyl and MSTFA. The ability to detect 55 fg on column (equivalent to 2.5 pg/mL in plasma) has been demonstrated.

### Experimental Conditions

The biological matrix used was depleted plasma (SFBC-Taylor Technology, Princeton, NJ, USA), and the samples were prepared by spiking various concentrations of the estradiol standard into 1.0 mL of blank plasma, using d4-estradiol as the internal standard. Sample preparation involved solid phase extraction clean-up, followed by derivatization with pentafluorobenzoyl chloride (for the aromatic hydroxyl group) and MSTFA (for the alkyl hydroxyl group). The final reconstituted sample volume was 45  $\mu$ L.

GC: TRACE GC Ultra™ and TriPlus™ Autosampler (Thermo Fisher Scientific)

Column: DB-17MS (J & W Scientific), 15 m  $\times$  0.25 mm i.d.  $d_f$  = 0.25  $\mu$ m

Injection: 1  $\mu$ L at 280 °C with Splitless mode (closed for 1 min) with transferline temp set to 280 °C

Oven: 210 °C (1 min), 40 °C/min to 290 °C (0 min), 4 °C/min to 305 °C (0 min)

Carrier: He, constant flow at 1.2 mL/min

Mass Spectrometer: TSQ Quantum GC (Thermo Fisher Scientific)

Ion Source Temp: 240 °C

Emission Current: 200  $\mu$ A

Ionization Mode: Negative CI (methane as reagent gas at 3.8 mL/min)

Scan Mode: SRM (Selected Reaction Monitoring)

Estradiol:  $m/z$  538 > 474; d4-Estradiol:  $m/z$  542 > 478

Scan Time: 0.15 s

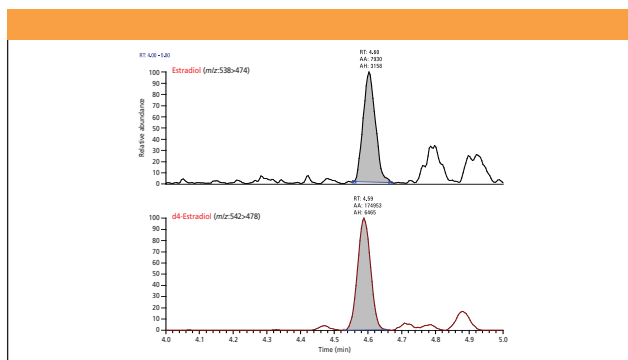
Peak Width: 0.7 Da (FWHM) for Q1 and Q3

Collision Gas: 1.5 mTorr (Ar)

Collision Energy: 13 V

### Results and Discussion

Figure 1 shows the representative chromatograms of a plasma



**Figure 1:** Representative SRM chromatograms for a plasma sample containing 2.5 pg/mL Estradiol (equivalent to 55 fg estradiol injected on column).

sample spiked with 2.5 pg/mL estradiol. With splitless injection of 1  $\mu$ L of the final extract and assuming 100% recovery, the total amount injected on column (55 fg) could be accurately quantified. Note that other peaks in the chromatograms are likely the isomers known to estradiol and its d4 analogue.

The assay also used three levels of quality control (QC) samples at 7.5 (Low QC), 50 (Mid QC) and 200 pg/mL (High QC). The recovery values were all within the range of 85 to 115%, indicating good method accuracy.

### Conclusions

A highly sensitive and accurate NCI GC-MS/MS method has been developed to assay estradiol in plasma from 2.5 to 250 pg/mL on the TSQ Quantum GC. Ongoing work is being conducted to further extend the assay method to include other estrogens and androgens in one single GC-MS/MS analysis.

### Acknowledgement

We thank Robert J. White of SFBC-Taylor Technology, Inc. for providing the extracted estradiol samples and many helpful suggestions.

### Reference

- (1) B. Sundarram, J.A. Settlege, S.K. Ohorodnik, and P.A. Taylor, A Combined GC/MS/MS and LC/MS/MS Bioanalytical Method for the Quantitation of Estradiol, Estrone, Estrone Sulfate, Testosterone and Androstenedione 51st ASMS Conference on Mass Spectrometry and Allied Topics, Montreal, Quebec, Canada, June 2003.

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