Application Note: 388

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

Hans Schweingruber, BC Cha, Ethan Chan, Kefei Wang, Thermo Fisher Scientific, San Jose, CA, USA

Key Words

- TSQ Quantum GC™
- Estradiol
- GC-MS/MS
- Negative CI
- SRM

Introduction

Estradiol is an endogenous hormone that has been implicated in many physiological effects for both genders. The detection of estradiol in biological fluids, such as plasma and urine, has important clinical application in the diagnosis of diseases and in monitoring the progress of disease states. Both LC-MS/MS and GC-MS methods have been widely used for the detection of estradiol in biological fluids, but both methods are often compromised as a result of endogenous matrix interference. While the LC-MS/MS method, with the advantage of avoiding the derivatization, can detect low pictogram (pg) level on-column, the GC-MS method has the advantage of being more sensitive, capable of detecting femtogram (fg) levels on column.[1]

In this note, a triple quadrupole based 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

Sample Preparation

The estradiol extract samples from plasma were kindly supplied by Taylor Technology, Inc. (Princeton, NJ, USA). The extracts were prepared by spiking into 1.0 mL of blank plasma the estradiol standard at the various concentrations and the d4-estradiol as the internal standard. The estradiol in plasma was then extracted with solid phase extraction using the Bond Elute cartridges, 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 µL.

GC-MS/MS Conditions

GC: TRACE GC Ultra™ and Triplus™ Autosampler (Thermo Scientific)
Column: (50% Phenyl)-methylpolysiloxane phase, 15 m × 0.25 mm i.d. df = 0.25 µm
Injection: 1 µL at 280 °C with Splitless mode (closed for 1 min)
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
Transferline: 280 °C
Mass Spectrometer: TSQ Quantum GC (Thermo Scientific)
Ion Source Temp: 240 °C
Emission Current: 200 µA
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 Width: 0.002 *m/z*
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

The purpose of this study was to develop an NCI GC-MS/MS method to assay estradiol in plasma samples from 2.5 pg/mL to 250 pg/mL.

Figure 1 shows the representative chromatograms of a plasma sample spiked with 2.5 pg/mL estradiol. With splitless injection of 1 µL of the final extract and assuming a complete recovery, the total amount injected on column is 55 fg. As shown, even with such a low amount, estradiol shows a well-defined peak that can be accurately quantified. Note that other peaks in the chromatograms are likely the isomers known to estradiol and its d4 analogue.

Figure 2 shows a 5-level calibration curve for plasma samples spiked with 2.5, 5.0, 25, 75 and 250 pg/mL estradiol. Each level of standard was injected in triplicates. Excellent linearity with a correlation coefficient $R^2 = 0.9976$ (weighting factor = 1/X) was obtained.

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.

Thermo SCIENTIFIC

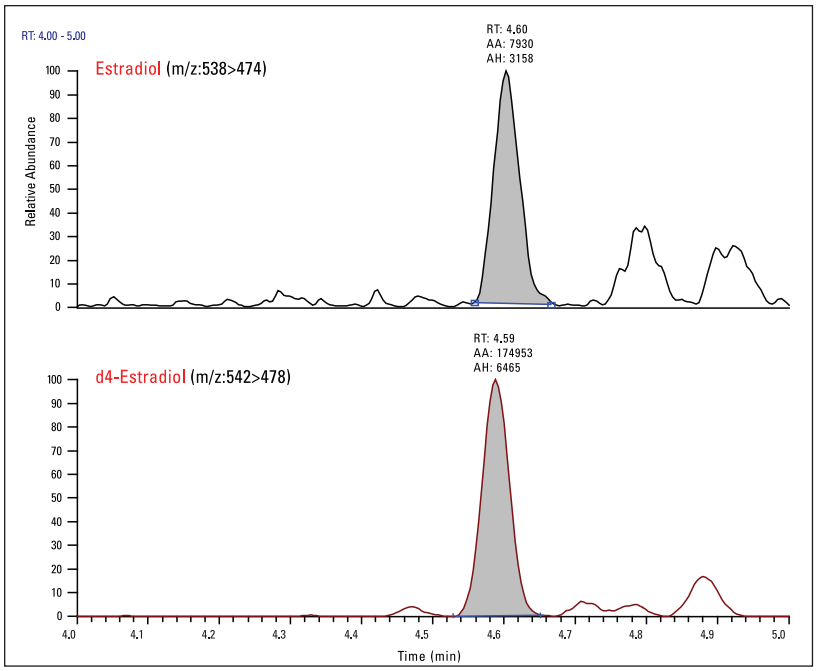

Figure 1: Representative SRM chromatograms for a plasma sample containing 2.5 pg/mL estradiol (equivalent to 55 fg estradiol injected on column)

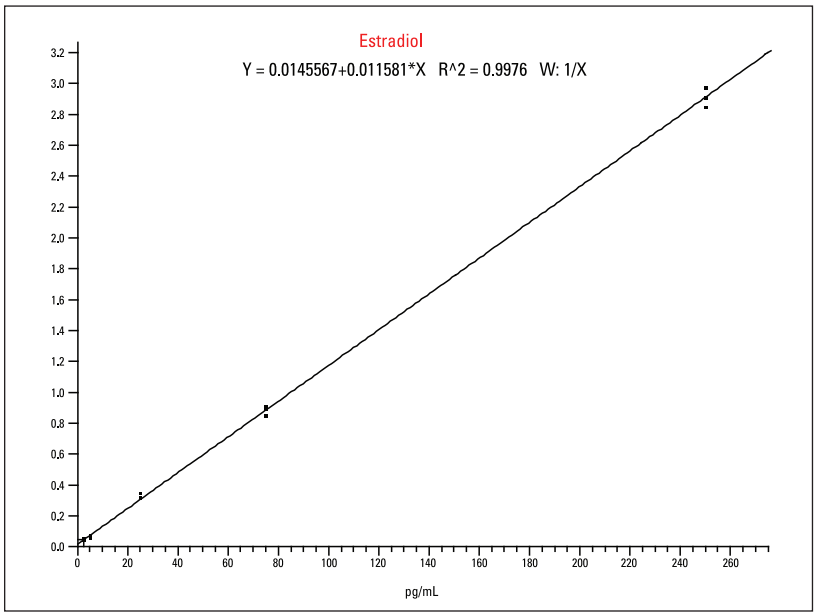

Figure 2: A 5-level calibration curve for estradiol in plasma from 2.5 pg/mL to 250 pg/mL (internal standard corrected)

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.

Acknowledgment

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

References

1. Bhaskar Sundarram, James A. Settlage, Susan K. Ohorodnik, Paul A. Taylor, *"A Combined GC/MS/MS and LC/MS/MS Bioanalytical Methods for the Quantitation of Estradiol, Estrone, Estrone Sulfate, Testosterone and Androstenedione"* 51st ASMS Conference on Mass Spectrometry and Applied Topics, Montreal, Quebec, Canada, June 2003.

In addition to these offices, Thermo Fisher Scientific maintains a network of representative organizations throughout the world.

Africa
+43 1 333 5034 127
Australia
+61 2 8844 9500
Austria
+43 1 333 50340
Belgium
+32 2 482 30 30
Canada
+1 800 530 8447
China
+86 10 5850 3588
Denmark
+45 70 23 62 60
Europe-Other
+43 1 333 5034 127
France
+33 1 60 92 48 00
Germany
+49 6103 408 1014
India
+91 22 6742 9434
Italy
+39 02 950 591
Japan
+81 45 453 9100
Latin America
+1 608 276 5659
Middle East
+43 1 333 5034 127
Netherlands
+31 76 587 98 88
South Africa
+27 11 570 1840
Spain
+34 914 845 965
Sweden/Norway/ Finland
+46 8 556 468 00
Switzerland
+41 61 48784 00
UK
+44 1442 233555
USA
+1 800 532 4752

www.thermo.com

Legal Notices
©2007 Thermo Fisher Scientific Inc. All rights reserved. All trademarks are the property of Thermo Fisher Scientific Inc. and its subsidiaries. This information is presented as an example of the capabilities of Thermo Fisher Scientific Inc. products. It is not intended to encourage use of these products in any manners that might infringe the intellectual property rights of others. Specifications, terms and pricing are subject to change. Not all products are available in all countries. Please consult your local sales representative for details.

***View additional Thermo Scientific LC/MS application notes at:* www.thermo.com/appnotes**

Thermo Fisher Scientific, San Jose, CA USA is ISO Certified.

AN62547_E 11/07S