

# Highly Repeatable Ultra Low Detection of Estradiol Using Triple Quadrupole GC/MS in NCI Mode

## Application Brief

Biomedical

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### Abstract

A method was developed on the Agilent 7890GC/7000 Triple Quadrupole GC/MS that provides an LOD of 1 pg/mL (2 fg on-column) and RSDs for quantification of <15% in the 1 to 600 pg/mL concentration range.

### Introduction

Estradiol, formed from developing ovarian follicles via the conversion of testosterone through the enzymatic action of aromatase, is the primary sex hormone of childbearing women. It is responsible for the development of female characteristics and sexual functioning. Although present in both genders, estrogen concentrations are significantly higher in females. While it is important to women's bone health, estradiol also contributes to most gynecologic problems such as endometriosis and fibroids, and even female cancers. The measurement of estradiol can be requested in a number of clinical scenarios, such as precocious puberty, infertility, assisted conception, and hormone replacement therapy.

This application brief describes a highly repeatable method for the quantification of estradiol, as it pertains to the analysis of human plasma, at concentrations as low as 1 pg/mL and as high as 600 pg/mL, using the Agilent 7000 Triple Quadrupole GC/MS.



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## Experimental

### Standards and Reagents

Ten derivatized estradiol calibration standard concentrations were prepared in dodecane from 1.0 to 600 pg/mL (Table 1). The deuterated estradiol (D3) internal standard (ISTD) concentration was 100 pg/mL in each sample. When estradiol is extracted from plasma, the theoretical concentration extracted from 1.0 mL of matrix at 100% recovery would provide approximately 20 fg of analyte, using a 2- $\mu$ L injection. Therefore, the 1.0 pg/mL standard in dodecane would represent 10% recovery (2 fg injected), the 2.5 pg/mL 25% recovery, and so forth.

The calibration standards were evaluated in a sequence of 82 consecutive injections. The injection sequence was set up such that six sets of levels EA 1-10 were run first, followed by four sets of levels EA 1-3. A blank was run after each set.

Table 1. Concentrations of the Estradiol Calibration Standards

Standard	Analyte 1 Estradiol (pg/mL)
EA-1	1.0
EA-2	2.5
EA-3	5.0
EA-4	10
EA-5	25
EA-6	50
EA-7	100
EA-8	150
EA-9	300
EA-10	600

### Instrument

Analysis was performed on an Agilent 7890GC/7000 Triple Quadrupole GC/MS system. The 7890 Series GC was configured with a Multimode Inlet (MMI) and equipped with a 15 m  $\times$  0.25 mm  $\times$  0.25  $\mu$ m film thickness DB-5MSUI analytical column. The 7000 Triple Quadrupole GC/MS was operated in the MS/MS-NCI (negative chemical ionization) mode. The instrument conditions are listed in Table 2. An Agilent 7693 automatic liquid sampler (ALS) was used, and injections were made with a 10- $\mu$ L syringe.

Table 2. Gas Chromatograph and Mass Spectrometer Conditions

GC Run Conditions	
Analytical column	15 m $\times$ 0.25 mm $\times$ 0.25 $\mu$ m Agilent J&W DB-5MS UI column (p/n 190915-433UI)
Injection	2 $\mu$ L using MMI Inlet
Carrier gas	Helium, constant flow, 1.2 mL/min
Oven program	160 $^{\circ}$ C (0.3 min hold), 30 $^{\circ}$ C/min to 290 $^{\circ}$ C (0 min); 2 $^{\circ}$ C/min to 295 $^{\circ}$ C (0 min); 10 $^{\circ}$ C/min to 305 $^{\circ}$ C (1 min)
Transfer line temp	280 $^{\circ}$ C
MS conditions	
Tune	Autotune
EMV Gain	100
Dwell	30 ms
Collision Energy	15 v
Acquisition parameters	NCI mode; 40% ammonia
Collision gas flows	Nitrogen at 1.5 mL/min, Helium at 2.35 mL/min
Solvent delay	2.85 minutes
MS temperatures	Source 150 $^{\circ}$ C; Quadrupoles 150 $^{\circ}$ C
Transitions	Estradiol: $m/z$ 538.1 $\rightarrow$ 474.1
Monitored	Internal Standard: $m/z$ 541.1 $\rightarrow$ 477.1

## Results and Discussion

### Sensitivity and Quantification

The method developed on the Triple Quadrupole GC/MS system provides excellent sensitivity. The limit of detection for the method, defined as the lowest concentration detectable at a signal-to-noise (S/N) ratio of 10, is 1 pg/mL (2 fg injected), as shown in Figure 1. Figure 2 illustrates the exceptional linearity obtained for quantification of estradiol from 1 pg/mL to 600 pg/mL, with an  $R^2$  value very close to 1.

### Peak Area Ratio Precision

The precision of the peak area ratio (analyte peak area divided by ISTD peak area) was determined for standards EA-1 to EA-5, and a minimum of five replicates was included in each calculation (Figure 3). The relative standard deviation (RSD) never exceeded 15%, illustrating the repeatability of the method. Table 3 shows the high level of precision of response ratio values obtained for each of the 10 replicates performed on the EA-1 standard, 2 fg on-column.

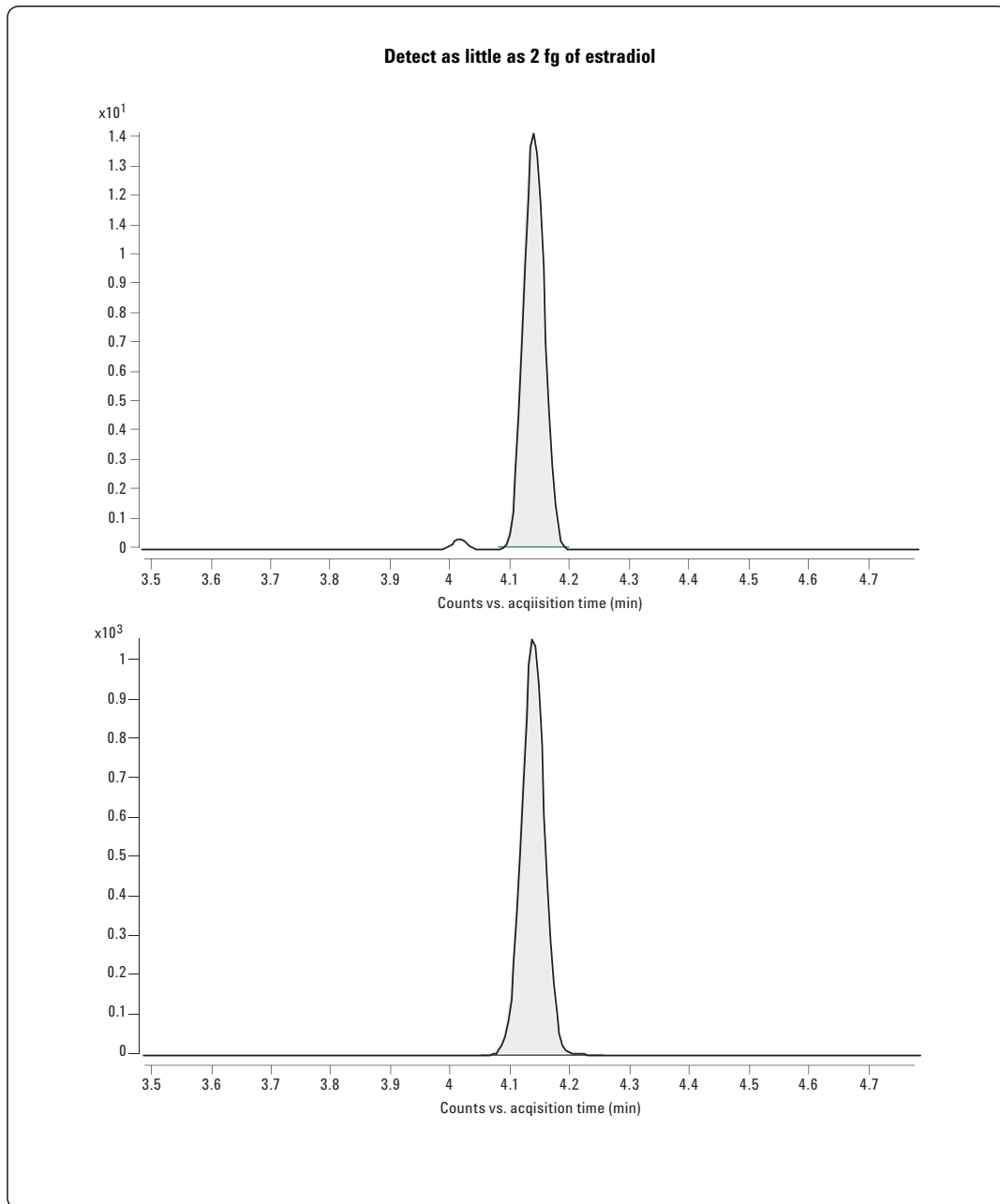


Figure 1. Selected Reaction Monitoring (SRM) analysis for 2 fg of estradiol injected on-column. The upper trace is estradiol ( $m/z$  538.1  $\rightarrow$  474.1) and the lower trace is that of the internal standard, estradiol-D3 ( $m/z$  541.1  $\rightarrow$  477.1).

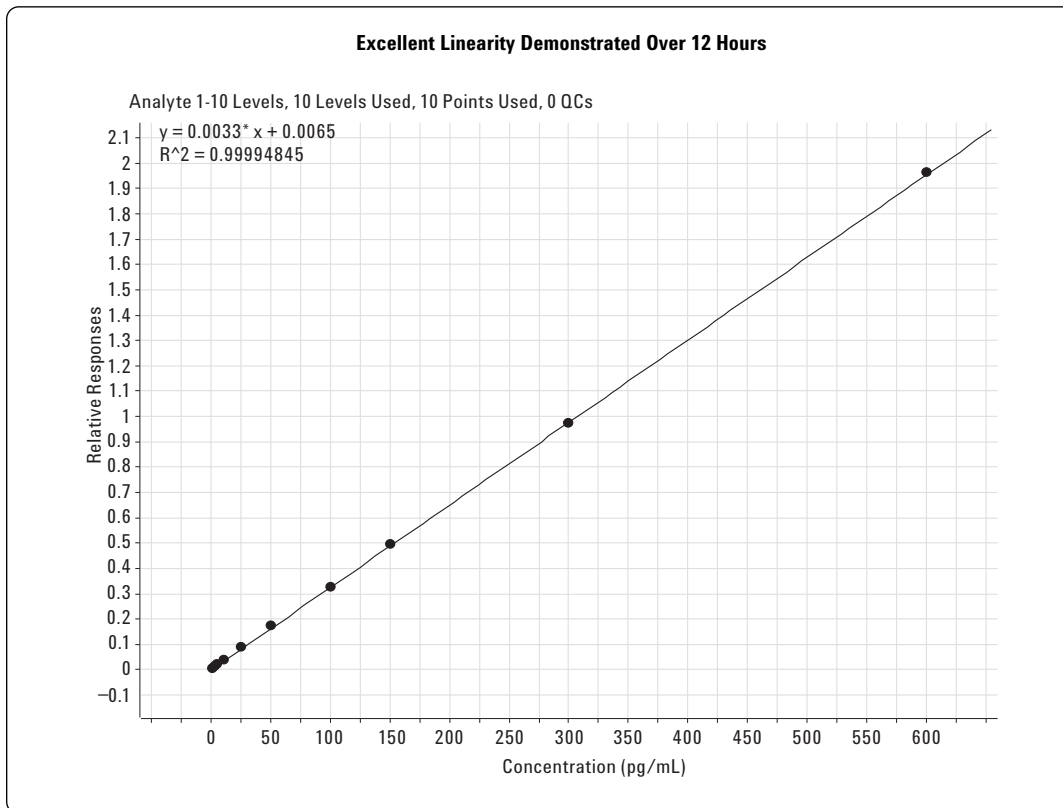


Figure 2. Calibration curve for quantification of estradiol from 1.0 to 600 pg/mL. For each of levels EA-4 through EA-10, N=6 and for levels EA-1 through EA-3, N=10. The correlation coefficient was 0.9999 using a 1/x linear regression. The total time required to run the samples was 12 hours.

Standard	Estradiol Concentration (pg/mL)	RSD (%)
EA-1	1.0	14.7
EA-2	2.5	14.6
EA-3	5.0	10.6
EA-4	10.0	10.0
EA-5	25	8.2

Figure 3. Precision of response ratios for five concentrations of estradiol. Order of injection: six sets of EA-1 through EA-10, then four sets of levels EA-1 through EA-3. The total time required to run the samples was 12 hours.

Table 3. Precision of the response ratio for the 1 pg/mL standard, EA-1, ten replicates.

Data File	N	Response Ratio
EA-1-1.D	1	0.0071
EA-1-2.D	2	0.0087
EA-1-3.D	3	0.0086
EA-1-4.D	4	0.0087
EA-1-5.D	5	0.0078
EA-1-6.D	6	0.0076
EA-1-7.D	7	0.0047
EA-1-8.D	8	0.0085
EA-1-9.D	9	0.0078
EA-1-10.D	10	0.0079
<b>Average</b>		<b>0.0077</b>
<b>Std. Dev.</b>		<b>0.0011</b>
<b>RSD (%)</b>		<b>14.69</b>

## **Conclusion**

The Agilent 7000 Triple Quadrupole GC/MS can extend the LOD for estradiol as low as 1 pg/mL (2 fg on-column), while providing highly repeatable quantification in the 1 to 600 pg/mL range. This makes it very suitable for the routine analysis of estradiol.

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