Low pg/mL Detection of Underivatized 17ß-Estradiol in Serum through Increased Ion Sampling Efficiency Using LC-MS/MS

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Introduction

17ß-estradiol (E2) is the subject of significant clinical research. Low levels of 17ß-estradiol have presented several challenges for traditional analysis of the molecule. Due to a lack of highly ionizable functional groups, mass spectrometric approaches have relied on laborious derivatization methods to achieve a sufficient limit of quantitation (LOQ). Through the use of dual ion funnel technology, ion sampling efficiency has been improved to the point that underivatized 17ß-estradiol can be quickly and accurately quantified at low levels using an LC/MS approach.

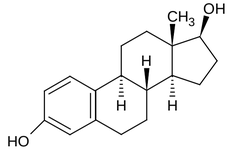


Figure 1. 17ß-Estradiol structure

Experimental

Agilent Jet Stream (AJS) technology consists of the addition of a concentric super-heated nitrogen sheath gas to the nebulizer. The super-heated sheath gas collimates the nebulizer spray producing efficient desolvation and ion generation. The use of an hexabore capillary increases the interface area of the capillary inlet within the AJS thermal ion rich zone. The bores spread across the central, ion rich part of the AJS thermal confinement zone. In this way the multibore capillary captures more ions but also more gas. This gas load would normally overwhelm the vacuum system and therefore a novel dual ion funnel was added to efficiently remove the gas, while focusing the ions into the entrance of the first quadrupole.

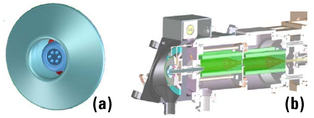


Figure 2. Agilent 6490 Technology – Hexabore capillary (a) and dual ion funnel (b)

Experimental

Sample preparation
Calibration standards were prepared by dissolving estradiol in charcoal stripped human serum. 600 µL of spiked serum was extracted with methyl t-butyl ether. Samples were vortexed, centrifuged and the organic layer (top) was evaporated under nitrogen until dry. Dried down extracts were reconstituted in water/acetonitrile (80/20) and injected onto the LC-MS/MS.

LC Method
Agilent 1290 and 1260 HPLC binary pumps (1 each), well plate sampler with thermostat, temperature-controlled column compartment, 2 position/6 ports switching valve

Parameter	Value
Trapping Column	Zorbax Extend C18, 2.1 x 12.5mm, 3.5µm
Analytical Column	Zorbax Extend C18, 2.1 x 50mm, 1.8µm
Injection Volume	10 or 100 µl
Autosampler Temp	4°C
Needle Wash	Flush port for 10 seconds
Mobile Phase A	Ammonium Hydroxide in Water
Mobile Phase B	Acetonitrile

Table 1. LC Parameters

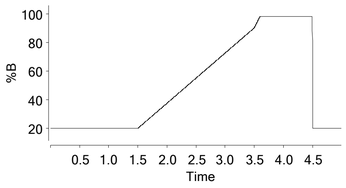


Figure 3. LC pump gradient

Online Sample Cleanup
Samples are injected onto a trapping column where the estradiol is retained and washed. The wash is sent to waste, reducing the amount of matrix sent to the mass spectrometer. After sufficient washing, a valve is switched and the analyte is eluted onto an analytical column where chromatographic separation is performed.

Experimental

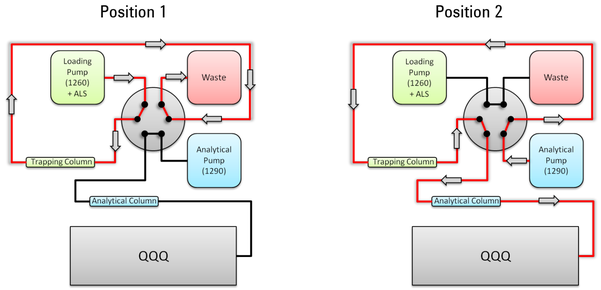


Figure 4. Back-Flush LC configuration for online sample cleanup

Results and Discussion

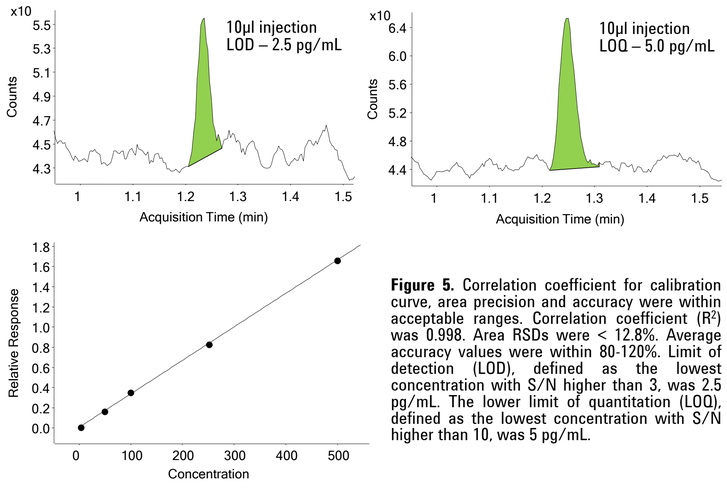
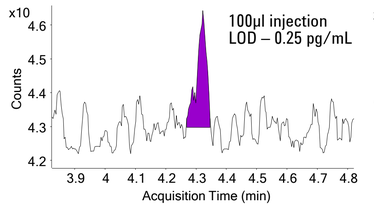
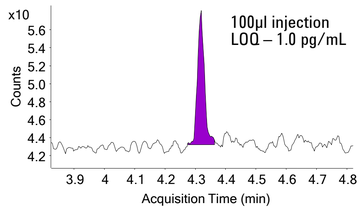


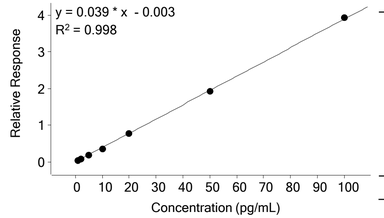
Figure 5. Correlation coefficient for calibration curve, area precision and accuracy were within acceptable ranges. Correlation coefficient (R^2) was 0.998. Area RSDs were < 12.8%. Average accuracy values were within 80-120%. Limit of detection (LOD), defined as the lowest concentration with S/N higher than 3, was 2.5 pg/mL. The lower limit of quantitation (LOQ), defined as the lowest concentration with S/N higher than 10, was 5 pg/mL.

Results and Discussion

Utilizing a conventional HPLC system consisting of a single column and a 10µl injection, the 6490 was capable of achieving an LOQ of 5pg/mL of underivatized estradiol. By implementing a two-column system (figure 4) quantitation was improved by a factor of 5 – LOQ down to 1pg/mL. By trapping and focusing the analyte on the first column and eluting onto the second column, it is possible to inject 100µl of sample on a short, narrow-bore column without significant band-broadening.







Level (pg/mL)	Measured (pg/mL)	Accuracy (%)
1.0	1.19	119.0
2.0	1.95	97.5
5.0	4.70	94.0
10.0	9.48	94.8
20.0	19.95	99.8
50.0	49.84	99.7
100.0	101.36	101.4

Table 2. Calibration Accuracy

Figure 6. Without the use of chemical derivatization, a detection limit of 0.25 pg/mL was reached. Quantitation was accurate down to 1.0 pg/mL and was tested up to 100 pg/mL.

Conclusions

Taking full advantage of 6490 Triple quadrupole increased sensitivity, it was possible to quantify underivatized estradiol at the low pg/mL level. Sensitivity of the method was demonstrated with LOQ at 1 pg/mL underivatized E2 using an LC configured for online sample cleanup capable of concentrating a 100 µL injection volume to avoid band broadening effects. This high quality estradiol assay with LOQ = 1 pg/mL and excellent performance (correlation coefficient for calibration curves, precision and accuracy) will be an important tool for clinical research where assay sensitivity is one of the most important factors

References

[1] M. M. Kushnir, A. L. Rockwood, J. Bergquist, M. Varshavsky, W. L. Roberts, B. Yue, A. Bunker, A. Meikle. High-sensitivity tandem mass spectrometry assay for serum estrone and estradiol. American Journal of Clinical Pathology 2008, 129, 530.