TRACE LEVEL MEASUREMENT OF STEROIDS WITH AGILENT 6490 TRIPLE QUADRUPOLE MASS SPECTROMETER

Measurement of estrone, estradiol and ethynyl estradiol in Environmental Waters

Pharmaceuticals are consumed in high quantities worldwide, and these amounts will continue to increase due to improving health care and longer life expectations. Administered pharmaceuticals are excreted by humans, as a parent compound or metabolites. They enter sewage treatment plants where they are not entirely removed and end up in the environment. These residual pharmaceuticals can negatively impact aquatic and terrestrial ecosystems. This technical flyer describes the optimization of a sensitive method for steroid detection on the Agilent 6490 Triple Quadrupole LC/MS System with iFunnel Technology. The method can detect sub part per trillion (ng/L) steroids in wastewater.

Sample Preparation Procedure

Sample collection in 2 L amber glas bottles, add 2 mL HCl (conc.) and 0.5 g of copper nitrate, Storage < 10°C.

Filter Sample through GF/D papers prior to extraction.

Add 20 µL internal standard solution (1 mg/L) to 1 L sample for crude sewage samples 100 mL sample was diluted with 900 mL water.

Solid Phase Extraction:

Cardridge Conditioning	Cardridge Landing	Wash Cardridge	Elute Cardridge
1. 5 mL ethyl acetate 2. 5 mL methanol 3. 3 mL water	Load cartridge with 250 mL sample	1. 3 mL 60% methanol 2. 3 mL water Dry Cartridge for 40 min	Elute column with 4 mL ethyl acetate Redissolve residue in 250 µL of cyclohexane : propan-2-ol (95:5)

Extracts were cleaned up with normal phase chromatography:
Column: 4.6 x 150 mm, 5µM, 55 °C, (Agilent ZORBAX Cyano Column)
Mobile Phase: cyclohexane : propan-2-ol (95:5), flow rate 1.0 mL/min
Fraction collection: 5.8 - 8.6 min
Evaporate extract under airstream (45 °C), redissolved residue in 250 µL methanol : water (90:10)

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Liquid Chromatography and Mass Spectrometry

LC Conditions	
Analytical column	Agilent C-18 Eclipse Plus, 2.1 × 50 mm, 3.5 µm (p/n 959763-902)
Guard column	Luna C-18, 4.0 mm × 2.0 mmCurabitur vehicular
Column temperature	40°C
Injection Volume	25 µL
Mobile phase	A = Water
	B = Acetonitrile
Runtime	16.0 min
Flow rate	0.3 mL/min: 0.1 mL/min postcolumn addition of 0.1% NH_3
Gradient program	Time (min) % Solvent A % Solvent B
	0 90 10
	0.5 60 40
	10.0 20 80
	10.2 0 100
	11.5 0 100
	11.6 90 10

| MS Conditions	
Acquisition parameters	ESI mode, negative ionization; Dynamic MRM
Sheath gas temperature	300 °C
Sheath gas flow rate	11 L/min
Gas temperature	180 °C
Drying gas	Nitrogen, 16 L/min
Nebulizer pressure	45 psig
Nozzle voltage	1,500 V
Vcap voltage	3,000 V
Cell accelerator voltage	Varied per optimization study
Delta EMV	Varied per optimization study
Low & high pressure ion funnel voltage	Varied per optimization study
Scan type dynamic MRM	Delta EMV 300 V

Analysis Parameters

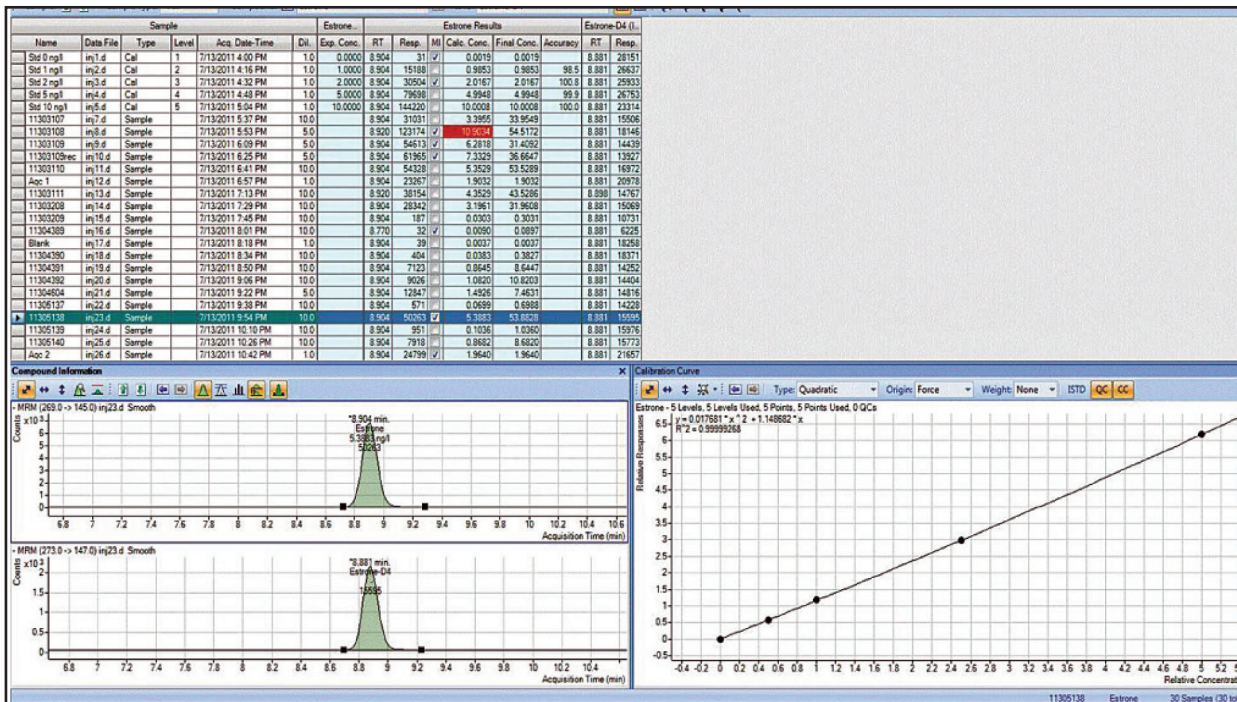| Time segment	Retention time (min)	Compound	Precursor ion	Product ion	CAV	Fragmentor Voltage	Collision energy
1	8.0	Estradiol	271.0	145.0	2	380	50
1	8.0	Estradiol-d5	276.0	147.0	2	380	50
1	8.5	EthynylEstradiol	295.0	145.0	2	380	52
1	8.5	EthynylEstradiol-d4	299.0	147.0	2	380	52
1	8.8	Estrone	269.0	145.0	2	380	45
1	8.8	Estrone-d4	273.0	147.0	2	380	45

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Performance Examples
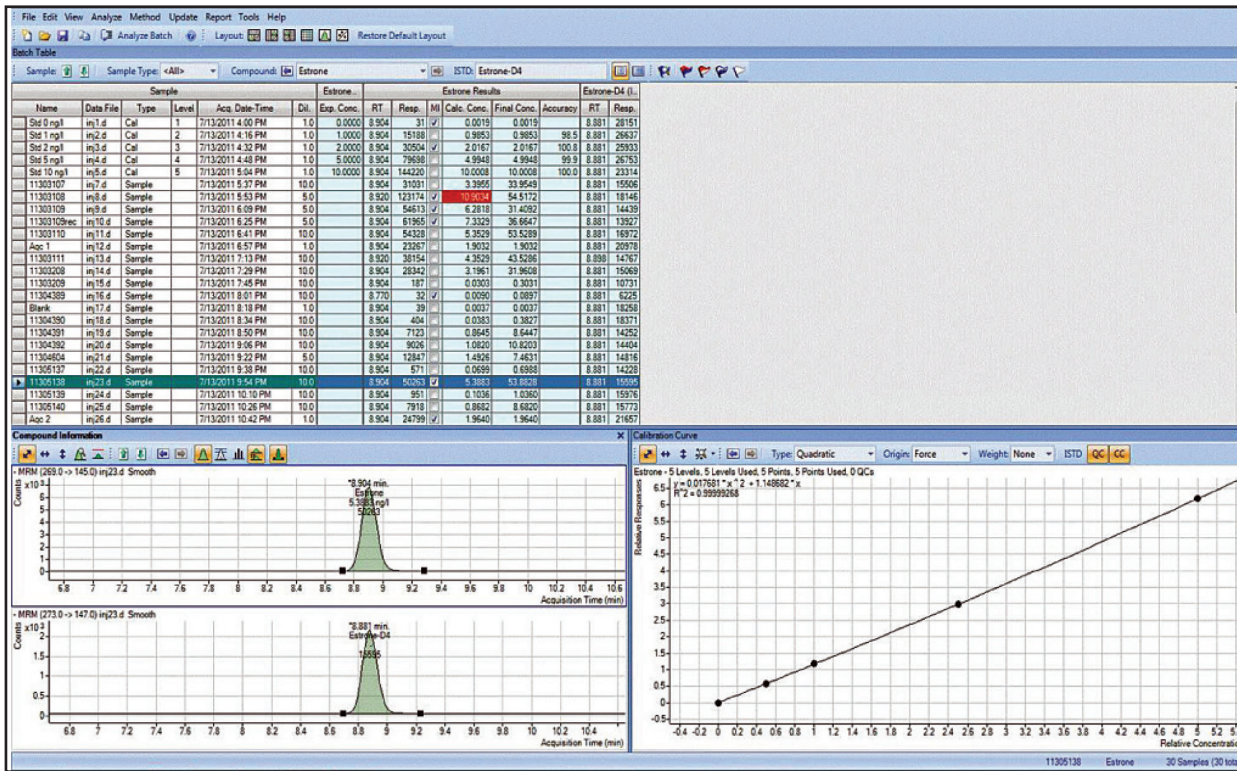
Analysis of 0.91 ng/L of ethynyl estradiol in crude sewage, showing the MRM chromatogram, as well as the chromatogram for the internal standard and the calibration curve with an R^2 value >0.9999

Analysis of 53.7 ng/L of estrone in crude sewage, showing the MRM chromatogram, as well as the chromatogram for the internal standard and the calibration curve with an R^2 value >0.9999

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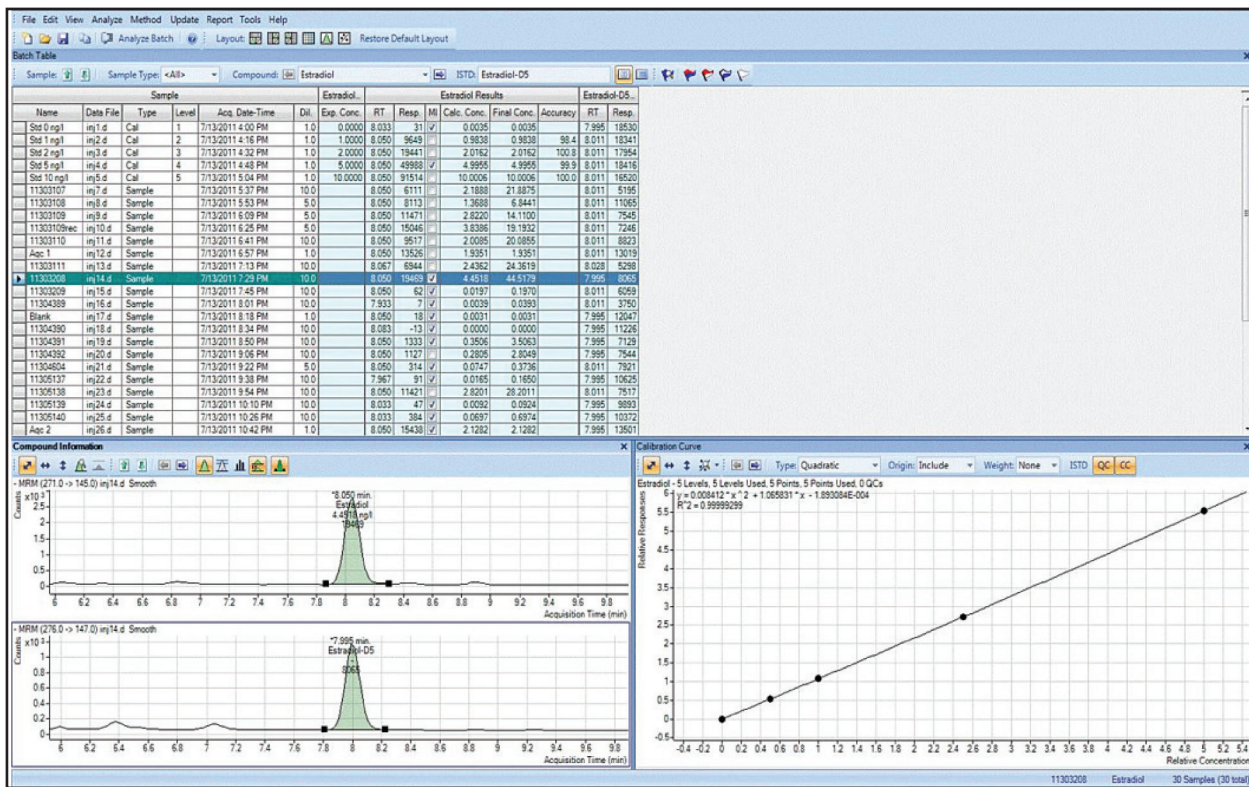
Analysis of 53.7 ng/L of estrone in crude sewage, showing the MRM chromatogram, as well as the chromatogram for the internal standard and the calibration curve with an R^2 value >0.9999

Conclusion

The Agilent 6490 Triple Quadrupole LC/MS System with iFunnel Technology provides exquisite sensitivity, making it the ideal choice for applications that require the detection of minute quantities of analyte. The optimized method can provide sub ng/L detection of steroids in wastewaters, which are very complex matrices.

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The Measure of Confidence

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