

# 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 glass 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:

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

#### 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)



**Agilent Technologies**

## 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 mm Curabitur 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 <sub>3</sub>		
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		

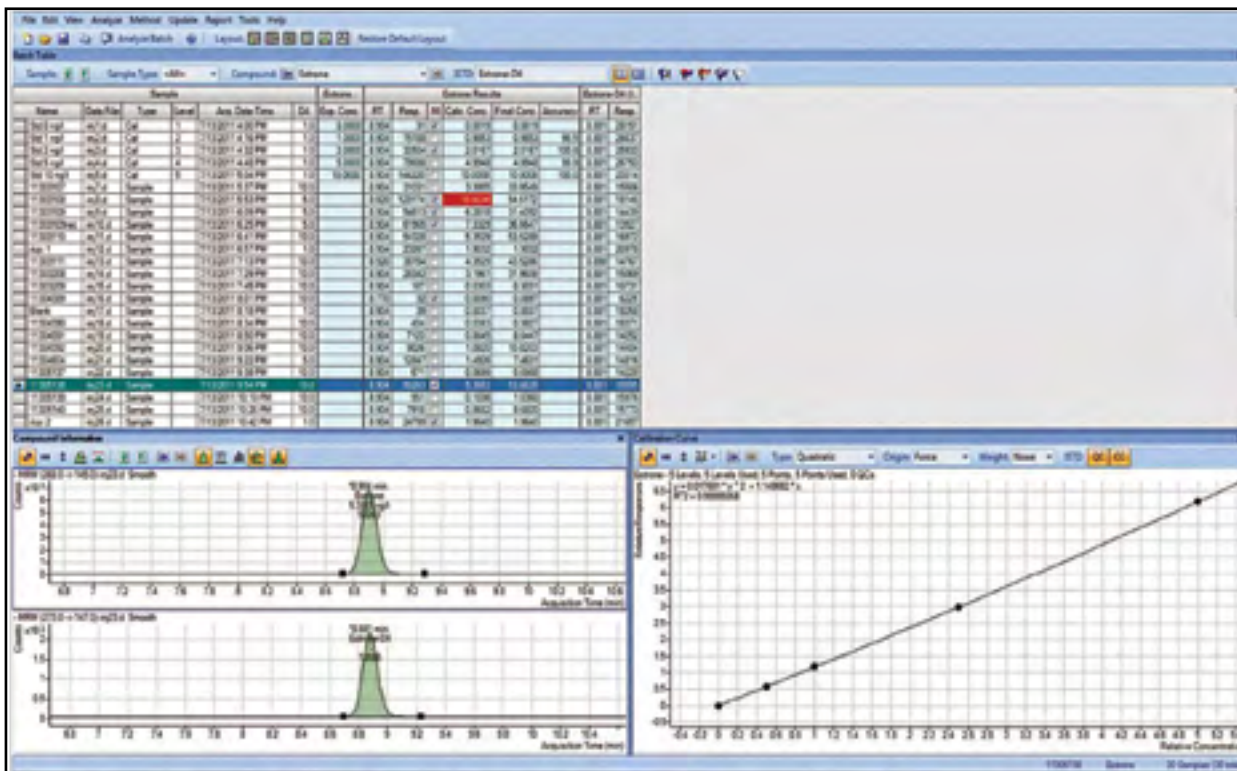
## Analysis Parameters

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

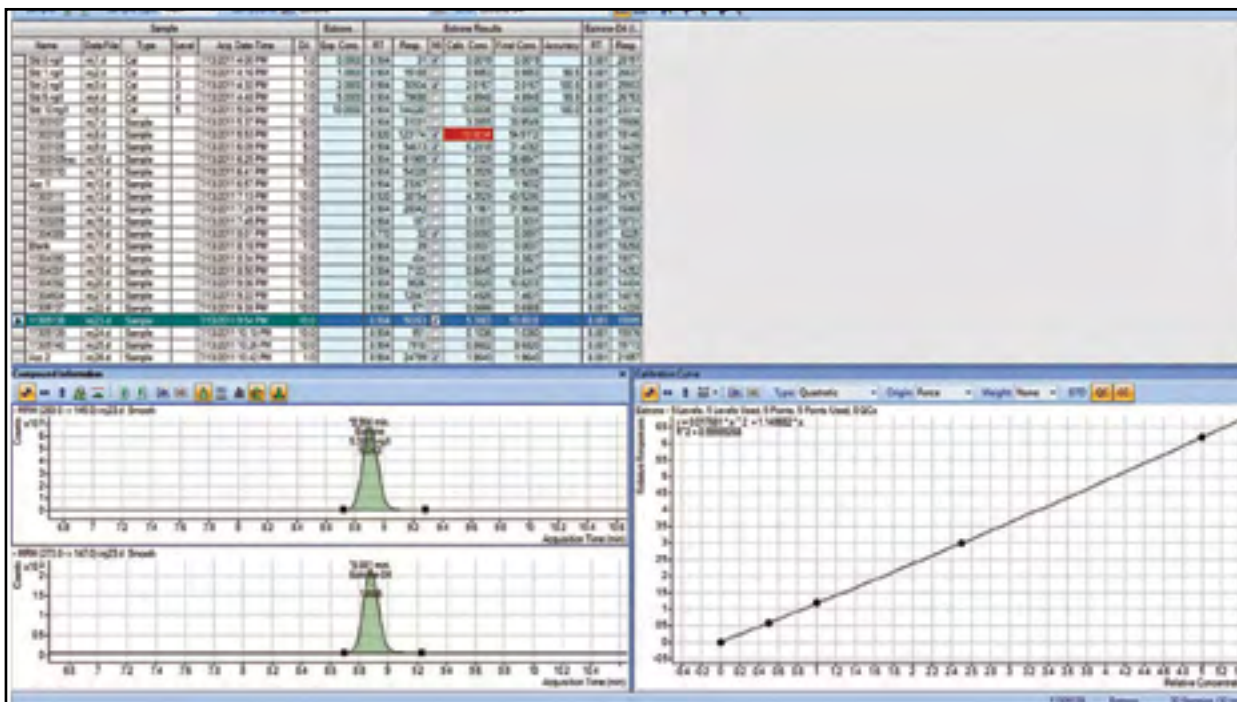


**Agilent Technologies**

## Performance Examples



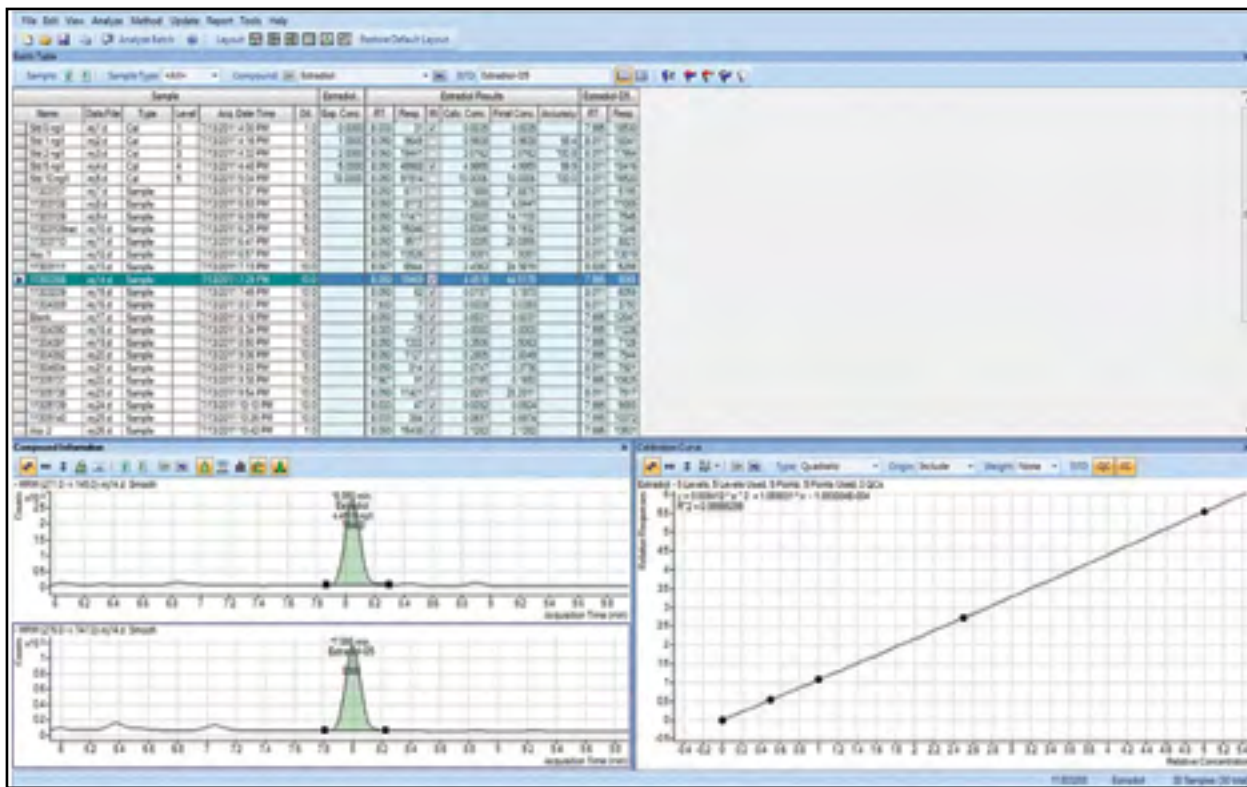
Analysis of 0.91 ng/L of ethynyl estradiol in crude sewage, showing the MRM chromatogram, as well as the chromatogram for the internal standard and the calibration curve with an  $R^2$  value  $>0.9999$



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  $>0.9999$



**Agilent Technologies**



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.

[www.agilent.com/chem/wateranalysis](http://www.agilent.com/chem/wateranalysis)

Agilent Products are for Research Use Only.  
Not for use in diagnostic procedures.  
Information, descriptions and specifications in this publication are subject to change without notice.

© Agilent Technologies, Inc. 2012  
Published in Germany, April 1, 2012  
5991-0271EN