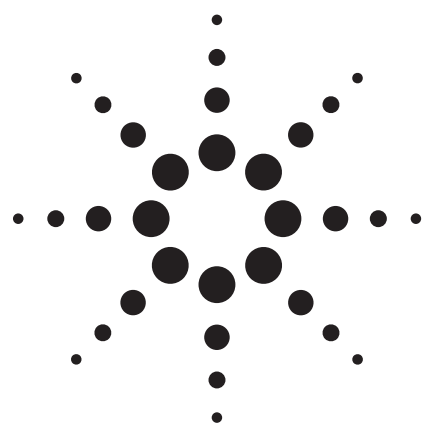


# Determination of Estrone-3-Sulfate in Water at Sub-ppt Level by LC-(ESI-)-MS/MS



Application

Environmental

## Authors

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## Abstract

Using solid phase extraction (SPE) and liquid chromatography/tandem mass spectrometry (LC-MS/MS), in negative electrospray mode, estrone-3-sulfate was analyzed down to sub-ppt level ( $< \text{ng L}^{-1}$ ).

## Introduction

The occurrence of steroids in the aquatic environment and their effects on normal endocrine function in aquatic organisms are subjects of current concern. Several studies have also shown that birds, reptiles, and mammals in polluted areas undergo alterations of the endocrine-reproductive system.

At present, a multitude of chemicals have been demonstrated to be endocrine disrupters. Among these, natural and synthetic estrogens already show adverse effects at the lower ng/L. Their efficient control in environmental waters is made possible nowadays thanks to numerous analytical approaches available in the literature. This is the case for estradiol (E2), estrone (E1), estriol (E3),

and ethinylestradiol (EE2) measurement. Estrone-3-sulfate (E1S) should also be highlighted, because of its stability in the environment, but until now fewer papers address this xenobiotic.

In this context, this application deals with the development of an analytical method dedicated to the quantification of estrone-3-sulfate in water. Identification relies upon the 2002/657/EC decision to confirm unambiguously the presence of steroids, even at ultra-trace levels ( $< 1 \text{ ng.L}^{-1}$ ).

## Experimental

### Sample Preparation Procedure

1. Add 10 ng internal standard (boldenone-sulfate- $\text{d}_3$ ).
2. If needed, filter water samples using 0.45- $\mu\text{m}$  glass fiber filters.
3. Load the water sample onto an MM4 SPE cartridge (1 g, 6 mL) (Interchim, France).
4. Elute the compounds with 12 mL MeOH/ $\text{NH}_4\text{OH}$  (98:2, v/v).
5. Evaporate the extract to dryness.
6. Reconstitute the extract in 50  $\mu\text{L}$  MeOH/ $\text{H}_2\text{O}$  (80:20, v/v).

### Calibration Curve

Six calibration samples were fortified at 0, 1, 2, 3, 4, and 5 ng/L (ppt) and extracted using the described sample preparation procedure.



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## LC-MS/MS Measurement (Negative Electrospray Mode)

**LC:** Agilent 1200  
**Column:** Gemini (50 mm x 2 mm, 3 µm) C18 110 A (Phenomenex)/Agilent equivalent: Extend-C18 3.5 µm, 2.1 x 50 mm (p/n 735700-902)  
**Column temperature:** 40 °C  
**Mobile phases:** A: Ammonium acetate buffer 25 mM, pH 9.2  
B: Acetonitrile (ACN)  
**Flow rate:** 0.3 mL/min

**Table 1. Elution Gradient**

Time (min)	%B
0	5
0.5	5
15.5	100
18.5	100
21	5
26	5

**Injected volume:** 10 µL

**MS:** Agilent 6410 LC/MS Triple Quadrupole  
**Ionization:** ESI (-)  
**Capillary:** 3500 V  
**Nebulizer pressure:** 40 psi  
**Drying gas:** 13 L/min  
**Gas temperature:** 275 °C

## MS/MS Parameters

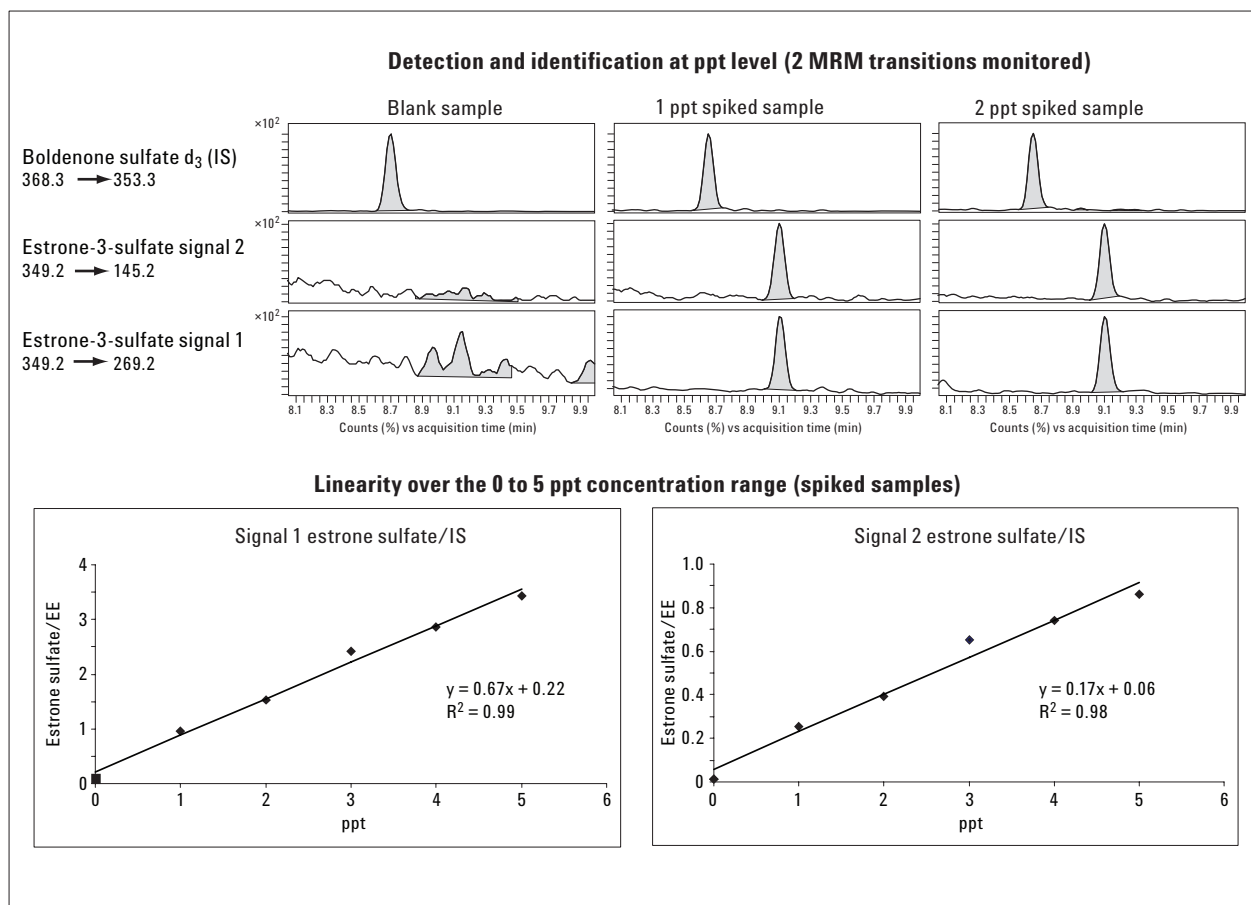
**Table 2. MS/MS Parameters**

Molecule	Precursor ion	MS1 resolution	Product ion	MS2 resolution	Dwell time (ms)	Fragmentor	Collision energy
Boldenone sulphate-d <sub>3</sub>	368.3	Wide	353.3	Widest	200	120	15
Estrone-3-sulfate T1	349.2	Wide	269.2	Widest	300	200	35
Estrone-3-sulfate T2	349.2	Wide	145.2	Widest	300	200	60

## Results and Discussion

### Analytical Performance

Validation relies upon the 2002/657/EC decision to assess the methodology performances at sub-ppt levels. Various water samples coming from different origins (surface water or groundwater) were analyzed as blank samples. Five calibration curves were realized from spiked samples (1, 2, 3, 4, and 5 ppt of estrone-3-sulfate). All these samples were extracted according to the sample preparation procedure described previously. Two diagnostic signals (MRM transitions) were monitored for estrone-3-sulfate and one for boldenone-sulfate-d<sub>3</sub>. Figure 1 illustrates the high specificity of the signals monitored and the good performance in terms of linearity.



**Figure 1.** Illustration of the main performance parameters for the developed method (specificity, sensitivity and linearity).

### Validation parameters

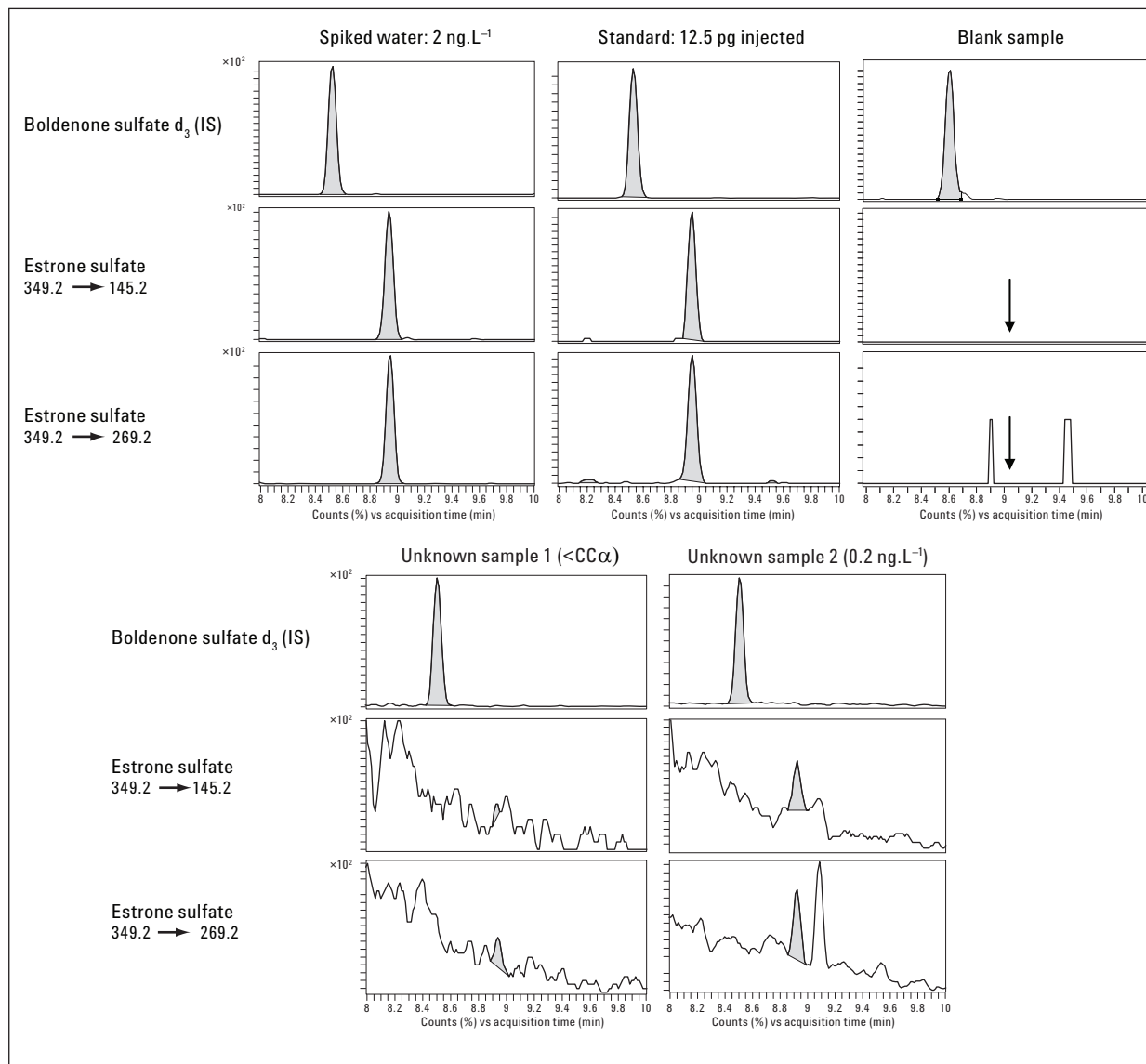
Table 3 presents the performances measured for each transition in term of linearity (for five different calibration curves), repeatability (at 1, 2, 3, 4, and 5 ppt fortified levels, on five different water), detection limit ( $CC\alpha$ ), and detection capability ( $CC\beta$ ).

**Table 3. Validation Parameters**

	<b>Linearity (range of <math>R^2</math> on five calibration curves)</b>	<b>Repeatability (n=25) (5 replicates for each of the 5 tested concentration levels)</b>	<b><math>CC\alpha</math> (ng.L<sup>-1</sup>)</b>	<b><math>CC\beta</math> (ng.L<sup>-1</sup>)</b>
<b>Estrone-3-sulfate</b>				
349.2 → 145.2	0.954 – 0.977	6.8 – 22.6 %	0.08	0.15
349.2 → 269.2	0.976 – 0.991	3.9 – 17.2 %	0.10	0.53

### Analysis of Unknown Samples

Figure 2 illustrates the results obtained after application of the developed method to a batch of surface and groundwater samples. These results confirmed the efficiency of the method, demonstrating sensitivity and specificity.



**Figure 2.** Typical MRM chromatograms obtained for routine analysis series (including spiked, standard, blank, and two unknown water samples).

## Conclusions

The developed method, focused on estrone-3-sulfate, associates a selective SPE preparation and a specific LC-MS/MS detection (Gemini column, Phenomenex, Agilent 6410 LC-MS/MS system). The performed validation according to 2002/657/EC criteria allowed unambiguous confirmation of steroid presence, even at ultra-trace levels ( $< 1 \text{ ng.L}^{-1}$ ).

## For More Information

For more information on this application, you may also contact Agilent Technologies, Inc., Attention:

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