Reduction of Ionization Suppression in Water Samples when Using LC-ESI-MS in Negative Ion Mode

Application

Environmental

Author

Neil Cullum
Anglian Water Services Laboratories
Hinchingbrooke Business Park
Kingfisher Way, Huntingdon, Cambs
UK

Abstract

Experimental modifications that inhibited ionization suppression when using LC-ESI-MS (liquid chromatography-electrospray ionization-mass spectrometry) in negative ion mode are described for two classes of materials: steroid estrogens and acidic herbicides.

Introduction

Ionization suppression is a problem when using electrospray ionization (ESI) in negative ion mode. It leads to an apparent reduction in analyte recovery from different matrices when, in fact, extraction efficiencies are very similar.

Ionization suppression effects are mostly due to the competition between matrix components and the analytes of interest to become ionized in the electrospray source, and their release from the droplets formed. This suppression is compound-dependent and appears to occur mainly in high-aqueous mobile-phase conditions.

There are several approaches to reduce the effects of ionization suppression and hence improve the apparent extraction efficiency of the analyte of interest.

- Matrix clean-up: removal of unwanted material from the sample extract. It may be possible to tailor a chemical clean-up procedure for a small suite of compounds, but this may not be possible when dealing with a larger suite of analytes due to their different physical and chemical properties. For instance, some of the more polar compounds may not be retained on a solid phase cartridge or eluted from the cartridge during the washing step.
- The use of extracted standards from the matrix of interest. This approach would work, providing only one matrix was being analyzed. If different matrices were being analyzed in small numbers, this would lead to low-throughput.
- Use of internal standards (ISTDs). This is a very useful approach when dealing with individual analytes (assuming a deuterated equivalent is available). However, when dealing with large suites of compounds, this could involve many ISTDs, adding complexity and cost to the method.
- Explore other ionization modes (if available) during method development; for example, atmospheric pressure chemical ionization (APCI) and/or atmospheric pressure photo ionization (APPI). Both of these sources are less prone to matrix suppression effects, but they may not be suitable for all analytes in an extended suite of compounds. Some analytes may be thermally sensitive, while others may not ionize. The use of positive/negative switching was shown to be useful when dealing with ionization suppression [1]. For example, imazapyr (an herbicide) showed varying recoveries

from different matrices [2]: borehole water = 73% recovery and tap water (derived from a surface water source) = 48% recovery when analyzed using ESI in negative ion mode. However, when analyzed in positive ion mode, the recoveries for both matrices were ≥90%. This approach may not be suitable to all analytes of interest, as they may not ionize in both modes.

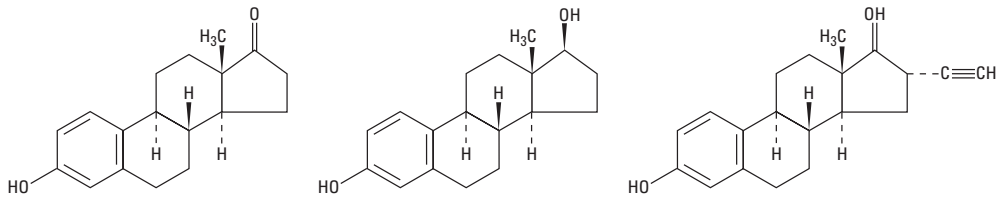
- Use more retentive LC conditions to elute compounds of interest away from matrix interferences or to shift the compounds into a higher organic solvent matrix.
- Other techniques tried: buffer and/or pH adjustment, post-column addition of an organic solvent, and the use nano-spray technology.

In this application note, the use of pH adjustment coupled with post-column addition of 2-propanol is demonstrated to reduce the effects of ionization suppression when analyzing acidic compounds. Two examples are given.

Experimental

The first example is the steroid estrogens. Compounds include estrone (E1), estradiol (E2), and ethynyl estradiol (EE2). See Figure 1.

Initially, a simple LC gradient method using HPLC-grade water and acetonitrile was used to separate the three steroid compounds under optimized negative ion electrospray conditions obtained from flow injection analysis (FIA) of the individual steroids. See Figure 2.



Estrone (E1)
[53-16-7]

Estradiol (E2)
[50-28-2]

Ethynyl estradiol (EE2)
[57-63-6]

Figure 1. Structures for the tested steroid estrogens.

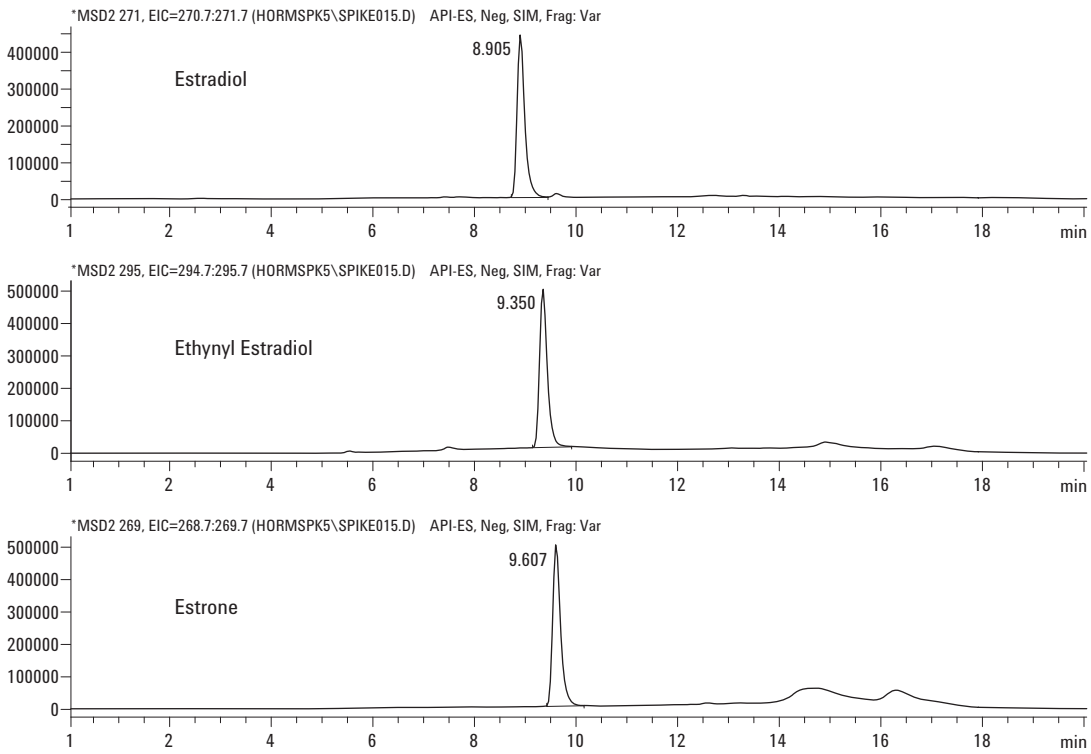


Figure 2. Extracted ion chromatograms (EICs) of the mixed standard; each standard at 25 ng/L.

Test A

Deionized water was spiked with the standards, each at 10 ng/L, and taken through a solid phase extraction (SPE) procedure, followed by LC/MS analysis.

The following results were obtained on six replicate samples:

E1 = 9.99 ±0.25 ng/L (100% recovery)
E2 = 8.97 ±0.40 ng/L (90% recovery)
EE2 = 9.61 ±0.35 ng/L (96% recovery)

The results obtained from Test A showed good recovery and reproducibility from deionized water.

Test B

Using the same extraction procedure and LC/MS conditions, tap water (derived from a surface water source) was spiked with the same standards, each at 10 ng/L, and analyzed as in Test A.

The following results were obtained on six replicate samples:

E1 = 4.93 ±0.27 ng/L (49% recovery)
E2 = 2.47 ±0.20 ng/L (25% recovery)
EE2 = 3.70 ±0.51 ng/L (37% recovery)

Compared to the deionized water results (Test A), the recoveries were much lower in Test B (in the range 25%–50%), and the precision was also reduced.

Test C

The objective of Test C was to see whether the low recoveries obtained in Test B, using tap water, were due to extraction efficiencies or to ionization suppression. Blank tap water was taken through the same extraction procedure, and the resulting extracts were spiked to 20 ng/L per standard and analyzed. See Figure 3.

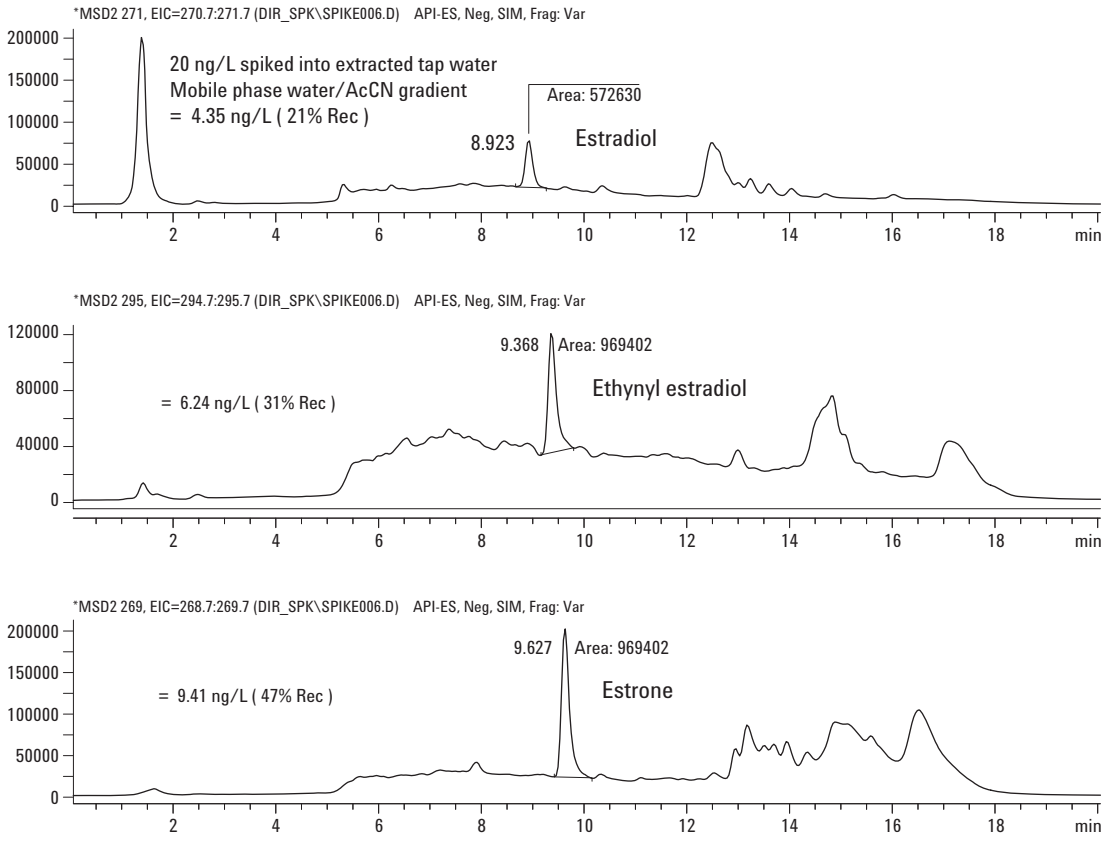


Figure 3. EICs for tap water extract, spiked with each standard at 20 ng/L.

The following results were obtained on six replicate samples:

E1 = 9.41 ±0.52 ng/L (47% recovery)
E2 = 4.35 ±0.41 ng/L (21% recovery)
EE2 = 6.24 ±0.29 ng/L (31% recovery)

The recoveries obtained were similar to those obtained in Test B. Direct spiking of the tap water extracts yielded reduced recovery values. The low recoveries obtained by direct spiking appear to be caused by ionization suppression, and not from poor extraction efficiency.

Test D

In the literature, there are examples of LC mobile phase conditions that use a small amount of ammonia (0.1%) in the water to enhance the ionization of the steroid analytes. The mobile phase was prepared, and a ZORBAX Extend LC column was fitted for use under high pH conditions. Tap water was spiked at 10 ng/L per standard, taken through the extraction procedure, and the resulting extracts were analyzed using the new mobile phase conditions. See Figure 4.

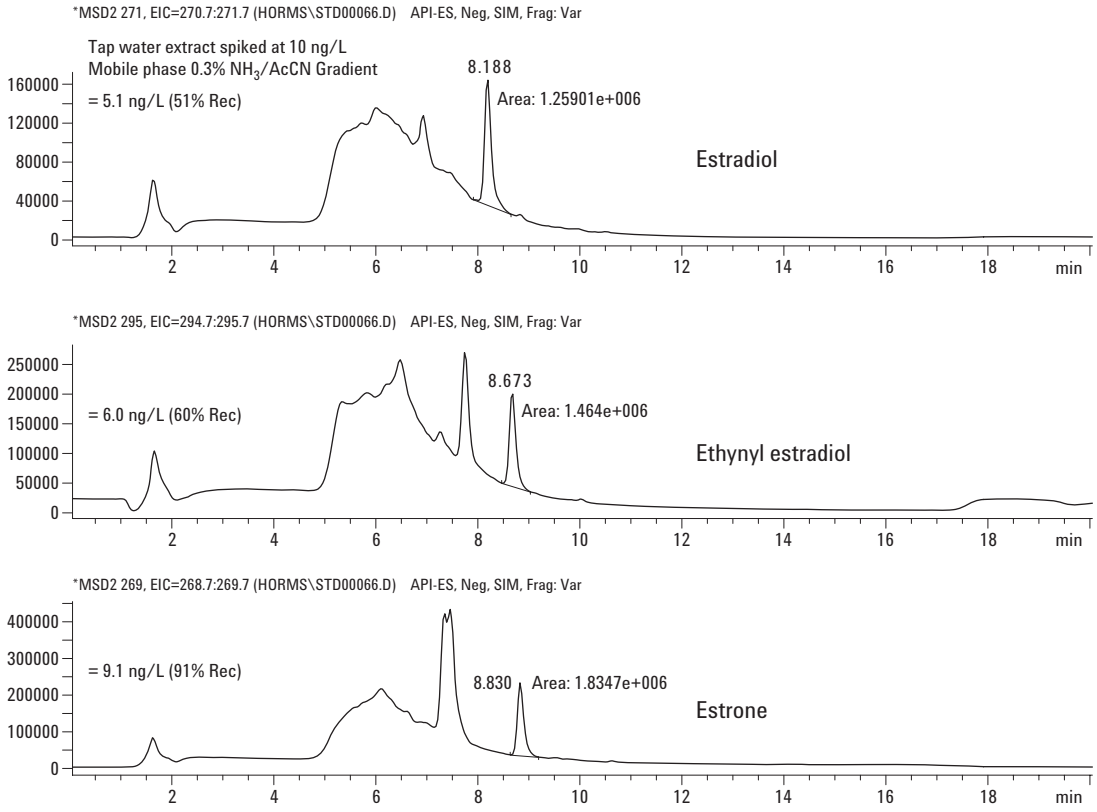


Figure 4. EICs of tap water extract spiked at 10 ng/L per standard, and using the new LC conditions.

The following results were obtained on six replicate samples:

E1 = 7.86 ±0.35 ng/L (79% recovery)
E2 = 6.24 ±0.24 ng/L (62% recovery)
EE2 = 6.37 ±0.49 ng/L (64% recovery)

A four- to five-fold increase in peak abundances for the standards was observed, with good linearity obtained for all calibration curves.

Compared to Test C, recovery values increased using Test D mobile phase conditions; the addition of ammonia in the mobile phase apparently reduced ionization suppression while still using the same extraction procedure.

Standard	Test C % recoveries	Test D % recoveries
E1	47	79
E2	21	62
EE2	31	64

Although a significant increase in recovery is observed for all three steroid analytes when using ammonia in the mobile phase, the recovery values are still low compared to the values obtained for deionized water (Test A).

Test E

The use of organic solvents (mainly alcohols) as postcolumn addition reagents to facilitate the reduction of ionization suppression is documented. Using the same LC conditions as in Test D and using a tee-piece after the analytical column, 2-propanol was added to the column eluent at a flow rate of 0.2 mL/min. Tap water, spiked at 10 ng/L per standard, was taken through the modified extraction procedure and analyzed. See Figure 5.

The following results were obtained on 12 replicate samples:

E1 = 9.44 ±0.26 ng/L (94% recovery)
E2 = 8.92 ±0.26 ng/L (89% recovery)
EE2 = 8.24 ±0.24 ng/L (82% recovery)

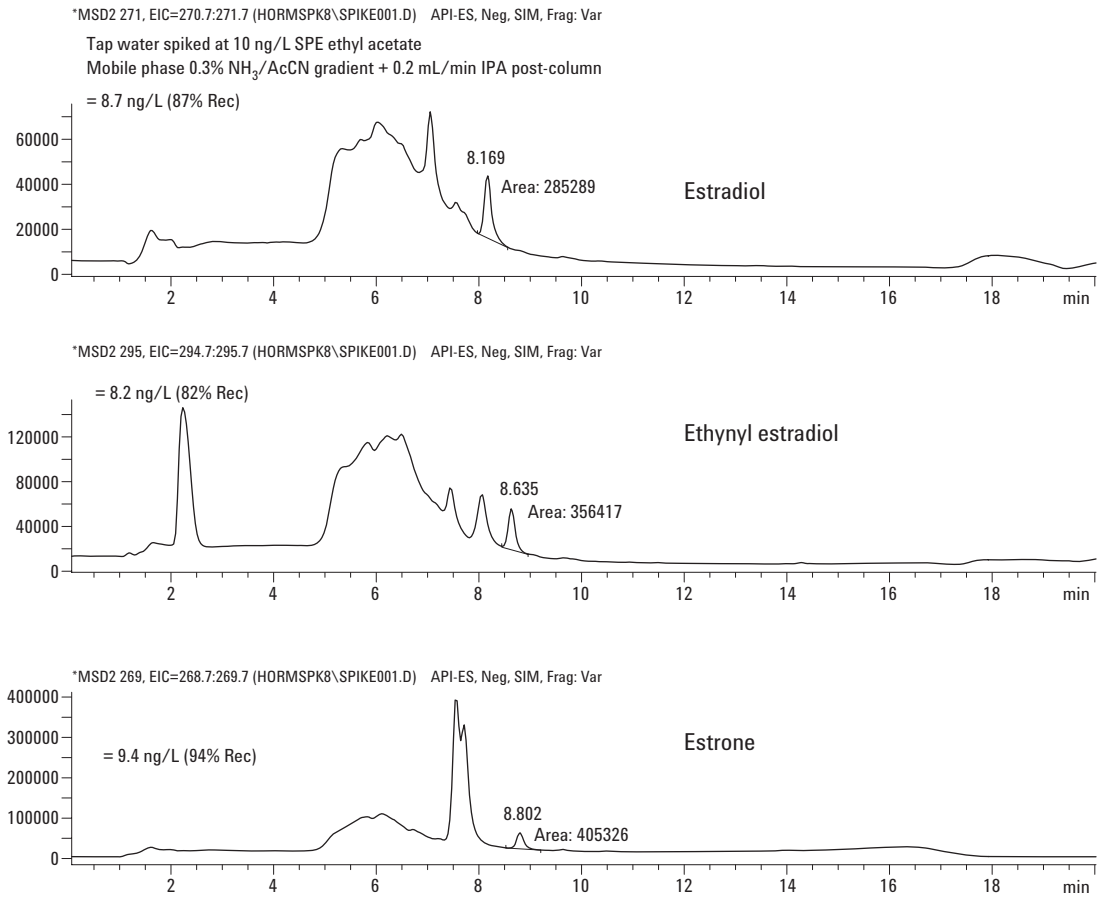


Figure 5. EICs of spiked (10 ng/L per standard) tap water with IPA postcolumn addition.

Compared to Test D, recovery values increased when using Test E mobile phase conditions. Therefore, post-column addition of 2-propanol to the mobile phase eluent further reduced ionization suppression while still using the same extraction procedure. See Table 1.

Table 1. Percent Recoveries for Different Test Conditions

Standard	Test A % recovery	Test B % recovery	Test C % recovery	Test D % recovery	Test E % recovery
E1	100	49	47	79	94
E2	90	25	21	62	89
EE2	96	37	31	64	82

Overall Conclusion for the Steroid Example

Test C results showed that the low recovery for the steroid analytes was caused by ionization suppression and not poor extraction efficiencies. By using the Test E extraction method and obtaining recovery values in excess of 80%, it is concluded that ammonia in the mobile phase and the post-column addition of 2-propanol reduce ionization suppression effects for the analysis of the steroid estrogens.

The second example is acidic herbicides; these are another class of compounds that are prone to ionization suppression effects. Compounds include 2,4-D, MCPA, dichloroprop, mecoprop, 2,4-DB, MCPB, and propyzamide.

Experimental

Separation of the acidic herbicides is achieved using 0.01% formic acid in HPLC grade water and acetonitrile gradient elution [2]. The acid is present in the mobile phase to aid the retention and separation of the compounds. Instead of using a weak solution of ammonia (0.1%) in the mobile phase, the ammonia was replaced with triethylamine added directly to the post column 2-propanol at a concentration of 0.01%. This mix was added post-column at 0.2 mL/min. All samples were spiked at 0.1 µg/L for each compound and matrix tested. Results shown in Table 2.

Both sets of data were obtained from fully validated methods, each data set comprising of 11 batches of samples analyzed in duplicate.

Final Test Conditions

LC Conditions			
Instrument	Agilent 110 Series HPLC		
Column	ZORBAX Extend-C18, 2.1 mm × 150 mm × 3.5 µm		
Temperature	60 °C		
Mobile phase A	0.1% Ammonia in HPLC grade water		
Mobile phase B	Acetonitrile		
Gradient	Initial	%B	5
	0.5 min	%B	5
	1.0 min	%B	40
	12.0 min	%B	80
	13.0 min	%B	80
	13.5 min	%B	5
Flow rate	0.3 mL/min		
Post-column addition	2-Propanol at 0.2 mL/min		
Mass spectrometer conditions			
Drying gas flow rate	13.0 L/min		
Drying gas temperature	350 °C		
Nebulizer pressure	50 psi		
V_{cap}	3000 V (negative)		
SIM ions	E1 = 269	Fragmentor = 190 V	
	E2 = 271	Fragmentor = 210 V	
	EE2 = 295	Fragmentor = 200 V	

Table 2. Acidic Herbicides Performance Data (Expressed as % recovery)

	Without post-column		With post-column*	
Compound	**Bore water (%)**	**Tap water (%)**	**Bore water (%)**	**Tap water (%)**
2,4-D	85.0	76.7	85.3	84.0
MCPA	91.5	83.2	84.9	84.4
Dichloroprop	95.8	89.8	84.3	85.0
Mecoprop	96.6	93.9	82.9	82.7
2,4-DB	90.7	81.6	87.1	87.8
MCPB	93.2	84.2	87.6	88.6
Propyzamide	86.8	87.5	82.6	84.6

* With post-column addition of 0.01% triethylamine in 2-propanol at 0.2 mL/min.

The results for the acidic herbicides, without post-column addition, showed the effects of ionization suppression, shown in Table 2. In particular 2,4-D, MCPA, 2,4-DB, MCPB and dichloroprop showed differences in recoveries between the two matrices approaching 10%.

When post-column addition of 0.01% triethylamine in 2-propanol was used, there was no significant difference in the recovery values from either matrix; hence the effects of ionization suppression are reduced.

References

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2. N. Cullum, P. Stephens, and S. Evans, “Determination of Acidic Herbicides in Groundwater and Potable Water by LC/MSD using Selective Ion Monitoring”, Agilent Technologies, publication 5988-5882EN, www.agilent.com/chem

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Printed in the USA
May 31, 2005
5989-2883EN

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