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Can ion suppression be quantitated?

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Abstract

Today, LC/MS/MS is without question the preferred platform for the analysis of organic molecules from complex matrices. Various fields such as pharmaceuticals, toxicology, environmental and clinical, have made the transition from the traditional one-dimensional system (i.e. LC/UV … etc) to hyphenated technologies (LC/MS, LC/MS/MS … etc). Most applications utilize the electrospray ionization (ESI) interface (about 80 % of published papers) versus atmospheric pressure chemical ionization (APCI). Several reasons can be presented to explain this trend. ESI is an interface that is relatively easy to use, exhibits low solvent consumption and can be used for large analytes (up to 100 KDa), wide polarity range and applicability to thermally labile compounds.

Unfortunately, ESI is prone to a phenomenon called "ion suppression"[1,2,3]. Ion suppression can lead to adverse effects during quantitation (i.e. poor accuracy and precision). Previous papers have reported that ion suppression is a direct result of endogenous material present in biological samples. However, the measured result is the combination of several operating conditions and parameters of an SPE/LC/MS/MS system. Little has been done to effectively monitor and/or choose optimized conditions for the extraction, clean up, separation and analysis. This work presents a simple setup for quantification of ion suppression/enhancement. Several mobile phase additives, ion pairing agents and SPE extracts were measured against a standard reference. The results demonstrated that a mixed mode ion exchange protocol leads to minimal ion suppression/enhancement (< 10 %) for compounds that were investigated.

1- Miller-Stein C, Bonfiglio R, Olah TV, King RC, *Am. Pharm. Rev.*, 2000, **54**
2- Matuszewski BK, Constanzer ML, Chavez-Eng CM, *Anal. Chem.*, 2003, **75**: 675
3- Mallet, CR, Lu, Z, Mazzeo, JR, Neue, UD, *Rapid Commun. Mass Spectrometry*, 2002, **16**: 805

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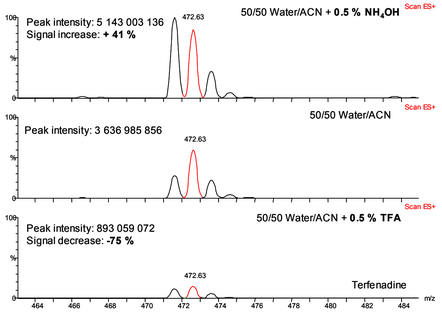
What is ion suppression or enhancement ?



Ion suppression or enhancement is a known phenomena that occurs with a mass spectrometer equipped with an electrospray interface (ESI). Several papers in the literature explain in detail the formation of ions with this type of source, but several parameters, effects or observations are still unclear. For example, is ESI concentration or mass flow dependent? Some papers suggest that ESI is concentration/mass flow combination. Most applications use the ESI source in combination with LC (mostly reversed-phase column). The problem of suppression or enhancement occurs when additives in the mobile phase can either increase or decrease the intensity of the target analyte. Sample extracts also produce the same effect.

Acidic additive

Trifluoroacetic acid
Acetic acid
Formic Acid

Basic additive

Ammonium hydroxide
Pyrrolidine

Detergents

Triton X100
SDS

Buffers

Ammonium formate
Ammonium bicarbonate
Ammonium biphosphate

Ion pairing additive

Tetraethylammonium hydroxide
Dimethylhexylamine

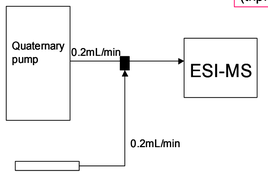
SPE extracts

protein precipitation
Reversed phase 1-D
Reversed phase 2-D
Mixed mode

Experimental setup

50/50 ACN/ H_2O
8 compounds

Compare 50/50 ACN/ H2O to additive stream signal (triplicates) blank, matrix, blank

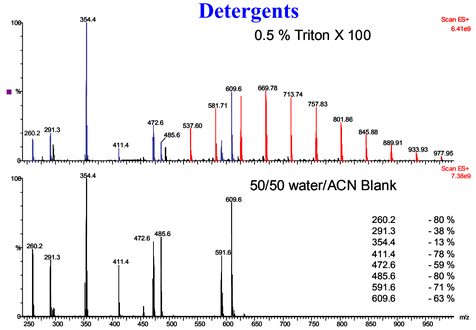


Infusion pump
Used to add range of modifiers, salts, ion pairs, pH additives, Matrix extracts

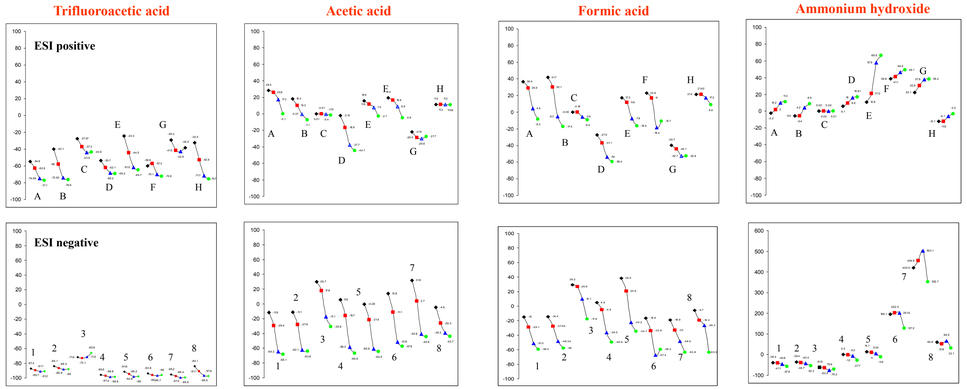
ES+	
260.2	Propranolol
291.3	Trimethoprim
354.4	Pipenzolate*
411.4	Resperidone
472.6	Terfenadine
485.6	Methoxy-Verapamil
591.6	Benextramine
609.6	Reserpine

*quaternary amine drug

Detergents



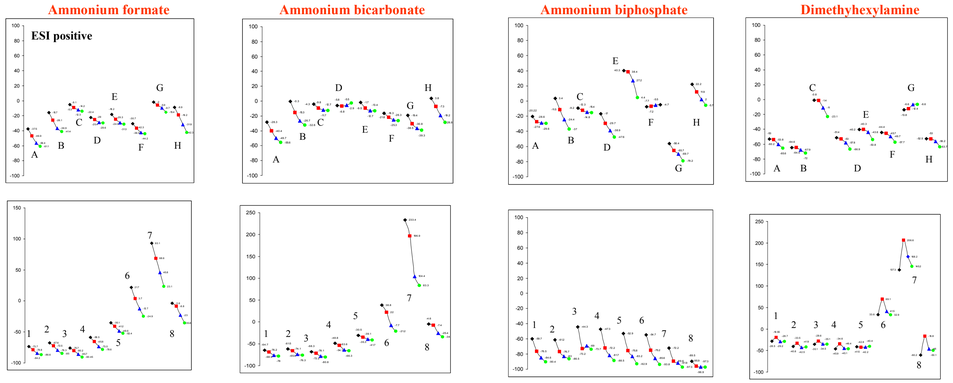
pH additives



◆ = 0.05 %
■ = 0.1 %
▲ = 0.5 %
● = 1.0 %

A = Propranolol B = Trimethoprim C = Pipenzolate D = Resperidone E = Terfenadine F = Methoxy-verapamil G = Benextramine H = Reserpine
1 = Fumaric Acid 2 = Malic acid 3 = Etidronic Acid 4 = Clodronic acid 5 = Niflumic acid 6 = Canrenoic acid 7 = Cholic acid 8 = Raffinose

Salt & Ion pairing additives*



◆ = 5 mM
■ = 10 mM
▲ = 20 mM
● = 50 mM

* pH not adjusted

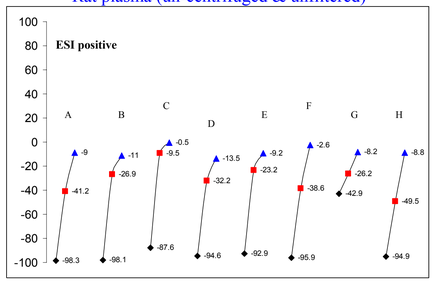
A = Propranolol B = Trimethoprim C = Pipenzolate D = Resperidone E = Terfenadine F = Methoxy-verapamil G = Benextramine H = Reserpine
1 = Fumaric Acid 2 = Malic acid 3 = Etidronic Acid 4 = Clodronic acid 5 = Niflumic acid 6 = Canrenoic acid 7 = Cholic acid 8 = Raffinose

Extraction Protocols*

Protein Precipitation	Reversed phase Method	Mixed mode Method
0.5 mL spiked plasma	Condition with 1.0 mL MeOH	Condition with 1.0 mL MeOH
Add 0.5 mL MeOH to precipitate plasma	Equilibrate with 1.0 mL H_2O	Equilibrate with 1.0 mL H_2O
Centrifuge the plasma at 5000 rpm for 5 minutes	Load 1.0 mL spiked rat plasma (diluted with H_2O 1:1)	Load 1.0 mL spiked rat plasma (diluted with H_2O 1:1)
Transfer the supernatant to a 2 mL 96-well container	Wash with 1.0 mL 5% MeOH in H_2O	Wash with 1.0 mL H_2O + 0.1 N HCl
	Elute with 0.5 mL MeOH	Wash with 1.0 mL Methanol
	Dilute with 0.5 mL H_2O	Elute with 0.5 mL MeOH + 2% NH_4OH
		Dilute with 0.5 mL H_2O

* These extraction protocols were designed for this ion suppression study using a 96-well plate packed with 10 mg of sorbent. The final steps and volumes are not optimized for LC/MS/MS applications.

Rat plasma (un-centrifuged & unfiltered)



◆ = protein precipitation ■ = reversed phase ▲ = mixed mode

A = Propranolol B = Trimethoprime C = Pipenzolate D = Resperidone
E = Terfenadine F = Methoxy-verapamil G = Benextramine H = Reserpine

Conclusions

On average, up to 95 % of the ESI signal was suppressed when rat plasma samples were prepared using a protein precipitation protocol for both solutions of both basic and acidic test analytes. The same conclusion can be drawn for human plasma. However, when a mixed mode SPE method is used to prepare plasma extracts, the results shows an average of only 10 % suppression and even an enhancement effect for some compounds. This is certainly not a novel observation, and has been reported in the literature since the introduction of LC/MS/MS for such analytical applications. It certainly shows that in order to gain lower LOQ and better accuracy, an optimized extraction and clean up protocol becomes crucial.