Determination of the Level of Ion Suppression from LC/MS Vials

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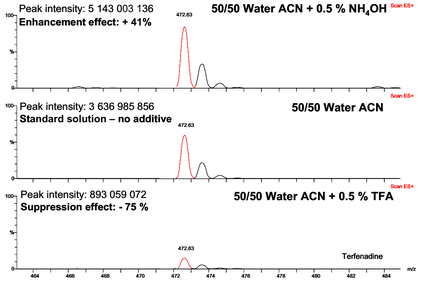
Abstract

Analyses are currently performed with the assistance of hyphenated instruments, especially SPE/LC/MS/MS systems. Many applications utilize electrospray ionization interface (ESI) versus a chemical ionization interface (APcI). This trend is easy to explain; the ESI interface is easy to use, has a large mass range (up to 100 kDa), a wide polarity range and is also applicable to thermally labile compounds. However, ESI is prone to a phenomena called "ion suppression"[1,2,3]. The mechanism of ion suppression is not well understood, but several sources were identified, such as the sample matrix and chromatographic conditions. In some cases, the suppression effect results in a 95 % loss of signal; in other situations, enhancement of the signal is observed.

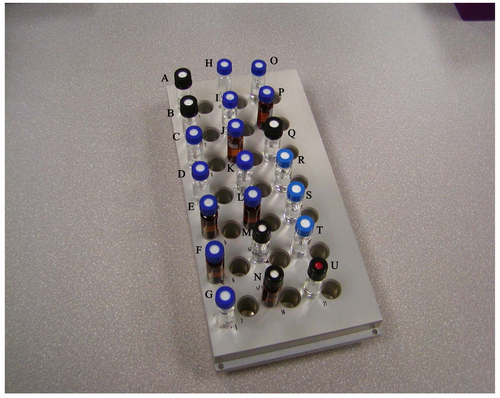
Mass spectra (100 – 1000 amu) were acquired for each test solution and ion intensities of each drug were measured against a common reference. The vials were subjected to rigorous testing conditions, such as various percentages of organic, strong acid and strong base. A previous paper[3] reported the effects of sample preparation techniques and mobile phase composition on the signal of ESI in positive mode using a simple setup for the quantification of ion suppression/enhancement. This work continues that study and focuses on another potential sources of suppression or enhancement, the sample vials. The concern is related to the potential leaching of material from various compositions of glass, septum and/or the presence of residues left behind from the manufacturing process.

[1] Miller-Stein C, Bonfiglio R, Olah TV, King RC, *Am. Pharm. Rev.*, 2000, **3**, 54.
[2] Matuszewski BK, Constanzer ML, Chavez-Eng CM, *Anal. Chem.*, 2003, **75**: 675.
[3] Mallet, CR, Lu, Z, Mazzeo, JR, *Rapid Commun. Mass Spectrometry*, 2004, **18**: 49.

What is ion suppression or enhancement?

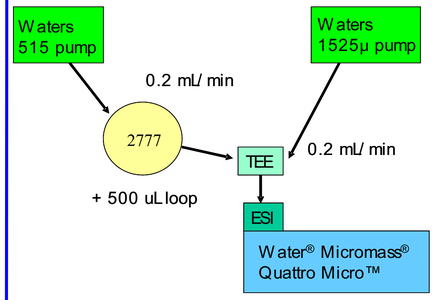


The ESI source is not a new technology. It was borrowed from the automotive industry and takes its origin from the principles of electro-painting. The fundamental role of ESI is relatively easy to understand. The liquid effluent is converted into fine droplets by a process called "pneumatic nebulization". This step is produced with a coaxial probe with the assistance of a nebulization gas (nitrogen) and a high voltage (kilovolts). As the droplets are produced, the presence of the high voltage ensures that static charges will be positioned on the surface of those droplets, hence the name "electrospray". With high temperature, the size of a droplet will shrink to a smaller size due to a desolvation effect, up to a point that the multiple charges on the surface can no longer co-exist and thus will create a "coulomb explosion". At that point, the charged species trapped in the droplet becomes a free ion in the gas phase. It is for this reason that ESI is also referred as to "solution chemistry", meaning that the ionization is produced by a chemical process in the solution rather than gas phase chemistry (e.g. electron impact, chemical ionization). The surface charges exhibit a pulling effect on ions present in the bulk of the droplets and moving those ions toward the outer surface. During that transition, multiple side-reactions (e.g. collisions) can occur. This is the core of the ion suppression/enhancement debate.

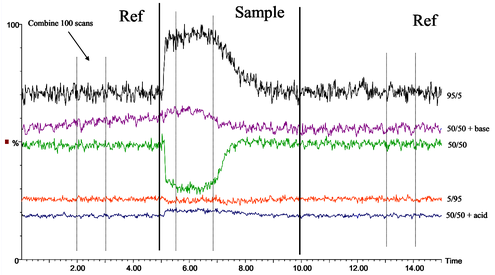


Waters:
A, B, C, D, E, F
Other vial manufacturers:
G, H, I, J & U – United Kingdom
K, L, M, N, O, P, Q, S & T – United States
R- China

Experimental setup

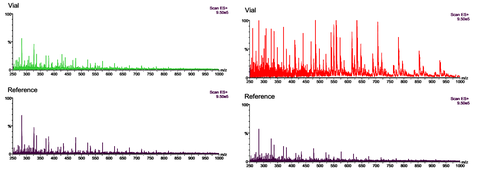


The experiment was conducted with the above two pump configuration. Both pumps are connected to the same mobile phase reservoir and both lines are connected to the mass spectrometer via a tee. An autosampler is inserted in one of the lines as seen in the above diagram. Several mobile phases compositions (5 %, 50 % & 95% methanol) and additives (formic acid & ammonium hydroxide both at 1 %) were evaluated to see if a particular condition would be suited for this task. The measurements were done following the following protocol: each vial was filled with 1.5 mL of a solvent mixture and let equilibrate for 4 hours. The measurements were made by recording the effluent of both pumps with the injection valve in the load position (reference), followed by the injection valve in the inject position (sample) and back to load position for confirmation. Each sequence was measured for 5 minutes for a total of 15 minutes. A hundred scans was combined for the reference and the sample as shown in the diagram below.

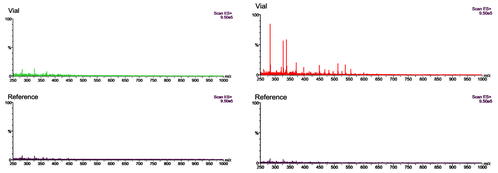


Note: each measurement was done with three vials and duplicate injection for a total of 6 runs per vial

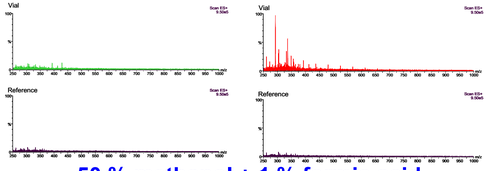
95 % methanol + no additive



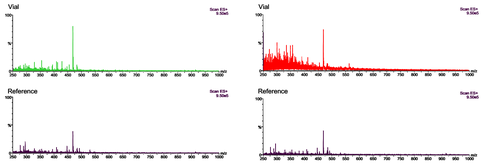
50 % methanol + 1 % ammonium hydroxide



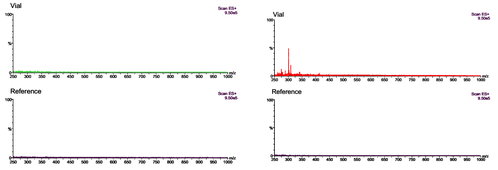
50 % methanol + no additive



50 % methanol + 1 % formic acid



5 % methanol + no additive



Vials	95%	50%+A	50%	50%+B	5%
A	Trace level	Trace level	Trace level	Trace level	Trace level
B	Minor contaminants	Minor contaminants	Minor contaminants	Intermediate contaminants	Minor contaminants
C	Intermediate contaminants	Trace level	Trace level	Minor contaminants	Trace level
D	Intermediate contaminants	Minor contaminants	Trace level	Minor contaminants	Trace level
E	Intermediate contaminants	Trace level	Trace level	Minor contaminants	Trace level
F	Major contaminants	Minor contaminants	Trace level	Major contaminants	Trace level
G	Trace level	Minor contaminants	Minor contaminants	Minor contaminants	Trace level
I	Intermediate contaminants	Minor contaminants	Minor contaminants	Trace level	Minor contaminants
J	Minor contaminants	Trace level	Trace level	Trace level	Trace level
K	Major contaminants	Minor contaminants	Intermediate contaminants	Trace level	Trace level
L	Intermediate contaminants	Minor contaminants	Intermediate contaminants	Trace level	Minor contaminants
M	Minor contaminants	Minor contaminants	Intermediate contaminants	Minor contaminants	Minor contaminants
N	Trace level	Trace level	Minor contaminants	Intermediate contaminants	Trace level
O	Trace level	Minor contaminants	Intermediate contaminants	Trace level	Trace level
P	Intermediate contaminants	Minor contaminants	Intermediate contaminants	Minor contaminants	Trace level
Q	Minor contaminants	Minor contaminants	Major contaminants	Minor contaminants	Minor contaminants
R	Intermediate contaminants	Minor contaminants	Major contaminants	Intermediate contaminants	Trace level
S	Intermediate contaminants	Minor contaminants	Intermediate contaminants	Intermediate contaminants	Minor contaminants
T	Major contaminants	Minor contaminants	Major contaminants	Intermediate contaminants	Minor contaminants
U	Major contaminants	Major contaminants	Major contaminants	Intermediate contaminants	Trace level

Trace level
Minor contaminants
Intermediate contaminants
Major contaminants

Conclusions

The mechanisms of ion suppression/enhancement are not well understood at this time. However, it is clear that in order to achieve quality results, it is necessary to identify the major sources of suppression or enhancement. Adding to a previous study of mobile phase additives and sample preparation (protein precipitation), vials were added to see the extent of their contributions. This preliminary study raised many questions, but one stood out: "how much suppression/enhancement is acceptable?". It becomes clear that the contribution from a contaminated vial can have a measurable effect during quantitation.

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