Mechanistic Investigation of Ionization Suppression in Electrospray Ionization

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We show results from experiments designed to determine the relative importance of gas phase processes and solution phase processes into ionization suppression observed in biological sample extracts. The data indicate that gas phase reactions leading to the loss of net charge on the analyte is not likely to be the most important process involved in ionization suppression. The results point to changes in the droplet solution properties caused by the presence of nonvolatile solutes as the main cause of ionization suppression in electrospray ionization of biological extracts. (J Am Soc Mass Spectrom 2000, 11, 942–950) © 2000 American Society for Mass Spectrometry

One of the most common analyses in the pharmaceutical industry is the analysis of biological fluids for a target analyte and related species. The use of liquid chromatography-tandem mass spectrometry (LC-MS/MS) has now become the standard technique throughout the pharmaceutical industry for quantitative analysis of drug compounds and related materials. The general applicability, with the inherent selectivity and sensitivity, has made LC-MS/MS the most important modern quantitative analytical technique in the industry. Routine analysis of samples from studies aimed at determining safety, efficacy, and pharmacokinetic and pharmacodynamic properties of new drug entities is now accomplished primarily by the use of atmospheric pressure ionization (API) LC-MS/MS sample analysis.

Even with the wide-spread use and remarkable success of API LC-MS/MS in quantitative analysis, there are still problems with the technique that can invalidate quantitative results [1, 2].

A number of researchers have shown that the response observed in electrospray can be affected by factors other than analyte concentration (Table 1) [3–12]. Most of these factors can be controlled to be reproducible from sample to sample, in which case they may affect overall sensitivity, but not the quantitative abilities of the technique. The solution pH, electrolyte concentration, and solution properties of the electrospray droplets will depend on the exact composition of the droplet which may vary from sample to sample. One of the often-observed consequences of unexpected changes in these factors is the variability in target compound response typically referred to as ionization suppression.

Sample molecules start out in the solution phase in the LC mobile phase. The liquid is formed into charged drops by the electrospray source. While in the solution phase in the drops, the analyte has several possible pathways. As the solvent evaporates, the analyte may precipitate from solution either as solid compound or as a coprecipitate with other nonvolatile sample components. Alternatively, the analyte may remain in the unevaporated portion of the liquid stream and consequently collect on the interface plate of the mass spectrometer. Analyte might also be transferred to the gas phase as an ion, a neutral gas phase molecule, or as part of a charged solvent cluster. Once in the gas phase as an ion or charged solvent cluster, the charge might be lost through neutralization reactions, charge stripping, or charge transfer to another gas phase species. Only in the case of an isolated ion or charged solvent cluster reaching the declustering region of the API source is there any possibility of observing the analyte ion in the mass spectrometer. Any mechanism that might decrease the production rate of small droplets, and ultimately gas phase analyte ions, could participate in ionization suppression [13–18].

The objective of the experiments described below was to determine the relative importance of gas phase and solution phase processes to the ionization suppression observed in typical study sample extracts. The information obtained about the causes of ionization suppression may lead to practical ways to eliminate the ionization suppression problems observed during analysis of study sample extracts.
The experiments described below were carried out with a variety of drug molecules. Data for experiments conducted with Urapidil, Phenacetin, and two Merck structural analogs are presented (Figure 1). Each experiment was repeated a minimum of three times to provide an assessment of the reproducibility of the reported results. Representative data are shown.

**Materials**

Phenacetin, caffeine, and urapidil were purchased from Sigma (St. Louis, MO) and used without further purification. The Merck compound and structural analog were synthesized by Merck (West Point, PA). All solvents used were high-performance LC (HPLC) grade or better.

**Extractions**

Three common sample preparation techniques were used to generate the blank extracts: methyl t-butyl ether (MTBE) liquid–liquid extraction, protein precipitation, and solid-phase extraction. The liquid–liquid extractions were performed by adding 3 mL of MTBE to 0.5 mL of control dog plasma buffered to pH 7.4 with 0.1 M K<sub>2</sub>HPO<sub>4</sub> in 13 × 100 mm glass test tubes with caps. After agitating in a shaker for 10 min, these samples were spun for 10 min at 3000 rpm in a Beckman (San Ramon, CA) GS-6 centrifuge. The top liquid layer was transferred into clean 12 × 75-mm glass test tubes. The tubes were then evaporated to dryness at 50 °C under dry nitrogen in a Turbo Vap (Zymark, Hopkinton, MA).

Solid-phase extractions were performed using Oasis HLB (30 mg) cartridges from Waters (Milford, MA) and Empore C8 disks from 3M (St. Paul, MN). Oasis cartridges were preconditioned with 1.0 mL of 100% methanol followed by 1.0 mL of distilled water. Blank plasma samples (0.5 mL) were then transferred from 10 × 75-mm glass test tubes to the cartridges and loaded on the cartridges at a flow rate of approximately 2 mL/min. The cartridges were then rinsed with 0.250 mL of distilled water and 1.0 mL of 5% methanol. The samples were eluted with 1 mL of 100% methanol. All samples extracted with SPE cartridges were evaporated to dryness in a Turbo Vap at 50 °C under dry nitrogen.

Protein precipitation samples were prepared by adding 1 mL of acetonitrile containing 0.1% TFA to 0.5 mL of control dog plasma in 2-mL plastic microcentrifuge vials. The samples were mixed on a vortex mixer for 1 min and centrifuged for 20 min at 11,000 rpm. The supernatant liquid was decanted into 12 × 75-mm glass test tubes and evaporated to dryness under nitrogen in a Turbo Vap at 50 °C. Dried samples from all three preparations methods were each reconstituted in 150 µL of 50/50 acetonitrile/water containing 0.1% formic acid, sonicated in an ultrasonic bath for 10 min, mixed on a vortex mixer for 1 min, and transferred to micro-injection vials.

**Infusion Chromatograms**

*Postcolumn infusions.* The postcolumn infusion system is schematically represented in Figure 2. The chromatographic portion system consisted of a Hewlett-Packard model 1050 HPLC and autosampler (Palo Alto, CA).