

# LC-MS Ionization Effect from Solid Phase Extraction Extractables

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## Abstract/Introduction

Ionization suppression (ion-suppression) in electrospray ionization (ESI) is an ever-growing concern for LC-MS users. Ion-suppression and/or -enhancement can greatly inhibit the ability for accurate quantitation. There have been many studies on the effect of matrix-induced ion suppression from biological samples and each use some form of sample preparation to remove interfering matrices. The process of solid phase extraction (SPE) itself can often induce ionization effects during MS analysis. This is potentially due to extractables endogenous to the SPE hardware (polypropylene cartridges/ 96-well plates) that co-elute with analytes of interest during SPE methodology. Although SPE is widely used in mass spectrometry, there have been few studies on the effect of extractables during the process of SPE on ionization. Known impurities in cartridges such as polyethylene glycols (PEGs) and

phthalates are readily extracted with commonly used organic solvents. Elution conditions for solid phase extraction typically require high organic solvents modified for pH or ionic strength. Although the SPE cartridges are designed for one time use, such elution conditions can cause deterioration of the stationary phase. These impurities are then eluted with the sample and may affect ionization either by suppression or enhancement.

The objective was to study what effect the SPE phases contribute to ionization suppression/enhancement in electrospray mass spectrometry. Several commonly used SPE phase chemistries were evaluated with a generic elution condition to measure the ionization effects from extractables. The phases that were evaluated were a standard C18, strong anion-exchange, strong cation-exchange, mixed-mode and a hydrophilic polymer. An empty polypropylene SPE cartridge with frits was also evaluated. A range of acidic, neutral and basic compounds (standard test mix) was used as a control to measure the effect of solid phase extraction extracts on MS signal suppression/enhancement.

## Methodology

The generic sample preparation procedure is detailed in Figure 1. Pure DI water was loaded onto each cartridge pre-conditioned with methanol and DI water. The cartridges were then washed with a 10% methanol followed by methanol elution. After collecting the eluate from the SPE, an aliquot of the eluate was spiked with an equal volume of the 1.0 µg/mL standard test mix. The post-SPE spiked eluate was injected directly into the LC-MS instrument for analysis (no analytical chromatography). All testing was performed on a Waters Micro Mass ZQ single quadrupole mass spectrometer with electrospray ionization in positive ion mode (ESI+). The sample was introduced directly into the MS by performing an automated loop injection with a carrier solvent of 50:50 methanol:water (LC-MS grade). The analysis was performed by taking a cumulative spectral scan across the entire chromatographic peak as shown in Figure 2. The resulting mass spectrum was then integrated, and the area for each standard ion was tabulated. The quantitation of samples was performed using a bracketed calibration table. This was necessary to account for drift in signal response that occurred due to contamination of the source.

Figure 1. System Diagram for Generic SPE Method

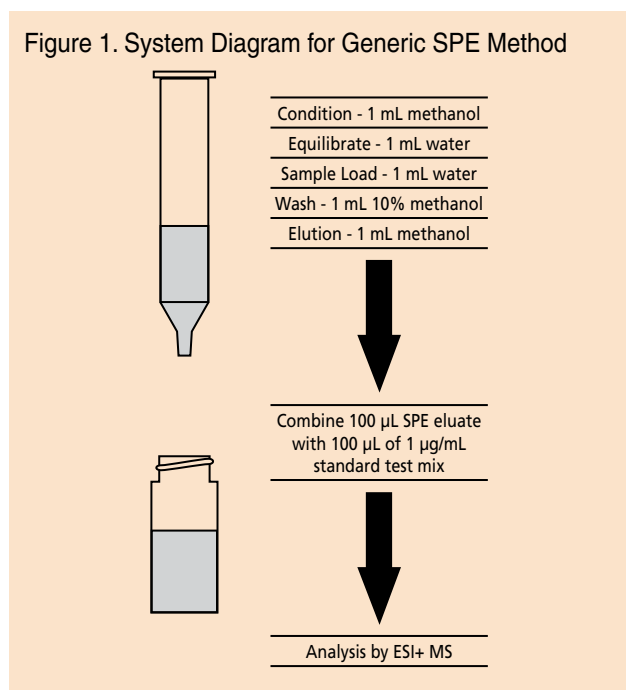
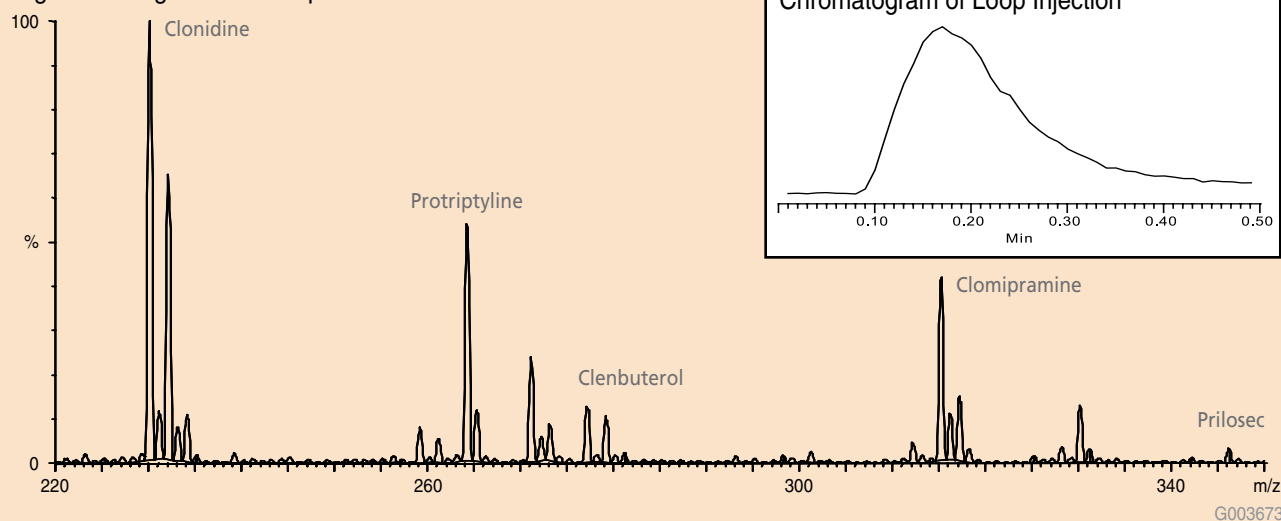


Figure 2. Integrated Mass Spectra of Standard Mixture



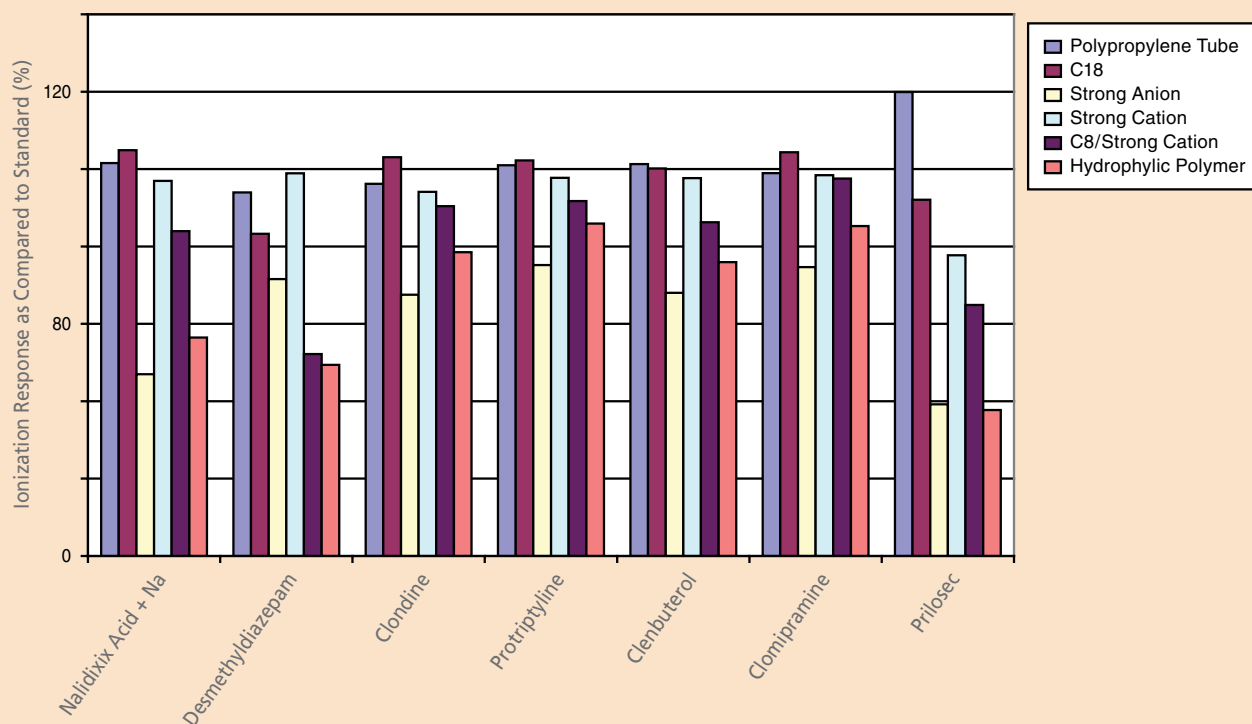
## Results

The results of the ion-suppression study are summarized in Figure 3. The effect on ionization varies significantly when comparing the response from the different phase chemistries. The non-polar hydrophobic C18 reversed phase chemistry contributed little effect on the ionization of the standard compounds, this was also observed with the extractables from the polypropylene tube. As to be expected, extractables from the strong anion-exchange phase had the greatest effect on ionization with up to

60% ion-suppression. This can be explained from the mechanism of charge competition in the ESI+ source as these extractables are readily ionizable in positive ionization mode. The strong cation-exchange stationary phase exhibited some ionization effect but not significant. The acidic functionality of this phase is not as readily ionizable in ESI+ mode as compared to the strong anion exchange phase. Though ion-suppression, due to ion-pairing from the strong cation-exchange SPE extractables

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Figure 3. Ionization Effect of SPE Extractables Using Generic SPE Method



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with the basic standard compound producing a neutral species was expected, this was not observed. The mixed mode C8/ strong cation exchange also contributed up to 50% ion-suppression. We suspect that this was due to the total concentration of extracted ionizable species. The high concentration of extractables increases the surface tension of the ESI+ droplet preventing charged ions from forming. The polymeric phases also exhibited up to a 60% ion-suppression. This can be due to the modification performed on the polymer to enhance the hydrophilic properties. These polar functional groups are also readily ionizable and act in competition with the test compounds.

### Conclusion

This observation supports the need for calibration standards to be prepared in the blank eluate derived from the SPE method. Often when measuring absolute recovery from SPE cartridges, the stock standard is used for calibration. By doing so, this does not account for the signal suppression from the phase bleed and is assumed to be due to low recovery from the SPE cartridge. Using

the eluate from the SPE cartridge to prepare the calibration standards enables a more accurate measurement of the absolute recovery from the SPE cartridge.

### References

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