An Investigation of the Impact of Common Experimental Parameters on Signal Intensity in SFC ESI MS

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Introduction

In the past decade, supercritical fluid chromatography (SFC) has experienced a steady growth in acceptance, particularly in pharmaceutical and chemical laboratories. In SFC, a “supercritical” fluid, most commonly CO₂, in combination with one or more polar organic solvents, such as alcohols, is used as mobile phase. Compared to HPLC, SFC offers better selectivity and shorter analysis time due to the low viscosity and high diffusivity inherent to supercritical fluids [1]. The ongoing acetonitrile (ACN) shortage has also stimulated an elevated interest in employing SFC as a possible alternative to the industry-dominating, ACN-reliant reversed phase LC (RPLC).

Advances in detection in SFC have contributed, at least in part, to its resurgence and increased acceptance by HPLC practitioners. The detection in SFC has gradually evolved to encompass many LC-type detectors, including the ultra-violet (UV) detector, the evaporative light scattering detector (ELSD), and the mass spectrometer (MS). As the general analytical philosophy gradually shifts from enhancing capacity and efficiency to generating high-quality and more informative data within a minimal time frame [2], SFC MS readily lends itself as an attractive complement to RPLC MS, mainly due to the combination of the high speed and unique selectivity of SFC and the intrinsic universality, sensitivity, and specificity of MS.
While much has been accomplished in LC MS, in both understanding its fundamentals as well as the practices, their counterparts in SFC MS remain limited in scope. We report herein part of our investigation of the impact of some common experimental parameters on signal intensity in SFC electrospray (ESI) MS. We hope that the observations from our study can stimulate more fundamental research activities in this area; and contribute, albeit small, to a better understanding of this emerging hyphenated technique.

**Experimental**

All chemicals were purchased from Sigma Aldrich (St. Louis, Missouri, USA) and used as received. HPLC grade methanol was purchased from Thermo Fisher Scientific. All samples were dissolved in methanol to make stock solutions of 0.25 mg/mL, with the exception of theobromine, where a saturated solution (estimated to be 0.02 mg/mL) was used instead. The chemical structures and molecular masses of the compounds used in this study are listed in Table 1.

A TharSFC SFC-MS Resolution II system (TharSFC, A Waters Company, Pittsburgh, Pennsylvania, USA) equipped with a 2998 photo diode array (PDA) detector and 3100 MS (Waters Corp., Milford, Massachusetts, USA) was used for all experiments. Electrospray mode was used for all experiments. The system was controlled by Masslynx® software. A 4.6 x 50 mm silica column (Kromasil, Bohus, Sweden) was used in this study. The effluent after the back pressure regulator (BPR) was directly flown into the inlet of the MS through a 50 µm ID PEEKsil tube (Upchurch, Oak Harbor, Washington, USA).

For all experiments, methanol was used as the modifier (co-solvent). Other general experimental parameters included: flow rate, 2.0 mL/min; injection volume, 5 µL (full loop injection); cone voltage, 40 V; source temperature, 150°C; cone gas flow rate, 70 L/hr; and mass scan, 100 to 400 amu. Investigated parameters were capillary voltage, 2 - 4 kV; desolvation gas temperature, 400 - 500°C; and mobile
phase composition, 10 - 50% methanol. Triplicate injections were performed under each condition and peak areas were averaged for each data point.

Results and discussion

Figure 1 shows representative extracted ion chromatograms (EIC) of the compounds from all four compound classes. The first parameter we studied was capillary voltage, and the results are summarized in Figure 2. Two neutral polar compounds, sulfamerazine and sulfaquinoxaline, exhibited the highest signal intensity among the group, most likely due to primary amine moiety in the molecules that are highly receptive to protons. Two acidic compounds, ketoprofen and acetaminophen, generated much lower signal intensity, as expected in the positive ionization mode; although the secondary amine group in acetaminophen seemed to significantly boost the MS signal. Across all compound classes, the peak area increased as the capillary voltage increased. Application of higher voltages to the electrospray needle results in the production of smaller droplets, thus, more efficient ionization. The “unexpected” ionization behavior, i.e. increased signal intensity in the absence of high voltage reported by Langley et al. [3] was not observed in our experiments. It is speculated that the reported unusual ionization behavior is somewhat compound specific. It is noted, however, the likelihood of sonic spray ionization, where ions are formed due to the collision of molecules in the free jet expansion region, is expected to be much higher in SFC MS than in LC MS. One of the major differences between SFC MS and LC MS arises from the mobile phase. In SFC, supercritical CO\textsubscript{2} in combination with one or more polar solvents, most commonly alcohol is used as the mobile phase. The supercritical state, or near-critical state, is maintained by regulating system pressure via a BPR, typically above 100 bar. On the other hand, nebulization and evaporation of the ionization process all take place at near atmospheric pressure. The dramatic pressure drop will cause CO\textsubscript{2} decompression to form a jet spray regardless of the applied capillary voltage. Even without jet spray ionization, the aerosol formation due to CO\textsubscript{2} decompression is beneficial for the electrospray ionization process in the context of either ion-evaporation model or
charge-residual model. However, it is also noted this decompression process is highly endothermic. The overall impact is the result of the competition of the opposing factors brought about by CO₂ decompression.

Figure 3 summarizes the peak area change as the function of desolvation gas temperature. In general, a higher desolvation temperature (typically 400 °C or higher) is required for SFC MS to compensate for the endothermic effect of CO₂ decompression. As shown in Figure 3, the MS signal appeared to slightly decrease as the temperature increased from 400 to 500 °C. This is likely due to the thermal degradation of the compounds.

And finally, we evaluated the signal dependence on mobile phase composition. In LC MS, mobile phase composition influences MS response by changing the number and size of droplets formed during nebulization and thus, ionization of the analytes, due to the changes in solvent physical properties including viscosity and surface tension. The scenario is vastly different for SFC MS. At lower percentage of modifier, there is more CO₂ expansion, which will lead to more efficient nebulization and possibly more small droplets. Desolvation of the droplets formed, and thus ionization of the analytes, is favored by the initial production of small droplets. This is evidenced by the observed decrease in peak area as the percentage of modifier increased (Figure 4). On the other hand, because of the CO₂ decompression, by the time the analyte reaches the ion source, it is surrounded by virtually 100% modifier regardless of the original percentage. The variation in modifier percentage essentially transpires to the change in flow rate, or more accurately, the rate of liquid flow into the SFC MS interface. The higher the modifier percentage, the higher is the rate of liquid flow. Figure 4 displays a concentration-sensitivity at low modifier percentage (< 20%) and mass-flow-sensitivity at higher modifier percentage (>20%), typical characteristic for ESI [4]. A more detailed analysis will be published elsewhere.

Conclusions
A systematic investigation of the impact of capillary voltage, desolvation temperature, and modifier percentage on MS signal intensity in SFC MS was reported. Similar to LC MS, increasing capillary voltage resulted in higher signal intensity, contrary to previous reports that high sensitivity can be achieved in the absence of high voltage in SFC MS. Desolvation temperature seemed to have negligible impact on signal intensity in the range studied; although caution should be exercised to minimize possible thermal degradation of the analytes. In general terms, the MS signal decreased as the percentage of modifier increased due to the decrease in CO2 expansion and the increase in liquid flow rate to the SFC MS interface.

References


Jacquelyn Cole graduated from Grove City College with a B. S. in Chemistry. Jacquelyn has been working for TharSFC, a Waters Company as an application chemist and technical support since February 2005. Prior to joining TharSFC, Jacquelyn worked in the environmental lab at Allegheny Energy, Greensburg, Pennsylvania and Flexsys in Monongahela, Pennsylvania.

Lakshmi Subbarao graduated from University of Delaware with a B.S. in Chemistry in 2004 and Illinois Institute of Technology in 2008 with a M. S. degree in Analytical Chemistry. Lakshmi joined TharSFC, a Waters Company in January 2009 as an application chemist. Prior to joining TharSFC, Lakshmi worked for Advanced Materials Technology (Wilmington, DE).

Rui Chen obtained his B.Sc. in Material Chemistry in 1993, and his M. Sc. in Polymer Physics and Chemistry in 1996, both from Fudan University, Shanghai, China. Rui obtained his Ph.D. in 2002 in analytical chemistry from the University of Alberta, Canada. In 2002, he became an Alberta Ingenuity Research Fellow. In 2004, Rui joined the Institute of Chemistry and Cell Biology at Harvard Medical School. Rui joined METTLER TOLEDO AutoChem in September 2005. Currently Dr. Chen is the global technical support manager for TharSFC, a Waters Company. Rui has published over 10 papers in peer-reviewed journals, and made numerous presentations at national and international conferences.
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*Table 1. Chemical structures and molecular masses of the compounds used in this study.*
Figure 1. Representative extracted ion chromatograms (EIC) of the compounds used in this study obtained with 15% methanol: A) progesterone (neutral non-polar); B) theobromine (base); C) acetaminophen (acid); and D) sulfamerazine (neutral polar).
Figure 2. Peak area vs. capillary voltage. Desolvation gas temperature: 450 °C; desolvation gas flow rate: 650 L/hr; modifier percentage: 15% MeOH.
Figure 3. Peak area vs. desolvation gas temperature. Capillary Voltage: 3.5 kV; desolvation gas flow rate: 650 L/hr; modifier percentage: 15% MeOH.
Figure 4. Peak area vs. modifier percentage. Capillary Voltage: 3.5 kV; desolvation gas flow rate: 650 L/hr; desolvation gas temperature: 450°C.