Preliminary investigation of supercritical-fluid-assisted nebulization for enhanced response in electrospray mass spectrometry

T.R. Baker[1] and J.D. Pinkston[2]

[1] *Procter & Gamble Pharmaceuticals, Health Care Research Center, PO Box 8006, Mason, OH 45040-8006, USA*
[2] *The Procter & Gamble Company, Miami Valley Laboratories, PO Box 538707, Cincinnati, OH 45253-8707, USA*

A preliminary investigation of the use of supercritical-fluid (SF) CO_2 to assist nebulization in electrospray HPLC/MS has been conducted. When used in conjunction with a nebulizing gas and a separately applied drying gas, a significant benefit was derived from the use of CO_2. The sensitivity enhancement from the SF-assistance was more significant for flow rates above 0.2 ml/min with highly-aqueous mobile-phase compositions. For example, using a 0.5 ml/min flow-rate (85 % aqueous), a seven-fold increase in peak height and area was observed with SF-assistance.

Keywords: LC/MS, assisted nebulization, assisted electrospray, electrospray

Introduction

The electrospray technique is a well-known means of interfacing high-performance liquid chromatography (HPLC) with mass spectrometry (MS). Electrospray HPLC/MS interfaces transfer a fraction of the analyte from the liquid phase to the gas phase in an ionized form, allowing sensitive detection by a mass spectrometer. An integral step in all theoretical treatments of electrospray is rapid evaporation of the tiny droplets produced in the spray [1,2].

In its purest form (unassisted) electrospray operates most effectively at low µl/min flow rates [1]. However, most HPLC techniques operate at far higher flow rates, requiring either effluent splitting or flow-rate reduction, as in capillary HPLC, to be compatible with "unassisted" electrospray ionization. A number of means of assisting the electrospray process have been devised to allow the effective use of higher flow rates. Prominent among these is pneumatically-assisted electrospray or IonSpray®. As the name implies, this version uses a concentric stream of gas (nitrogen or air) to assist the nebulization process [3]. This allows the effective use of flow rates from the tens of µl/min to approximately 200 µl/min. A further enhancement of this approach is termed TurboIonSpray®. This involves the addition of a second stream of gas that is heated and directed perpendicularly to the eluent spray plume, between the sprayer tip and the vacuum orifice [4]. This permits effective use of HPLC flow rates from hundreds of µl/min up to 1 ml/min.

Other means of assisting the electrospray desolvation/ionization process are also widely utilized. Electrospraying eluent through a heated capillary tube [5,6], a multi-channel tube, or the so-called Z-spray® [7,8] has been used as a means of separating the ambient-pressure electrospray region from the lower-pressure region of the mass spectrometer. These arrangements are also assisted by a nebulizing gas and allow flow rates up to about 1 ml/min. Other less-widely-utilized means of assisting the electrospray process have also been reported. These include thermally-assisted electrospray [9] and ultrasonic nebulization [10].

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During our development of a modified electrospray interface for open-tubular [11] and then packed-column [12] supercritical-fluid chromatography/mass spectrometry (otSFC/MS and pcSFC/MS, respectively), we noted analyte response exceeding our expectations. We speculated that the enhanced response might be due to an improved spray plume (finer droplets) caused by a mobile phase consisting primarily of CO_2 with a lesser amount of polar organic solvent such as methanol, an essentially "self-nebulizing" mobile phase. One aspect of this unique nebulization was the appearance of the spray-cone formed by the SFC effluent in the pneumatically-assisted electrospray interface. We had previously made the empirical observation that one factor in obtaining strong and steady signal for an HPLC/IonSpray-MS experiment was a "proper" spray-cone. Specifically, a fine, symmetrical, and straight spray-cone produced the best results. The spray-cone produced by our pcSFC/MS interfacing effort visually appeared to be superior to any we had ever produced during HPLC/MS experimentation.

These observations led us to conduct a preliminary investigation of the addition of supercritical CO_2 upstream of the nebulization zone in the conventional IonSpray interface (with TurboIonSpray gun) operating with HPLC eluents. Analytes were injected in the flow-injection-analysis (FIA) mode using reversed-phase HPLC (aqueous) eluents. This limited investigation suggests that some benefit may be derived from supercritical-fluid (SF)-assisted HPLC/IonSpray-MS.

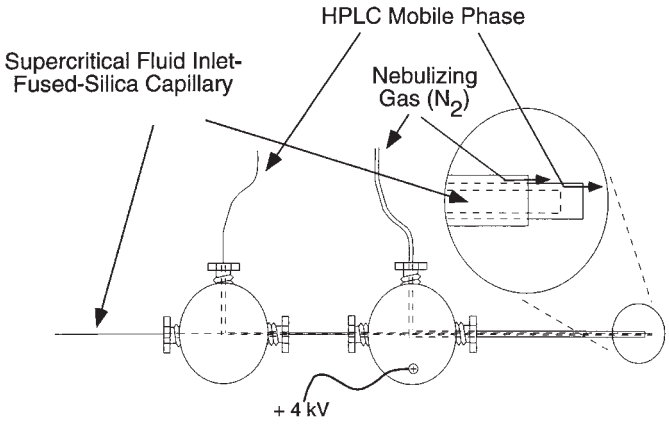

Figure 1. Modified IonSpray interface used for SF-assisted nebulization HPLC/MS.

Experimental

HPLC (FIA system)

The FIA flow was provided by Waters 600MS pumps (Milford, MA, USA). The FIA flow was connected to the "sheath-flow" inlet of the interface (Fig. 1), as originally designed for SFC/MS.

Supercritical CO_2 delivery

The supercritical CO_2 was introduced, as described below, using a Model 260D syringe pump from Isco (Lincoln, NE, USA) operated under pressure control. The cylinder of the pump was equipped with a cooling jacket and was cooled to approximately 10 °C using a Model RTE-111 circulating chiller from Neslab (Portsmouth, NH, USA). This ensured that the pump was efficiently filled with, and pumped, liquid CO_2. While the pump delivered a liquid, the CO_2 was heated beyond its critical temperature (31.08 °C) in the IonSpray interface (with TurboIonSpray gun on). We previously reported that the temperature of the components inside the IonSpray interface rises to ~60 °C during operation [11] in this mode.

Mass spectrometry

The experiments were conducted using a Model API III+ tandem quadrupole mass spectrometer from PE-Sciex (Concord, Ontario, Canada). The same ultra-high purity N_2 source (liquid N_2 boil-off) was used for curtain, nebulizer, and TurboIonSpray gases. The sprayer potential was held at 4 kV. Values used for the orifice ring voltage (45 V), curtain gas flow (1.4 liter/min), TurboIonSpray gun parameters (8 liter/min, 500 °C), nebulizer gas pressure (46 psi, 0.32 MPa), and interface plate temperature (55 °C) were typical of conventional HPLC/MS experiments. Values were as indicated by the instrument. The mass spectrometer was tuned in the conventional manner with an aqueous solution of poly(propylene glycol) (PPG) (1×10^{-4} M PPG 1000, 2×10^{-4} M PPG 2000, 1×10^{-3} M ammonium acetate) using the unaltered IonSpray interface. Direct infusion of the tuning solution was accomplished using a Model 22 syringe pump from Harvard Apparatus (South Natick, MA, USA) set at 10 µl/min.

Modified IonSpray interface

The IonSpray interface was modified for use as a pcSFC/MS interface, as previously described [12]. For these experiments we used the modified interface, as shown in figure 1. The liquid CO_2 was introduced through a fused-silica transfer line (deactivated fused-silica, 50-µm ID, 200-mm OD, Dionex, Sunnyvale, CA, USA). The transfer line extended through both tees and the stainless-steel tubing (see below) to the tip of the sprayer. The FIA or HPLC mobile phase entered the first tee (left in diagram). This liquid ran coaxially to the fused-silica CO_2 line through a 0.41-mm (0.016-in.) ID, 0.71-mm (0.028-in.) OD, 11.4-cm long, stainless-steel capillary (Small Parts Inc., Boca Raton, FL, USA). The mobile phase flowed in the space between the stainless-steel capillary and fused-silica transfer line and did not interact with the supercritical CO_2 until both reached the tip of the spray device. The nebulizer gas was added at the sprayer tee (right in figure 1), in the conventional manner. This gas flowed coaxially to the HPLC mobile phase and to the

supercritical CO_2 through a 0.84-mm (0.033-in.) ID, 1.27-mm (0.050-in.) OD, stainless-steel nebulizer tube. The fused-silica and stainless-steel capillary connections to the tees employed graphite-filled-Vespel capillary ferrules (Valco Instruments, Houston, TX, USA) or PEEK fittings (Upchurch Scientific, Oak Harbor, WA, USA). The stainless-steel mobile-phase capillary protruded past the end of the stainless-steel nebulizer-gas tube by approximately 1 mm. While the flow-path of the mobile phase was not optimized for low extra-column volume, this arrangement did serve to test the effect of SF assistance.

Upstream CO_2 addition

The HPLC effluent and liquid CO_2 were mixed approximately 30 cm from the standard API-III+ IonSpray sprayer tee using a low-dead-volume, stainless-steel, 1/16-inch Valco tee. The mixed effluent flowed from the mixing tee to the spray device through 50-µm ID, 150-µm OD fused-silica tubing.

Materials and sample preparation

Solvents and samples

All solvents were HPLC grade or better (J. T. Baker, Phillipsburg, NJ, USA). Carbon dioxide was SFC/SFE grade (Air Products, Allentown, PA, USA). Solutions of the analytes were prepared by dissolution in methanol. Norepinephrine was obtained from Lancaster Synthesis (Windham, NH, USA). The quinolonyl-lactam antibacterial (QLA) was synthesized in-house.

Mobile phases

The mobile phase for the norepinephrine analyses consisted of 85 % (v/v) 10-mM heptafluorobutyric acid in water with 15 % methanol. The water was deionized and purified with a Milli-Q Reagent Water System (Millipore, Bedford, MA, USA). For the QLA, the mobile phase was a 34/66 mixture of water/methanol with 0.1 % formic acid and 2 mM ammonium acetate overall.

Results and discussion

Our ultimate goal was to investigate the utility of SF CO_2-assisted HPLC/MS/MS as a means of improving sensitivity for the analysis of pharmaceutical compounds. For this work, we typically use reversed-phase eluents and 2-mm ID, 5 to 15-cm long HPLC columns. Due to the specificity of the tandem mass spectrometry detection scheme (selected reaction monitoring or SRM), these separations are typically rapid and use eluent flow-rates between 200 µl/min and 500 µl/min. The first compound we tested was norepinephrine. The L-form of this drug is used therapeutically as an adrenergic (vasoconstrictor) agent. FIA of this compound, using the aqueous mobile phase described above and monitoring the collisionally-induced dissociation (CID) reaction m/z 170 → m/z 107, was conducted using the modified interface shown in figure 1, but without SF CO_2 addition. A reproducible FIA profile was obtained in this manner. The typical example displayed in figure 2 (top) resulted from a 50-µl injection of 73 ng of norepinephrine (eluent flow 500 µl/min). Within minutes this experiment was repeated. The only difference was that SF CO_2 flow was added. With the SF CO_2 transfer line described above, 20 MPa provided approximately 2.0 ml/min fluid flow, or ~2.0 g/min, resulting in an expanded gas flow of ~1.2 liter/min in the source. The result with SF-assisted nebulization is shown in the lower portion of figure 2. A better than 7-fold signal enhancement was obtained with the SF-assistance, based on either peak height or area.

A similar experiment was conducted with a much larger amount of a different analyte, using a different mobile phase. QLAs have been investigated as antiinfective agents. We had developed an HPLC/MS/MS assay for one of these compounds that required an isocratic aqueous mobile phase as described above. Using a 500-µl/min flow of this aqueous mobile phase, FIA with MS/MS detection was performed with and without SF-assisted nebulization. The results for

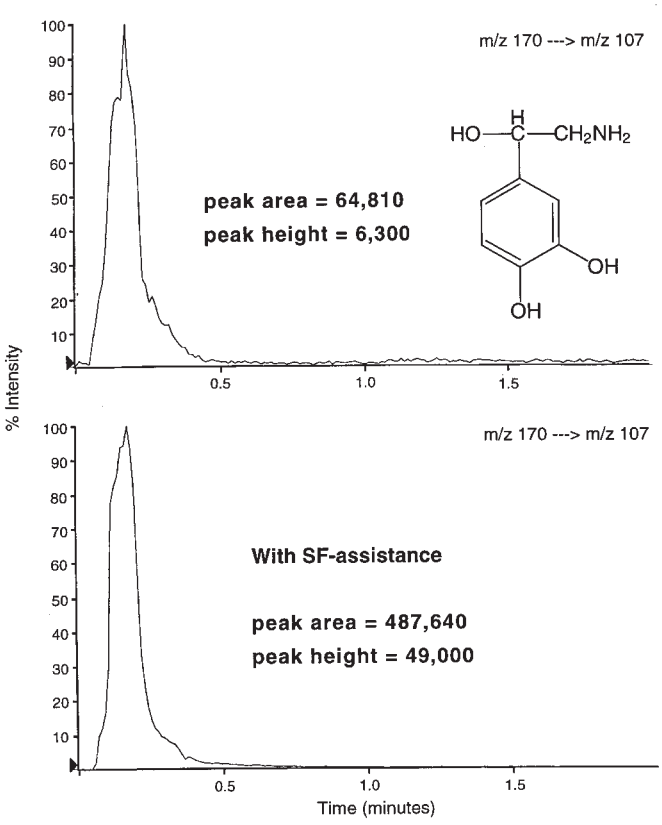

Figure 2. Flow-injection profiles of 73 ng of norephinephrine without (top) and with (bottom) SF-assisted nebulization. At this 0.5 ml/min flow-rate (85 % aqueous), over a seven-fold increase in peak height or area was observed with SF-assistance.

1.5-mg FIA injections are displayed in figure 3. Note that the instrument was not optimized for detection of this compound and it appeared to be absorbing onto the tubing walls (i.e., peak tailing). In this case, a better than 2-fold benefit (peak height or area) was obtained. It's important to note that we attempted to repeat this FIA experiment using a mobile-phase flow rate of 1 ml/min. The SF-assisted leg of the experiment proceeded as expected and provided results equivalent to those shown in the lower portion of figure 3. However, we were not able to complete the unassisted (no CO_2 addition) leg of the experiment, because the spray-cone was not stable, and we felt the amount of liquid on the walls of the source and near the vacuum orifice was excessive.

We believe that the differences in benefits obtained using the SF-assisted nebulization in these two cases are a function of mobile-phase composition and are not related to analyte differences. The first eluent contained 85 % water, while the second only contained 34 %. We also observed that the benefit due to SF-assisted nebulization decreased noticeably as we reduced the mobile phase flow-rate (i.e., from 1 ml/min to 200 µl/min). These observations are not surprising and are analogous to the benefits derived from the use of the TurboIonSpray gun in pneumatically-assisted electrospray. The benefits derived from the heated nitrogen stream applied orthogonally to the spray-cone clearly increase as mobile-phase flow rate and aqueous content increase. One must also consider that addition of CO_2 to aqueous mobile phases will result in the formation of carbonic acid, with a concomitant drop in pH. We have observed that the pH of the aqueous phase in a liquid CO_2/water mixture at a pressure of approximately 8 MPa drops rapidly to approximately 4 [13]. This effect may also affect ionization efficiency in pneumatically-assisted electrospray desorption/ionization.

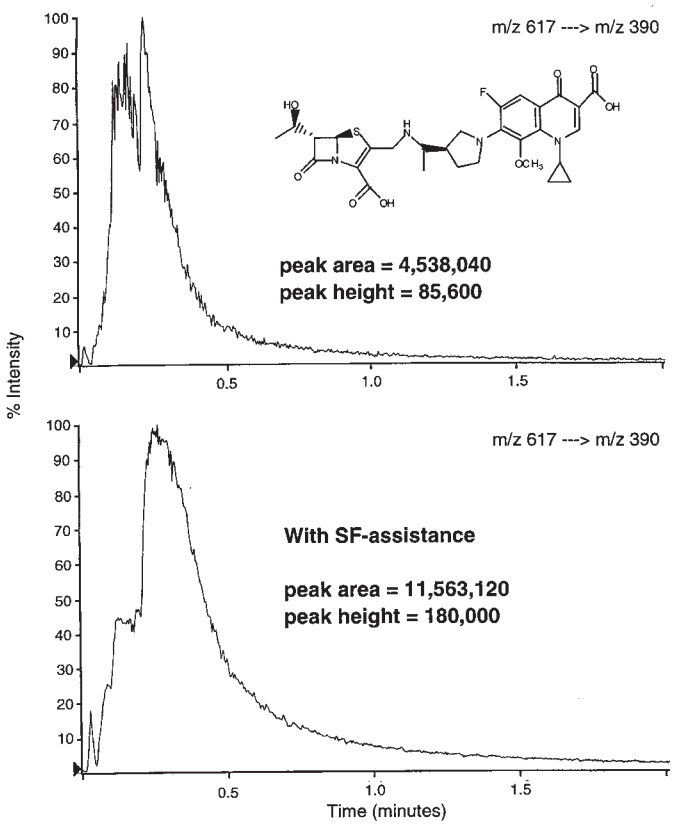

Figure 3. Flow-injection profiles of a QLA without (top) and with (bottom) SF-assisted nebulization. The improvement for this compound and mobile phase (34 % aqueous, 0.5 ml/min) is greater than a factor of two, based on peak height or area.

Optimization of CO_2 pressure

A rudimentary optimization of the CO_2 delivery pressure was conducted. This work was performed using FIA of norepinephrine, as described earlier. Raising the CO_2 delivery pressure to 20 MPa resulted in a 50 % improvement in response when compared to 10 MPa. With the transfer-line configuration and temperatures described above, these pressures correspond to flows of liquid CO_2 from the pump of 2.3 g/min (~1.4 liter/min expanded gas in the source) and 1.7 g/min (~1.0 liter/min expanded gas), respectively. At a pressure of 40 MPa (flow of 3.4 g/min or ~2.0 liter/min expanded gas), the spray-cone was disrupted. This caused a dramatic decrease in signal and signal-to-noise ratio (S/N), as compared to 20 MPa.

Position of the fused-silica CO_2 line in the spray tip

The position of the tip of the fused-silica CO_2 capillary relative to the stainless-steel mobile-phase capillary was optimized. The position providing the best signal and S/N was achieved by withdrawing the CO_2 capillary inside the mobile-phase capillary by approximately 0.5 mm. This position is identical to that found most suitable for SFC/MS experiments [12]. Withdrawing the tip of the CO_2 capillary any further inside the metal tubing resulted in lower signal and S/N.

Upstream CO_2 addition

Further experiments were conducted in which the liquid CO_2 and HPLC effluent streams were mixed approximately 30 cm from the IonSpray device, as described in the Experimental section. Note that this mixture was likely 2-phase for the more highly aqueous mobile phase. No improvement in signal or S/N was observed over our earlier results with addition of CO_2 at the sprayer tip.

Conclusions

This preliminary investigation leads us to believe that SF-assisted nebulization of HPLC eluent may find utility, particularly when used with highly aqueous mobile phases at flow rates typical of 4.6-mm-ID columns (i.e., ~1 ml/min).

It is possible that benefits beyond those we observed might be realized with an interface specifically designed for SF-assisted nebulization. Furthermore, Joule-Thompson cooling of the IonSpray tip, due to the rapid expansion of the CO_2, may be beneficial in the analysis of thermally-labile analytes. Finally, the substitution of other dense gases for CO_2, such as NH_3, SF_6, etc., may result in benefits not observed with CO_2. Ammonia, for example, may enhance ionization of acidic analytes. SF_6 would likely allow the use of higher IonSpray voltages before a corona discharge occurs.

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