Development and Application of Packed-Column Supercritical Fluid Chromatography/ Pneumatically Assisted Electrospray Mass Spectrometry

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A pneumatically assisted electrospray liquid chromatography/mass spectrometry (LC/MS) interface has been modified for use with packed-column supercritical fluid chromatography (pcSFC). The modifications include the addition of a concentric sheath-flow liquid to the spray device. This allowed the addition of modifiers at the sprayer tip that promote ionization of neutral, pcSFC-separated components. Post-column chromatographic fidelity was preserved by adding a “pressure-regulating fluid” (supplied under pressure control) to the effluent just ahead of the sprayer. The modified interface has been used to characterize a variety of mixtures including emollients, modified polysiloxanes, and pharmaceutical agents. The spectra produced by using this pcSFC/MS interface are similar to electrospray LC/MS spectra.

Interfacing supercritical fluid chromatography (SFC) to mass spectrometry (MS) presents both opportunities and challenges. For example, the common SFC mobile phases are more easily pumped by typical vacuum systems than are high performance liquid chromatography (HPLC) mobile phases, and mobile-phase flow rates in open-tubular SFC (otSFC) are relatively low (a few mL/min measured at STP). This allows the direct coupling of otSFC with common, differentially pumped mass spectrometers using traditional electron ionization (EI) or chemical ionization (CI) ion sources. Similarly, the “self-volatilizing” effect of packed-column SFC (pcSFC) mobile phases is often an advantage in pcSFC/MS interfacing [1]. On the other hand, most otSFC and some pcSFC separations involve pressure programming, where the mobile-phase pressure is raised over the course of the separation. When pressure programming is used with fixed flow restriction, the flow rate of mobile phase from the column rises over the course of the separation. In SFC/MS, this results in increased flow into the ion source. This increased flow can be deleterious to the desired electron or chemical ionization conditions and to ion transmission [2–4]. Additional pumping, such as liquid-nitrogen cryopumping [5–7], can, at a minimum, reduce the effect of mobile-phase flow on ion transmission. However, a more universal solution to these challenges is the use of atmospheric-pressure ionization (API) techniques. These methods introduce the effluent to an atmospheric-pressure “buffer” region between the high-pressure chromatograph and the low-pressure mass spectrometer, reducing the effect of mobile-phase flow on mass spectrometer performance.

Two primary API interface/ionization methods have been used for SFC/MS: the electrospray (ESI) [8–10] and the atmospheric pressure chemical ionization (APCI) [11–17] interfaces. The APCI interface has most frequently been used for open-tubular SFC/MS, although it is compatible with packed-column flow rates [13]. Electrospray ionization differs significantly from APCI. Rather than generating ions in a gas-phase, chemical-ionization process as in APCI, most workers believe that “preformed” ions are desorbed from evaporating droplets in ESI [18, 19]. One commonly used form of ESI is “pneumatically assisted ESI,” also known as IonSpray (PE Sciex, Ontario, Canada) [20], in which a flow of nebulizing gas is introduced coaxially to the expanding plume of electrospray droplets. An additional stream of heated gas is sometimes directed orthogonally to the expanding plume (sometimes called “TurbolonSpray”® (PE Sciex, Ontario, Canada) [21, 22]. The additional gas flows serve to enhance the desolva-
tion of analytes. Sadoun and Arpino described an ESI interface for packed-capillary SFC/MS [8]. The technique provided detection limits in the low-pg range. But the authors also described a number of difficulties. The mobile-phase composition significantly affected the ionization process. This complicated the results during composition-gradient programming, the most common programming method in pSFC. Also, less-volatile analytes produced tailing peaks, presumably from analyte accumulation on the electrospray needle during mobile-phase decompression. We later described a sheath-flow interface for otSFC/pneumatically assisted ESI-MS that addressed these problems [10]. The sheath-flow fluid (containing methanol, water, and a buffer such as NH₄OAc) provided enhanced and more uniform ionization conditions. The sheath flow also eliminated tailing of nonvolatile analytes. We demonstrated that the sheath-flow interface, combined with TurbolonSpray, is compatible with expanded-gas flow rates in the milliliter-per-minute range encountered in otSFC. Encouraged by these initial results, we have proceeded to investigate the use of our sheath-flow interface for coupling pSFC with mass spectrometry.

Packed-column SFC has a number of advantages over otSFC. Analyses are much faster, volumes and flow rates are such that connections are easier to make, the use of mobile-phase “modifiers” allows the analysis of more polar analytes, and the range of column selectivities is far greater than in otSFC. Accordingly, the challenges associated with coupling pSFC with MS are somewhat different from our otSFC experiences. First, the volume of mobile phase delivered to the IonSpray interface is orders-of-magnitude greater for 4.6-mm-i.d. packed columns (typically between 1.5 and 2.5 \times 10^{-2} \text{ g CO}_2/\text{s}, or 1 to 1.5 L/min measured at STP) than for 50-\mu m-i.d. open-tubular columns (typically 1.5 \times 10^{-5} to 1.5 \times 10^{-4} \text{ g CO}_2/\text{s}, or 1 to 10 mL/min measured at STP). Second, the pSFC mobile phase usually contains a polar “modifier” (such as methanol) and sometimes an “additive” (such as a weak acid or base) in addition to \text{CO}_2. Open-tubular SFC is most commonly performed with unmodified \text{CO}_2.

Another important difference is that column pressure in pSFC is most commonly (and most successfully) regulated “downstream” (i.e., at the column or detector exit) rather than at the head of the column, as in otSFC [23]. The column-outlet pressure is held sufficiently high to ensure that the effluent is a single phase. This, in turn, ensures that the entire chromatographic system upstream of the pressure-regulating point remains in a single phase (super- or subcritical). Most commercial pSFC instruments are equipped with ultraviolet absorbance (UV) detection and a computer-controlled, post-detector pressure-regulation device located downstream from the detector. Some investigators have reported that simply linking the outlet of this pressure regulator to an APCI nebulizer provides good pSFC/MS performance [24]. However, we found that this arrangement introduced an unacceptable amount of extra-column variance (band broadening) for the analytes we used as probes. This may be due to the internal volume of the pressure-regulating device and associated fittings. Or the band broadening may be the result of poor mass transfer in the line linking the pressure-regulating device to the IonSpray interface. Recall that the pressure (and the resulting phase behavior) within this transfer line is not regulated in this arrangement. Therefore, we employed a novel means of post-column pressure regulation, using a “pressure-regulating fluid.” This approach is summarized in the Experimental section and described in greater detail elsewhere [25]. Not only does this provide a low-dead-volume means of regulating pressure, but it enriches the mobile phase in the polar organic fluid during the pressure-reduction step, ensuring good mass transfer through the interface. This is, at least on the surface, similar to the addition of a “particle-forming solvent” to the effluent in pSFC/particle-beam mass spectrometry [26, 27]. However, the goal of this latter work was the formation of particles of appropriate size for the particle-beam interface. The particle-forming solvent was pumped under flow control, rather than pressure control. In our work, the goals of post-column solvent addition are pressure regulation and assurance of efficient mass transfer.

We report here our development of a modified IonSpray interface for pSFC/MS, and its application to the characterization of a variety of mixtures.

**Experimental**

**Supercritical Fluid Chromatography**

pSFC was performed by using a Model G1205A supercritical fluid chromatograph (Hewlett-Packard, Little Falls, DE). The injector was equipped with a 10-\mu L external loop. A “pressure-regulating fluid,” such as methanol, was added to a “pressure-regulating tee” located in-line between the UV-detector cell and the sheath-flow interface (see Figure 1). The pressure regulating tee replaced a number of components of the G1205A: the pressure transducer, pressure regulating valve, two unions, one filter, and the transfer lines between these components. The pressure (200 bar) and temperature (ambient) of the pressure-regulating tee were such that the column effluent and the pressure-regulating fluid mix would form a single phase in the pressure-regulating tee. (We recommend that the pressure-regulating fluid be the same as the mobile-phase modifier in order to avoid phase-separation uncertainties.) The fluid was supplied by a Model 260D syringe pump (Isco, Lincoln, NE) operated under pressure control. A second line extended from the Isco pressure-regulating pump to the post-column pressure transducer of the HP G1205A SFC instrument. This allowed the HP instrument-control system to sense the post-column pressure regulated by the Isco pump. The outlet...