A Brief Review of Interfacing Supercritical Fluid Chromatography with Mass Spectrometry

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In the past decade, supercritical fluid chromatography (SFC) has experienced a steady growth in acceptance, particularly in pharmaceutical and chemical laboratories. Compared to HPLC, SFC offers better selectivity and shorter analysis time because of the low viscosity and high diffusivity inherent to supercritical fluids. Due to the intrinsic universality, sensitivity, and specificity of MS, SFC MS readily lends itself as an attractive complement to RPLC MS. This brief review deals primarily with the applications of SFC MS to show the versatility and broad applicability of this hyphenated technique, with a heavy emphasis on pharmaceutical related applications.

Keywords: SFC; MS; pharmaceutical; high throughput; pharmacokinetics; mass-directed.

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 CO_2, in combination with one or more polar organic solvents, such as alcohols, is used as mobile phase. While CO_2 itself is relatively non-polar, the addition of polar organic solvents enables the mobile phase to retain the polarity and solvating power of the polar organics. 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. From the original gas chromatographic (GC) detector, the detection in SFC has evolved to encompass more 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.

This brief review deals primarily with the applications of SFC MS to show the versatility and broad applicability of this hyphenated technique, with a heavy emphasis on pharmaceutical related applications. It is not meant to be an exhaustive nor in-depth technical discussion. Reviews of SFC MS applications in other fields, (e.g., food additives, natural products, polymer/oligomer, organometallics, fuel), which are too numerous to list in this review, can be found elsewhere [2-7].

Ionization and Mass Analyzers

Similar to LC MS, the most commonly used ionization methods for SFC MS are atmospheric pressure chemical ionization (APCI) and electrospray ionization (ESI). In SFC, the supercritical state, or near-critical state, is maintained by regulating system pressure via a back pressure regulator (BPR), typically above 100 bar. On the other hand, nebulization and evaporation of APCI and ESI processes all take place at near atmospheric pressure. The pressure drop from greater than 100 bar to atmospheric pressure causes CO_2 decompression to form aerosols, thus assisting nebulization of the analyte. It has been speculated that SFC is more amenable to atmospheric pressure ionization (API) MS source integration than LC [7]. However, CO_2 decompression is also a highly endothermic process. It is intuitive that a higher nebulizer and source temperature is required for SFC than HPLC.

APCI

APCI can accommodate relatively high flow rates (typically up to 2 ml/min) and is better suited for compounds with low to moderate polarity. Coincidently, SFC has a higher optimal flow rate, typically 3 to 5 times higher than HPLC, and better retention for compounds of low to moderate polarity. APCI was therefore perceived as the choice of ionization for SFC MS; this notion, however, has somewhat subsided with the emergence of ample successful applications generated by SFC ESI MS.

Anacleto *et al.* [8] employed SFC APCI MS for the analysis of poly aromatic hydrocarbons (PAHs) with 100% CO_2 as the mobile phase. Sjoberg and Markides [9] reported the SFC APCI MS analysis of steroids. Dost and Davidson [10] reported analysis of atropine using SFC APCI MS. Ventura *et al.* demonstrated using SFC APCI MS for high speed screening of pharmaceutically relevant compounds [11]. In this study, for routine high-throughput analyses of 500-1000 samples per 24-h period, SFC MS was shown advantageous over LC MS, not only in overall throughput, but also in chromatographic efficiency.

ESI

ESI, on the other hand, has been the predominant choice of ionization for LC MS, mainly due to its ability to generate intact molecular ions and to ionize high molecular weight species [12]. Sadoun *et al.* [13] were among the first to report the use of an ESI interface for SFC MS. In this study, a detection limit in the low-pg range was achieved using a custom source design. Baker *et al.* [14] developed a pneumatically assisted ESI source for SFC MS to accommodate flow rates up to 4 ml/min. Arguably the best chromatographic tool for chiral separation, SFC was also found to couple to ESI MS for the analysis of chiral mixtures. Garzotti and Hamdan [15] demonstrated the potential of high throughput chiral analyses on selected diverse molecules. The use of MS was proven essential for an unambiguous assignment of the eluting components, particularly in the case of complex chiral mixtures.

Mass Analyzers

Single quadrupole is the most commonly used mass analyzer for SFC MS. However, there is no limitation on the type of mass analyzers that can be coupled to SFC, other than practicality and capital expense. Morgan *et al.* [16] demonstrated the coupling of SFC to both ion trap and triple quadrupole MS. Xu *et al.* [17] reported use of SFC ion trap MS in separating 15 estrogen metabolites in less than 10 min. Garzotti *et al.* [15, 18] coupled a Q-TOF II MS to SFC to take advantage of the mass accuracy and high speed of the Q TOF MS for fast and unambiguous peak identification. Coe *et al.* [19] illustrated coupling a triple quadrupole MS to SFC for fast bioanalysis of R/S-warfarin in human plasma. Another noteworthy effort was described by Bolanos *et al.* [20]. By coupling SFC with a time-of-flight (TOF) MS, considerably faster than any scanning type mass analyzer, the chromatographic integrity for peaks as narrow as 2 s was preserved in ultra-fast SFC separations of pharmaceutical compounds.

SFC MS Applications

The pharmaceutical industry has been the driving force for the development and advances of SFC. SFC MS has become a powerful tool for qualitative and quantitative analyses at two key stages of drug discovery: hit-to-lead and lead optimization. Often used in parallel with other separation techniques, SFC MS has been extensively employed in purity assessment, structure confirmation, in-process monitoring, structure elucidation, degradation profiling, scale-up purification and method transfer for bioassays [2].

SFC is typically superior to LC in separating structurally related compounds, isomers and enantiomers. This is manifested by the high adoption rate of SFC in chiral separation/purification in nearly all major pharmaceutical companies. For example, Alexander and Staab [21] demonstrated the applicability of SFC MS for the profiling of isomeric products in chiral drug synthesis. Xu *et al.* [17] demonstrated the separation of 15 estrogen metabolites by SFC in less than 10 min, significantly faster than the 70-min run using RPLC. The limit of detection (LOD) and limit of quantitation (LOQ) were 0.5 and 5 pg, respectively, both comparable to those from RPLC.

High Throughput Analysis by SFC MS

Pursuing high throughput has been one of the most consistent themes in the analytical arena in pharmaceutical sectors. SFC holds great potential to become the choice of chromatography for such an endeavor, owing to its higher optimal flow rate and short re-equilibration time.

One prerequisite for any technique employed in a high throughput fashion is its broad applicability for diverse compounds. SFC has been demonstrated to cover as wide a range of compounds in both functionality and polarity as RPLC, but also compliments RPLC in selectivity, particularly for extremely polar and non-polar analytes due to its normal phase separation mechanism. Pinkston *et al.* [22] compared SFC MS with LC MS in analyzing a total of 2266 diverse, pharmaceutical relevant compounds. The percentages of eluted and correctly identified compounds from both methods were statistically equivalent. The results unequivocally justify the use of SFC MS for high throughput screening of large and diverse libraries of drug-like molecules.

Bolanos *et al.* [20] described improving analytical throughput by using a high speed TOF MS coupled to SFC. Four pharmaceutical compounds were eluted within 6 s, with a base peak width of less than 2 s. Hoke *et al.* [23] described SFC MS/MS applications in high throughput bioanalysis. By using a 2.1×10 mm i.d. column and a flow rate of 7.5 ml/min, the time for target compound quantitation in plasma extracts was reduced to 10.2 min for a 96-well plate, a significant improvement over existing LC MS/MS methods.

While most efforts in improving throughput were directed into implementing fast analysis and automated column/solvent switching on SFC, MS can be another source for throughput improvement. Zhao *et al.* [24] reported a SFC MS based screening strategy, sample pooling, for rapid chiral method development. Taking advantage of the selectivity and specificity of MS, using sample pooling substantially improved the overall throughput by running a pooled sample mixture followed by reconstruction of ion chromatograms based on each constituent's specific m/z. Zeng *et al.* [25] reported a custom-made automated parallel four-column SFC/MUX MS system for high-throughput enantioselective method development in support of drug discovery. The improvement in throughput was achieved by parallel screening four columns, simultaneous detection of all eluents by MUX, and intelligent software-controlled method optimization.

Pharmacokinetics Analyses by SFC MS/MS

An emerging application field for SFC MS within pharmaceutical sectors is pharmacokinetics (PK). Hoke *et al.* [26] were among the first to employ SFC MS/MS for such studies. In the first study, a LC-comparable sensitivity of ppt level was reported for bio-analytical quantification. In a subsequent study [22], a throughput of 10 min per 96-well plate was achieved. Hsieh *et al.* [27-29] reported the determination of pharmaceutical compounds in metabolic stability samples. Coe *et al.* [19] also reported fast bioanalysis of R/S-warfarin in human plasma using SFC MS/MS.

Overall, SFC MS/MS applications are still limited in scope. This is due, in part, to the lack of commercially available integrated SFC MS/MS instruments. As chiral drugs, in the form of single enantiomers or stereoisomers, overtake achiral ones in the percentage of approved drugs [30], it is expected that SFC MS/MS, a superior chiral separation technique with demonstrated ultra-high detection sensitivity, will find more uses in PK analyses and other bio-analytical applications.

Protein/Peptide Analyses by SFC MS

Peptide separations are of great economic, human, and environmental importance in pharmaceutical and other industries. SFC MS may allow faster determinations of targeted "biologics" (i.e., peptide-based pharmaceuticals) in physiological fluids. The major limitation, though, is the low solubility of these biomolecules in organic solvents typically required for SFC MS. Literature on this subject is scarce. Bolanos *et al.* [7] described the analyses of both protein and peptides by SFC ESI MS, and the purification of gramicidin by SFC. In collaboration with Marshall *et al.* [31], SFC MS was also applied to alleviate Hydrogen/Deuterium (H/D) back exchange in solution phase in proteomic analyses. Taylor *et al.* [32] recently demonstrated the elution and detection of polypeptides up to 40-mers using SFC MS.

MS Directed Preparative SFC

Until recently, mass-directed purification capabilities were limited to RPLC based techniques for high throughput purification of diverse libraries of drug-like compounds. The ability to collect a single fraction per injection in an open-bed format from a sequence of complex matrix purifications is standard for such high throughput library applications. While SFC has proven advantageous to RPLC in terms of speed, efficiency and cost-savings [33], open bed collection has been problematic for SFC due to aerosol formation caused by depressurization of CO_2, particularly at high flow rates. Over the years, many groups have attempted to custom-design a mass-triggered preparative SFC in open-bed collection, with limited success. Wang *et al*. [34] developed a semi-preparative SFC that consisted of a single quadrupole MS, a binary SFC, and a mass-triggered fraction collection. The flow rate was 15 ml/min and the average recovery was 77%. Zhang *et al*. [35] reported development of a MS directed preparative SFC with recovery greater than 85% at flow rates up to 30 ml/min. It is noted that the flow rates of these systems were limited due to aerosol formation. The challenge of managing aerosols in collection at high flow rates was finally overcome with the development of the TharSFC™ SFC-MS Prep 100 system (www.tharsfc.com) using a proprietary gas/liquid separator prior to collection. This high throughput, MS-directed purification platform boasts a maximum flow rate of 100 g/min, with recovery greater than 85% and purity above 95%.

Conclusions

As SFC continues to gain momentum as a viable chromatographic technique, SFC MS finds wide applications in such fields as purity assessment, structure confirmation, structure elucidation, pharmacokinetic profiling, and library purification. Compared to RPLC, SFC offers better selectivity and a shorter analysis time. Due to significant cost-saving associated with SFC, the realization of mass directed preparative SFC with open bed collection marks an important milestone for SFC in gaining mainstream acceptance. Because of the lack of commercial instrumentation, application of SFC MS/MS lags behind its counterpart in HPLC. It will require a concerted effort from both vendors and practitioners to harness the full potential of this hyphenated technique.

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