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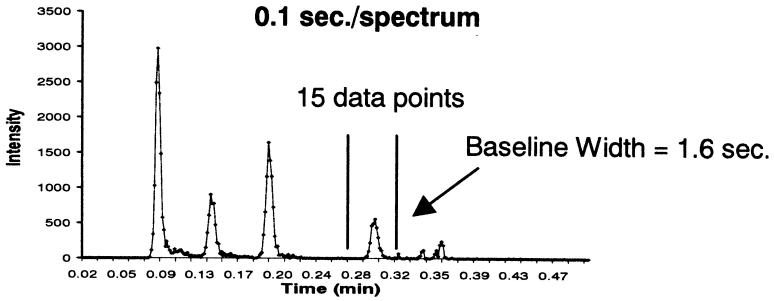

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Preserving the Chromatographic Integrity of High-Speed Supercritical Fluid Chromatography Separations Using Time-of-Flight Mass Spectrometry

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The advantage of high-speed time-of-flight-mass spectrometry (TOF-MS) detection for ultrafast qualitative supercritical fluid chromatography/mass spectrometry (SFC/MS) applications allows the superior resolving power of SFC to be exploited in high-throughput analysis. A chromatographic comparison of quadrupole MS and TOF-MS shows high-speed TOF total ion current data point sampling to be more indicative of fast SFC separations and corresponding short (1−2 s) baseline peak widths. Results shown for analysis of a six-compound mixture with two peaks eluting at 0.86 and 0.89 min exhibit >50% resolution by high-speed TOF data sampling, whereas the same peaks appear to coelute using quadrupole MS data sampling. Additionally, a marked improvement in the peak baseline widths is afforded by fast TOF data acquisition of 0.1 s/spectrum, resulting in a reduction in the baseline width, 1.6 s, of sulfanilamide in a four-compound mixture that is more than 2-fold greater than that achieved at the slower data acquisition of 0.5 s/spectrum. The resulting increase in resolution and improved peak shapes allow automatic integration routines to perform more effectively. For most classes of compounds amenable to high performance liquid chromatography, including druglike species, steroids, and polymers, the union of SFC with TOF-MS provides the maximum density of chemical information per unit time available with any high-speed chromatographic/mass spectrometric method.

Introduction

The use of high-throughput chemistry has pushed the analytical chemist to respond with similar success in rapid analysis of these compounds. Strategies for fast separations, such as capillary electrochromatography and ultrafast gas chromatography coupled with mass spectrometry, have recently been demonstrated.[1,2] Modern liquid chromatography/mass spectrometry (LC/MS) methods also provide quick separations, and supercritical fluid chromatography/mass spectrometry (SFC/MS), an inherently fast technique, as discussed below, is quickly gaining popularity in the pharmaceutical industry.[3−5] For detection of the narrow chromatographic peaks obtained with these high-speed separations, the need for much higher data point resolution requires a high mass spectral data acquisition rate.[6] Conventionally, a chromatographic peak is considered adequately defined if there are 15−20 data points across its profile.[7] For chromatographic peaks having a baseline width of 5 s or more (typical even with fast LC/MS techniques), adequate digital resolution can usually be achieved using full-scan quadrupole mass spectral acquisition. In these cases, total ion current (TIC) data points are separated by several hundreds of milliseconds for mass/charge (m/z) scan ranges in the neighborhood of 1000.

The use of a time-of-flight (TOF) mass spectrometer can provide much greater data point resolution due to its higher rate of data acquisition.[8,9] A sample eluting from the column is transferred to the atmospheric pressure ionization (API) source. A continuous beam of ions from the API source is accelerated into a "pulser" region. A packet of ions is accelerated from the pulser region at a right angle to the original direction of flight into a field-free drift tube. Since all ions created are imparted a kinetic energy from the accelerating potential equal to zeV, where e is the charge on the electron and V is the accelerating potential, ions of different mass-to-charge ratios (m/z) are separated over a flight distance L in time t by the inverse square proportionality with velocity, v.

$$v = (2zeV/m)^{1/2}$$

Since $v = L/t$, the time interval between the arrival of pulses from ions of mass m_1 and m_2 at the end of the flight tube is proportional to $L[(m_1)^{1/2} - (m_2)^{1/2}]$. A transient for an m/z scan range of 1000 is acquired on a time scale of ∼100 μs for conventional length flight tubes ($L \sim 1$ m). TOF-MS systems can theoretically acquire around 10 000 transients/s, which are subsequently averaged and stored to provide a single mass spectrum. This speed of spectral acquisition is far in excess of the digital resolution required by any current chromatographic time scale. The exact spectral acquisition rate depends on the upper limit of the mass range monitored and the duty cycle of the pulsing circuitry. TOF is also inherently more sensitive for wide mass range applications in that the majority of ions generated are extracted from the

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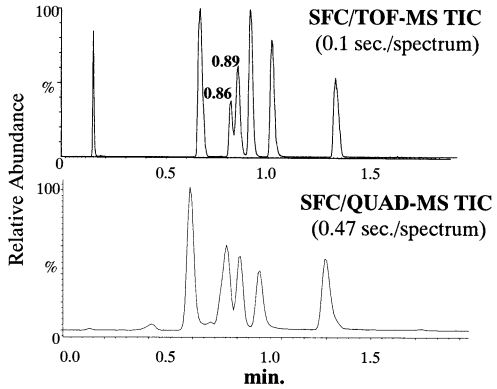

Figure 1. Comparison of SFC/MS total ion chromatograms for a six-compound mixture analyzed by both TOF-MS and quadrupole MS. A dramatic improvement in peak resolution was demonstrated by high-speed TOF sampling rates of 0.1 s/spectrum over the manufacturer recommended quadrupole rate of 0.47 s/spectrum.

source with each pulse, whereas with scanning instruments, a particular ion is filtered through and detected for only a small fraction of the total scan time.

As stated, TOF-MS is especially advantageous when coupled with high-speed separation techniques. Supercritical fluid chromatography (SFC) is a normal-phase chromatographic technique, which because of its properties can provide much faster separations than HPLC for a similar range of compounds. The SFC mobile phase consists of a densely compressed mixture usually consisting of CO_2 and a polar organic solvent, such as methanol. The lower viscosities of supercritical relative to liquid mobile phases results in lower pressure drops across a column in SFC,as compared with HPLC.[10] Further, since diffusion coefficients are an order of magnitude higher in supercritical fluids than in liquids, the number of theoretical plates generated per unit time increases ~3-fold for SFC.[11] Van Deemter plots show that the range of optimum linear velocities (μ_0) are increased with SFC relative to HPLC. This allows higher flow rates and longer columns to be used in SFC, resulting in faster separations with a greater number of theoretical plates than HPLC.[12]

Experimental Section

Two SFC/MS configurations were used in the initial data point sampling studies described. A conventional SFC (Berger Instruments, Newark, DE) was coupled to an Agilent quadrupole MSD as previously described.[13] For subsequent TOF-MS work, the Agilent MSD was replaced with a Micromass LCT system (Micromass, Manchester, U.K.). A fraction of the flow through an SFC system was diverted through a 40-μm fused-silica capillary directly into the APCI

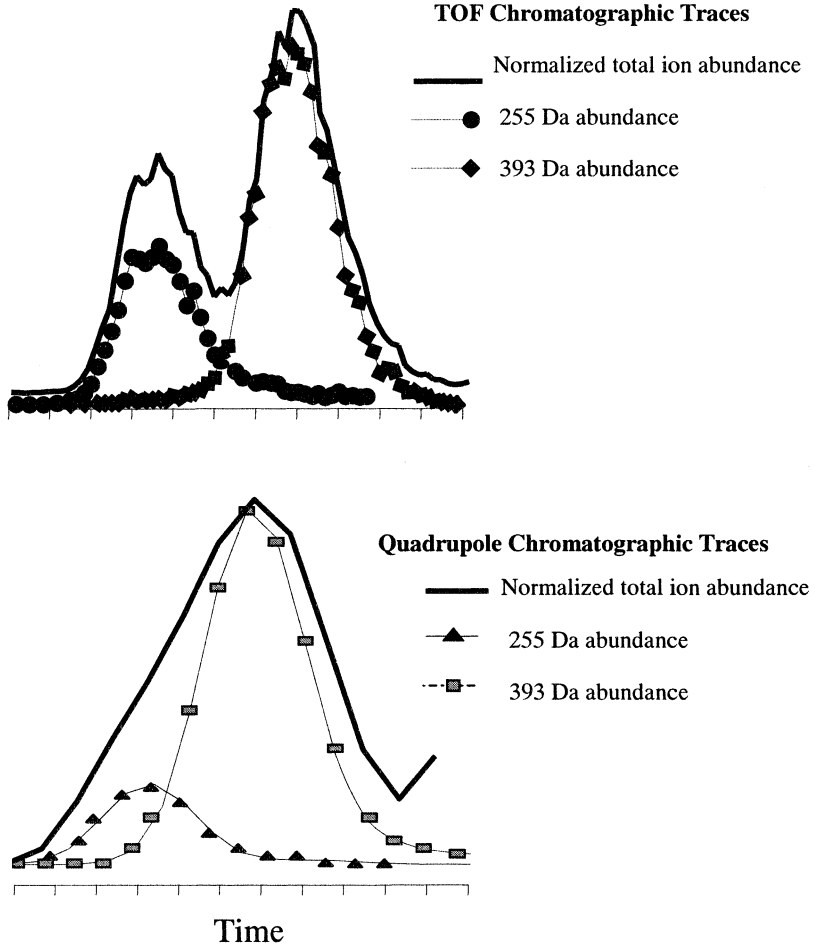

Figure 2. Mass chromatograms for *m*/*z* 255 and 393 compounds eluting at 0.86 and 0.89 min (from sample in Figure 1), respectively. The faster data sampling provided by the TOF exhibited >50% resolution of both compounds in the TIC, whereas the corresponding peaks appeared to coelute in the quadrupole-MS TIC.

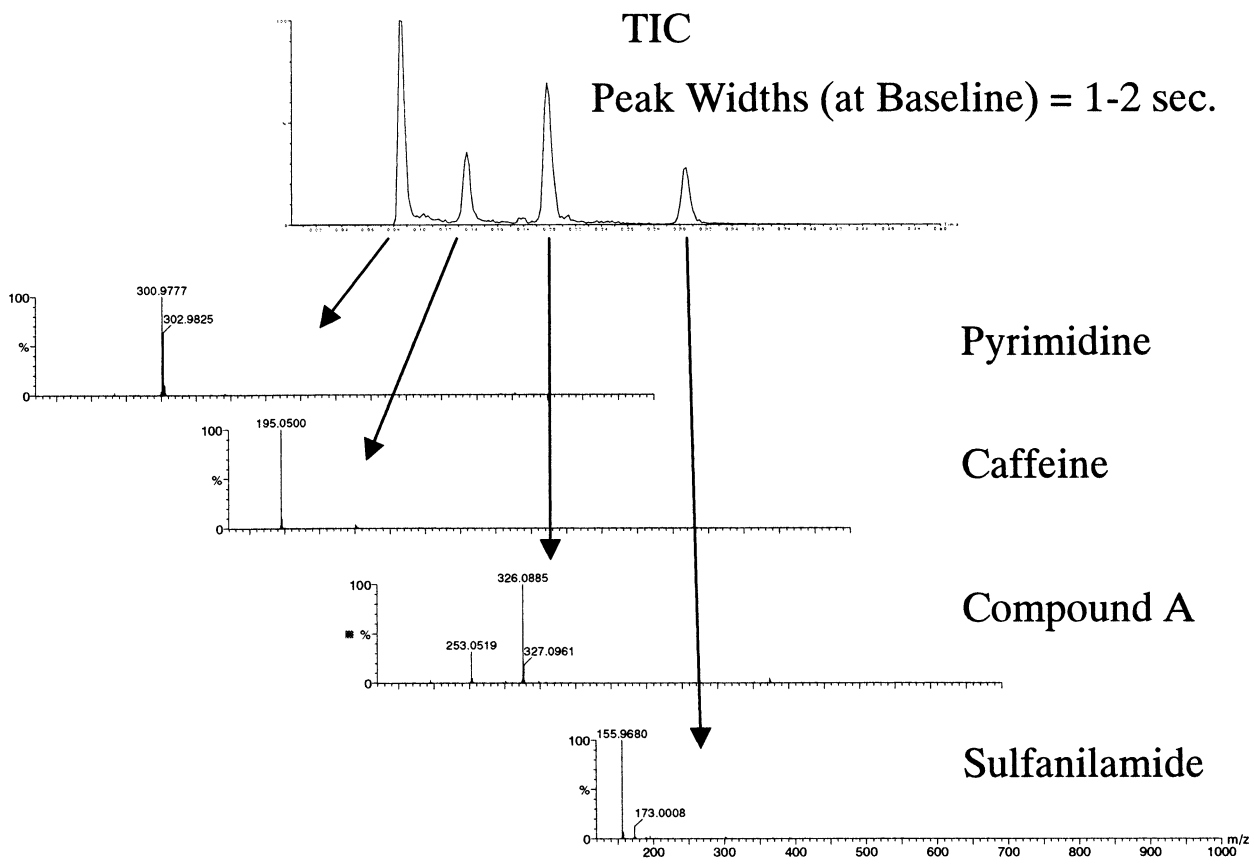

Figure 3. A mixture of pyrimidine, caffeine, proprietary compound A, and sulfanilamide was separated via ultrafast SFC in 15 s with baseline peak widths of 1−2 s. The integrated mass spectrum for each peak shows only the compound of interest present, with the exception of fragmentation occurring in the APCI source.

source of the TOF mass spectrometer, while the remainder of the flow was directed to the back-pressure regulator. The standard nebulizer needle was removed from the source, along with a filter cartridge, to allow the capillary to pass directly through the source assembly to the end of the nebulizer. The end of the capillary was positioned ~0.5 mm inside the opening of the heated nebulizer chamber.

The chromatographic conditions for the Berger SFC system separations employed a Zymor Zyrosil-Hybrid (Zymor, Wayne, NJ) packed column with the bonded stationary phase linked to 5-μm particles with 60-Å pore size. The column tube inner dimensions were 4.6 × 50 mm, and the column was maintained at a temperature of 35 °C. The mobile phase was a gradient of methanol in CO_2 from 5 to 60% over 1.67 min. The flow rate for the analysis was 5 mL/min, and the column backpressure was maintained at 200 bar. Samples for analysis were dissolved in methanol at a final concentration of 0.2 mg/mL. Injections were made by a LEAP HTS Pal autosampler (LEAP Technologies, Inc., Carrboro, NC) by over-filling a 10-μL loop.

Ultrafast SFC separations with a total cycle time of 40 s (injection-to-injection) were performed using a Jasco 1580 SFC system (Easton, MD) with a mobile-phase flow rate of 10 mL/min. The Jasco SFC system was operated from within the Micromass MassLynx software, which also controlled the LCT/TOF mass spectrometer and LEAP autosampler. The Leap autosampler was modified to incorporate a valve self-wash module to provide a fast (10 s), independent methanol rinse of the autosampler injector amenable to the ultrafast method. The same 5−60% methanol in CO_2 gradient was performed over a 30-s ramp time. System backpressure was regulated at 130 bar. A 30 × 4.6 mm Zymor Zyrosil-Hybrid stationary-phase chromatographic column with 3-μm particles and 60-Å pore size was used for all ultrafast methods.

Results

A fast SFC gradient was used to analyze a mixture consisting of five steroids plus one proprietary polar species, using both quadrupole and TOF mass spectrometers. The flow rate used was 5 mL/min with a fixed outlet pressure of 200 bar. Both mass spectrometers' APCI source conditions (positive mode) were tuned for maximum sensitivity with minimal fragmentation for the compounds studied. Aside from the physical API interface differences, all other experimental conditions were duplicated as closely as possible for the comparison of the two systems. The quadrupole mass spectrometer was set to scan the *m*/*z* range of 100−1000 at the maximum rate (0.47 s/scan) recommended by the system software for good performance with *m*/*z* 0.10 accuracy. In the total ion chromatogram, 128 spectra/min were recorded with the quadrupole system. Alternatively, the TOF acquisition provided 600 spectra/min, consisting of individual transients (50 μs) summed over data collection times of 0.05 s with a required 0.05-s delay after each averaging event for data transfer. A faster data collection

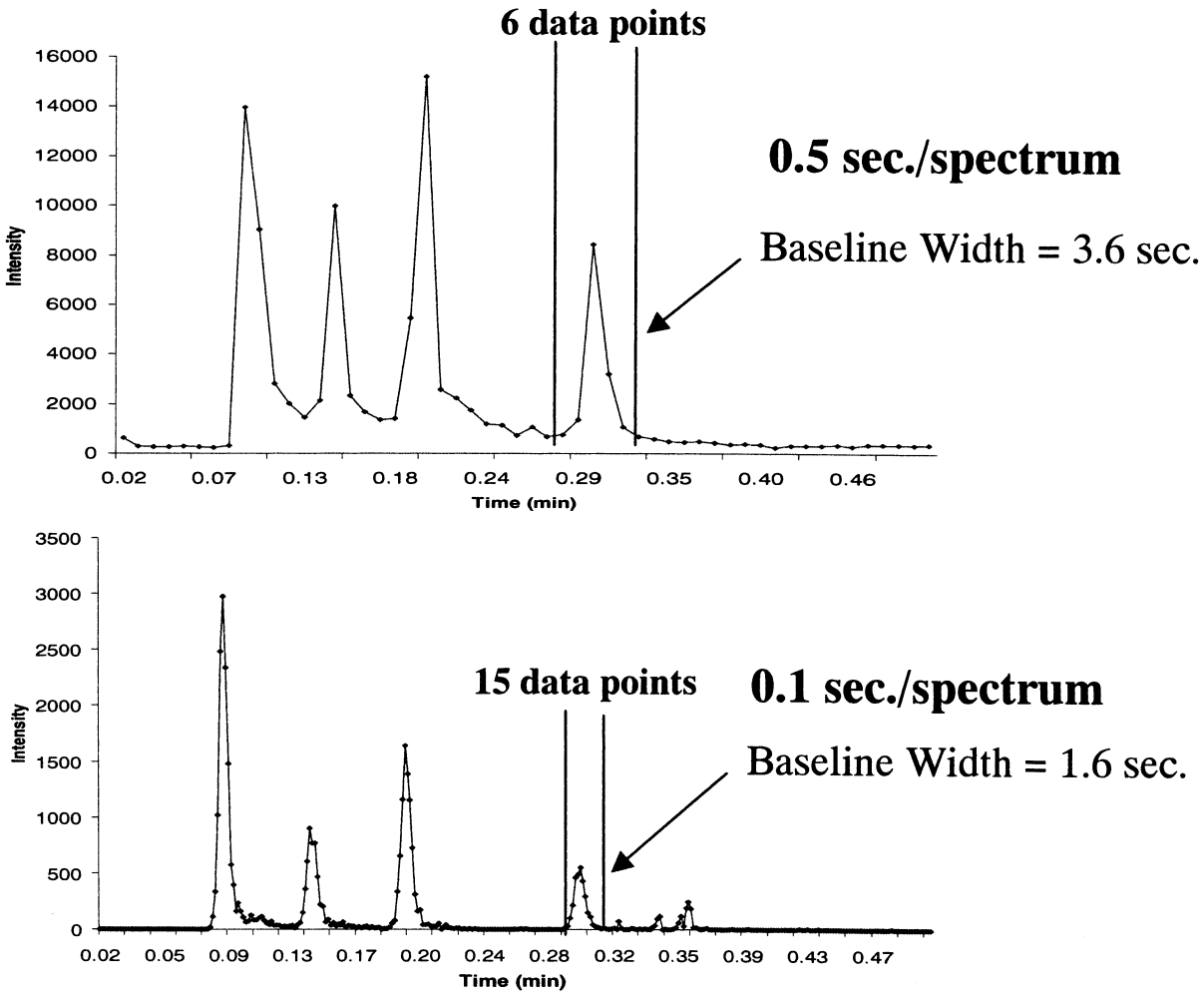

Figure 4. A comparison of TOF data point sampling rates and the effect on resolution and the peak baseline width. As a consequence of a slow TOF sampling rate (0.5 s/spectrum), the TIC resulted in a broad sulfanilamide peak with a baseline width of 3.6 s. A much narrower peak width, 1.6 s., was observed when the data point sampling was increased to 0.1 s/spectrum.

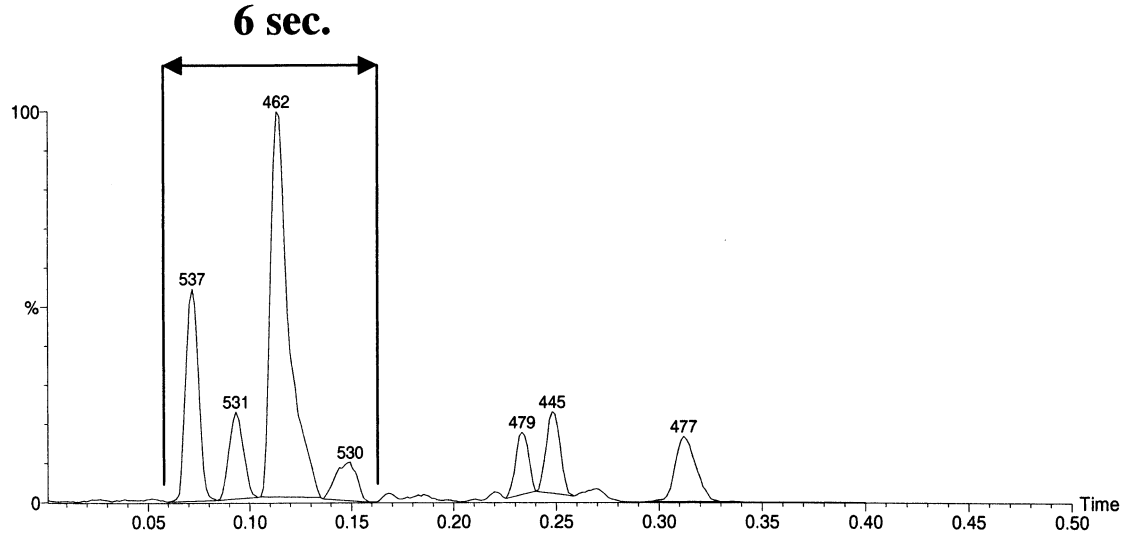

Figure 5. Analysis of a complex mixture by the ultrafast SFC TOF method. Measurement of baseline separation of four eluting compounds within a window of 6 s was made possible in large part as a result of fast 0.1 s/spectrum data point sampling.

rate of <0.1 s is possible with less spectral averaging per datum point and correspondingly larger data files, but diminishing returns in chromatographic improvement beyond that to be described below, were discernible.

The dramatic improvement using TOF-MS high-speed sampling rates is exhibited in the chromatographic comparison with the quadrupole-MS system acquisition (Figure 1). For the six-compound mixture, the baseline peak widths from

the quadrupole-MS TIC were no less than 6 s. However, major chromatographic peaks from the TOF-MS TIC exhibited baseline widths as short as 1 s, more indicative of the separations provided by SFC.

The difference in data point frequency is evident from a comparison of the TOF and quadrupole single ion chromatographic traces shown in Figure 2. The faster data sampling provided by the TOF resolved the TOF TIC peaks marked at 0.86 and 0.89 min, but the corresponding peaks using the quadrupole TIC appear to coelute. A close-up view of the 6.6-s "coelution region" of the TOF and quadrupole-MS chromatograms with underlying contributions from principal ions of the closely eluting species is shown in Figure 2. Because of the lower data sampling frequency of the quadrupole based system, the TIC trace shows no separation between the two peaks. In contrast, the TOF TIC preserves the integrity of the fast SFC chromatography. Because of the increased data point density of the TOF, the two compounds with principal ions at 255 and 393 Da are observed as fully resolved by their mass chromatograms and the TIC exhibits greater than 50% resolution between them, as well.

Proper TIC data point sampling becomes exceedingly important as separation times are shortened, as with "ultrafast" SFC methods. The ability to achieve baseline resolution of mixtures via ultrafast SFC at a flow rate of 10 mL/min is demonstrated in Figure 3. A steep 5−60% methanol gradient was adapted to the ultrafast method to cover separation of more polar species. This gradient in concert with a shorter (30 × 4.6 mm) packed column has allowed the analytical cycle time to be reduced to 40 s. For example, in Figure 3, a mixture of pyrimidine, caffeine, proprietary compound A ($m/z = 326$), and sulfanilamide is separated within 15 s. The separation of these compounds is validated in Figure 3, where the summed mass spectra show ions corresponding to only the compound of interest plus APCI-induced fragmentation (m/z 253). The ultrafast run produces fast, sharp peaks that have baseline peak widths on the order of 1−2 s. Such sharp chromatographic peaks require fast data point sampling to maintain the integrity of the peak.

A data point comparison for varied TOF acquisition rates using the previously mentioned four-component mixture is demonstrated in Figure 4. As shown in Figure 4, applying a typical 0.45-s data acquisition rate with the addition of the 0.05-s interscan delay provides only 2 data points/second sampling. As a consequence of this slow sampling rate, the TIC reveals a broader sulfanilamide peak with a baseline width of 3.6 s. A much narrower sulfanilamide peak was observed, 1.6 s, when data point sampling was increased to 0.1 s/spectrum. Although chromatographic integrity was better maintained by fast MS data sampling, there was also a corresponding loss in MS signal intensity. This is a drawback to be aware of, which could potentially be overcome by adjusting detector settings.

Application of this ultrafast method was also shown to extend beyond model compound studies. Shown in Figure 5, a complex sample was analyzed by the ultrafast SFC method. Here again, fast data point sampling of 0.1 s/spectrum is critical to maintain the fidelity of the eluting compounds. In fact, baseline separation of the first four compounds is shown within a window of 6 s, in large part as a result of the complimentary data point sampling.

Conclusions

These examples clearly demonstrate the advantage of TOF-MS for ultrafast qualitative SFC/MS applications. High-speed MS detection allows the superior resolving power of SFC to be exploited in high-throughput analytical settings. Additionally, the resulting increase in resolution and better peak shapes allows the automatic integration routines in various software packages to perform more effectively. Automated purity evaluation routines are more precise with sharper peaks, allowing higher throughput with less human interaction. For classes of compounds amenable to HPLC, including druglike species, steroids, and polymers, the union of SFC with TOF-MS provides the maximum density of chemical information per unit time available with any high-speed chromatographic/mass spectrometric method.

References and Notes

(1) Choudhary, G.; Horváth, C.; Banks, J. F. *J. Chromatogr., A* **1998**, *828*, 469.
(2) van Deursen, M. M.; Beens, J.; Janssen, H.-G.; Leclercq, P. A.; Cramers, C. A. *J. Chromatogr., A* **2000**, *878*, 205.
(3) Berger, T. A.; Fogelman, K.; Staats, T.; Bente, P.; Crocket, I.; Farrell, W.; Osonubi, M. *J. Biochem. Biophys. Methods* **2000**, *43*, 87.
(4) Hoke, S. H.; Tomlinson, J. A., II; Bolden, R. D.; Morand, K. L.; Pinkston, J. D.; Wehmeyer, K. R. *Anal. Chem*. **2001**, *73*, 3083.
(5) Morgan, D. G.; Villeneuve, M. S.; Frick, L. W. Use of Basic Additives with SFC/MS. Poster presented at the 10th International Symposium and Exhibition on Supercritical Fluid Chromatography, Extraction and Processing, Myrtle Beach, SC, August 19−22, 2000.
(6) Dyson, N. *J. Chromatogr., A* **1999**, *842*, 321.
(7) Novak, J. *Quantitative Analysis by Gas Chromatography*; Chromatography Science Series; Marcel Dekker: New York, 1988, Vol. 41, p 187.
(8) Holland, J. F.; Enke, C. G.; Allison, J.; Stults, J. T.; Pinkston, J. D.; Newcome, B.; Watson, J. T. *Anal. Chem.* **1983**, *50*, 997A.
(9) Karas, M.; Hillenkamp, F. *Anal. Chem.* **1988**, *60*, 2299.
(10) Berger, T. A. *Packed Column SFC*; The Royal Society of Chemistry: Cambridge, U.K., 1995; p 45.
(11) Berger, T. A. *Packed Column SFC*; The Royal Society of Chemistry: Cambridge, U.K., 1995; p 11.
(12) Gere, D. R. *Application Note 800−3*; Hewlett-Packard Company: Wilmington, DE, 1983.
(13) Ventura, M. C.; Farrell W. P.; Aurigemma, C. A.; Greig, M. J. *Anal. Chem*. **1999**, *71*, 4223.

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