Comparison of LC/MS and SFC/MS for Screening of a Large and Diverse Library of Pharmaceutically Relevant Compounds

ARTICLE *in* ANALYTICAL CHEMISTRY · DECEMBER 2006

Impact Factor: 5.64 · DOI: 10.1021/ac061033l · Source: PubMed

5 AUTHORS, INCLUDING:

Kenneth Morand
Procter & Gamble
22 PUBLICATIONS **734** CITATIONS
SEE PROFILE

Debra A Tirey
Procter & Gamble
4 PUBLICATIONS **192** CITATIONS
SEE PROFILE

David T Stanton
Procter & Gamble
34 PUBLICATIONS **1,604** CITATIONS
SEE PROFILE

Anal. Chem. **2006,** *78,* 7467−7472

Comparison of LC/MS and SFC/MS for Screening of a Large and Diverse Library of Pharmaceutically Relevant Compounds

J. David Pinkston,*,† Dong Wen,‡ Kenneth L. Morand,† Debra A. Tirey,§ and David T. Stanton||

Mason Business Center, Procter & Gamble Pharmaceuticals, 8700 Mason-Montgomery Road, Mason, Ohio 45040, Dayton Site, Sandoz Inc., 2400 Route 130 North, Dayton, New Jersey 08810-1519, Winton Hill Business Center, The Procter & Gamble Company, 6083 Center Hill Avenue, Cincinnati, Ohio 45224, and Miami Valley Innovation Center, The Procter & Gamble Company, P.O. Box 538707, Cincinnati, Ohio 45253

The search for greater speed of analysis has fueled many innovations in high-performance liquid chromatography (HPLC), such as the use of higher pressures and smaller stationary-phase particles, and the development of monolithic columns. Alternatively, one might alter the chromatographic mobile phase. The low viscosity and high diffusivity of the mobile phase in supercritical fluid chromatography (SFC) allows higher flow rates and lower pressure drops than is possible in traditional HPLC. In addition, SFC requires less organic, or aqueous−organic, solvent than LC (important in preparative-scale chromatography) and provides an alternative, normal-phase retention mechanism. But fluids that are commonly used as the main mobile-phase component in SFC, such as CO_2, are relatively nonpolar. As a result, SFC is commonly believed to only be applicable to nonpolar and relatively low-polarity compounds. Here we build upon recent work with SFC of polar and ionic compounds and peptides, and we compare the LC/MS and SFC/MS of a diverse library of druglike compounds. A total of 75.0% of the library compounds were eluted and detected by SFC/MS, while 79.4% were eluted and detected by LC/MS. Some samples provided strong peaks that appeared to be related to the purported compound contained in the sample. When these were added to the "hits", the numbers rose to 86.7 and 89.9%, respectively. A total of 3.7% of the samples were observed by SFC/MS, but not by LC/MS, and 8.1% of the samples were observed by LC/MS, but not by SFC/MS. The only compound class that appeared to be consistently detected in LC/MS, but not in SFC/MS under our conditions, consisted of compounds containing a phosphate, a phosphonate, or a bisphosphonate. The SFC/MS method was at least as durable, reliable, and user-friendly as the LC/MS method. The APCI source required less cleaning during the SFC/MS separations than it did during LC/MS.

Speed of analysis has been one of the driving factors in the modern evolution of chromatographic methods. Speed is especially prized for areas such as high-throughput bioanalytical determinations,[1] confirmation of hits from high-throughput assays,[2] and screening samples in large repositories for purity and stability.[3,4] Reversed-phase high-performance liquid chromatography (HPLC)/mass spectrometry (LC/MS) and LC/MS/MS are arguably the most widely used chromatographic methods in these fields. The drive for speed has led to many chromatographic innovations, a few examples of which are the use of monolithic columns,[5,6] the use of higher column temperatures,[7−9] and the use of higher operating pressures coupled with smaller stationary-phase particles.[10−12] The need for speed has also fueled changes in the practice of mass spectrometry, such as increased use of flow injection analysis[13,14] and "multiplexed" mass spectrometry.[15] An alternative approach to achieve greater speed of analysis is to alter the chromatographic mobile phase. The low viscosity and high diffusivity of the mobile phase in supercritical fluid chromatography (SFC) allows higher flow rates and lower pressure drops than is possible in traditional HPLC. The "green" nature of SFC is an added benefit, since it requires a smaller amount of organic solvent than is required in LC. This is especially appealing for

* To whom correspondence should be addressed. E-mail: pinkston.jd@pg.com.
† Procter & Gamble Pharmaceuticals.
‡ Sandoz Inc.
§ Winton Hill Business Center, The Procter & Gamble Co.
|| Miami Valley Innovation Center, The Procter & Gamble Co.

(1) Alnouti, Y.; Srinivasan, K.; Waddell, D.; Bi, H.; Kavetskaia, O.; Gusev, A. I. *J. Chromatogr., A* **2005**, *1080*, 99−106.
(2) Smalley, J.; Kadiyala, P.; Xin, B.; Balimane, P.; Olah, T. *J. Chromatogr., B* **2006**, *830*, 270−7.
(3) Kozikowski, B. A.; Burt, T. M.; Tirey, D. A.; Williams, L. E.; Kuzmak, B. R.; Stanton, D. T.; Morand, K. L.; Nelson, S. L. *J. Biomol. Screening* **2003**, *8*, 205−9.
(4) Kozikowski, B. A.; Burt, T. M.; Tirey, D. A.; Williams, L. E.; Kuzmak, B. R.; Stanton, D. T.; Morand, K. L.; Nelson, S. L. *J. Biomol. Screening* **2003**, *8*, 210−5.
(5) Klodzinska, E.; Moravcova, D.; Jandera, P.; Buszewski, B. *J. Chromatogr., A* **2006**, *1109*, 51−9.
(6) Tanaka, N.; Kobayashi, H.; Ishizuka, N.; Minakuchi, H.; Nakanishi, K.; Hosoya, K.; Ikegami, T. *J. Chromatogr., A* **2002**, *965*, 35−49.
(7) Claessens, H. A.; van Straten, M. A. *J. Chromatogr., A* **2004**, *1060*, 23−41.
(8) Nawrocki, J.; Dunlap, C.; Li, J.; Zhao, J.; McNeff, C. V.; McCormick, A.; Carr, P. W. *J. Chromatogr., A* **2004**, *1028*, 31−62.
(9) Dolan, J. W. *J. Chromatogr., A* **2002**, *965*, 195−205.
(10) MacNair, J. E.; Opiteck, G. J.; Jorgenson, J. W.; Moseley, M. A., III. *Rapid Commun. Mass Spectrom.* **1997**, *11*, 1279−85.
(11) Wren, S. A. C. *J. Pharm. Biomed. Anal.* **2005**, *38*, 337−43.
(12) Eschelbach, J. W.; Jorgenson, J. W. *Anal. Chem.* **2006**, *78*, 1697−706.
(13) Cheng, X.; Hochlowski, J. *Anal. Chem.* **2002**, *74*, 2679−90.
(14) Wang, T.; Zeng, L.; Strader, T.; Burton, L.; Kassel, D. B. *Rapid Commun. Mass Spectrom.* **1998**, *12*, 1123−9.
(15) Bayliss, M. K.; Little, D.; Mallett, D. N.; Plumb, R. S. *Rapid Commun. Mass Spectrom.* **2000**, *14*, 2039−45.

10.1021/ac061033l CCC: $33.50
Published on Web 10/06/2006

preparative-scale chromatography since less time and energy are required to remove solvent and isolate products. Fluids that are commonly used as the main mobile-phase component in SFC, such as CO_2, are relatively nonpolar. As a result, SFC has been generally limited to separations of nonpolar and relatively low-polarity compounds.

However, it has become clear over the past few years that a much broader range of analyte polarities are amenable to SFC and SFC/MS if appropriate mobile-phase modifiers, additives, and columns are used. For example, anionic analytes are well behaved when volatile ammonium salt additives are used at low-to-submillimolar levels.[16,17] Cationic analytes can even be eluted without additive with the proper choice of chromatographic stationary phase, such as the 2-ethylpyridine phase.[18] Large, hydrophilic peptides, long thought absolutely incompatible with SFC, have been eluted using low levels of trifluoroacetic acid (TFA) as an additive in a CO_2/methanol mobile phase.[19] These recent results have made it clear that previous assumptions about the incompatibility of SFC with polar and ionic analytes are unfounded. "Pharmaceutically relevant" compounds (i.e., drugs, their synthetic precursors, new chemical entities synthesized for testing, and other compounds contained in compound repositories maintained by pharmaceutical companies) span a wide range of compound polarities and incorporate a great variety of functional groups. We wondered how SFC/MS would compare with standard LC/MS methods for a wide range of pharmaceuticals. Might the advantages of SFC/MS be applied on a more widespread basis in the world of pharmaceuticals? This question has been addressed on a more limited basis in previous work.[20] Some groups in the pharmaceutical industry have enthusiastically adopted SFC, SFC/MS, and semipreparatory-scale SFC and have achieved impressive results.[21,22] However, many pharmaceutical scientists remain reluctant to use SFC, despite its advantages, due to fears that it is not applicable to a large fraction of druglike molecules. In the work presented here, we selected a large and diverse library of pharmaceutically relevant compounds, performed SFC/MS and LC/MS on these compounds using "universal" screening methods, and compared the results.

EXPERIMENTAL SECTION

Safety Considerations. The mobile phase used in this work consists in large part of compressed CO_2. Researchers should note that mobile-phase leaks will result in significant cooling of the region surrounding the leak and can result in freezing of unprotected skin. All columns, tubing, and fittings must be rated to withstand the pressures and temperatures applied. Column effluent should be vented to an appropriate fume hood.

Chemicals. HPLC-grade methanol (MeOH), acetonitrile (ACN), and distilled methyl sulfoxide (DMSO, 99.96%) were purchased from EM Science (Gibbstown, NJ). Water was purified using a Milli-Q system (Millipore, Bedford, MA). TFA (99%) was obtained from Aldrich (Milwaukee, WI), and ammonium acetate (NH_4OAc, 97%) was purchased from J. T. Baker (Phillipsburg, NJ).

Analyte Selection and Sample Preparation. We used a six-parameter, bin-based sampling method to chose a diverse library of analytes representing the compounds in Procter & Gamble Pharmaceuticals' Haystack Repository. The compounds in the Repository were either purchased from sources outside the company or produced in-house. The compounds in the Haystack were stored as sealed dry powders at room temperature. The similarity/diversity analyses were performed using the DiverseSolutions program (version 4.0.9, from Prof. R.S. Pearlman, University of Texas at Austin). The chemistry space consisted of six "BCUTs". The BCUTs are complex molecular descriptors that capture the properties of individual atoms in a molecule along with a measurement of the "distance" between all pairs of atoms. The BCUTs we chose included one based on electronegativity, one based on partial atomic charges (Gasteiger-Huckel), one based on H-bond donating ability, one based on H-bond accepting ability, and two that rely primarily on polarizability. We chose a total of 2266 compounds for our diverse library. The chosen compounds incorporated a wide variety of functional groups, including nonpolar aliphatics, aromatics, carotenoids, amine hydrohalides, quaternary ammonium salts, multicarboxylate salts, sulfonates, sulfates, sulfamic acid salts, phosphates, phosphonates, multiphosphonate salts, polyhydroxy compounds, and nitro compounds. Figure 1 shows a superposition of our 2266-compound library on top of the September 2000 version of the World Drug Index (WDI), containing 55 720 structures. The graph shows the score plot of the first two principal components resulting from the application of principal component analysis of our library and of the WDI in the original six BCUT descriptor space.

Samples were submitted for analysis in a 96-deep-well plate format. Three milligrams of each sample was dissolved in 500 μL of DMSO (6 mg/mL). Thirty microliters of each solution was evaporated to dryness under a stream of dry N_2 and taken up in MeOH to give a final concentration of 0.2 mg/mL. This solution was then subjected to SFC/MS. Sixty microliters of the original 6 mg/mL solution was evaporated to dryness and diluted with DMSO to give a final concentration of 0.4 mg/mL. This solution was analyzed by LC/MS.

SFC/MS System. The SFC experiments were performed using a Berger Analytical SFC system (Mettler-Toledo-Autochem, Newark, DE) equipped with an Agilent 1100 series DAD detector (Agilent Technologies, Palo Alto, CA). The Berger system incorporated an Alcott autosampler (Alcott Chromatography, Inc. Norcross, GA). The system was controlled by BI 3D ChemStation software. The column for SFC/MS was a Deltabond Cyano 50 × 4.6 mm, 5-μm particle size (Thermo Hypersil, Bellefonte, PA). The primary mobile-phase component was SFC/SFE-grade CO_2 (Air Product, Allentown, PA). The mobile-phase modifier consisted of MeOH containing 1 mM NH_4OAc. The total mobile-phase flow rate was 2.5 mL/min (measured in the liquid phase). The mobile-phase gradient began with a 0.5-min hold at 1% modifier. The modifier concentration then rose from 1 to 60% at 40%/min, was

(16) Zheng, J.; Glass, T.; Taylor, L. T.; Pinkston, J. D. *J. Chromatogr., A* **2005**, *1090*, 155−64.

(17) Zheng, J.; Taylor, L. T.; Pinkston, J. D.; Mangels, M. L. *J. Chromatogr., A* **2005**, *1082*, 220−9.

(18) Zheng, J.; Taylor, L. T.; Pinkston, J. D. *Chromatographia* **2006**, *63*, 267−76.

(19) Zheng, J.; Pinkston, J. D.; Zoutendam, P. H.; Taylor, L. T. *Anal. Chem.* **2006**, *78*, 1535−45.

(20) Berger, T. A.; Fogleman, K.; Staats, T.; Bente, P.; Crocket, I.; Farrell, W.; Osonubi, M. *J. Biochem. Biophys. Methods* **2000**, *43*, 87−111.

(21) Bolanos, B.; Greig, M.; Ventura, M.; Farrell, W.; Aurigemma, C. M.; Li, H.; Quenzer, T. L.; Tivel, K.; Bylund, J. M. R.; Tran, P. *Int. J. Mass Spectrom.* **2004**, *238*, 85−97.

(22) Ventura, M.; Farrell, W.; Aurigemma, C.; Tivel, K.; Greig, M.; Wheatley, J.; Yanovsky, A.; Milgram, K. E.; Dalesandro, D.; DeGuzman, R. *J. Chromatogr., A* **2004**, *1036*, 7−13.

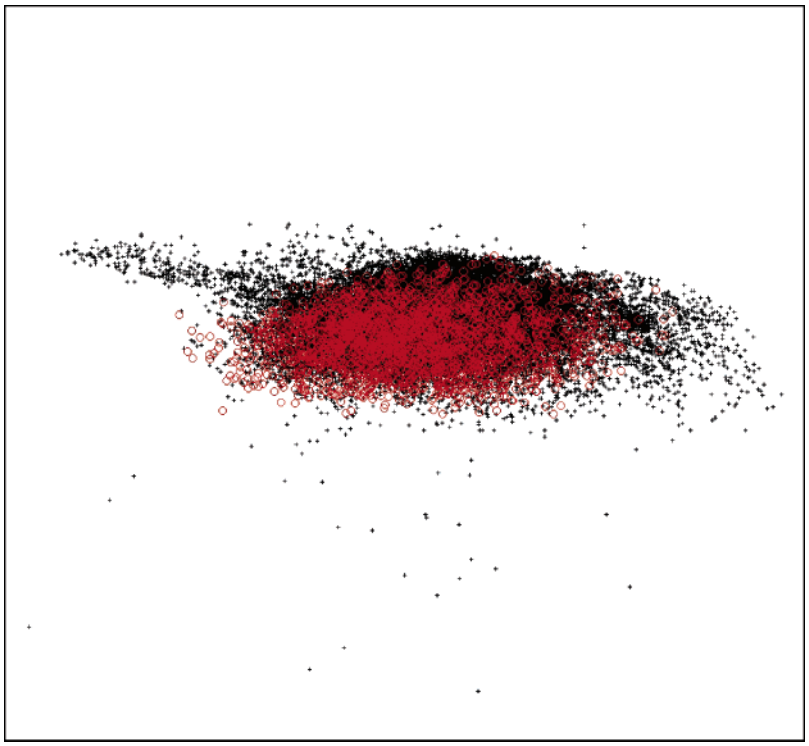

Figure 1. Scores plot of the two principal components from the principal components analysis of our diverse library and of the WDI (September 2000 version containing 55 720 members) in the six BCUT descriptor space. +: Member of the WDI; ○: member of the diverse library chosen from P&G Pharmaceuticals' Compound Repository.

Table 1. Experimental Conditions for SFC/MS and LC/MS

	SFC/MS	LC/MS
flow rate	2.5 mL/min	3 mL/min
injection volume	5 μL	10 μL
UV wavelength	220, 280 nm	254 nm
ELSD	n/a	gain = 9, 2.8 bar, 41 °C
split ratio	none	5:1 (UV/ELSD:MS)
ionization/data type	APCI, alternating + and -, centroid data	APCI, alternating + and −, centroid data
mass range	100 − 800 (+, -)	130−1000 (+, -)
scan rate	0.60 s/scan, 0.05-s interscan delay	1.75 s/scan, 0.175-s interscan delay
desolvation gas	N_2, 750 L/h	N_2, 750 L/h
cone gas	N_2, 25 L/h	N_2, 25 L/h
probe temperature	450 °C	450 °C
source temperature	150 °C	150 °C
cone voltage	7 V	8 V

held 1 min at 60%, and then returned to the starting concentration of 1% at −60%/min. The column oven was held at 35 °C. Other chromatographic conditions are listed in Table 1.

The Berger SFC instrument was interfaced to a Micromass ZMD mass spectrometer from Waters (Waters Co., Milford, MA) using the pressure regulating fluid interface.[23−25] The downstream (i.e., post-UV detector) pressure of the SFC system was regulated at a constant 200 bar using a model PU-1580 pump (Jasco Co., Hachiojishi, Tokyo, Japan) running in the constant-pressure mode. The pump flow and the chromatographic effluent mixed in a near-zero dead volume (ZDV), 0.0625-in. (1.59-mm) chromatographic tee (Valco Instruments, Houston, TX). The PU-1580 pump delivered ~0.2 mL/min of MeOH. The effluent from the tee was directed to the mass spectrometer using a short piece of 0.127-mm-i.d. PEEK tubing (Upchurch Scientific, Oak Harbor, WA).

Atmospheric pressure chemical ionization (APCI) was chosen as the method of ionization. The Micromass ZMD APCI probe was modified by replacing the small inlet filter with a ZDV 1.59-mm chromatographic union (Valco Instruments) containing a piece of deactivated fused-silica tubing of 300 μm o.d. × 100 μm i.d. (Dionex, Sunnyvale, CA) which was held in a F-230 PEEK sleeve (Upchurch Scientific) by a stainless steel nut and ferrule (Valco Instruments). Mass spectrometric conditions are shown in Table 1.

LC/MS System. The LC/MS system was already in use for screening repository hits, for quality control for repository acquisitions, and to study compound stability under the repository storage conditions. The LC system was a model 2790 Alliance (Waters). The column was a Symmetry Shield Rx C8, 4.6 × 50 mm, 2.5-μm

(23) Baker, T. R.; Pinkston, J. D. *J. Am. Soc. Mass Spectrom.* **1998**, *9*, 498−509.
(24) Chester, T. L.; Pinkston, J. D. *J. Chromatogr.* **1998**, *807*, 265−73.
(25) Pinkston, J. D. *Eur. J. Mass Spectrom.* **2005**, *11*, 189−97.

particle size (Waters). Mobile phase A was 95% ACN/5% H_2O/0.005% TFA, and mobile phase B was 3% ACN/97% H_2O/0.005% TFA. The mobile-phase gradient went from 100% B to 100% A over 3 min. The final condition was held for 1.5 min, then the composition returned to 100% B, and equilibrated at that level for 1.5 min (total cycle time was 6.0 min). The LC system was interfaced to the Micromass ZMD mass spectrometer through the standard APCI interface. Other chromatographic and mass spectrometric conditions are shown in Table 1.

RESULTS AND DISCUSSION

The goal of this work was to compare the results of screening by SFC/MS and by LC/MS using "universal" methods. In earlier work, we found that the addition of a low level of a volatile salt, such as ammonium acetate, to the mobile-phase modifier made a dramatic improvement in the elution of polar and ionic molecules by SFC/MS.[26] We therefore added 1 mM ammonium acetate to the methanol modifier in the SFC/MS work. It is likely that further improvements in the ability to elute polar and ionic druglike molecules could be obtained by applying advances in stationary-phase and additive technologies from recent research.[16−19] Similarly, the universal LC/MS method incorporated a low level of TFA in the mobile phase. APCI was the ionization method used in the existing universal LC/MS screening method. Since we did not want the mass spectrometric detection scheme to be a confusing factor in the results, we used the same mass spectrometer and the same ionization method, APCI, for the SFC/MS portion of this work.

It is important to note that the sample solvent for SFC/MS (methanol) differed from that used for LC/MS (DMSO). We found that ionization suppression from DMSO was a greater problem with the SFC/MS method we used than with the standard LC/MS method. We performed a series of SFC/MS experiments with model analytes, caffeine, dibenzyloxyacetophenone, hydralazine hydrochloride, and ibuprofen, dissolved in methanol containing 0, 1, 5, and 10% DMSO. We observed ionization suppression in both positive and negative ion modes with 10% DMSO and ionization suppression in positive ion mode with 5% DMSO. Ultimately, we decided to remove as much DMSO as practical by simple evaporation under a stream of dry N_2 and to redissolve in methanol for SFC/MS. A low level of DMSO (<1%) remained in the SFC/MS sample solutions. A greater number of SFC sample solutions treated in this manner displayed a precipitate in the bottom of the well than did the LC/MS sample solutions dissolved in DMSO. The implications of this observation on our results are discussed below.

A few other notable differences between the LC/MS and SFC/MS methods include the presence or absence of effluent flow splitting, the concentrations of the sample solutions, the injection volumes, and the mass spectral scan rates and ranges. For simplicity, we choose not to use the ELSD for the SFC/MS work. The full SFC effluent flowed through the UV cell and to the APCI interface. The mass spectrometric response was therefore greater in SFC/MS mode than in LC/MS mode, the latter of which operated with a 5:1 (ELSD vs MS) split. For this reason, we chose to reduce the SFC injection volume and the concentration of the SFC sample solutions. Because of these changes, the mass spectral responses in LC/MS mode (5:1 split) and in SFC/MS mode (no split) were roughly comparable. The SFC peaks were narrower than were the LC peaks. We increased the mass spectral scan rate in SFC/MS mode to adequately capture the SFC peak profile (0.6 vs 1.75 s/scan). We also reduced the mass-to-charge ratio (m/z) scan range in SFC/MS mode to accommodate the increased scan rate (m/z 100−800 vs 130−1000). The great majority of analytes in the library fell within the m/z range covered by both LC/MS and SFC/MS (m/z 130−800). Overall, we do not believe that these differences between the LC/MS and SFC/MS methods adversely affected our comparison of how many compounds could be eluted and detected using the two methods.

The LC/MS system had been in use for some time performing quality control for compounds present in, or acquired for, Procter & Gamble Pharmaceuticals' Compound Repository. The LC/MS data in this work were analyzed and presented to the investigator using Micromass/Waters' OpenLynx Browser. The most common question asked of the data was whether the compound purportedly present in the repository sample was detected in the LC/MS analysis. The browser provided an indication of whether ions related to the compound of interest were detected (green spot), were not detected (red spot), or the presence of the analyte was uncertain (yellow spot). The user may set parameters that categorize the results in one of these three categories. The investigator would typically review results that fell into the third, uncertain, category, but was also at liberty to review other result sets to make sure that detection parameters were set correctly and that the browser was performing as expected.

As might be expected, a wide variety of chromatographic behaviors were observed for the library compounds, in both the LC/MS and the SFC/MS modes. Figure 2 provides some typical chromatograms of compounds that were classified as "hits" (i.e., present in the sample). These separations were all performed in the SFC/MS mode, but similar results were obtained in LC/MS mode. Figure 2A illustrates the chromatogram of a multifunctional compound that was best detected in positive-ion mode. The compound is well retained and is relatively well-behaved chromatographically, though some tailing is observed in the ion chromatogram at m/z 575 ($[M + H]^+$). Similarly, Figure 2B shows the chromatogram of a well-retained, multifunctional compound that contains a carboxylic acid and is best detected in negative-ion mode (m/z 319, $[M - H]^-$ of the most abundant isotope). Figure 2C illustrates the somewhat unusual case for nitro-containing compounds. This compound, like most nitro-containing compounds, was detected as the negative molecular ion ($[M]^-$). (This is only unusual in the sense that most molecules produce predominantly the protonated or deprotonated molecule by APCI.) This compound is well retained. The shoulder on the front of the peak most likely indicates an unresolved isomer. Figure 2D shows the chromatogram of an unretained, multfunctional compound that is best detected as its protonated molecule at m/z 533. Figure 2E illustrates an example of a case that was more common than we would have preferred. In this situation, the target compound was flagged as a hit, but was only detected at a low level as its deprotonated molecule, in the negative-ion mode. It is also clear from the ultraviolet absorbance trace that the sample contains a number of other major components. Nevertheless, following the standard protocol in place for LC/MS screening, this sample was

(26) Pinkston, J. D.; Stanton, D. T.; Wen, D. *J. Sep. Sci.* **2004**, *27*, 115−23.

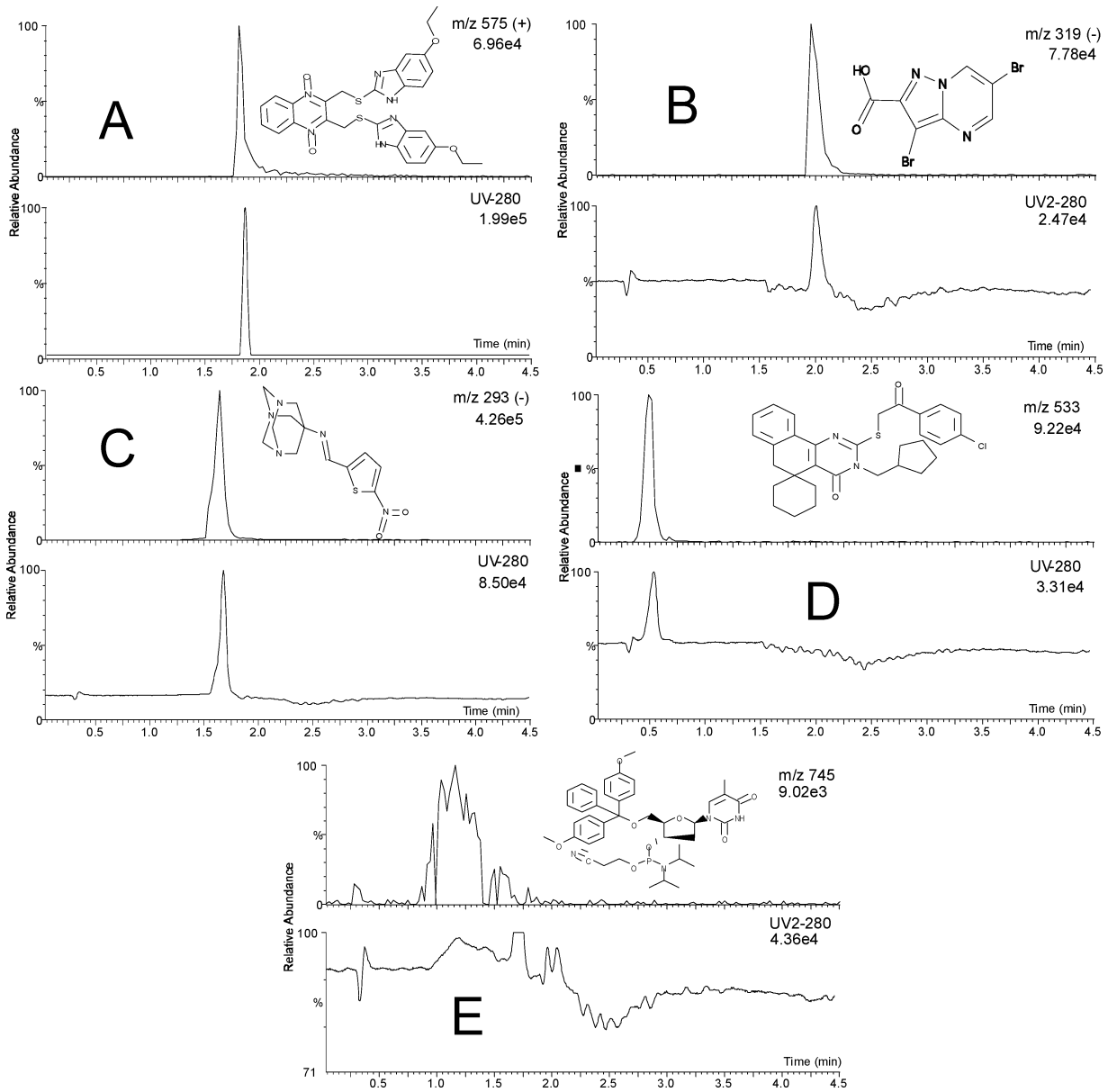

Figure 2. Typical chromatograms acquired in SFC/MS mode. The upper trace in each pair is a mass chromatogram. The lower trace is a UV absorbance chromatogram at 280 nm. See the text for further details.

classified as containing the compound which it purportedly contained, and this compound was reported to have been successfully eluted and detected by SFC/MS. This type of situation arose with roughly equal frequency in SFC/MS and LC/MS modes.

A note about the quality of large compound repositories is in order at this point. The samples contained within the P&GP compound repository were acquired from a variety of sources. Some were synthesized and purified in-house. Others were purchased from external organizations. A subsample of these compounds was often assayed for identity and purity during the normal course of building the repository. Many of the groups of samples purchased externally were of high quality. However, the quality of some of the groups of samples was not as high as advertised. It has been estimated that 5−10% of samples in most large compound repositories do not contain the compound that they are reported to contain. Other samples contain one or more other compounds, in addition to the compound they are purported to contain. This is an accepted state of affairs in the world of high-throughput screening.[27] Anecdotally, we confirmed this situation. For example, many samples in our library that were not hits (i.e., did not contain the compound of interest) exhibited one or more large peaks upon closer examination. It was not uncommon for these peaks to be shifted in mass by, for example, 2 or 16 Da, from the target compound, suggesting that the compound of interest had undergone a simple reaction. This was a situation that we fully expected to encounter during our comparison. Since the criteria for establishing whether a sample in our library was a hit were identical for LC/MS and SFC/MS, we do not feel that the relatively small percentage of samples which were impure or incorrectly labeled greatly altered our conclusions. Every result was independently confirmed by examination of the raw data by

(27) Gibboney, K. personal communication, 2006.

at least two investigators. Occasionally, incorrect results reported by the browser were corrected.

Of the 2266 samples chosen for our library, 2153 were screened. (Some samples were not present in sufficient quantity to allow screening.) A total of 1615 samples (75.0% of the samples screened) were judged to be "hits" (compound reportedly present in the sample was actually detected) by SFC/MS screening. Another 252 samples were not judged hits but provided a strong peak by SFC/MS. This peak could often be easily related to the compound that the sample was reported to contain. Thus, 1867 samples (86.7%) were either hits or contained a strong peak by SFC/MS. Importantly, only 18% of the SFC/MS-screening samples judged "not detected" (i.e., not containing the compound they were purported to contain, also known as "no's") displayed a precipitate. So we were able to conclude that low solubility in methanol did not appear to be a strong factor in generating negative results in the SFC/MS screening.

A total of 79.4% (1709 samples) of samples screened by LC/MS were judged to be hits. Similarly to what was observed by SFC/MS, 89.9% (1935 samples) of the screened samples were either hits or displayed a large peak by LC/MS screening. The similarity between the number of hits by SFC/MS and by LC/MS was surprising to us. While LC/MS is more widely applicable than SFC/MS, the differences in hit rates, at 4.4% for confirmed hits, and 3.2% for "hits + large peaks", were smaller than we expected. Interestingly, 3.7% of the samples were hits by SFC/MS but not by LC/MS. Conversely, 8.1% of the samples were hits by LC/MS but not by SFC/MS. This result speaks to the complimentary nature of the two methods.

In reviewing the results, a few trends emerged. The no's fell roughly into four categories. The largest fraction of no's were compounds that appeared to be good candidates for ionization by APCI (i.e., contained an amine or other suitable heteroatom), were within the m/z range examined, and had structures that appeared to be compatible with elution by SFC and LC, but were not detected by either LC/MS or SFC/MS. We suspect that these samples simply did not contain the compound they were purported to contain, as discussed earlier. A second group of no's included compounds that were within the m/z range examined, but were primarily aromatic or saturated hydrocarbons, and contained few, if any, heteroatoms. A few members of this group were large ethers. These types of molecules are known to not be efficiently ionized by APCI. We were not surprised that these compounds were not detected by either LC/MS or SFC/MS. A third group of no's consisted of compounds containing one or more phosphates, phosphonates, and bisphosphonates. These compounds were consistently not detected by SFC/MS, while they were often hits (i.e., detected) by LC/MS. This is the only functional group that appeared to consistently prevent elution of an analyte under our SFC/MS conditions. This result was consistent with SFC/MS results we have observed in unrelated studies.[28] Finally, the smallest group of no's consisted of compounds whose protonated or deprotonated molecule, or adduct ions, were simply above or below the m/z ranges examined in this study (m/z 100−800 for SFC/MS and m/z 130−1000 for LC/MS).

It is important to note the SFC/MS method was at least as durable, reliable, and user-friendly as the LC/MS method in this work. The APCI source required less cleaning during the SFC/MS separations than it did during the LC/MS work. Column durability was approximately equal for the two methods.

CONCLUSIONS

The difference in the percentage of hits between the LC/MS and SFC/MS screening of a large and diverse library of druglike molecules was 4.4% (79.4% by LC/MS vs 75.0% by SFC/MS), a smaller difference than we initially expected. The difference was even smaller (3.2%) when samples providing a large signal, but not at the expected m/z, were added to the hits. For the best possible coverage in a universal SFC/MS method, it is important that the SFC modifier contain an additive such as ammonium acetate to facilitate the elution of very polar and ionic analytes. With this proviso, it appears that SFC/MS is suitable for screening large and diverse libraries of druglike molecules. The advantages of SFC—speed, environmental friendliness, orthogonal selectivity to reversed-phase HPLC, reduced cost of operation (especially in preparatory-scale separations)—will be welcome in the pharmaceutical and related industries.

Received for review June 6, 2006. Accepted August 30, 2006.

AC061033L

(28) Pinkston, J. D.; Mangels, M. L. unpublished work, 2003.