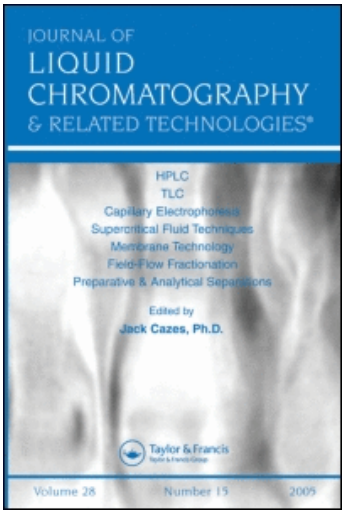

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Purification of HTOS Libraries by Supercritical Fluid Chromatography

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ABSTRACT

A commercially available preparative scale supercritical fluid chromatography (SFC) system has been customized and applied to the purification of high throughput organic synthesis (HTOS) libraries. Pilot reactions allow the triage between HPLC and SFC as the most appropriate purification technique, and when applicable, SFC has been proven to offer advantages in terms of decreased purification and sample dry-down time and purification capabilities, somewhat complementary to HPLC. Standardized chemistry-specific SFC methods have been determined and HTOS pilot reactions allow refinement of these standard methods to optimal conditions for a specific library core.

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Key Words: Supercritical fluid chromatography (SFC); High-throughput organic synthesis (HTOS); Preparative chromatography.

INTRODUCTION

The High Throughout Purification (HTP) group in Pharmaceutical Discovery at Abbott Laboratories offers purification support to the High Throughput Organic Synthesis (HTOS) group. The role of the HTOS group is to elaborate core chemical structures for Discovery scientists into libraries of analogs using standardized chemistries and monomer sets. Preparative scale supercritical fluid chromatography (SFC) has recently been added to the HPLC-based purification capabilities available in HTP for HTOS, and has been fully integrated into the purification service. As preliminary synthetic investigations proceed with each reaction type in the HTOS group, the material generated is used by HTP to triage between HPLC and SFC, selecting the most appropriate purification method for each functional group set. When core compounds are submitted to the HTOS group by medicinal chemists, it is common practice to perform pilot reaction runs, attaching a diverse set of monomers to each core to assess the likely success of library production for that core. These pilot reaction sets are then used by the HTP group for SFC methods development, where appropriate column, buffer, and gradient conditions can be developed for the purification of the subsequent full libraries (sets of 48 samples and multiples thereof) to be synthesized.

A UV and ELSD-triggered fraction collection RP-HPLC with mass spectrometry validation procedure has been the method of choice for the purification of combinatorial chemistry libraries for five years. This philosophy of UV-trigger, followed by MS validation, allows the accommodation of several preparative HPLCs by a single mass spectrometer (the faster and more expensive instrumentation relative to HPLC). During this time, a highly automated system has been developed, including custom software for data tracking, sample validation by flow injection mass spectrometry, report generation, and sample labeling. The bottleneck of this system has always been sample dry-down and in an attempt to eliminate this bottleneck, preparative scale SFC, with a carbon dioxide based solvent system, was investigated. Although, the use of SFC for the purification of high-throughput libraries has previously been reported,[1–6] no commercially available instruments met our needs, which included; the ability to collect an unlimited number of fractions/sample in a software environment and hardware format compatible with the current HPLC sample tracking, validation and labeling capabilities, and the ability to collect at atmospheric pressure with high recovery. To meet

these needs, a "Manual Preparative" SFC system was purchased from Berger Instruments (A subsidiary of Mettler-Toledo, Greifensee, Switzerland) and customized by the Automation Engineering group at Abbott Laboratories. The engineering details of this work can be found in a companion article.[7]

EXPERIMENTAL

Abbott Laboratories' engineers[7] have modified a Berger Instruments (Newark, DE) preparative SCF and have developed software for the integration of this system into the existing preparative HPLC purification processes, as outlined in Fig. 1.

To a "manual" version of the Berger SFC was integrated a Gilson (Middleton, WI) 232 auto-sampler with a Gilson 720 keypad for sample injection, and a Cavro MiniPrep™ (Sunnyvale, CA) pipetting instrument customized in-house to serve as a fraction collector. Custom designed "collection shoes" (Fig. 2) attached to the pipettor arms enable collection of samples at atmospheric pressure, and a methanol wash system is incorporated into the fraction collection lines to assure high recovery and eliminate cross-contamination between fractions.

Communication between the proprietary Berger SFC control software, the Gilson auto-sampler scripts, and the custom fraction collector control software, each, of which runs on a separate computer, is accomplished through a series of digital control lines that indicate the status of a particular subsystem to its two companions. In addition, analog and digital input lines running from sensors on the Berger SFC to the fraction collection computer are used to track all fractions, to log their timing, and to record the overall chromatograms. Fraction collection is UV-triggered, and post-purification validation is accomplished offline by mass spectrometry on a Finnigan (San Jose, CA) LCQ MS fitted with APCI and ESI probes, acquired in the positive or negative ion mode as is most appropriate to the structural class being isolated. At present, a single mass spectrometer adequately accommodates four preparative scale HPLCs and one preparative scale SFC for post-purification validation. An already existing custom software package—Post Purification Analysis, used for tracking tube positions, fraction collection data, and mass spec results from UV-triggered, MS-validated preparative HPLC, functions equally well for appropriately formatted SFC data.

The application tracks sample and fraction information using a Microsoft Access database. The user can review chromatographic data, select fractions for MS analysis, and export a sample list for acquiring flow-injection MS of these fractions. Flow-injection MS (most often +APCI), are acquired for selected fractions, sampling directly from the fraction racks. The MS data can

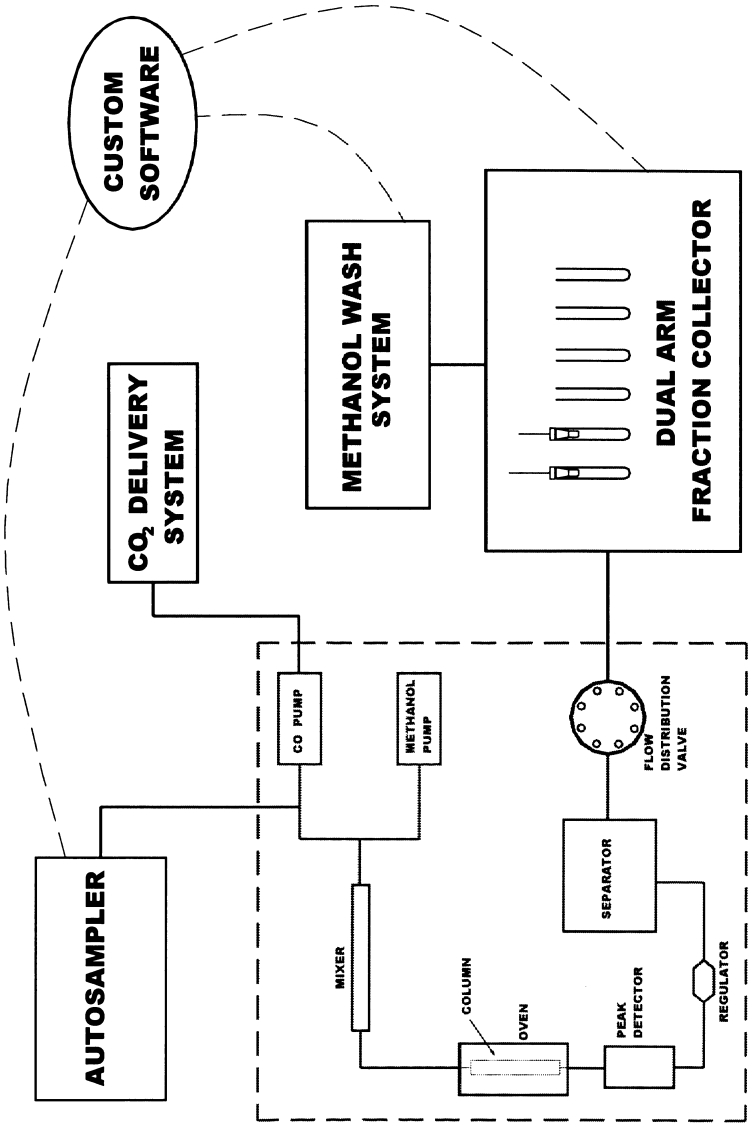

Figure 1. Schematic representation of customized system.

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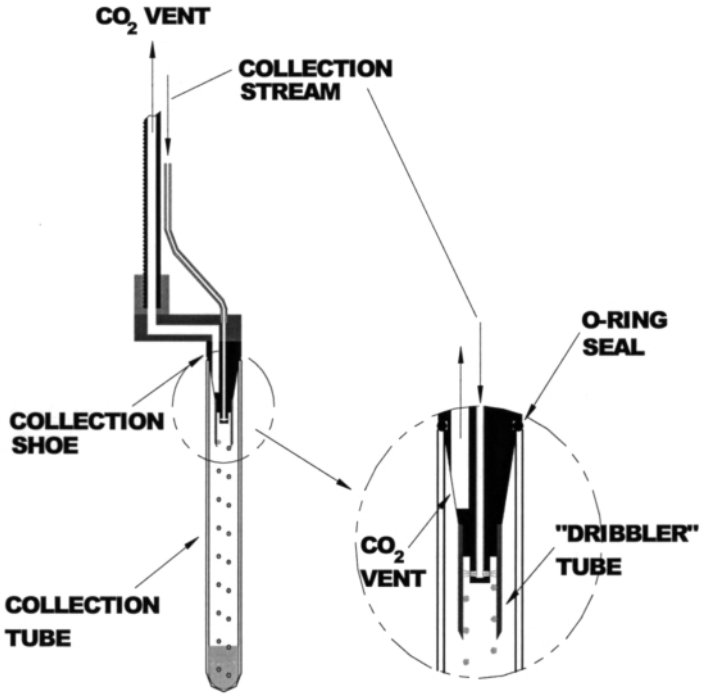

Figure 2. Collection shoe design.

then be reviewed in the same software application, and fractions of interest selected for labeling and dry-down. The software also provides label and report printing functions. A screen capture of the SFC-integrated PPA package is shown in Fig. 3.

RESULTS AND DISCUSSION

Triage Between Supercritical Fluid Chromatography and HPLC

The HTOS group develops and implements standardized chemistries that can be applied to a wide variety of "core" compounds submitted to this service group by medicinal chemistry clients. This reaction development step generates a range of reaction mixtures of similar functional groups and varying polarity, which can be used for the purpose of triaging between HPLC and

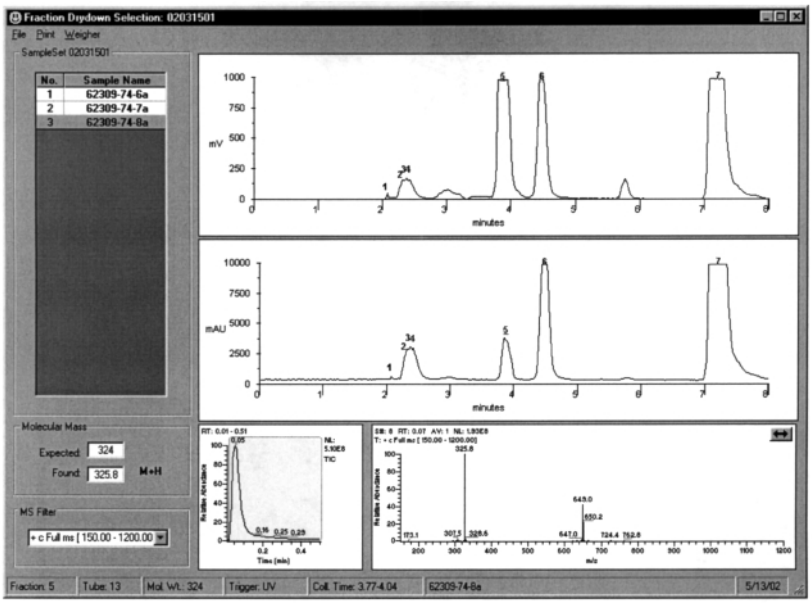

Figure 3. Screen capture of PPA (post purification analysis) software. "Expected MW" field is populated from information provided by the synthetic chemist from a sample submission excel sheet. "Found MW" is populated from data imported from the post-purification MS run and changes as each peak in the chromatogram is selected to provide MS results for the corresponding collection tube. Chromatograms are displayed for two selected UV wavelengths (220 nm and 254 nm in this case for upper and lower chromatograms, respectively).

SFC as the optimal purification technique. Large numbers of libraries will be generated subsequently via these reaction protocols, and the initial analysis of functionality vs. optimal method from pilot samples is generally predictive of optimal technique for most libraries thus generated. Reaction mixtures from various "practice runs" are split, and one-half of the available material is purified by each of HPLC and SFC under standard general conditions. ("General conditions" it should be noted are iteratively identified from previous experience of the functional groups to be separated, and are reaction dependant.) The chromatograms from each technique, for each reaction mixture, are then compared to assess which method is the better able to accommodate the functional groups generated in products and, also, those present in un-reacted starting materials for a particular reaction type. Figure 4 depicts a case in which SFC was selected as the purification technique of

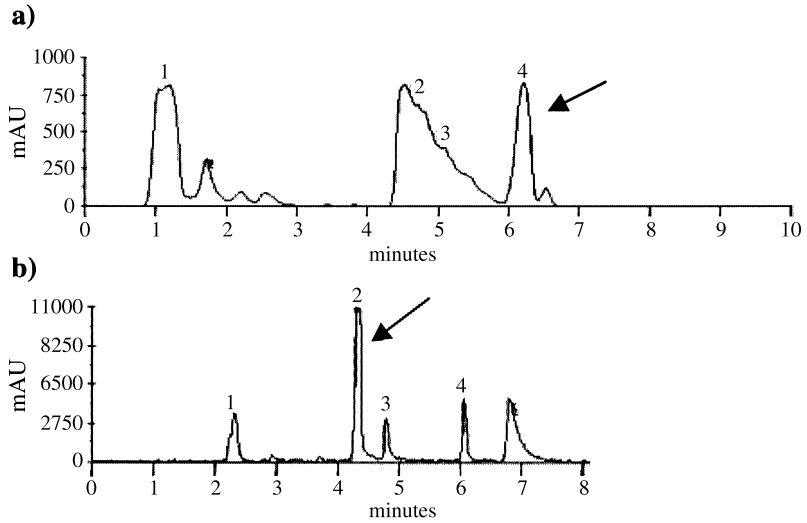

Figure 4. (a) HPLC chromatograms for Reaction Mixture A. HPLC conditions: C18 (20×100 mm Nova-Pak^{TM}), gradient 5–95% CH_3CN/H_2O with 0.1% TFA. (b) SFC chromatogram for Reaction Mixture A. SFC conditions: Diol column (21.2×150 mm, Berger Instruments), gradient 5–60% MeOH with 0.5% dimethylethylamine in CO_2.

choice. This selection was made due to the relatively narrow peak width in SFC and the fact that elution of the desired compound was well separated from (and prior to the elution of) most other reaction components. Figure 5, in contrast, depicts a case in which HPLC was selected as the optimal purification technique. This selection was made due to the relatively sharp peak width in HPLC and the fact that the desired compound was well separated from most other reaction components.

In many cases, SFC can give better resolution than can HPLC for specific reaction products, particularly amines, which typically are broad in the latter technique. (It should be noted that column lengths are not equivalent for the two techniques, SFC allowing the use of longer columns at the same flow rate as HPLC with lower backpressure due to the low viscosity carbon dioxide primary mobile phase component.) In some cases, HPLC is selected as the method of choice due to the presence of highly polar inorganic reaction bi-products, which can be easily accommodated by HPLC washing off with the column dead volume solvent front.

Solubility of the reaction mixture in an appropriate solvent for injection can limit the availability of SFC in certain cases. In order to retain good

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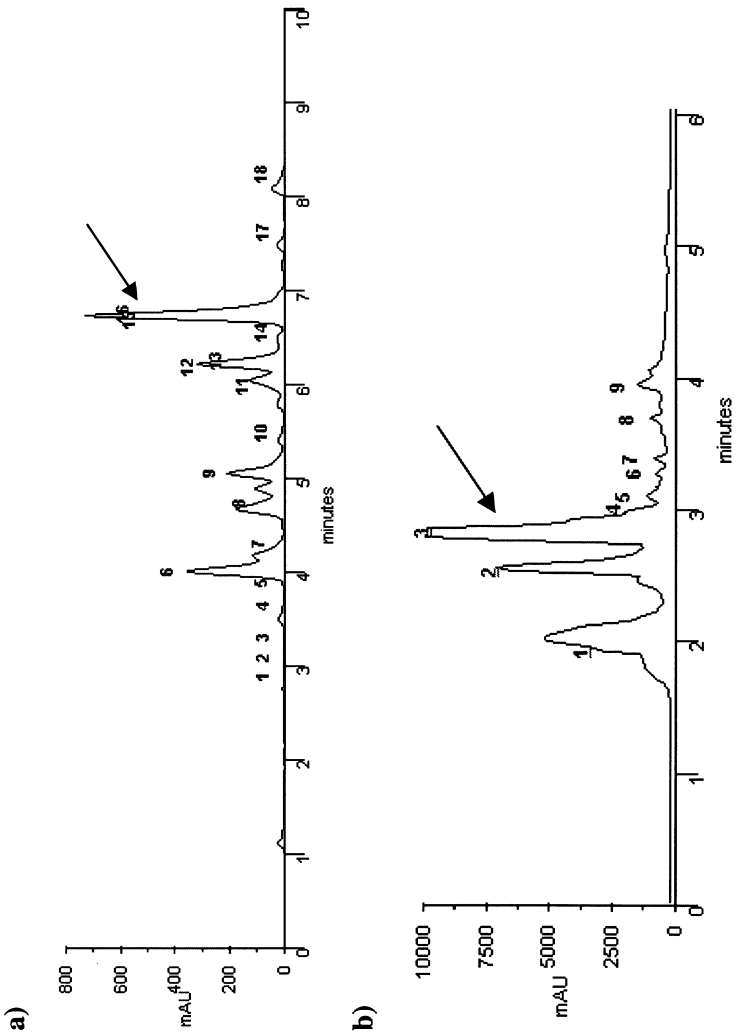

Figure 5. (a) HPLC chromatograms for Reaction Mixture B. HPLC conditions: C18 (20 × 100 mm Nova-Pak™), gradient 5–95% CH_3CN/H_2O with 0.1% TFA. (b) SFC chromatogram for Reaction Mixture B. SFC conditions: Diol column (21.2 × 150 mm, Berger Instruments), gradient 5–60% MeOH with 0.5% triethylamine in CO_2.

resolution, the sample must be dissolved in a relatively small amount of injection solvent. Typically, a 50 mg sample is dissolved in up to 1.5 mL, and gives good chromatographic behavior. Acceptable solvents have been; pure methanol, methanol/dichloromethane (2 : 1), and methanol/acetonitrile (1 : 1). Methanol/DMSO mixtures of 1 : 1 give poor resolution, though lower percentages of DMSO can be used. For HPLC, a 50 mg sample is dissolved in 1.5 mL of DMSO/methanol, which is more widely universal than any of the above mentioned SFC injection solvents, therefore, a larger percentage of samples are not amenable of SFC purification due to this solubility limitation.

Supercritical Fluid Chromatographic Method Development

Once SFC has been selected as the method of choice for the purification of samples resultant from a particular HTOS reaction protocol, HTOS libraries prove particularly amenable to the development of, and accommodation by, these chromatographic methods. This is due to the procedure of HTOS to perform pilot reactions on each new core (typically reaction with a set of six structurally diverse monomers) provided for purification "practice" before a full 48 member library is subsequently synthesized. This protocol generates pilot reaction mixtures, which can be used for methods development in SFC, assessing the effects of: different stationary phase columns, solvent system gradient slopes, and the effect of various tertiary additives to the basic methanol/carbon dioxide system. Columns, which we have found to be most appropriate for the purification of the desired compound types required by HTOS, have been: diol, aminopropyl, and cyanopropyl. It has been our experience, that the stationary phase packing material has the largest effect of all parameters studied on the retention and resolution of various compounds. Figure 6 for example, illustrates the effect of a diol, vs. a cyanopropyl vs. an aminopropyl column for the separation of components of a typical reaction mixture. For the separations, which we have been asked to accommodate, we have found cyanopropyl to be a very general column from which most components can be eluted, but offering poor separation capability, particularly for structurally related components. Diol has proven, by far, to be the best general column for the accommodation of these libraries, with aminopropyl proving superior only when all components of the reaction mixture are basic in nature.

Tertiary modifiers added to the methanol/CO_2 mixture have the second largest effect on peak shape and retention. For neutral or acidic compounds, binary component-methanol and carbon dioxide solvent gradients, appear to give excellent chromatography with no tertiary modifier included. For the

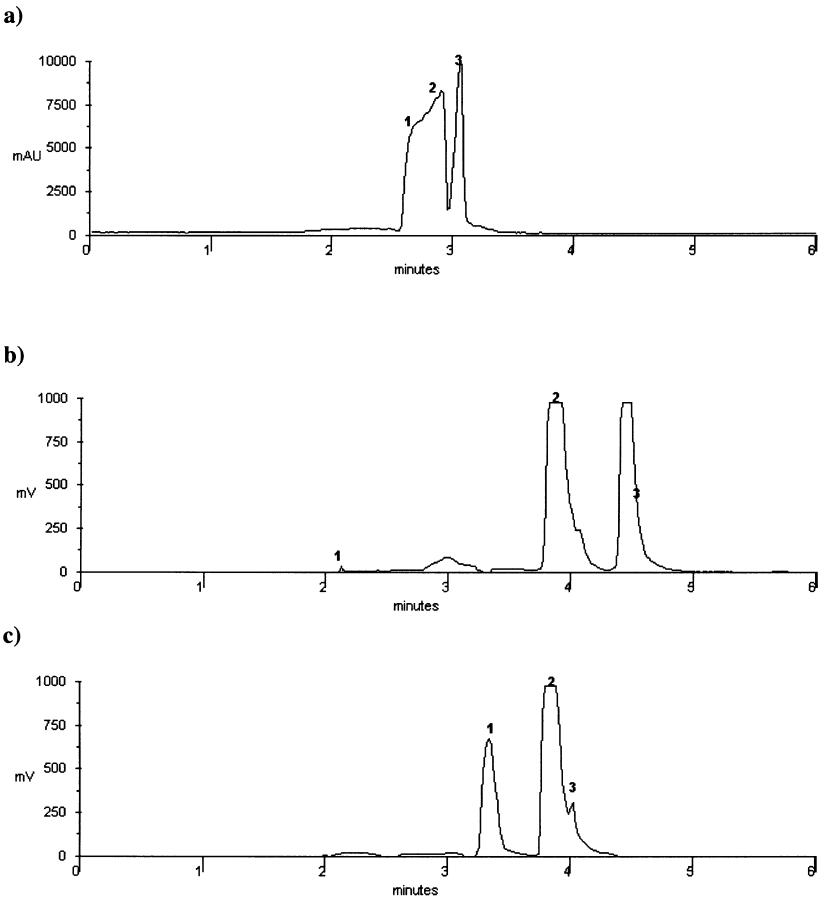

Figure 6. (a) SFC chromatogram for Reaction Mixture C. SFC conditions: cyanopropyl column (21.2 × 150 mm, Berger Instruments), gradient 5–60% MeOH with 0.5% triethylamine in CO_2. (b) SFC chromatogram for Reaction Mixture C. SFC conditions: Aminopropyl column (21.2 × 150 mm, Berger Instruments), gradient 5–60% MeOH with 0.5% triethylamine in CO_2. (c) SFC chromatogram for Reaction Mixture C. SFC conditions: Diol column (21.2 × 150 mm, Berger Instruments), gradient 5–60% MeOH with 0.5% triethylamine in CO_2.

purification of basic components, small amines added at 0.1% to 0.5% by volume to the methanol component of the mobile phase give better resolution and an earlier elution profile. Figure 7 contrasts the chromatography achieved for an amide formation reaction from an amine and a

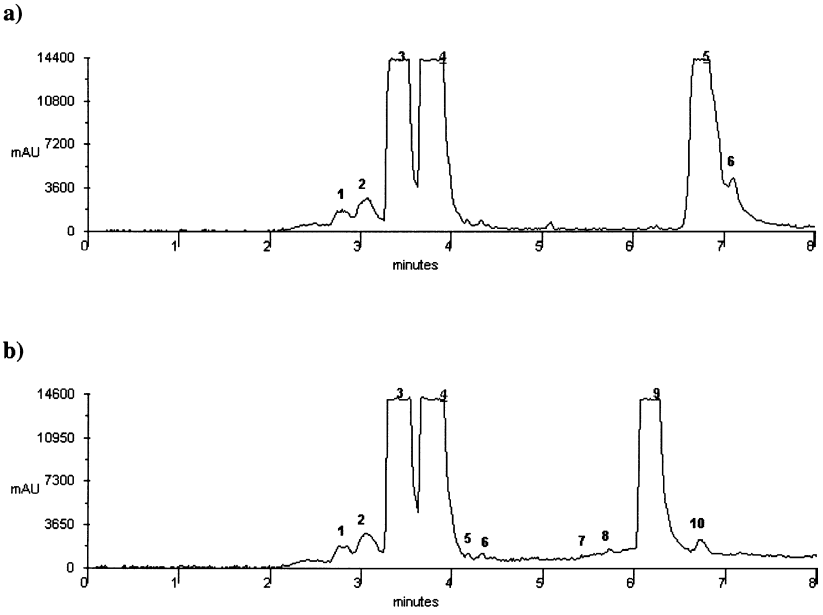

Figure 7. (a) SFC chromatogram for Reaction Mixture D. SFC conditions: Diol column (21.2 × 150 mm, Berger Instruments), gradient 5–60% MeOH. (b) SFC chromatogram for Reaction Mixture D. SFC conditions: Diol column (21.2 × 150 mm, Berger Instruments), gradient 5–60% MeOH with 0.5% triethylamine in CO_2. In each of (a) and (b), the un-reacted amine starting material was identified as the latest eluting peak in the chromatogram.

carboxylic acid in binary vs. tertiary solvent systems. The addition of the basic modifier has little effect on the resolution or retention of the amide reaction product and carboxylic acid starting materials, but has the effect of peak sharpening and reduced retention of the excess amine starting material present in the reaction mixture.

We have employed, successfully, several different small amine tertiary modifiers, including: triethylamine, diethylmethylamine, and 1,4-dimethylpiperazine. The identity of the amine tertiary modifier seems to have only a small effect on chromatographic behavior for most compounds studied. One exception has been superior chromatographic resolution observed with 1,4-dimethylpiperazine modifier for the purification of piperazine and morpholine containing compounds. Figure 8 illustrates the typical differences observed by the addition to the methanol component of the mobile phase of different amine modifiers.

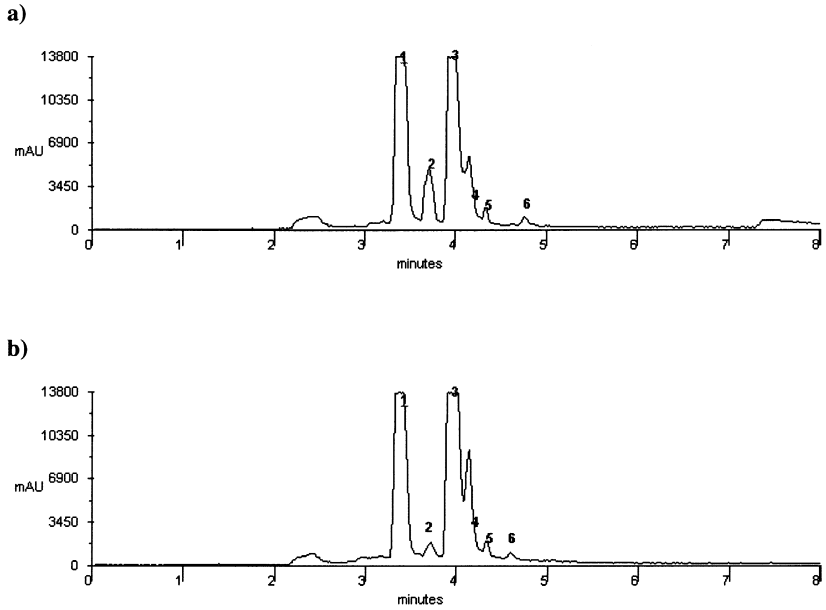

Figure 8. (a) SFC chromatogram for Reaction Mixture E. SFC conditions: Diol column (21.2 × 150 mm, Berger Instruments), gradient 5–60% MeOH with 0.5% triethylamine in CO_2. (b) SFC chromatogram for Reaction Mixture E. SFC conditions: Diol column (21.2 × 150 mm, Berger Instruments), gradient 5–60% MeOH with 0.5% 1,4-dimethylpiperazine in CO_2.

The effect of gradient slope seems to have only a small effect of the resolution achieved for the components of a particular reaction mixture. The major effect observed is in retention time, as the gradient hits various percentages of carbon dioxide modifier. Figure 9, for instance, demonstrates the effect of a steep vs. shallow gradient. Typically, we simply employ a very steep slope to the gradient of from 5–10% methanol (with or without tertiary modifier) to 50–60% methanol. An extra 2 min of isocratic 50–60% methanol in carbon dioxide is often employed at the end of the gradient, the extra time occasionally being well spent to elute more polar materials and thus increase column life.

Once an SFC method has been optimized for the members of a set of pilot reactions, this chromatographic method provides good purification success for all members of a subsequently synthesized 48-member library. As can be seen in Fig. 10, the desired component from each reaction set tends to elute in a predictable pattern relative to the starting materials and reaction side products.

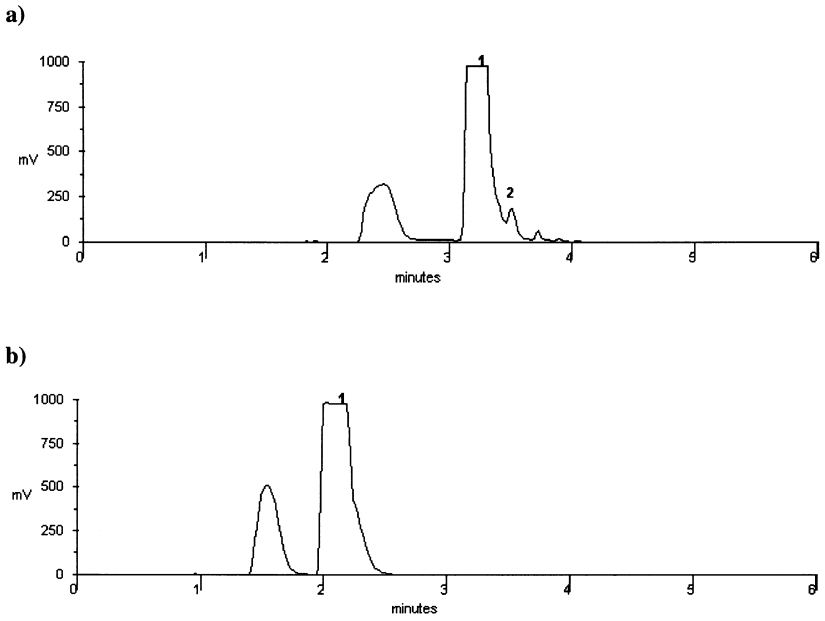

Figure 9. (a) SFC chromatogram for Reaction Mixture F. SFC conditions: Diol column (21.2 × 150 mm, Berger Instruments), gradient 5–60% MeOH with 0.5% triethylamine in CO_2. (b) SFC chromatogram for Reaction Mixture F. SFC conditions: Diol column (21.2 × 150 mm, Berger Instruments), gradient 20–60% MeOH with 0.5% triethylamine in CO_2.

This is of particular advantage in our UV-triggered fraction collection scheme, as it limits the number of fractions of which mass spectra must be acquired to identify the correct peak for return to the synthetic chemist.

Examples from the Purification of Different Reaction Type Libraries

As has been noted, the HTOS group excels at the development of standardized chemistry types, which can be generally applied to the modification of a wide range of "core" library structures. At present, 10 standard chemistries are in place, and we have developed chromatographic methods amenable to each of these. The sole reaction, with which we have not had success at applying SFC, has been Suzuki chemistry, which generates boronic acid side products too polar for elution from any column type by the methanol

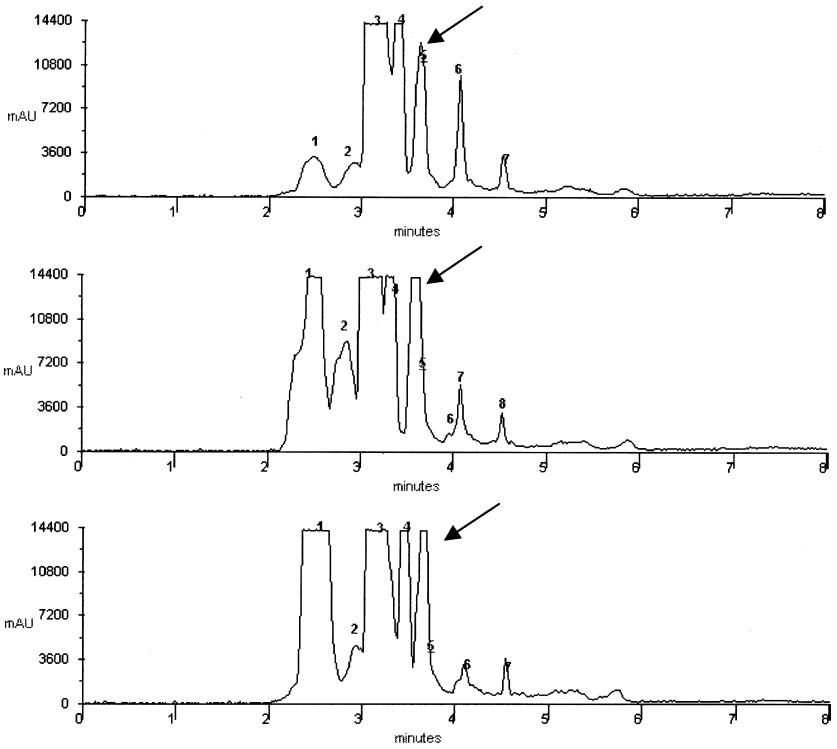

Figure 10. Chromatograms for the purification of three individual library members purified from an amide-formation HTOS library on a Diol column eluted with a gradient of: 5–60% MeOH in CO_2 ramping over 6 min, followed by 2 min hold at 60% MeOH/CO_2. Desired component from each reaction indicated by an arrow on the chromatogram.

and carbon dioxide solvent system employed in SFC. As each new reaction type is brought on-line, SFC vs. HPLC triage experiments are performed, followed by methods development in SFC if such is the chromatographic technique selected. Thereafter, when different cores are subjected to the new standard reaction, the most general best SFC method is first run, and based upon the chromatograms obtained, an optimizing modification is generally obvious. Table 1 provides a good first choice of conditions based upon structural types to be purified. For instance, for the purification of amide formation reactions from amines and carboxylic acids, a diol column eluted with 5–60% methanol with 0.5% triethylamine in carbon dioxide is first

employed. If this particular library core imparts high hydrophilicity to the reaction product components and hence, late elution of peaks, a cyanopropyl column may be substituted for diol. Cyanopropyl is not the first column of choice, as it does not perform as well for the separation of most structural types from one another, as does diol. For the purification of product from amine and aldehyde components from a reductive amination reaction, the same diol column eluted with 5–60% methanol with 0.5% triethylamine in carbon dioxide is also the first method of choice. However, if several closely related amines must be separated from this reductive amination reaction mixture, an aminopropyl column can be substituted for the diol. Aminopropyl is not the first method of choice, as it tends to be more retentive than diol for amines and risks losing elution for the most polar materials. Figures 11–13

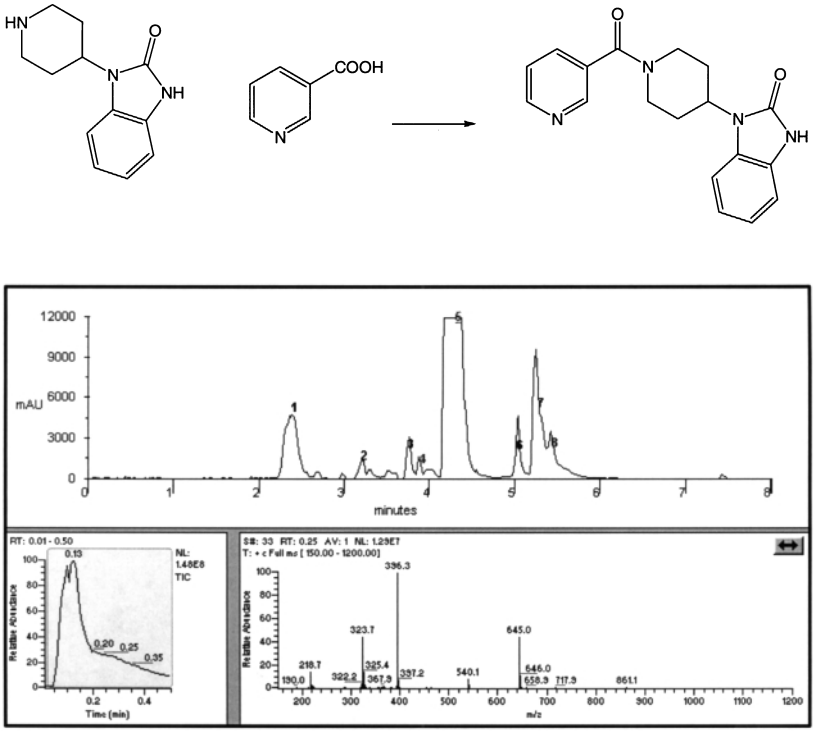

Figure 11. (a) Amide formation reaction for chromatogram 11b. (b) SFC chromatogram for reaction 11a. SFC conditions: Diol column (21.2 × 150 mm, Berger Instruments), gradient 5–60% MeOH with 0.5% triethylamine in CO_2, ramp gradient for 6 min followed by hold at 60% for 2 min.

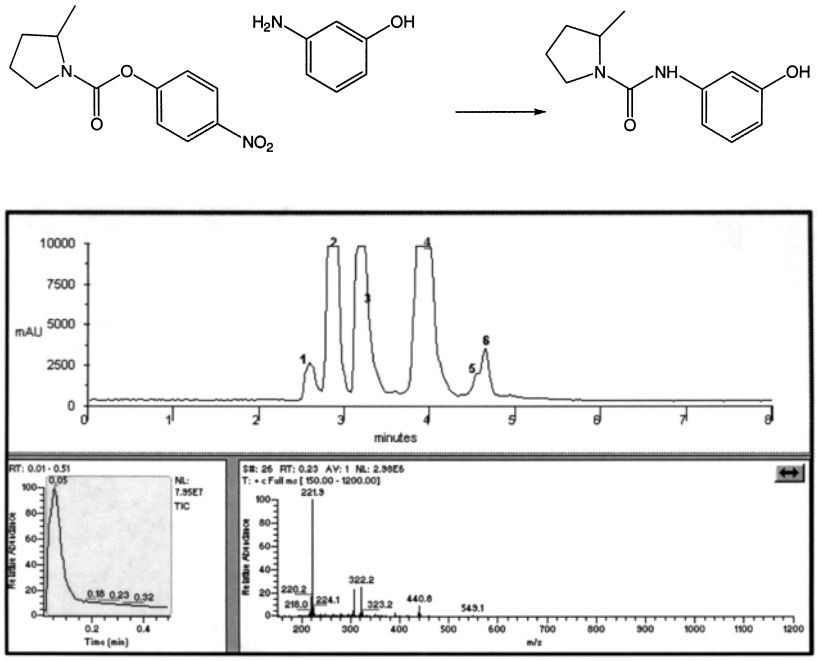

Figure 12. (a) Urea formation reaction for chromatogram 12b. (b) SFC chromatogram for reaction 11a. SFC conditions: Diol column (21.2 × 150 mm, Berger Instruments), gradient 5–60% MeOH with 0.5% triethylamine in CO_2, ramp gradient for 6 min followed by hold at 60% for 2 min.

show chromatograms for the purification of single members from 48 member libraries from three different chemistry types; amide formation, urea formation, and Mitsunobu reaction, and are typical of chromatographic results which we have obtained for the purification of a wide range of HTOS structural types. Table 1 depicts typical conditions selected for the purification of reaction mixtures based upon the structural types present in both the products and any un-reacted starting materials.

CONCLUSIONS

The employment of SFC for the purification of HTOS libraries offers decreased turn-around time, both in terms of chromatography and sample dry-down. Chromatographically, due to the low viscosity and high diffusivity of carbon dioxide, faster chromatography is possible for SFC relative to

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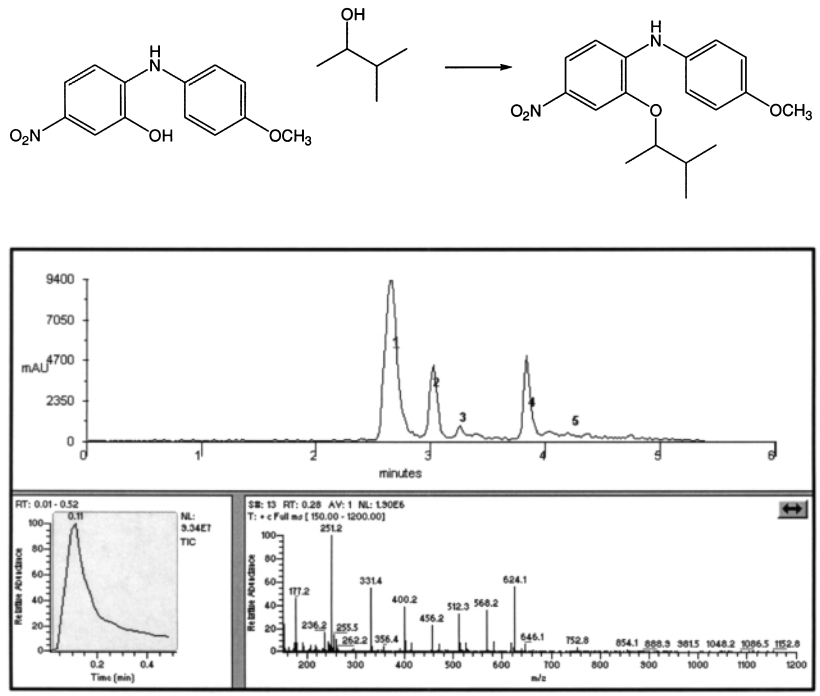

Figure 13. (a) Suzuki reaction for chromatogram 13b. (b) SFC chromatogram for reaction 13a. SFC conditions: Diol column (21.2 × 150 mm, Berger Instruments), gradient 5–60% MeOH with 0.5% triethylamine in CO_2, ramp gradient for 6 min.

HPLC. For a typical sample weight of 50 mg applied to a 20–25 mm × 100–150 mm column, run times in HPLC are 10–15 min and SFC run times are 6–8 min. Rapid re-equilibration in SFC relative to HPLC chromatography theoretically allows, also, a shorter time between sample injections. In practice, SFC suffers somewhat in our hands by both injection overhead (due to the fact that the SFC system must wait for the next sample to be applied), and also due to the fact that the SFC occasionally requires time to stabilize temperature and pressure settings between runs. In practice therefore, a typical HPLC sample run requires ~13–18 min, and a typical SFC run anywhere from 9 to 15 min.

Sample dry-down time for samples generated by SFC is similarly reduced relative to that for samples generated by RP-HPLC. A typical 50 mg sample purified by HPLC will elute from the stationary phase in approximately 20 mL of a mixture of acetonitrile and water of various gradient

compositions. The dry-down time for these aqueous organic fractions is approximately 12 hours in a centrifugal evaporation system. In contrast, a 50 mg sample purified by SFC will elute from the stationary phase in approximately 3 mL of methanol. The dry-down time for this pure methanol sample is less than 1 hour. In addition to the time saving for 3 mL of methanol relative to 20 mL of aqueous acetonitrile, an added advantage is achieved in that the shorter dry-down time is gentler on a labile sample, as heat is applied during dry-down.

Another advantage of the solvent systems employed in SFC relative to HPLC lies in the generation of vastly lower quantities of hazardous waste for disposal; a typical HPLC run generating 600 mL of aqueous acetonitrile vs. a typical SFC run generating 260 mL of methanol. Methanol can, thereafter, be burned as fuel, whereas the mixed organic/aqueous generated by HPLC purification is disposed of as hazardous waste. Hence, environmental considerations and impact are an added advantage of SFC.

Supercritical fluid chromatography can often, but not always, be optimized to provide better resolution for the separation of a given set of compounds than can HPLC. This is due to the nature of high pressure carbon dioxide, which is a low viscosity solvent, allowing relatively high mobile phase flow rates along with the employment of relatively longer stationary phase columns. By example, Fig. 14 depicts the separation of three commercially available amines under optimized conditions by each of SFC and HPLC. These results are typical of the relative resolution often achieved in the two techniques.

Recovery of material by SFC is found to be, on the average, comparable to that achieved by HPLC. As recovery in any type of liquid chromatography is dependant upon how a particular component interacts with a given stationary phase column, there will be examples where each offers superior recovery to the other. For chromatographically well-behaved compounds, such as ketoprofen (Table 2), excellent recovery is achieved by either technique. For Quinine, a compound that traditionally gives poor recovery by RP-HPLC, the recovery is dramatically improved in SFC. In contrast, *N*-benzylbenzamide gives excellent recovery by HPLC, yet suffers much poorer results by SFC. These mixed results are typical of what we have seen for the relative recovery between the two techniques.

An added advantage of SFC relative to HPLC that was not anticipated is the finding that SFC chromatographic results are frequently acceptable in solvent systems run with no tertiary modifier (i.e., in pure carbon dioxide/methanol gradients). It is almost never the case that HPLC can be run without buffer or some tertiary modifier, the most common being trifluoroacetic acid. When a tertiary modifier is employed in SFC, it's purpose is to convert the compounds to be separated into their least polar, or salt free

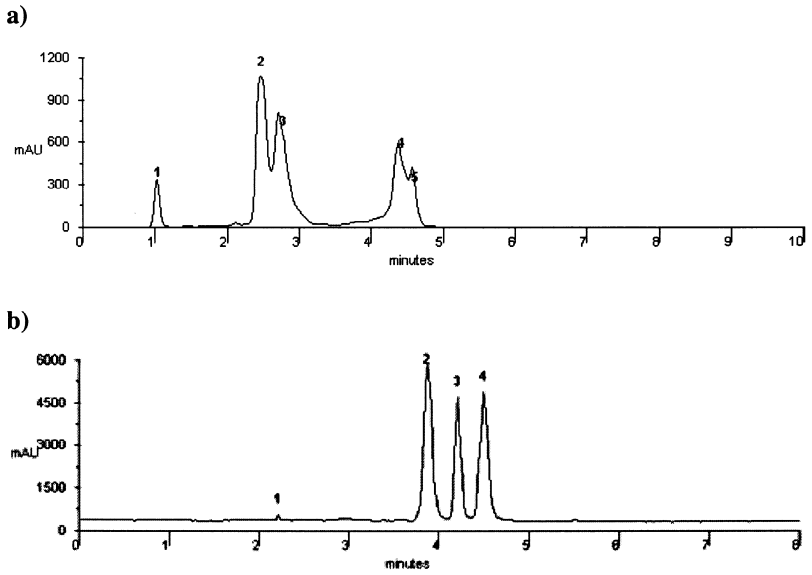

Figure 14. (a) HPLC chromatogram of the separation of 10 mg each of; quinine, 5-anilino-1,2,3,4-thiatriazole, 1-(4-methylphenyl)-2-methylpiperazine on C18 (20×100 mm Nova-Pak^{TM}), gradient 5–95% CH_3CN/H_2O with 0.1% TFA. (b) SFC chromatogram of the separation of 10 mg each of; quinine, 5-anilino-1,2,3,4-thiatriazole, 1-(4-methylphenyl)-2-methylpiperazine on an Aminopropyl column (21.2×150 mm, Berger Instruments), gradient 5–60% MeOH with 0.5% triethylamine in CO_2.

form, which is done by the addition of a base such as triethylamine, as most drug-like compounds contain a nitrogen. Triethylamine and similar small bases are highly volatile and removed from the sample during dry-down. Therefore, samples purified by SFC are generated as the non-salt form. This is not the case with TFA, where dry-down for HPLC samples leave behind, at best, the TFA salt of any basic amine and, additionally, it has been our experience, excess amounts of TFA up to one or two equivalents.

One limitation to SFC relative to HPLC lies in the necessity to dissolve samples for injection onto the former, in a solvent system compatible with methanol/carbon dioxide. As was noted in the results and discussions section of this paper, we have been able to accommodate reaction mixtures soluble in methanol and mixtures of methanol/dichloromethane or methanol/acetonitrile, the latter two of which do dissolve a certain percentage of materials not soluble in pure methanol. The addition of DMSO by contrast

Table 1. Typical SFC methods employed by structural type to be purified.

Structural type	Column	Gradient	Buffer
Neutral/Acidic	Diol	5–50% MeOH	None
Neutral/ Acid polar	Cyanopropyl	5–60% MeOH and "Hold"	None
Basic	Diol or Aminopropyl	5–50% MeOH	Et_3N or DMP
Polar Basic	Diol	5–60% MeOH and "Hold"	Et_3N or DMP

Note: Et_3N, Triethylamine; DMP, 1,4-dimethylpiperazine.

does appear to degrade chromatographic behavior and is not used in our laboratory for SFC purification.

In our particular experience with SFC equipment, the level of robustness has not been as good as that for HPLC. We have experienced periods of "down-time" at a greater frequency than with our existing HPLC instrumentation. This is not surprising, since the widespread implementation of SFC vastly lags that of HPLC. HPLC enjoys the benefit that countless more customer-years of experience have been gained with both the technique itself and associated equipment and software. As with any technology, this has led to a greater level of equipment refinement and reliability for HPLC. In terms of hardware, the engineering challenges associated with SFC are somewhat more challenging. Mechanically, the pumps used for SFC are similar to those used by HPLC. But in the case of SFC, two high-pressure pumps are normally required rather than one. Moreover, the CO_2 delivery system must ensure that

Table 2. Comparison of recovery of quinine, ketoprofen, and *N*-benzylbenzamide by semi-preparative scale SFC and HPLC respectively.

	Ketoprofen	Quinine	*N*-Benzylbenzamide
SFC recovery	98%	79%	72%
HPLC recovery	97%	54%	93%

Note: Chromatography conditions: HPLC, Nova-Pak™ C18 column (20×100 mm) developed in a solvent gradient of 5–95% aqueous CH_3CN with 0.1% TFA; SFC, Diol (21.2×150 mm) column developed in a solvent gradient of 5–60% MeOH in CO_2 with 0.5% Et_3N. Recovery is generated for 50 mg samples, average value of triplicates.

the CO_2 is in a liquid state at the pump inlet. This normally requires a combination of pressure and cooling. And liquid CO_2 is much more compressible than most other liquids, calling for greater compressibility compensation from the pump. Other differences include the need for a dynamically adjustable back pressure regulator to maintain proper conditions throughout the column and the requirement that the UV detector must withstand pressures as high as several hundred Bar. Also, collection of the eluted sample is more difficult, because at the collection vial conditions must ultimately return to ambient pressure and temperature. Decompression of the CO_2 results in an approx. 500/1 volumetric expansion and the ensuing high velocities and volumetric flow rates must be dealt with. Mishandling of this step can cause poor yields due to formation of aerosols or blockages due to freezing within the lines. In spite of these factors, there is no inherent reason why the reliability of SFC equipment should not attain that of HPLC. As the technique becomes more widely adopted and more vendors jump into the fray, continuous progress will be made.

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