Molecular Diversity **3:** 61–70, 1997.

High throughput analysis and purification in support of automated parallel synthesis

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Received 19 June 1997; Accepted 29 June 1997

Key words: combinatorial chemistry, combinatorial library, purification, HPLC, preparative HPLC

Summary

Rapid reverse-phase analytical and preparative HPLC methods have been developed for application to parallel synthesis libraries. Gradient methods, short columns, and high flow rates allow analysis of over 300 compounds per day on a single system, or purification of up to 200 compounds per day on a single preparative system. Hardware and software modifications allow continuous unattended use for maximum efficiency and throughput.

Introduction

The drug discovery process constitutes a continuum beginning with high-throughput screening in support of target validation and lead discovery, proceeding through lead optimization with respect first to intrinsic target activity, then optimization with respect to pharmacokinetics and other related parameters, and finally to candidate selection. Since the introduction of combinatorial chemistry, a variety of automated synthesis techniques have been described [1]. Each of these has strengths and weaknesses with respect to support of various parts of the drug discovery continuum. For example, split and pool methods [2] allow access to very large numbers of compounds for high-throughput screening, but deconvolution requirements make the method less suitable for lead optimization. Similarly, encoded libraries [3] are often highly suitable for optimization of intrinsic activity but may be less suitable for optimization of pharmacokinetics, toxicology, etc. Finally, parallel synthesis arrays of individual compounds [4] provide useful information throughout the process but are more labor intensive to prepare and assay. An optimized drug discovery effort may thus consist of more than one technology, each applied to the part of the process where it works best.

Automated parallel synthesis provides compounds suitable for late-stage studies including intrinsic activity comparison, pharmacokinetic studies, toxicology studies, and screening in true disease models (in vivo testing), but *only if compounds are provided in sufficient amount and sufficient purity to support the assay requirements*. Solid-phase synthesis techniques provide a mechanism for automatically removing soluble reagents from reaction mixtures, but offer no way to remove resin-bound reaction by-products. After several sequential synthesis steps on solid-phase, the small amounts of by-products from each step lead to substantial impurities in the final synthesis product. This problem has been largely overcome in peptide synthesis by many years of reaction optimization. Considering the potential complexity of automated solid-phase organic synthesis (infinite monomers, infinite reaction types and conditions, many potential protecting groups) compared with peptide synthesis (20 monomers, one significant bond-forming reaction, limited protecting

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groups) it is unlikely that the same level of generalized optimization will be achieved quickly. As a result, chemists must spend considerable time and effort optimizing synthesis methods before executing a library in order to achieve a high level of compound purity. As an alternative to optimizing the synthesis methods, we have developed an automated, high-throughput, high-performance analysis and purification procedure with broad applicability to combinatorial organic synthesis. The ability to rapidly analyze and purify large numbers of compounds allows chemists to execute libraries with less synthesis optimization and dramatically increases overall library synthesis throughput in cases where pure compounds are required.

Analysis of all members of a large and diverse parallel synthesis array requires a fast analytical method of wide applicability that is easily automated. We selected gradient reverse-phase high-performance liquid chromatography (RP-HPLC) as our analytical method because of its adaptability to automation and its proven track record for performing separations of a wide variety of structural types. Gradient elution is required in order to attain wide applicability to all compound types without tedious method development. Gradient reverse-phase chromatography, though, is inherently a serial process and is generally slow due to gradient time, required column cleaning, and reequilibration cycles. Our first goal was thus to develop rapid universal reverse-phase gradient elution methods for the analysis and purification of large parallel synthesis arrays.

To achieve our goal of highly efficient analytical and preparative HPLC methods required reinvestigation of standard gradient reverse-phase chromatography. For a typical gradient elution method, each sample component experiences three distinct environments. In the first phase, the sample is loaded onto the column which has been pre-equilibrated with a non-eluting mobile phase. Under these conditions, the sample components do not elute and are retained at the top of the column in a narrow band; conventional rules of partition chromatography do not apply since the compounds do not migrate. As the mobile phase composition changes (due to the gradient change), a point is reached where one or more sample components begin to migrate down the column. At that point, conventional partition chromatography occurs and the rules of partition chromatography apply. The compound spends some time in the mobile phase and some time in the column media. As the mobile phase composition continues to change, a third point is reached where the component in question no longer partitions into the media but rather remains in the mobile phase. At this transition point, the conventional partition chromatography rules do not apply. We reasoned that as gradient times become shorter and shorter, the second 'partition chromatography' phase becomes less significant than the first and third phases (totally retained or totally unretained). Taken to the extreme, if little or no partition chromatography occurs, then the conventional rules of partition chromatography governing resolution such as flow rate and particle size no longer apply and separation is solely based on the composition of the eluant at the point where components begin to elute. The question we asked was whether or not sufficient separating power would remain under such extreme conditions to provide useful chromatography.

Our second goal was to develop a fully automated preparative RP-HPLC system with detector-based fraction collection and sufficient throughput to support automated parallel array synthesis. Chromatographic methods for preparative purification fall into two distinct categories which we refer to as *collect-before-detect* and *detect-before-collect.* In the former case, fractions are collected without linkage to a real-time detection system and are assayed in a subsequent step. Samples collected in this way will be 'purified' with respect to the starting mixture from which they were obtained, but they may not be 'pure' since closely eluting peaks will not necessarily be separated except by serendipitous fractionation. In the detect-before-collect case, real-time detection ensures optimal fractionation and samples will be as pure as the chromatographic separation allows. We and others [5] have reported collect-before-detect purification systems for parallel synthesis arrays using solid-phase extraction methods. While these methods are rapid and efficient, they do not necessarily provide material of sufficient purity for late-stage drug discovery projects.

Existing commercially available systems for automated HPLC purification are optimized for repetitive purification of sequential batches of the same sample whose chromatographic properties have been well studied. An optimized system for the automated purification of samples from high-throughput synthesis requires no prior knowledge of the chromatographic properties, but rather collects product based on input from a suitable detection device (e.g., UV or mass detector). Additionally, automated sampling, adequate fraction collection capacity, and accurate fraction tracking are essential properties of such a detect-before-collect system. Once suitable chromato-

graphic methods were in hand, we then developed a fully automated preparative HPLC system optimized for the specific needs of high-throughput automated synthesis.

This paper summarizes our analytical and preparative HPLC method development efforts as well as the architecture of our preparative HPLC system.

Materials and Methods

HPLC systems

Our analytical HPLC systems are generally configured as described previously [6]. The analytical system consists of two Shimadzu LC-10AS solvent pumps, an SCL-10A system controller, an SPD-10A variable-wavelength UV detector, an SIL-10A autosampler, and an FCV-14AH multiposition valve for column selection. Individual components of the system are linked to the SCL-10A controller via a fiber optic link for system control and feedback. The SCL-10A controller, in turn, is controlled (via RS-232 serial port interface) by a personal computer (HP-Vectra XM) running proprietary BMS software written in the Microsoft Visual Basic™ environment. Data capture and analysis is done using Shimadzu Class-VP™ version 4.2 software that is also controlled by the Visual Basic™ interface. All user interaction is via the Visual Basic™ graphical user interface. The overall architecture of the Visual Basic™ software is the same as that which we previously described [6] for an older software package, except that specific import and export links have been added to facilitate data transfer from our combinatorial chemistry database.

Our preparative HPLC systems consist of the following components: two Shimadzu LC-8A solvent pumps, an SCL-10A system controller modified as described below, an SPD-10A variable-wavelength UV detector equipped with a preparative flow cell, an SIL-10A autosampler modified by incorporation of a Valco model CS-3006 two-position six-port valve, an FCV-11AL solvent selection valve, an FCV-14AH multiposition valve for column selection, a C-R7A$^+$ integrating computer, and up to six FRC-10A fraction collectors.

The parent circuit board of the SCL-10 system controller employs a microcontroller that is equipped with a single data port to communicate with solvent delivery units, a column oven, detectors, an auto injector and a fraction collector. The microcontroller utilizes data multiplexers to connect its data port to any one of these peripheral devices. Each device is connected to a designated multiplexer port by way of fiber optic transmission cabling. Shimadzu system controller firmware assigns the auto injector (SIL) and the fraction collector (FRC) to port numbers one and two respectively. In order to facilitate communications with additional devices, without modifying the microcontroller firmware, it was necessary to equip the SCL-10A with additional multiplexing capabilities. We modified the parent circuit board of the SCL-10A so that we can selectively communicate with as many as eight fraction collectors. A small circuit board was designed and fabricated whose data input is switched among as many as eight data outputs by way of four data selection lines. The circuit board is housed in the SCL-10A system controller cabinet in the space directly behind the rear panel remote optical port expansion slot. Our circuit board utilizes one or two Shimadzu PC-4 optical connector expansion modules. Each PC-4 provides four pairs of fiber optic cable connectors and conversion of the serial data stream between electrical and light energy. The data input to our board emanates from the output of the SCL-10A multiplexer which is designated for fraction collector interface. The data select lines are connected to the PC-16 digital interface and controlled by our preparative HPLC software package using simple TTL logic.

HPLC columns

All HPLC columns were provided by YMC, Inc., Wilmington, NC. Similarly configured analytical columns provided by Jones Chromatography and MacMod Analytical performed qualitatively similarly in limited tests. Columns were prepared and packed with spherical ODS (C_{18}) media at slurry pressures greater than would be routinely experienced during the rapid gradient separations. The 4.6×150 mm columns typically provide 15 000–18 000 plates per column and the 4.6×50 mm columns provide 5000–6000 plates per column. The short 20×50 mm, 20×100 mm, and 30×100 mm columns were also slurry packed at higher than normal pressure.

Test chromatograms

All analytical test chromatograms were run using 5 μl injections of a test mixture containing 4 mM concentration of each of the following components in 95% aqueous methanol: 1-hydroxy-7-azabenzotriazole [39968-33-7], 4-methoxybenzene sul-

fonamide [1129-26-6], methyl-3-amino-2-thiophenecarboxylate [22288-78-4], and 4-aminobenzophenone [1137-41-3]. Gradient conditions were all linear from 10% aqueous methanol to 90% aqueous methanol with both A and B solvents containing 0.2% phosphoric acid. Flow rates and gradient times were as described in the Results section. Monitoring of UV absorbance was done at 220 nm.

Preparative test chromatograms were run using 2.0 ml injections of a filtered test solution containing 40 mM concentration of each of the following components in 1:1 methanol:water: 1-hydroxy-7-azabenzotriazole [39968-33-7], 4-methoxybenzene sulfonamide [1129-26-6], methyl-3-amino-2-thiophenecarboxylate [22288-78-4], and 4-aminobenzophenone [1137-41-3]. Gradient conditions were linear from 10% aqueous methanol to 90% aqueous methanol with both A and B solvents containing 0.1% trifluoroacetic acid in place of 0.2% phosphoric acid. Flow rates and gradient times were as described in the Results section. Except as noted, monitoring of UV absorbance was done at 220 nm.

Preparative load and recovery experiments were done in triplicate. For 10, 50, and 100 mg injections on 20 and 30 mm diameter columns, 2.0 ml of solutions containing the stated amounts of both 4-nitrobenzoic acid [62-23-7] and 1-(4-chlorophenyl)-1-cyclobutanecarboxylic acid [50921-39-6] in 64% aqueous methanol were prepared and transferred to autosampler vials. The autosampler was instructed to inject the entire 2.0 ml solution. Gradient chromatograms were run at 20 (for a 20 mm diameter column) and 45 (for a 30 mm diameter column) ml/min over 10 min. Samples were collected automatically and concentrated in a Savant Speed-Vac concentrator overnight. The residue was weighed and compared with the original sample amount. For 200 mg injections, the same procedure was followed, except that the total injection volume was 4.0 ml. For injections on the 10 mm diameter column, the same procedure was followed except that the samples were dissolved in 55% aqueous methanol, the flow rate was 5 ml/min, and data acquisition was extended to 15 min.

Results

The HPLC conditions that we have chosen are 'universal'. The mobile phase is suitable for acidic, neutral, and basic compounds and does not require further methods development time to optimize resolution. Our gradient reverse-phase methods for analytical HPLC are summarized in Figure 1. We created a standard test mixture containing four components which are well separated under gradient elution conditions. Figure 1a shows a typical gradient chromatogram of the test mixture using a standard 4.6×150 mm spherical 5 μm ODS column operated under widely reported gradient conditions (30 min gradient time, 1.0 ml/min flow rate). Excellent separation is obtained. Higher flow rates are limited on the 150 mm length column due to excessively high back pressures generated during the gradient operation, while shorter gradient times are not practical on this column due to the inherently large void volume (2 ml). The 30 min gradient time represents 15 multiples of column void volume at the flow rate of 1.0 ml/min. Figure 1b shows a chromatogram of the same test mixture on a 4.6×50 mm column packed with spherical 3 μm ODS material. The flow rate has been increased to 2.5 ml/min, the void time has been reduced to under 20 s, and the gradient time of 8 min represents 28 multiples of void time. Excellent chromatography and resolution of all components is still obtained. The flow rate of 2.5 ml/min is once again limited by high back pressure of the column due to the small 3 μm media. Figure 1c shows a chromatogram of the same test mixture using an identical gradient and identical column except that it is packed with spherical 5 μm ODS material. As seen from Figure 1c, there is

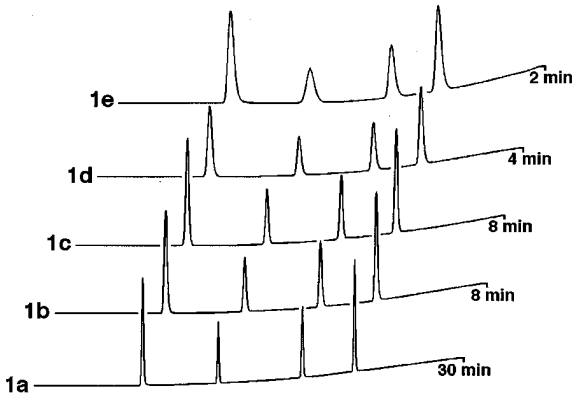

Figure 1. Effect of column length and flow rate on analytical separation. Gradient test mixture chromatograms using a linear gradient from 10 to 90% aqueous methanol containing 0.2% H_3PO_4. UV absorbance was monitored at 220 nm. (a) 4.6×150 mm column, 1.0 ml/min flow rate, 30 min gradient time. (b) 4.6×50 mm column, 3 μm particle size, 2.5 ml/min flow rate, 8 min gradient time. (c) 4.6×50 mm column, 5 μm particle size, 2.5 ml/min flow rate, 8 min gradient time. (d) 4.6×50 mm column, 5 μm particle size, 4.0 ml/min flow rate, 4 min gradient time. (e) 4.6×33 mm column, 5 μm particle size, 5.0 ml/min flow rate, 2 min gradient time.

little or no effect of particle size on chromatography under these conditions. Using the larger 5 μm particle size sorbent significantly reduces the back pressure, allowing us to increase the flow rate to 4 ml/min and shorten the gradient time to 4 min with little compromise of chromatography (Figure 1d). Finally, we shortened the column once again (4.6 × 33 mm, spherical 5 μm ODS), increased the flow rate, and shortened the gradient time to 2 min with some peak broadening but little effect on overall separation (Figure 1e).

With our shortest gradient method, the chromatographic cycle time is under 4 min (including reequilibration of the column for the next sequential injection). With rapid cycle times, autosampler rinse and injection times can become significant. To overcome this problem, our Visual Basic™ software contains an algorithm to determine whether a sample can benefit from an 'inject ahead' method. The software compares the gradient method used for the current injection with that to be used for the next injection. If the two are identical, then the software instructs the autosampler to make the first injection normally, but then to proceed directly (via a pretreatment file) to rinse the probe and then aspirate the sample for the next injection. The sample is loaded into the injector loop and retained there until it is time for the next injection. When the first chromatogram is complete and the column is reequilibrated, a 'Start' signal is sent to the autosampler from the SCL-10A and the first action is valve movement to inject the sample onto the column. The system then proceeds to 'inject ahead' the next sample if appropriate. Using this algorithm, the autosampler time (from start signal to inject) is reduced from over 90 s to under 5 s and sample throughput is increased by as much as 25%.

Having demonstrated that short columns operated at high flow rates can result in rapid and efficient analytical separations under gradient elution conditions, we then sought to extend the method to preparative separations. An optimized preparative mobile phase should minimize sample clean-up after collecting the samples. The 0.1% TFA preparative buffer system that we use provides a volatile buffer allowing rapid evaporation of the collected samples, thus eliminating the need to subsequently isolate the collected sample compounds from non-volatile buffer salts. Figure 2 shows chromatograms of our test mixture under preparative HPLC conditions. Figure 2a shows a chromatogram run on a conventional 20 × 250 mm spherical 5 μm ODS column operated at a flow rate of 10 ml/min. with a gradient time of 30 min. Figure 2b shows the same

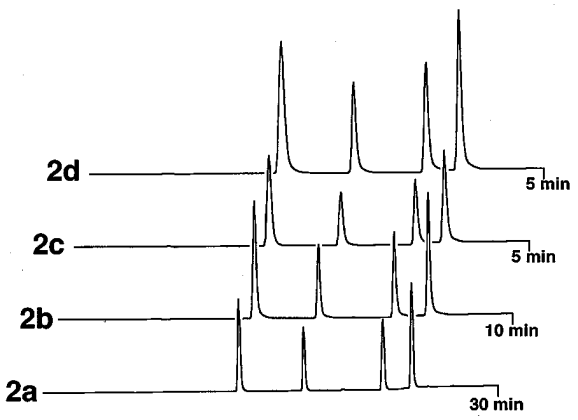

Figure 2. Effect of column length and flow rate on preparative separation. Gradient test mixture chromatograms using a linear gradient from 10 to 90% aqueous methanol containing 0.1% trifluoroacetic acid. UV absorbance was monitored at 220 nm. (a) 20 × 250 mm column, 10 ml/min flow rate, 30 min gradient time. (b) 20 × 100 mm column, 20 ml/min flow rate, 10 min gradient time. (c) 20 × 100 mm column, 40 ml/min flow rate, 5 min gradient time. (d) 20 × 50 mm column, 20 ml/min flow rate, 5 min gradient time.

mixture separated on a 20 × 100 mm spherical 5 μm ODS column operated at 20 ml/min with a gradient time of only 10 min; excellent resolution of all compounds is still obtained. Figure 2c shows the same column operated at double the flow rate (40 ml/min) and half the gradient time (5 min) with acceptable results. Similar results are obtained by shortening the column to 20 × 50 mm and maintaining a flow rate of 20 ml/min with a 5 min gradient (Figure 2d). Particle sizes less than 5 μm resulted in increased back pressure, reduced column life, and no improvement in chromatographic results (not shown).

Column load and sample recovery are important parameters in preparative chromatography. Column load capacity is related to column diameter, as shown in Figure 3. Injections of 10, 50, 100, and 200 mg each of a two-component test mixture were made on 10, 20, and 30 mm diameter (× 100 mm length) spherical 5 μm ODS columns and eluted under rapid (10 min) gradient conditions at similar linear flow rates. As seen from Figure 3a, the 10 mm diameter column overloaded significantly at 50 mg. The poor chromatogram shown in Figure 3a probably represents a combination of massive column overloading and extracolumn effects. The 20 mm diameter column, on the other hand, showed only moderate signs of overloading at 200 mg (Figure 3b), while the 30 mm diameter column did not overload even at 200 mg (Figure 3c). Samples from the above experiments were collected by the automatic

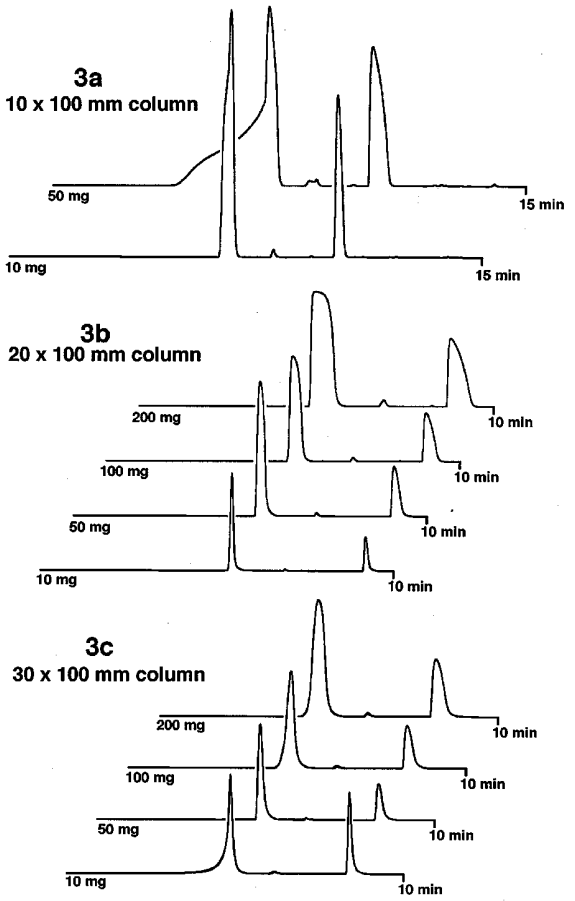

Figure 3. Effect of column diameter on loading capacity.

detect-before-collect routine and recovered by evaporation, with results as shown in Table 1. The data show excellent recovery independent of sample or column size. Stated percent recovery represents the entire process including drawing the entire sample from the vial into the autosampler, peak detection and collection, and post-collection sample transfers.

Our requirements for a fully automated preparative HPLC package included accurate fraction collection and tracking based on detector feedback (detect-before-collect), fully automated unattended operation, and adequate injection volume and fraction capacity. The overall architecture of our preparative HPLC system designed to meet these needs is shown in Figure 4. Fingertight fittings and in-line filters are used to facilitate maintenance. We previously described an instrument control software package designed for open access use by organic chemists [6]. All features of that

Table 1. Recovery of samples from preparative HPLC

Sample	Percent recovery from		
	10×100 mm column	20×100 mm column	30×100 mm column
10 mg, component 1	95±3	99±1	94±7
10 mg, component 2	92±3	90±13	94±7
50 mg, component 1	92±6	94±2	91±6
50 mg, component 2	89±3	92±3	86±9
100 mg, component 1		91±3	85±1
100 mg, component 2		83±3	82±1
200 mg, component 1		92±1	93±2
200 mg, component 2		94±1	91±2

Component 1: *p*-nitrobenzoic acid; component 2: 1-(4-chlorophenyl)-1-cyclobutanecarboxylic acid. Results are from triplicate experiments, with standard errors representing one standard deviation from the mean.

original package have been retained in our new preparative HPLC software package.

The standard Shimadzu SIL-10A autosampler is capable of up to 2 ml injection volume for preparative use. In practice, though, the standard injector clogs frequently with viscous or heterogeneous preparative samples. To overcome this problem, we replaced the standard Shimadzu SIL-10A two-position six-port injector valve with a Valco model CS-3006 two-position six-port valve having a large 0.030″ through-bore. At the same time, we replaced all tubing leading from the injector port to the valve with 0.040″ through-bore tubing. These modifications eliminated the injector clogging problem.

The standard Shimadzu SCL-10A system controller is capable of controlling a single FRC-10A fraction collector containing up to 64 18 × 150 mm test tubes. Since this clearly does not offer adequate fraction capacity for unattended operation, we had to develop a method to 'chain' fraction collectors. The SCL-10A system controller communicates with the FRC-10A fraction collector via a fiber optic link. We modified the parent circuit board of the SCL-10A as described in the Materials and Methods section so that we can select which fraction collector of a series is 'on-line' by using simple TTL logic. A six-position multiport valve is used to simultaneously direct the eluant flow path when the FRC control is switched, thus maintaining full fraction collector control of up to six fraction collectors connected in parallel. The total fraction capacity is thus expanded to 6 × 64 = 384 18 × 150 mm test tubes.

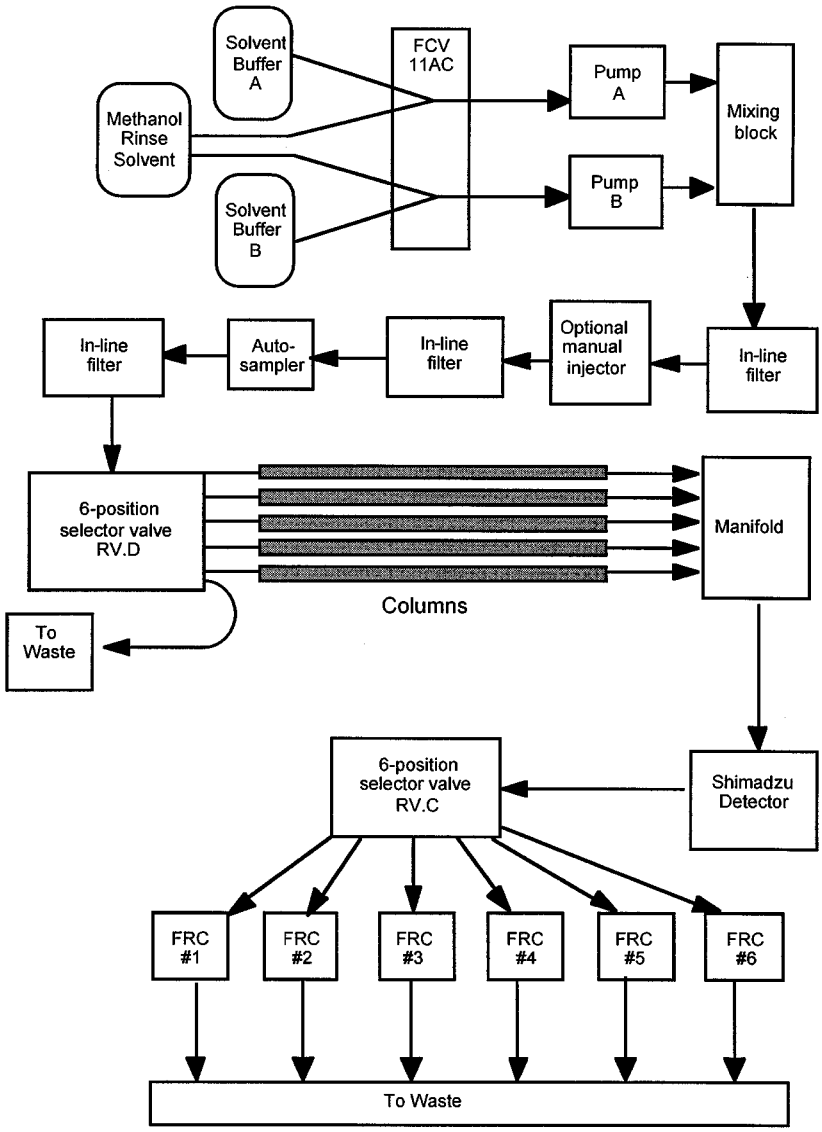

Figure 4. Schematic diagram of automated preparative HPLC.

Our standard HPLC control software [6] was modified to incorporate fraction collector control and fraction tracking. A limitation of the fiber optic control system is that we are unable to switch fraction collectors while a chromatogram is running. Accordingly, before each run the software polls the fraction collector to determine the number of unused tubes in the current FRC-10A. If the number of available tubes exceeds a preset threshold (located in a preference file), then fractions are collected in the next available tube of the current fraction collector. If the number of available tubes is below the threshold, then the next FRC-10A is selected and fraction collection begins with the first tube. The system undergoes an automatic shutdown if all fraction collectors have been 'used up' before all samples have been injected. A 'reset' provision allows users to replace tubes in fraction collectors and add them back into the queue without causing system shutdown.

The standard Shimadzu fraction collector firmware (built into ROM on the SCL-10A) allows user-definable slope and threshold values to be entered to trigger fraction collection. In our case, the parameters are entered automatically into the SCL-10A by our

CHROMATOPAC BASIC software so that users do not need to know suitable values. Fractions are collected only when both slope and threshold minima are met by the input signal from an analog detector. We use the Shimadzu SPD-10A variable-wavelength UV detector with a preparative flow cell, but in principle any real-time analog detector signal will suffice. The preparative flow cell provides minimal flow restriction and decreased sensitivity to eliminate the detector signal from blanking out due to high sample concentrations. The standard Shimadzu SCL-10A firmware provides a real-time chromatogram with tick marks corresponding to fraction collector movement. In addition, an ASCII table showing start and end times for each collection tube used in a run is provided and stored to disk.

As described previously [6], the overall software package provides automatic column selection, conditioning and flushing, monitors methods as they are input to make sure they fall within predefined limits, and is very user friendly.

Discussion

Compounds synthesized for pharmacological studies in late-stage drug discovery programs must be of known purity and must meet purity requirements of the assays in which they will be studied. Automated synthesis methods now provide the means to synthesize large numbers of compounds in sufficient amount for late-stage studies. As a result, it has become a significant analytical challenge to assay the purity of large numbers of compounds from automated synthesis and to purify such compounds when required. The use of shorter than normal analytical HPLC columns for rapid gradient analysis has been reported previously [7]. We have taken that approach to the extreme and found that specially prepared very short analytical and preparative HPLC columns operated at high flow rates with very short gradient times provide rapid and efficient analysis and purification of combinatorial libraries. The columns, packed under higher than normal slurry pressure, withstand the severe conditions of high-speed gradient runs including extremely high flow rates, high linear velocity, high pressures and rapid changes of pressure and mobile phase composition.

High flow rates are limited on conventional length columns due to excessively high back pressure generated during gradient operation, while shorter gradient times are not practical due to the inherently large void volume of conventional columns. Shorter columns, on the other hand, can be operated at higher flow rates, which allows shortening of the gradient time without loss of resolution. The capacity factor, and therefore the resolution [8], is proportional to the number of column void volumes passed through the column during the gradient time as shown by the following formula [8]:

$$k' \cong CV = GT \times FR/V$$

where CV is the number of void volumes passed through the column during the gradient time, GT is the gradient time, FR the flow rate, and V the column void volume. For a conventional 4.6×150 mm column operated at 1.0 ml/min the number of column volumes passed through the column is represented by

$$CV = (30\ \text{min}) \times (1.0\ \text{ml/min})/2.0\ \text{ml} = 15$$

As seen from the equation, resolution can be maintained with a shorter gradient time only if the flow rate can be increased proportionately. On the other hand, by reducing the column length to 50 mm, increasing the flow rate to 4.0 ml/min, and reducing the gradient time to 4 min, resolution can be maintained:

$$CV = (4\ \text{min}) \times (4\ \text{ml/min})/0.66\ \text{ml} = 24$$

Resolution is maintained on the short column since the gradient change occurs over 24 column volumes. This change is greater than the original separation on the 4.6×150 mm column where the change was accomplished over only 15 column volumes. In addition to maintaining separation, the cycle time is dramatically reduced from nearly 60 min to about 6 min, and solvent consumption per sample is reduced from 60 ml to about 24 ml. These principles apply to both analytical and preparative separations using gradient elution on short columns.

The analytical gradient HPLC methods described above, in combination with the inject-ahead autosampler modification, allow the analysis of one compound approximately every 4 min on the 33 mm length column. This amounts to 15 samples per hour or 360 samples per 24 h of instrument time (in practice, scheduling inefficiency may reduce the daily throughput below the theoretical level). In comparison, conventional methods allow the analysis of only 1 sample per hour or 24 samples per day, with considerable solvent consumption per sample. The improvements we have described

make it possible to quickly analyze all members of moderate-sized libraries. The availability of this analytical tool, in turn, expands the utility of automated parallel synthesis methods by allowing their application even in late-stage drug discovery programs.

While the availability of a high-throughput analytical method allows the estimation of purity for compounds made by automated synthesis, it still provides no way to salvage samples whose purity does not meet required standards. The preparative methods that we described greatly magnify the power of our analytical methods. Our shortest preparative gradient methods have a 7 min cycle time, thus allowing high-throughput purification. The automated system we describe provides automated unattended purification of up to 80 samples at a time (limited by autosampler capacity) with theoretical throughput of up to 200 samples per 24 h day. (Scheduling inefficiencies resulting from pre- and post-purification sample manipulation reduce practical throughput somewhat.) At this rate, moderate-sized libraries are purified when required to support late-stage drug discovery programs. By scaling the diameter of the preparative column to suit the size of the sample to be purified, a wide range of sample sizes are accommodated with excellent separation and recovery (Figure 3 and Table 1). For most combinatorial chemistry applications, though, we have found the 20×100 mm column configuration to be adequate. The primary limitations of our system are solubility (the sample must be soluble in 2 ml of an appropriate injection solvent) and detection (we currently use UV detection). In the latter case, there is no clear reason why evaporative light scattering or mass detection [9] cannot be used to expand the scope of our system.

We believe that our automated systems have their greatest impact if they are placed directly in the hands of the chemists synthesizing the compounds. Our software was created from the start as 'open access' software. Our systems reside in common laboratory areas and are shared by organic synthesis chemists. The systems operate as a queue (rather than batchwise) so that users simply add samples into the queue at any time. An analyst does not control access to these systems. We have found that these systems, designed specifically to support combinatorial chemistry, are also heavily used by chemists practicing conventional organic synthesis. The rapid analytical gradient methods provide real-time reaction monitoring, while the preparative systems are useful to purify target compounds from both individual and combinatorial syntheses. By making these systems available for general use, their power has been magnified significantly and overall productivity has been improved.

In summary, we have developed highly automated analytical and preparative HPLC systems and corresponding high-throughput gradient HPLC methods to support the analysis and purification of compounds from automated parallel synthesis. Using our methods and systems, chemists routinely analyze and purify moderate-sized compound libraries. These have proven to be powerful tools that have expanded the utility of automated syntheses into the very latest stages of the drug discovery process.

Acknowledgements

The authors wish to thank the following for valuable advice and technical assistance: Mr. Dennis J. Becker and Mr. Jeffrey M. Boyer (BMS); Mr. Jack Frank (Valco Instruments); Mr. Robert Cooley Jr., Mr. J. Robert Bickler, and Ms. Teri-lynne Brown (YMC Inc.); and Mr. Thomas Moran (Shimadzu Scientific).

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