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Development and Practical Use of a High Performance Preparative Liquid Chromatographic System

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DEVELOPMENT AND PRACTICAL USE OF A HIGH PERFORMANCE PREPARATIVE LIQUID CHROMATOGRAPHIC SYSTEM[1]

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ABSTRACT

The application of an analytical high performance liquid chromatograph to preparative tasks is discussed. Large capital investments are the norm when purchasing modern high performance liquid chromatographic equipment. In many cases an analytical HPLC system can be made to do double duty by also serving as a preparative liquid chromatograph. Often, with a small additional capital investment and minor equipment modifications a more versatile chromatograph is obtained. This paper will briefly review the process required to convert an analytical liquid chromatograph into a preparative liquid chromatograph. The resulting unit is fully automated and is capable of continuous unattended operation for periods of up to several weeks. Finally, the use of this system is illustrated by showing the scale up of a separation from an analytical to a preparative mode resulting in the isolation of high-purity standard materials.

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INTRODUCTION

Preparative chromatography refers to the practice of isolating one or more sample fractions from a chromatographic system. So called "preparative" techniques have been applied to all types of chromatography. In this paper we will confine our discussions to high performance preparative liquid chromatography where milligram to less than ten gram quantities of material are isolated. Verzele and Geeraert[2] classified preparative liquid chromatography into five categories. The equipment and techniques discussed are applicable to the first two types of preparative liquid chromatography discussed by these authors. Since they involve large scale equipment not found in an analytical laboratory, types 3-5 are beyond the scope of this review.

Verzele and Geeraert describe Type 1 preparative liquid chromatography as that carried out on analytical high performance liquid chromatographic (HPLC) columns. Typically, analytical high performance liquid chromatographic columns range in length from 5 to 50 cm with internal diameters in the range of 1 to 5 mm. Although most liquid chromatographic systems, on the market today, are directly applicable to this mode of preparative liquid chromatography, it is often not used because the amount of material which can be isolated, in a single run, is confined to the microgram range. This mode tends to be tedious, if milligram or gram quantities are required. An exception is the isolation of small microsamples for analysis by techniques such as mass spectroscopy.

Type 2 preparative liquid chromatography is described as scaling HPLC to a load factor 2-10 times that normally used in Type 1 analytical systems. In this technique milligrams are usually isolated and therefore, over a reasonable period of time with the collection of a number of fractions, gram quantities can be easily obtained. This of course makes the technique attractive to many workers for the isolation of high purity standard materials and extremely expensive or rare chemicals. This paper will confine itself, in the greater part, to Type 2 preparative liquid chromatography.

Type 2 preparative liquid chromatography has experienced a growth in use over the past 2 to 3 years. Several manufacturers have introduced detectors or modified detectors, for use in this mode. Also, other manufacturers have directly scaled up from analytical liquid chromatographic columns to preparative columns of similar length and separation efficiency in the 9 mm to 22 mm internal diameter range. These columns are packed with high efficiency materials identical to those found in analytical liquid chromatographic columns.

Preparative liquid chromatography in the milligram range is attractive in that it can be performed quite easily on analytical liquid chromatographic systems with only minor modification. When one considers the initial investment for a liquid chromatographic system of research grade may range anywhere from 20,000 to $40,000, the cost of adding a few components such as a fraction collector or preparative pumping heads is quite minor.

In that many, if not most, research grade modern liquid chromatographs incorporate microprocessor controls, the interfacing of alternate components for automated preparative liquid chromatography often requires no more than connecting 2 or 3 wires for the control of a fraction collecting device. Normal analytical sampling valves are easily modified by substituting a larger sample loop in order to work in the milligram range. Perhaps the most commonly available loop on analytical sampling valves is the 10 microliter loop. These loops are easily removed and replaced with loops in the 0.5 to 5 ml volume range. This allows the use of these valving systems in the preparative mode.

We will concern ourselves with the modification and application of an analytical high performance research grade liquid chromatograph in the preparative mode. The equipment will be discussed and illustrated in some detail. A high resolution isomer separation will be shown as an example of system utility.

INSTRUMENTATION BEFORE MODIFICATION

One tenet of our laboratory has been to purchase a variety of equipment types in order to address the broadest range of possible liquid chromatographic problems. In line with this philosophy we have usually purchased component-based liquid chromatographic systems as opposed to packaged systems. Although there are many fine units from a variety of manufacturers in both classes, selection of a component system gives one the ability to change detectors, pumps, injection valves, or other related

units. The choice of this type of equipment makes the modification and integration of a number of components into a high performance preparative liquid chromatograph a relatively simple matter.

The equipment discussed below is typical of what can be used for high performance preparative liquid chromatography. In no way is the description of any particular piece of equipment an absolute endorsement of that equipment beyond the fact that it was found to be satisfactory and functionally applicable. Many units can be used in a similar manner; the only limitation is the ingenuity of the chromatographer.

At the time the construction of an automated preparative system was being considered certain equipment was available. (1) An Altex model 420 binary liquid chromatographic pumping system equipped with two model 110-A pumps with microprocessor control. In their initial configuration these pumps were capable of producing a binary gradient at flow rates up to 10 ml/minute. The pump controller had 4 contact closures for the control of external equipment and also contained 4 pneumatic control valves which could accommodate gas pressures up to 100 psi. (2) A Rheodyne model 70-10 loop injector, equipped with a 10 microliter loop and pneumatic actuator. (3) A Gilson Holochrome® variable wavelength UV/visible detector unit. The prime advantage of this detector, in this application, was its ability to use a variety of flow cells of varying path length and volume. (4) A Gilson model CPR®peak fraction collector. This unit monitors the output

of the liquid chromatographic detector and upon elution of a peak, indicated by the appropriate signal from the detector, initiates the fraction collection sequence. The CPR® can be controlled by an external contact closure device and has the advantage, as is with all peak fraction collection devices, of collecting only the peaks and discarding the intermediate solvent to waste. (5) A FMI low pressure liquid pump for filling the injection valve loop.

CONSIDERATIONS FOR EQUIPMENT MODIFICATION AND INTEGRATION

As we contemplated integrating and/or modifying the components discussed in the previous section, we had to consider what type of columns were to be used and what separations were to be performed on this system. Table I lists a brief summary of flow rate as a function of internal diameter of a number of liquid chromatographic column types. If the 4.6 mm internal diameter analytical column operating at 1 ml/minute is considered standard, we see that use of preparative 9 mm bore columns requires an average flow rate of 3.8 or approximately 4 ml/minute. This is well within the range of today's modern liquid chromatographic pump. Doubling the flow rate, a common practice, with a 9 mm bore column presents no functional problems for most modern HPLC pumps. The next most commonly used preparative liquid chromatographic column for this class of liquid chromatography is the 22 mm bore, often called 1 inch columns. As can be seen from Table I, the average flow rate, in relative proportion to a 4.6 mm bore column operating at 1

TABLE I
Typical Flow Rates in Liquid Chromatography as a Function of Column Diameter*

Column Type	ID mm	Flow Rate ml/min
microbore	1	0.05
analytical	2.1	0.21
analytical	4.6	1.0
preparative	9.0	3.8
preparative	22.0	22.9

*See Figure 1 for a pictorial view of column type.

ml/minute, would be 22.9 ml/minute for a 22 mm column. Such flow rates are beyond the range of most modern liquid chromatographic pumps without some modification. The flow rate proportions given are designed to maintain a constant linear velocity through each column regardless of internal diameter. Velocity (flow rate) changes are proportional to cross sectional area ratios of the columns.

It is fortunate that several manufacturers offer preparative liquid chromatographic pumping heads for their analytical pumping systems. The system described used a relatively inexpensive, commercially available, pair of preparative heads, providing flow rates of up to 28 ml/minute each. Although it is not necessary to have such heads installed when using 9 mm internal diameter columns, it will be shown later that it is advisable that if one is to use high flow rates, over 4 ml/minute for any extended period of time, that such heads be purchased. Their use

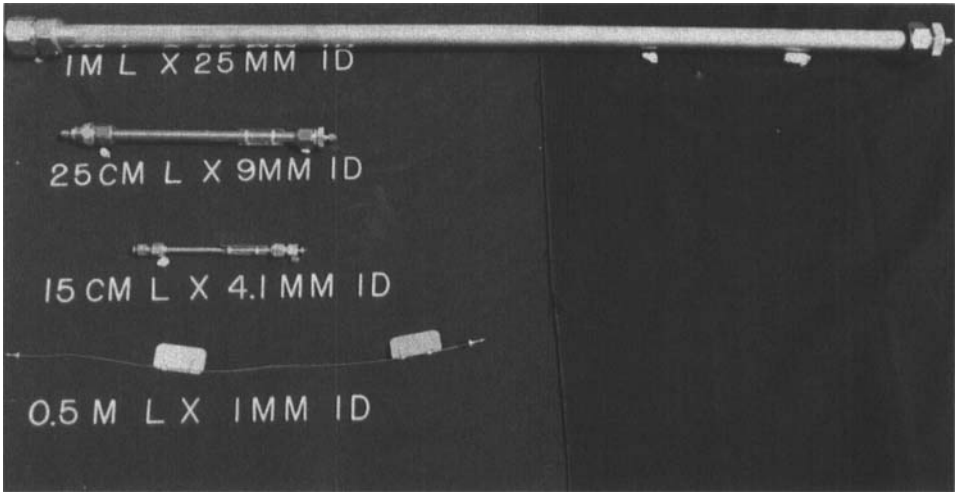


FIGURE 1

Typical Analytical and Preparative
HPLC Columns

eliminates a great degree of strain on the pumping system, and in the case of the system described the pumps were found to run both cooler and with greater reliability.

The system utilized in our lab, when equipped with the two preparative pumping heads, was capable of providing a binary gradient at flow rates of up to 28 ml/minute. If one needed to approach flow rates up to 56 ml/minute, in the isocratic mode, the microprocessor was defeated and each pump run independently.

The Rheodyne model 70-10 analytical sampling valve was easily modified by cutting a piece of the appropriate tubing so that its sampling loop was 0.5 ml. In the study described, this 500 microliter loop was used for all injection purposes. This is perhaps the easiest modification described in this paper.

Most UV/visible liquid chromatographic detectors are not geared towards preparative liquid chromatography. The cells are too small to accommodate the high flow rates involved and they tend to be swamped by the sample (signal) intensity, assuming compounds with reasonable molar extinction coefficients are used. Several approaches to the use of UV/visible detectors in liquid chromatography have been taken. I will briefly mention two. A very interesting approach, not used in this work, but effectively used by many workers, is the utilization of detectors such as the Gow-Mac model 80-800 preparative LC/UV detector.[3] This detector uses a reduced path length of a factor of approximately 100 by eliminating the use of a flow-through cell. Instead, a thin film of column effluent is run across the quartz sampling plate, which allows for sensitivity in the proper range with high sample through-put.

The detection system employed in this paper permits the use of a number of interchangeable flow cells. The Gilson Holochrome® detector was fitted with a preparative liquid chromatographic cell with a 2 mm path length. This reduced the detector's sensitivity by approximately a factor of 5. Also, since this is a variable wavelength detector, the detector can be detuned from the optimum analytical wavelength allowing reasonable on-scale detection and tracking for the automatic peak fraction collector.

As with all liquid chromatographic systems, sample introduction is key. One of the most important aspects of any automated system is the ability to bring samples in accurately and repetitively when the system is unattended. An FMI lab

metering pump, adjustable to flow rates up to 20 ml/minute, or with a low flow rate option, 10 ml/minute, was used in series with the Rheodyne model 70-10 valve. A tube from the inlet end of the pump is placed in the sample reservoir while the outlet side is hooked to the inlet of the injection valve. With the desired starting signal the pump is activated for a set period of time with the proper amount of sample being introduced. Since the Altex model 420 controller did not have any provision for AC switch output control, a means of initiating a controlled pumping system for loop filling operations had to be found. The Altex 420 controller has a number of processor controlled relays, which cannot handle AC switched line voltages, but can provide low voltage, low current switching control to appropriate systems. From the variety of delay relays available, we chose a variable time unit which could be set to activate its contacts for a period between 0.6 and 120 seconds by a simple dial on the top. All that was required to activate the relay was a momentary contact closure to its inputs, however one must select a relay capable of switching AC line voltage. The relay we used is manufactured by Omnetics Inc. Figure 2 illustrates the simple sample draw configuration utilizing this relay system. The external relay, flag, is wired into the control unit box of the Omnetics relay. A 0.05 second contact closure from the Altex 420 controller to the relays external input lead contact, triggers the relay for a determined period as set by the control dial on top. This provides AC power to the FMI lab metering pump shown on the right. As can be seen in the picture, the draw side of

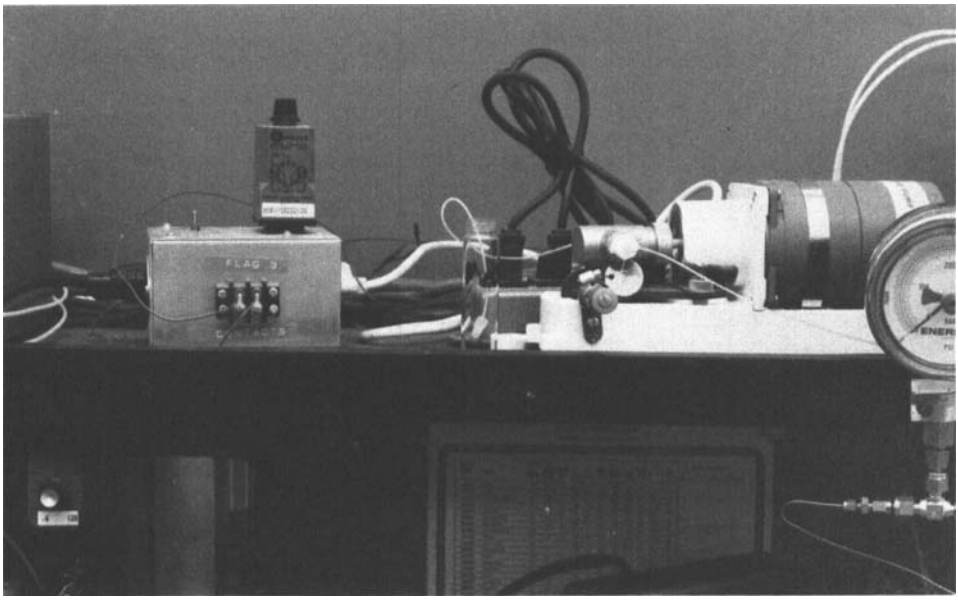


FIGURE 2

Sample Draw Control via Time Delay Relay
Control of an FMI Lab Metering Pump

the pump is in a small sample bottle ready to pump sample into the liquid chromatographic sampling valve.

The use of the time delay relay and FMI pumping system allows for precise and accurate control of the sampling process. Both partial and full loop operations are possible using this system. In the partial fill mode variable volumes may be introduced by controlling the time of sample introduction by either the time delay relay or by holding the external relay controlling the time delay relay from the Altex model 420 controller open. In both partial and full loop operations the

external pneumatic relays from the model 420 controller are used for controlling the Rheodyne 70-10 pneumatic injection valve for sample injection and return to the load position. By having a high degree of microprocessor control we are able to effect utilization of "backcut" injection techniques. In many high performance applications it has been shown that backcutting techniques greatly improve and maintain resolution, especially when scaling analytical to preparative systems, by eliminating the long sample tail encountered when a full loop flush is utilized. This technique is well described in the literature and will not be discussed in any detail here.[4-5]

The Gilson model CPR® peak fraction collector is directly controllable via a relay contact from the model 420 liquid chromatographic processor controller. A simple two-wire hook up completes this connection. This closure was used to indicate the beginning of the collection cycle. The automatic peak fraction collector has controls to set peak collection threshold, peak width sensitivity and time slicing of peaks. In the application that will be shown later four peaks were collected. Since we are interested in collecting only these four peaks, which elute sequentially, the peak fraction collector is set automatically to recycle to a null state after the final, lower peak threshold was passed. It would take a cycle activation closure from the processor control program to begin another automatic fraction collection cycle.

So far we have looked at considerations for system modification and how some of the components were integrated. In

the next section of this paper we will discuss the assembly of these units into a functional, automated, preparative liquid chromatograph.

FUNCTIONAL SYSTEM ASSEMBLY

Figure 3 shows a picture of the entire automated preparative liquid chromatograph. In the upper right hand corner is the control module from the Altex model 420 processor controller; to the left of that is a high pressure gauge and still further to the left can be seen the relay control box and sample introduction pump previously described. Directly below the 420 processor controller can be seen the Rheodyne model 70-10 pneumatically controlled injection valve. The incubator compartment, sitting on top of the Gilson Holochrome detector, was modified to act as a column oven for high performance liquid chromatography. Its temperature control device was removed and replaced with an external high performance temperature controller. Additional heating elements were installed internally along with a fan for circulation purposes. The oven is capable of accomodating columns up to 40 cm in length. The internal configuration of this oven is illustrated in Figure 4. Returning to Figure 3 we see, in the lower right hand corner, two Beckman 110 pumps equipped with high flow rate preparative liquid chromatographic heads sitting on top of the Gilson peak fraction collector. Figure 5 shows the pumps and the peak fraction collector in more detail. Figure 6 diagramatically shows the entire system. As can be seen, the controller is the heart of

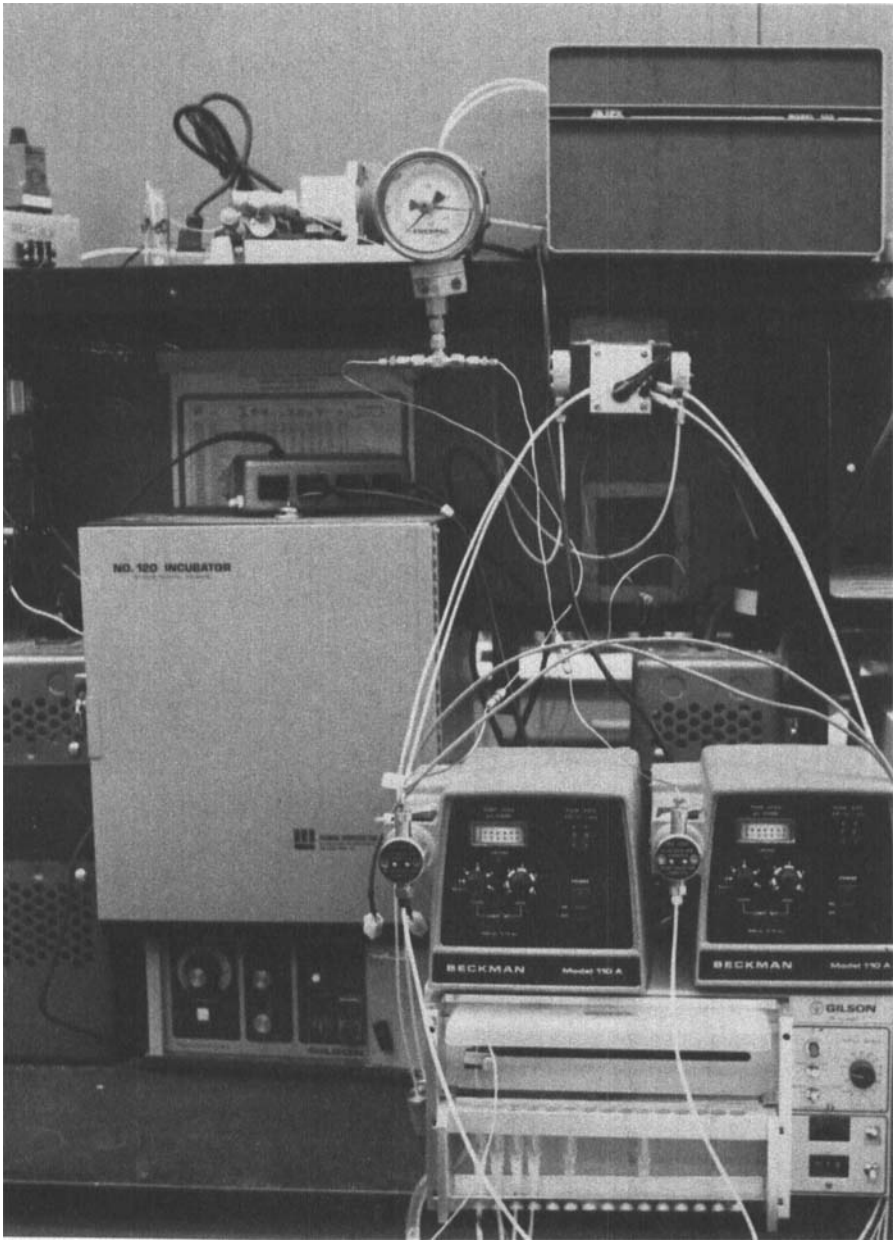


FIGURE 3

The Automated Preparative Liquid Chromatograph

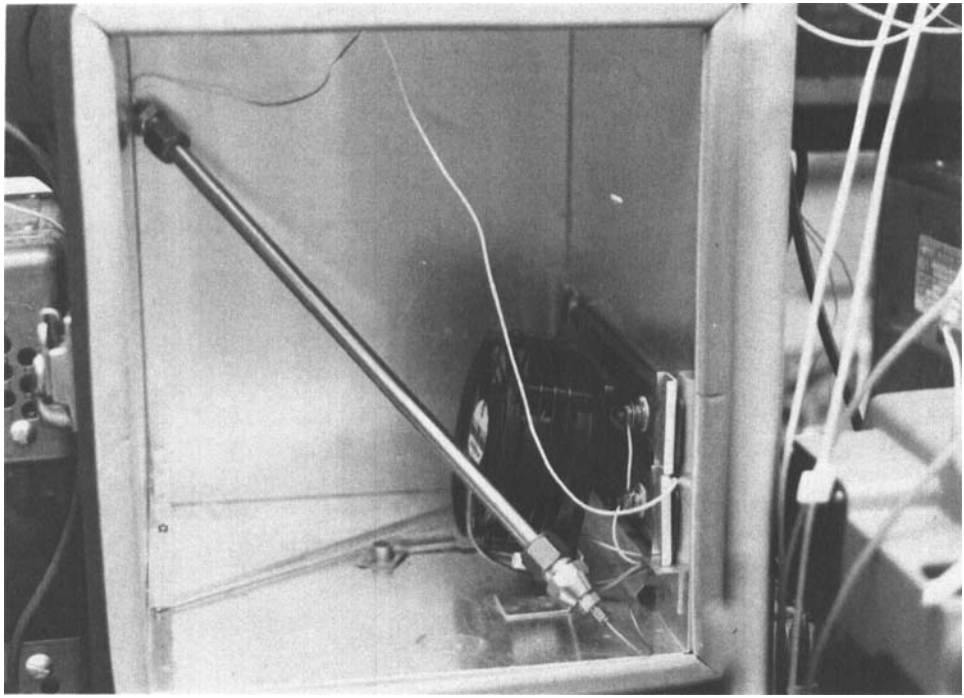


FIGURE 4

Modified Incubator for Use as an HPLC Column Oven

the entire automated system. It controls not only the binary pumping system, via direct hook up, but four of its relay flags provide pneumatic control along with all other major system control functions. Programming such a system is quite simple. A minimum number of programming steps are required to make this unit functional. Table II shows a typical control program, of 7 steps, for the control of a simple preparative liquid chromatographic operation. Step 1 sets the solvent composition to 80% solvent B. By default solvent A is set to 20%. Step 2 sets

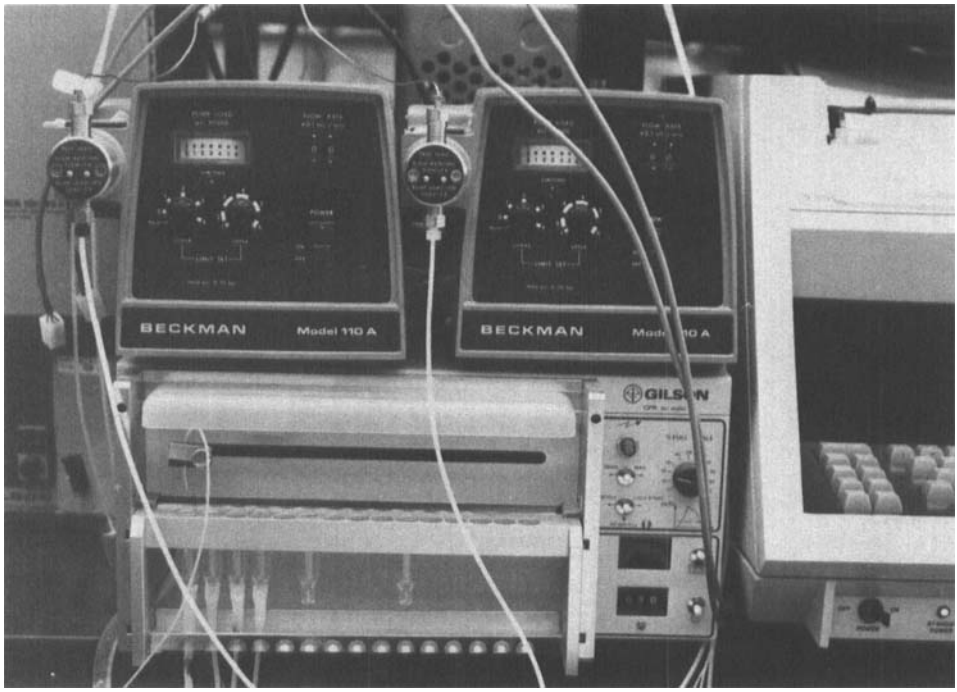


FIGURE 5

Binary Preparative Pumping System and
Peak Fraction Collector

the flow rate to 10 ml/minute. Step 3 fires external flag 3 for a short duration. This activates the time delay relay. The sampling pump is activated generating the loop filling operation for the sample valve. Step 4 fires external flag 6, a pneumatic flag, which performs the sample injection. Step 5, two tenths of a minute later returns the sample valve to the load position. Step 6 at 20 minutes fires external flag 1 which activates the automatic peak fraction collector. Step 7 defines the end of the program. If a single cycle of this program is performed, flow

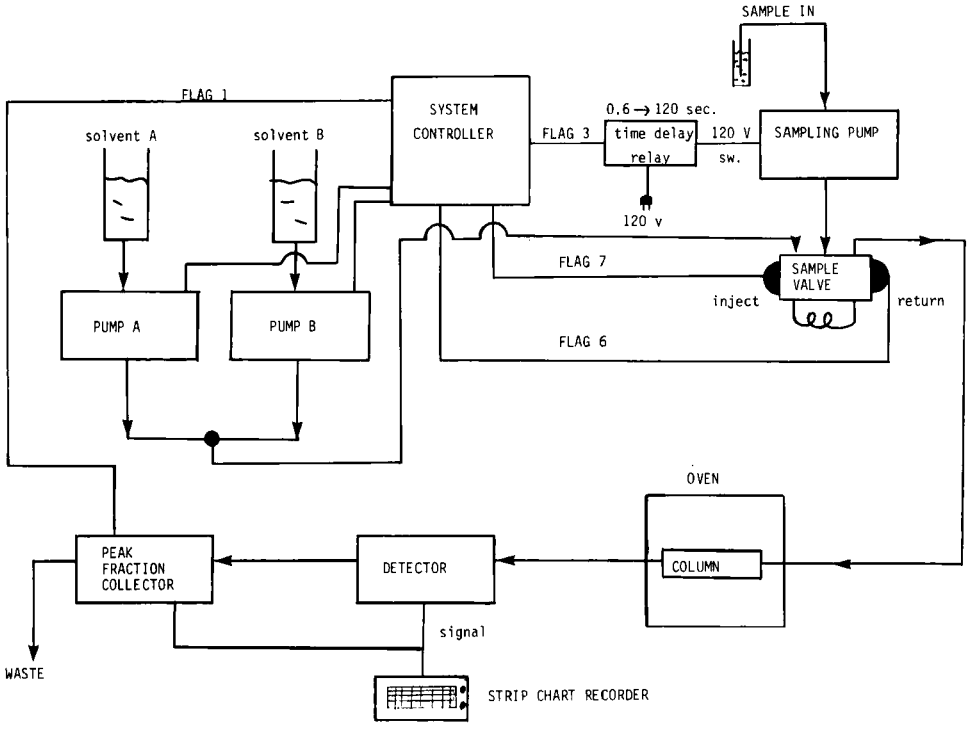


FIGURE 6

Block Diagram of the Preparative Automated Liquid Chromatograph

would be set to zero and the system shut down. With the model 420 controller we can program the system to cycle many times. Therefore, if 7 injections were to be made, we could set a cycle duration of 7. When the last step in the program, Step 7 at time 30, was reached, flow rate would be set at zero for a few milliseconds, but the program would automatically recycle to Step 1 for the second cycle, resetting the flow rate to

TABLE II
A Typical Control Program

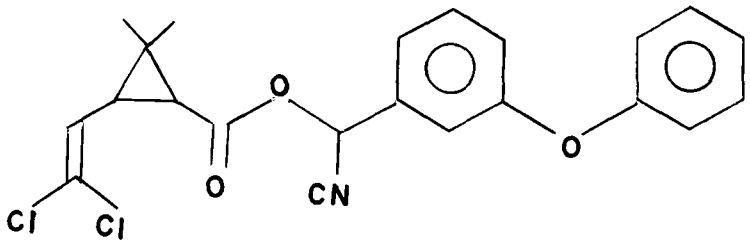
Step #	Time	
1	0	% B 80
2	0	Flow 10
3	0	Ext Flag 3 Duration 0.05
4	1	Ext Flag 7 Duration 0.10
5	1.2	Ext Flag 6 Duration 0.10
6	20	Ext Flag 1 Duration 0.05
7	30	Flow 0

10 ml/minute at time 0. Flow interruption would be so slight that the column would not see any significant pressure changes, and an uninterrupted cycle would be the apparent result.

So far we have gone through the description and integration of the preparative liquid chromatograph system. In the following sections we will discuss an analytical separation of interest, its transfer to the preparative system, and subsequent isolation of an analytically pure standard material.

THE ANALYTICAL SEPARATION

Of interest in our laboratory was the analytical separation of cypermethrin. Chemically cypermethrin is (±)-α-cyano-3-phenoxybenzyl (±)-*cis*-*trans*-2,-2-dimethyl-2-(2,2-dichlorovinyl) cyclopropane-1-carboxylate. The structural formula for cypermethrin is shown below.



As can be seen from the structure, there are three centers of optical activity in this compound. Therefore, a maximum of eight isomers exists. If one uses nonchiral specific separation techniques, one would expect to separate, at the maximum, four isomer pairs. This is indeed the case that was encountered in the development of a variety of procedures by FMC and other laboratories[6-8]. The separation which is described in this section is based on FMC Test Method ACG-48[7].

The separation of interest is performed in the normal phase chromatography mode. Table III summarizes the analytical conditions used for the separation, and Figure 7 shows a typical chromatogram obtained in the analytical mode. As one may guess, from examining Table III, the heptane/acetonitrile ratio is extremely critical. This of course will vary from column to column and must be adjusted to meet local conditions. Another critical factor in the separation is the residual water content of the heptane. To guarantee consistent low levels of water in the heptane we pass it through an activated silica gel column. One of the key indications of a poorly performing column, after the optimum acetonitrile ratio has been obtained, is its

TABLE III
Analytical Conditions

Column:	DuPont Zorbax Sil 25 cm L x 4.6 mm id
Mobile Plan:	99.825% n-heptane/0.175% acetonitrile
Temp:	45°C
Flow Rate:	2.4 cc/mm
Detection:	254 nm, 0.05 AUFS

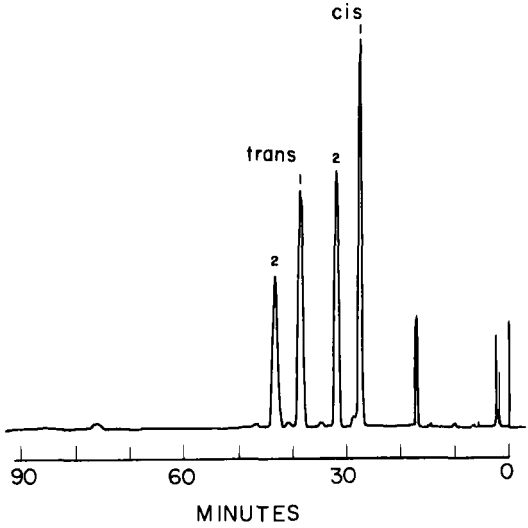


FIGURE 7

Analytical Separation of Cypermethrin Isomers

inability to provide baseline resolution of the isomers. Assuming the column exhibits the proper efficiency (N≃30,000 plates/m), poor performance is usually traced to high water content in the heptane.

Our goal was to obtain a gram or two of high purity, analytical reference-grade material by preparative liquid chromatography. This was considered an easier task than

performing isomer specific synthesis, since a good analytical method for separation was already available. Therefore, it was to a modified version of this method that we applied the automated preparative liquid chromatograph.

The next section will discuss scale up and subsequent results of the isolation study. This will provide a typical example of the capabilities of automated preparative liquid chromatography.

THE PREPARATIVE SEPARATION

When developing any preparative separation it is always useful to have good background data for the chemical and physical properties of the compound of interest. In the case of cypermethrin we have an excellent, well resolved analytical separation.

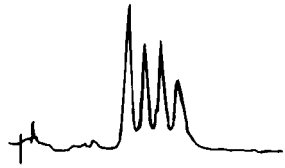
When performing scale up from the analytical separation to the preparative mode usually only minor changes in separation parameters are required. Once one knows the required quantity of sample, it is fairly easy to determine how many separations and fractions must be collected to obtain the desired amount. Of course, one must know the sample load capacity of the system of interest as a key parameter in this determination.

Theoretical considerations[9] can be invoked to determine sample load capacity. On the other hand, when one is concerned with a practical application a few quick empirical checks often tell one the same information much more quickly and absolutely than extensive calculations. One of the first changes made in

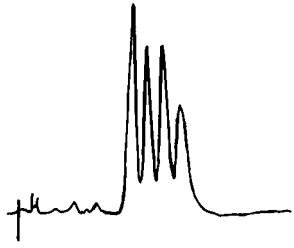
going to the preparative liquid chromatographic mode was to modify the mobile phase. A few tenths to a few percent of methylene chloride, added to the mobile phase, greatly stabilized the separation and, although decreasing the base resolution somewhat, was still satisfactory for the preparative requirements.

Figure 8 shows the results of a preparative separation using 0.5 ml injected onto a Whatman Partisil® 10 column of 9 mm internal diameter by 25 cm length. The column operating temperature was 50°C, and flow rate was 9.9 ml/minute. The stock solution concentrations were varied from 0.1822 g/ml to 0.0456 g/ml. As can be seen from Figure 8, inadequate resolution was obtained at the higher concentrations due to loading factors. The best separation for the 4 isomers, and one usable in a heart cut slicing routine, was at 0.0456 g/ml initial concentration. As was stated in the analytical discussion, solvent impurities can have a large affect on the quality of the separation. The main impurity which effects this separation is the residual water content of heptane. Figure 9 illustrates how improper mobile phase control can grossly effect the separation. A separation on a Partisil® 10 column is shown in this figure. Chromatogram #1 shows the separation with heptane having a water concentration of under 20 parts per million. Separation #2, utilizing higher water content heptane, shows the peaks somewhat sharpened but also moved in towards other impurities giving inadequate resolution for cutting isomers out in the proper mode.

Another consideration, relative to how long one can run such a separation, is the effect of impurities building up on the front



0.0456 g/ml



0.0911 g/ml

0.1822 g/ml

FIGURE 8

Effect of Mass Injected on the Separation

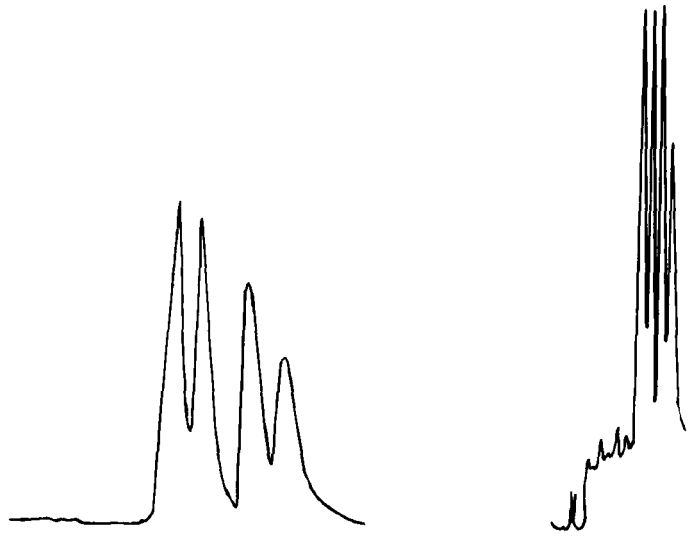
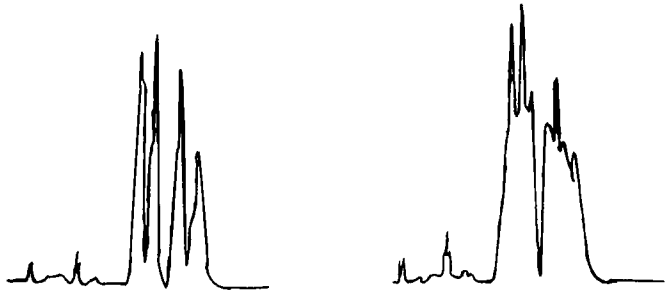


FIGURE 9

Effect of Solvent Impurities

of the column. As impurities build up, the ability of the column to take additional load and to perform the separation properly is diminished. Chromatogram #1 in Figure 10 shows a column which is beginning to deteriorate. Notice that the peaks are getting fatter and showing bulges on their sides. Although this column and the separation obtained is probably still useful for preparative cutting purposes, not many more injections could be performed on it before a clean up would be required. Separation #2 in Figure 10 shows the results of perhaps 10 or 15 more injections on this column. Resolution has been lost, and a totally inadequate separation has been obtained.



1 2

FIGURE 10

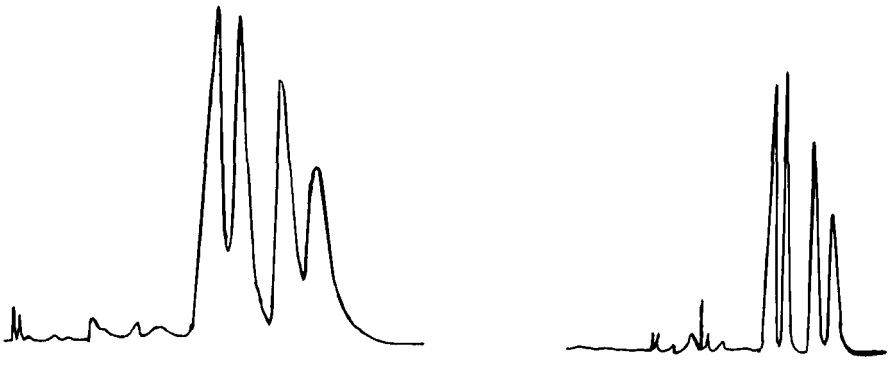
Results of Front End Column Build-Up

The preparative liquid chromatographic automated system provides an excellent solution to column contamination problems. The methylene chloride was determined to be an excellent solvent for purging the column and hence returning to the separation activity required. Since we are dealing with a dual pump system, pump A was normally used to pump the mobile phase of interest for the separation. Pump B was dedicated to the pumping of methylene chloride. Periodically, even in an automated run, the program could be cycled to a system purge of 100% methylene chloride. This purged impurities built up on the column and upon subsequent recycle to the primary mobile phase, given some time for reequilibration, the preparative separation could continue as before.

Another consideration in any analytical or preparative separation is the particle size of the column packing being used. Most scouting work, as illustrated in Figures 8 through 10, was

performed with 10 micron silica gel columns 25 cm length by 9 mm internal diameter. The described analytical separation was performed on a DuPont Zorbax® Sil column which nominally contains a 6 micron particle size material and is therefore a higher efficiency material. For our final preparative separation we wanted to use a stationary phase of greatest efficiency, hence simulating the analytical system to the greatest extent. Figure 11 illustrates the simple change in going from a 10 micron Partisil® column to a 5 micron Partisil column. The mobile phase in this case is 98.85% n-heptane, 1% methylene chloride and 0.15% acetonitrile, operating at a temperature of 50°C and a flow rate of 9.9 cc/minute. Much more satisfactory results, more closely resembling the analytical separation, were obtained using the 5 micron material. Good resolution, nearly baseline, is obtained for all the isomers as was the separation of early eluting impurities.

Cypermethrin has almost equivalent molar absorptivity at 254 and 280 nanometers. Even when decreasing the detector to its minimum sensitivity, with the 2 mm path length flow through preparative cell, the use of either of these standard wavelengths still grossly overloaded the detector in our concentration ranges of interest. One technique, very useful with a variable wavelength detector, is the ability to go off wavelength. Therefore, setting the analytical sensitivity at 0.5 nominal absorbance units full scale (AUFS), the switch setting on the detector, which in reality is 2.5 AUFS, because of the use of a 2



10 micron 5 micron

FIGURE 11

Effect of Particle Size on Separation

mm cell instead of a 10 mm cell, at 290 nanometers brought the detector into a range where we could see the entire peaks without overloading it.

Once we have scaled the analytical separation into the preparative mode and all conditions are optimized one only needs to set the peak fraction collector. In the case of this separation the collector is set to activate its collection cycle just before the elution of the first peak of interest. Since this is a peak fraction collector, which monitors the output of the of 9.9 cc/minute. Much more satisfactory results, more closely resembling the analytical separation, were obtained using the 5 micron material. Good resolution, nearly baseline, is obtained for all the isomers as was the separation of early eluting impurities.

Cypermethrin has almost equivalent molar absorptivity at 254 and 280 nanometers. Even when decreasing the detector to its minimum sensitivity, with the 2 mm path length flow through preparative cell, the use of either of these standard wavelengths still grossly overloaded the detector in our concentration ranges of interest. One technique, very useful with a variable wavelength detector, is the ability to go off wavelength. Therefore, setting the analytical sensitivity at 0.5 nominal absorbance units full scale (AUFS), the switch setting on the detector, which in reality is 2.5 AUFS, because of the use of a 2 mm cell instead of a 10 mm cell, at 290 nanometers brought the detector into a range where we could see the entire peaks without overloading it.

Once we have scaled the analytical separation into the preparative mode and all conditions are optimized one only needs to set the peak fraction collector. In the case of this separation the collector is set to activate its collection cycle just before the elution of the first peak of interest. Since this is a peak fraction collector, which monitors the output of the detector, a peak collection threshold of 20 to 30% was set with the front switch. This allows a good heart cut of each peak of interest. Each purified isomer solution was collected into a separate collection vessel with the intermediate waste being discarded.

Approximately 2 grams of final combined weights of all 4 isomer pairs of greater than 99% purity were prepared by this

system. Once the separations were completed and fractions collected, it was a simple matter to evaporate the mobile phase, perform a rapid recrystallization, and end up with very pure analytical standard reference material. In order to prepare this material the preparative liquid chromatograph operated 24 hours a day, 7 days a week, for approximately 1 month. Operator attention and intervention was very minimal. Usually this consisted of replenishing the mobile phase every other day. Once, during an intermediate period in the study, a column clean up as previously mentioned was performed with methylene chloride. This resulted in no more than 2 hours total down time of the system.

CONCLUSIONS AND SUMMARY

The previous discussions illustrated that minor modifications and additions to a research grade analytical liquid chromatograph can make it a viable tool for preparative liquid chromatography, when milligram or low gram quantities of material are to be isolated. Work can be performed reliably and can be cost effective and, the utility and versatility of one's liquid chromatograph can be greatly expanded. Perhaps one of the factors discouraging many workers from using high performance preparative liquid chromatography is the cost of the media. An analytical column typically costs $250, preparative columns in the 25 cm by 9 mm internal diameter range often cost between $500 and $1,000. For many smaller operations these costs become prohibitive. Fortunately several manufacturers now offer high efficiency preparative media at a cost approximately 1/10 of the analytical

media. As an example, 10 grams of a 10 micron analytical silica gel may cost approximately $50, however, high efficiency 10 micron materials are now available for preparative work, with high plate count and near analytical performance, at a cost of approximately $50 per 500 grams. If one wants to do reversed phase work, such materials are also available at a price of approximately $200 per 100 grams. Greater savings can be effected, if individuals perform their own surface derivatization. The resulting price reductions make it attractive to consider packing your own preparative liquid chromatographic columns. When dealing with 9 mm bore preparative columns, packing is not much more difficult than for analytical columns. The literature is replete with papers, including one of our own[10], dealing with packing and preparation of columns for high performance liquid chromatography.

One final consideration is the type of fittings and interconnecting tubings used for the system. The use of 9 mm bore columns, and even 22 mm bore columns, often allows the use of the same types of tubing as in an analytical system. The fittings at the front and back end of the preparative column do become critical, since a good smooth flow outlet is required for the complete flushing of the column. Flat ended outlet fittings generate unswept volumes, which are then cleared by diffusion, and hence peak tailing is generated. Several manufacturers sell both prepacked columns or loose end fittings with outlet tapers. The use of this type of fitting is suggested.

Mass scale up to the preparative mode, an order of magnitude greater than encountered in the analytical mode, tends to be a rather simple, straight forward matter. It should be well realized by many analytical chromatographers and users of analytical chromatography that when they perform an analytical separation, in most cases they are throwing out highly purified standard grade material.

Chromatographers and scientists in general are only beginning to realize the utility of analytical liquid chromatographs as isolation tools. Hopefully this paper has illustrated how such systems can do double duty extending their value and effectiveness.

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