Fast Preparative Column Liquid Chromatography (PCLC)

Application Note 224

Joan Stevens, Ph.D. and Gary Scharrer

Introduction

Preparative HPLC is recognized as a prime method for obtaining pure compounds from complex mixtures. It is often expected that prep HPLC can be satisfactorily performed via low-efficiency, large-diameter columns used under overload conditions. Evaluation of this preparative HPLC, low-tech route is not the best option. Short, fine-particle columns permit baseline resolution of closely-eluting compounds. Incorporating gradient elution and multicycle automation, the 1-inch bore can isolate 1 mg of each of several substances, but can also be employed to obtain up to 200 grams of a single compound.

This technique, called FAST PCLC (preparative column liquid chromatography), reduces analysis time and increases throughput. For example, a 5-µm column can handle a throughput of 1 gram in about one-sixth of the time required for a 30-µm column. Complete preparative separations can be developed on the same system with the same column. Having completed method development, the user can leave the system to operate unattended. Repetitive cycles, rather than scale-up, substantially reduce labor and column costs. The instrument can then be used to check for purity of collected fractions.

Materials & Methods

Instruments and Accessories

305/306 Pumps: binary-gradient solvent delivery (25 mL/min), 4,060 psi max

Automated Injector: 5-mL syringe, 2-mL sample loop

155 UV/VIS Dual-Wavelength Detector: 0.2-mm flow cell

Fraction Collector: programmable and signal-based

Column: 3 µm, 100 Angstrom, ODS, 21.4 mm ID, 10 cm length, permanent axial compression fittings

Description of the Procedure

- Dissolve sample in minimum volume, typically 10 mg/mL
- Determine gradient profile by using variables in the control method for the initial and final % of organic and for the injection volume
- Start an unattended, multicycle program with repetitive injections under the required conditions (mobile phase gradients, gradient slopes, flow rates, etc.)
- Fraction collection can occur in individual tubes, or, in the case of a large sample where the impurity is significantly separated from the main peak of interest, into a large vessel throughout the multiple runs

Software

- The software is extremely versatile: one method can be set so that numerous conditions are easily modified by simply filling in the blanks
- This type of flexibility allows walk-up users to vary conditions without having to compose a new method by using only the operation list
- Initial % of Pump B, End % of Pump B, Gradient Time, Chromatography Run Time, Start Fraction Collection, Stop Fraction Collection and Sample Volume are just a few of the options available to the user

Software Options

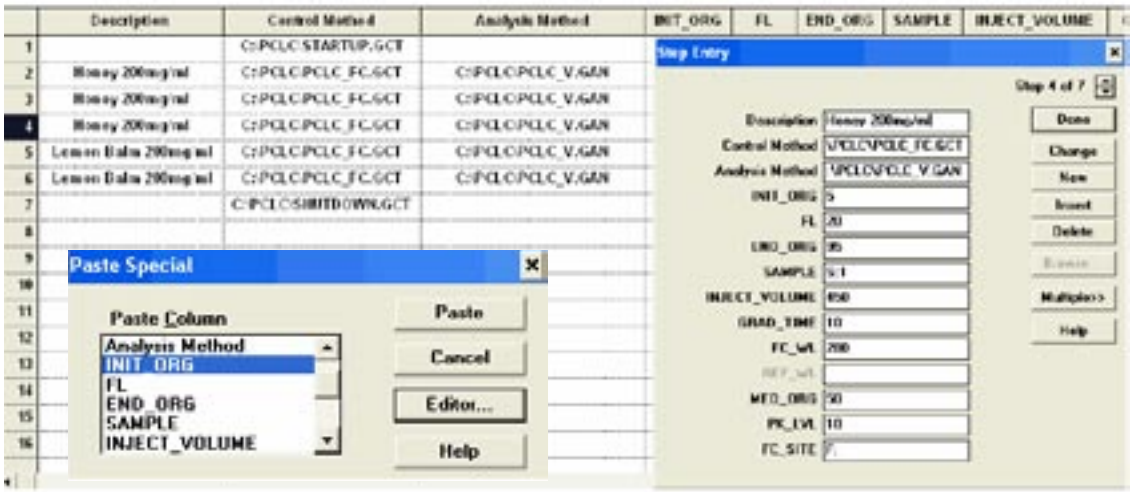
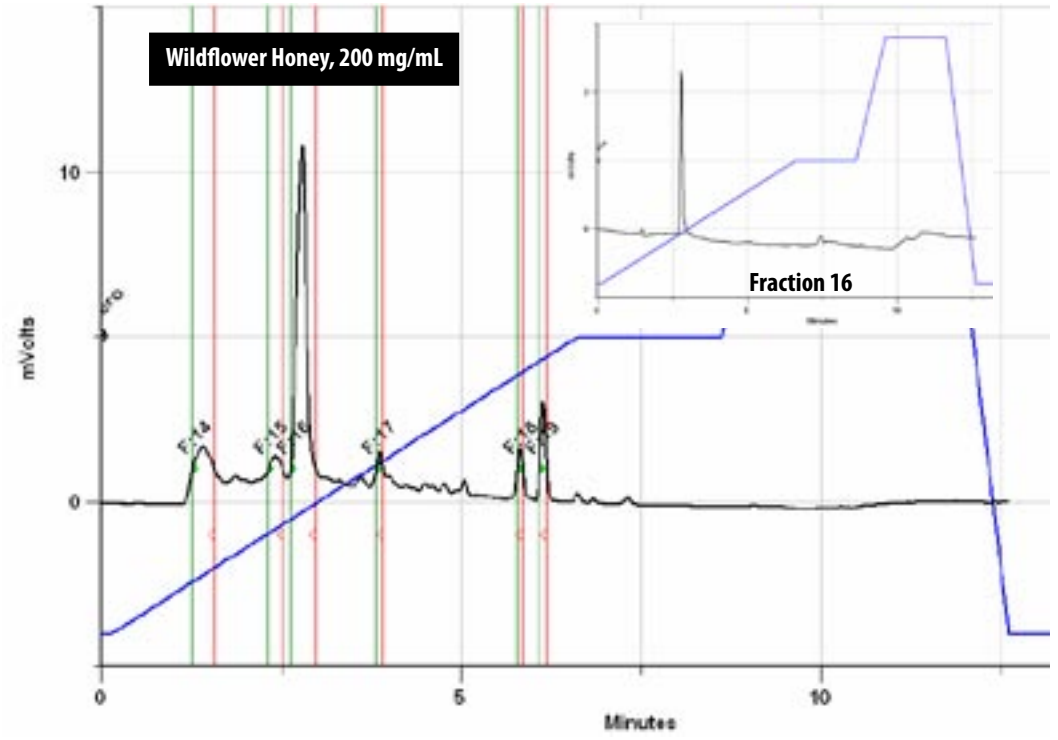


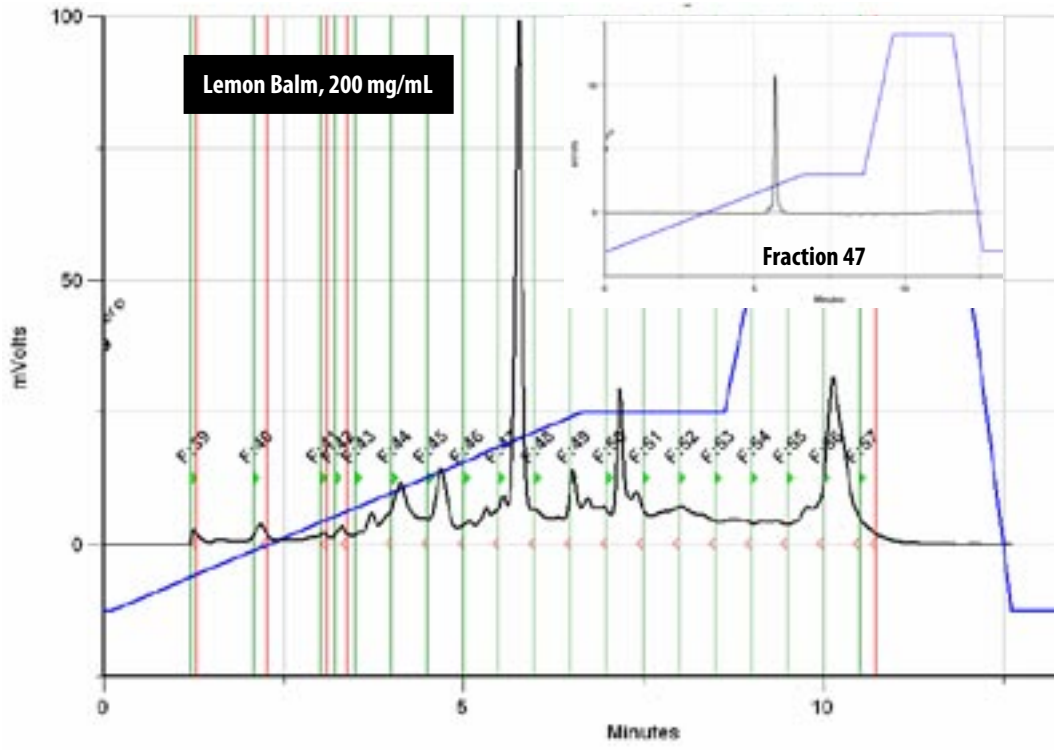
Table 1. Representation of the "Step Entry" screen that allows the end user to input parameters for each sample or to populate multiple samples with the same or incremented parameters. Text file formatted parameters can also be directly imported into a particular cell for population via the "Paste Special" option.

Table 2. Represents the ease of use within UniPoint Software to change the initial, end gradient, slope of the gradient and overall run time, fraction collection (including injection volume) and flow rate for all samples in one screen without the need for additional methods.

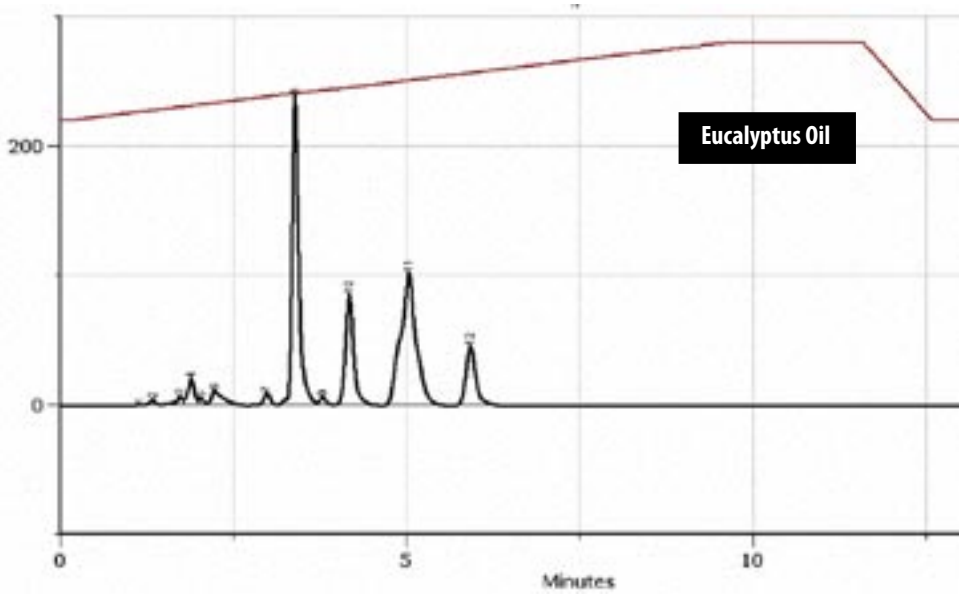
	Time	Device(s)	Command
1	GRAD_TIME+3	Aqueous / Organic	FL (ml/min): 100% Aqueous, INIT_ORG% Organic
2	GRAD_TIME	Aqueous / Organic	FL (ml/min): 100% Aqueous, END_ORG% Organic
3	GRAD_TIME+2	Aqueous / Organic	FL (ml/min): 100% Aqueous, END_ORG% Organic
4	GRAD_TIME+4	Aqueous / Organic	FL (ml/min): 100% Aqueous, INIT_ORG% Organic
5	GRAD_TIME+3	Data Channels	Stop Chromatogram Channels
6	START_FC	Fraction Collector	Start Collection
7	GRAD_TIME+2.5	Fraction Collector	Stop Collection
8	0.01	Aqueous / Organic	FL (ml/min): 100% Aqueous, INIT_ORG% Organic
9	0.05	Partial Loop Injection	<start> SAMPLE, INJECT_VOLUME
10	0.10	Detector 1B	Set Dual Wavelength 1 FC_WL
11	0.12	Detector 1B	Set Dual Wavelength 2 REF_WL

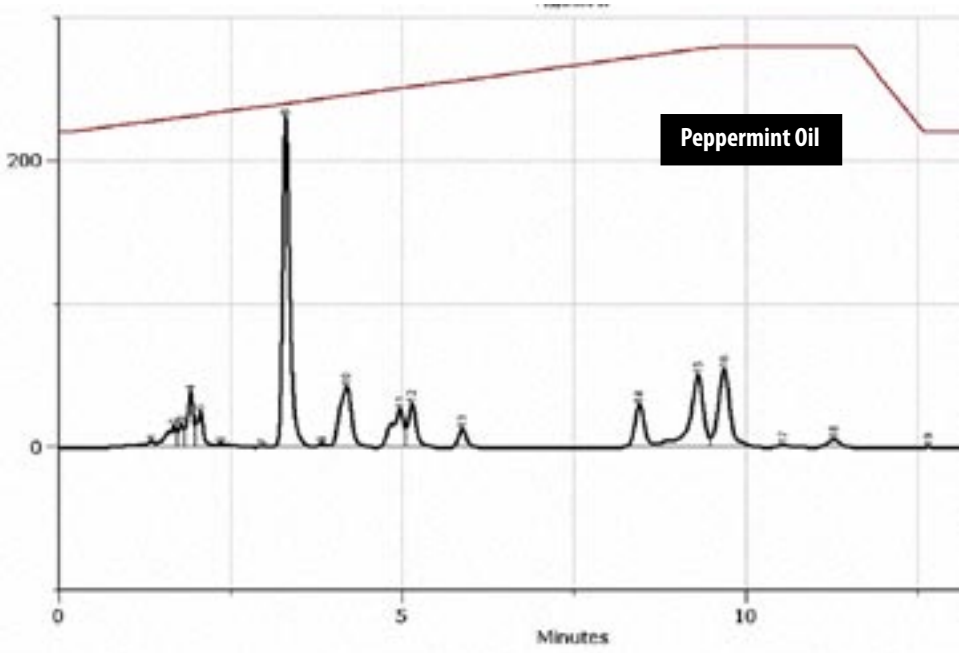
Table 3. Represents the generic control method that allows multiple users to use the same method without the need to create new methods for different gradients or conditions associated with the samples. Using the variables for "GRAD_TIME", "FLOW", "% ORG" and "START_FC", etc., allows complete customization for individual needs.





Chromatograms 1 & 2. Representation of the chromatographic separation available on the 3 micron, 100 Angstrom, ODS particle, 900 µL injections. Using the multicycle approach, sample can be purified in a short cycle time (7–15 min), offering lower solvent consumption per cycle (100–200 mL/cycle), high-substance concentration per liter (50–500 mg/L) and high-peak capacity per cycle (20–40 detected). Insets represent the injection of the collected fraction, 95% and 96% recovery respectively.





Chromatograms 3 & 4. Additional representations of the separation of natural products that often contain multiple peaks of interest.

Summary

- High-pressure mixing provides greater accuracy when compared to low-pressure mixing systems, for any composition, even at extreme ends of the gradient. The delay volume is smaller; bubbles will not form in the pump.
- Better reproducibility because flow rate accuracy is independent of pressure changes. Using the pressure value and compressibility coefficient of the liquid being pumped, the pumping modules adjust the piston speed for each pump.
- Employing the use of the high-performance, 3 micron particles allows for increased peak capacity, shorter cycle times and reduced solvent consumption.
- The use of variables makes the PCLC system a walk-up user friendly HPLC for multicycle runs and unattended operation.

Conclusion

Fast PCLC is a general laboratory technique for preparative separations from off-line sample preparation (1 mg) up to purification at the pilot scale (200 g). The high performance of the 3 micron particles is combined with the advantages of gradient elution and automation. Repetitive cycles instead of scale-up substantially reduces labor and column costs. The use of variables offer the end user a tremendous amount of flexibility for all types of samples and compounds. Applications that could employ this technique include: natural products, pharmaceutical compounds, organic chemistry, synthetic oligopeptides, environmental pollutants and plant protective agents.

Gilson, Inc. World Headquarters
Middleton, WI 53562 USA
Telephone: 800-445-7661 or 608-836-1551• Fax: 608-831-4451

Gilson S.A.S.
19, avenue des Entrepreneurs, F-95400 VILLIERS LE BEL France

www.gilson.com

sales@gilson.com, service@gilson.com, training@gilson.com