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Closed-loop recycling with periodic intra-profile injection: a new binary preparative chromatographic technique

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Abstract

Closed-loop recycling with periodic intra-profile injection (CLRPIPI) is a binary chromatographic separation technique. It is similar to simulated moving bed chromatography in that sample is injected into the interior of the circulating chromatographic profile. Although the CLRPIPI process is repetitive, it is different from SMB in that CLRPIPI is not continuous. It is shown that the CLRPIPI process reaches a steady state and that high purity fractions can be collected.

Keywords: Recycling; Closed-loop; Injection methods; Preparative chromatography

1. Introduction

Binary separations are those in which the entire sample is separated into two fractions or product streams. Such separations usually involve samples that have more than two components. For example, in the purification of *p*-xylene by simulated moving bed (SMB) chromatography, pure *p*-xylene is collected in one product stream (the extract) and the other three components (*o*-xylene, *m*-xylene and ethylbenzene) are collected in the other product stream (the raffinate) [1]. However, with chiral separations, in which racemates or enriched mixtures of enantiomers are separated into the pure enantiomers, the sample usually will consist of only the two enantiomers, with perhaps small amounts of impurities that can be removed easily in later separation steps. On the large scale, the most cost-effective binary chromatographic technique is usually SMB [2]. SMB is a continuous process; the sample or feed is continuously injected and two product streams are continuously collected.

Chiral separations are the most important type of binary separation in the pharmaceutical industry. Generally, only one enantiomer of a racemic drug will be beneficial. From the body's viewpoint, the other enantiomer is a completely different compound and, thus, should be regarded as an impurity [3]. It has become policy with most pharmaceutical companies that new drugs with stereogenic centers must be enantiomerically pure [4].

This paper discusses a new preparative chromatographic technique for performing binary separations. It is similar to SMB chromatography in that fresh sample is injected into the interior of the circulating chromatographic profile, however, unlike SMB, it is not continuous. The new technique uses high-performance liquid chromatography (HPLC) with closed-loop recycling and can therefore be described as closed-loop recycling with periodic intra-profile injection (CLRPIPI).

Bailly and Tondeur [5] describe a recycling chro-

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matographic process that involves thoroughly mixing the entire recycled chromatographic profile with fresh sample and reinjecting. In this process, the resolution that developed in the chromatographic profile as a result of its passage through the column is destroyed by mixing the entire profile with fresh sample prior to reinjection. The CLRPIPI process, however, utilizes a precise injection of fresh sample at a precise location in the circulating chromatographic profile. No significant mixing occurs between the fresh sample and the circulating chromatographic profile prior to or during the injection process.

Before discussing the CLRPIPI process in detail, let us review closed-loop recycling and SMB.

1.1. Closed-loop recycling

HPLC with closed-loop recycling has been known since at least 1959 [6]. Fig. 1 shows a schematic diagram of a commonly used closed-loop recycling system. The chromatographic profile, after eluting from the column and passing through the detector, is directed to the suction side of the mobile phase pump through the recycle valve (RV). The profile is then sent through the column again, i.e., it is recycled.

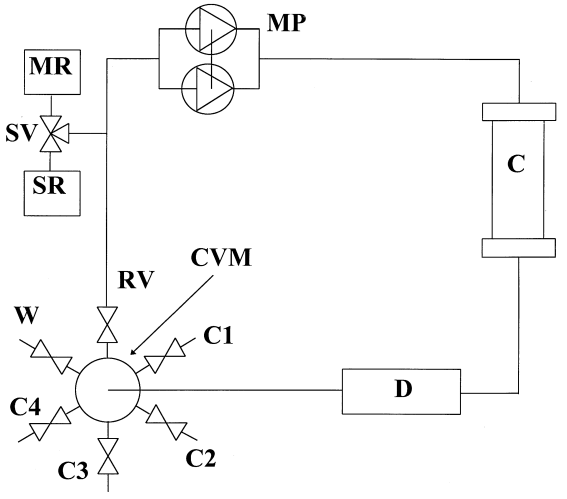

Fig. 1. Schematic diagram of a closed-loop recycling system. MP, mobile phase pump; C, column; D, detector; CVM, collection valve manifold; C1, C2, C3, C4, fraction collection valves; W, waste valve; RV, recycle valve; SR, sample reservoir; MR, mobile phase reservoir; SV, three-way selection valve.

This recycling increases the number of theoretical plates available for the separation, because in essence closed-loop recycling simulates the use of a longer column. Thus, resolution is increased after each cycle.

Since mixing occurs in the pump, some of the separation that occurred in the column is destroyed. Therefore, closed-loop recycling is feasible only when the resolution gained each time the profile passes through the column is greater than the loss in resolution due to mixing each time the profile passes through the pump. In practice, this means that the volume of the column must be much larger than the volume of the pump.

Fig. 2 shows a recycling chromatogram. Here, 150 mg each of methyl and propyl *p*-hydroxybenzoate were injected onto a 250×25 mm I.D. column packed with LiChrospher C_{18}, 12 μm particle size, 100 Å pore size (see Section 3 for details). Note that the resolution increases with each cycle, indicating that the mixing that occurs in the pump is not sufficient to destroy the separation produced in the column.

During any cycle in which sufficient resolution has been attained, fractions can be collected or shaved from the leading and trailing edges of the chromatographic profile using collection valves or a stand-alone fraction collector. A review article, discussing the use of closed-loop recycling in the separation of enantiomers, has been published recently [7].

1.2. SMB chromatography

SMB chromatography was invented in the early 1960s by Broughton et al. [8]. As mentioned, SMB chromatography is a continuous process, in which feed is continuously injected into the interior of the circulating SMB profile, two product streams (extract and raffinate) are continuously collected and fresh mobile phase is also added continuously. The process is illustrated schematically in Fig. 3, which depicts the circulating SMB profile in a twelve-column SMB system. The profile is moving to the right as indicated. At the appropriate time, all injection and collection points will be switched simultaneously one position to the right in order to maintain their correct positions relative to the profile.

Injecting into the interior of the profile makes

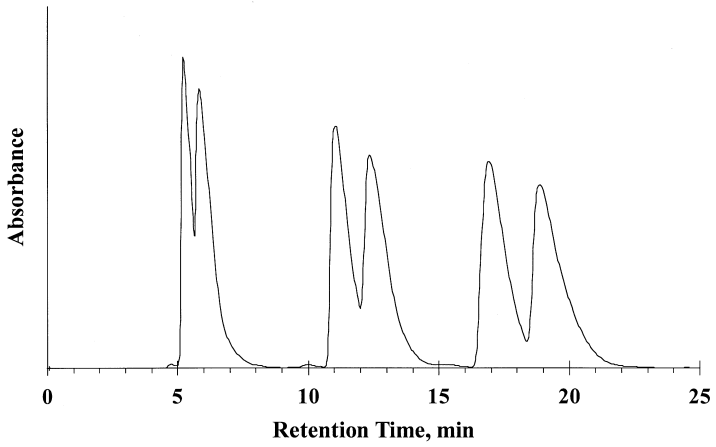

Fig. 2. Closed-loop recycling chromatogram. Sample, methyl and propyl *p*-hydroxybenzoates, 30 mg each/ml, dissolved in methanol–water (80:20, v/v). Injection volume, 5.0 ml, containing 150 mg of each component. Mobile phase, methanol–water (90:10, v/v). Flow-rate, 20 ml/min.

SMB inherently a binary separation process. The central part of the profile must be a mixture of components because it is near the injection point. When the conditions are optimized, purified components can be collected from either end of the profile. In the multicomponent case, only one component can be collected in pure form in one of the product streams; all of the other components are collected as a mixture in the other product stream. However, if only two components are present in the feed (as in chiral separations and as depicted schematically in Fig. 3), it is theoretically possible to collect both components in a pure form [9].

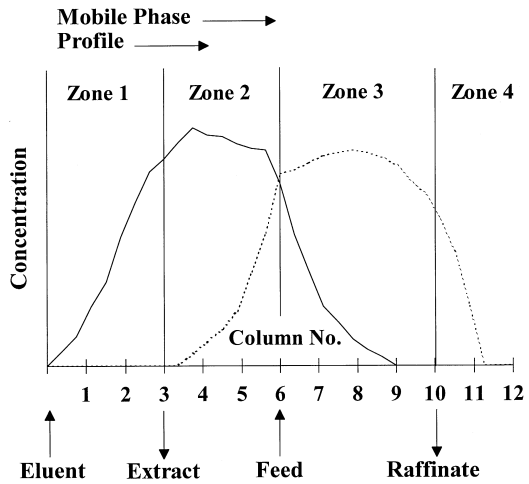

Fig. 3. Schematic representation of an SMB chromatographic profile.

1.3. The CLRPIPI process

In the CLRPIPI process, closed-loop recycling is transformed into a binary separation technique that is similar to SMB. Fresh sample is injected into the interior of the circulating chromatographic profile, and fractions are collected from the leading and trailing edges of the profile during each cycle. At steady state, the total mass of components collected in the fractions from each cycle will equal the total mass injected, i.e., a mass balance will develop. Again, the CLRPIPI process is similar to SMB in that sample is injected into the interior of the profile. It is different from SMB in that the process, although repetitive, is not continuous.

2. CLRPIPI operating principles

All events, such as valve switching and turning pumps off and on, are initiated by the control software. There are two methods that can be used to control the timing of these events:

Method 1: Absolute time. For example, event 1

might occur at 0.0 min in the cycle; event 2, at 0.10 min in the cycle; event 6, at 3.50 min in the cycle, etc.

Method 2: Relative time. With this method, event times are referenced to the detection of the chromatographic profile's leading edge. Thus, a time window is set in which the control software will look for some indicator of the profile's leading edge, such as a specific value of the ascending slope or the absolute value of the detector output. The detection of the leading edge of the profile in essence starts a clock. All collection and injection events then occur at specific times after the detection of the profile's leading edge. For example, a relative time of 0.0 min for the collection of Fraction 1 means that the collection of Fraction 1 begins simultaneously with the detection of the profile's leading edge; a relative time of 0.90 min for the injection of fresh sample means that at 0.90 min after detection of the profile's leading edge, the injection valve is switched so that fresh sample is injected into the interior of the profile, etc.

Method 2 (the relative time method) treats the circulating profile as an object. As soon as the object is detected, the important work of collection and injection begins. It is assumed that the profile will always be the same size and shape; thus, the collection and injection events can be safely referenced to the detection of its leading edge. The advantage of using the relative time method is that, if the profile drifts for any reason (such as temperature fluctuations), the collection and injection events will still occur in the correct parts of the profile.

Fig. 4 shows the schematic diagram of a CLRPIPI system. It is similar to the closed-loop recycling system shown in Figure l, except that an injection valve (IV) and an injection pump (IP) have been added between the detector (D) and the collection valve manifold (CVM). At the beginning of a run, the injection loop (IL) is filled with sample. To accomplish this, the injection valve is placed in the load position, indicated by the dotted-line flow path, and the injection pump is switched on. Sample is thus drawn from the sample reservoir (SR) and pumped into the injection loop. When the injection loop is filled, the injection pump is switched off. Mobile phase previously in the loop and any excess

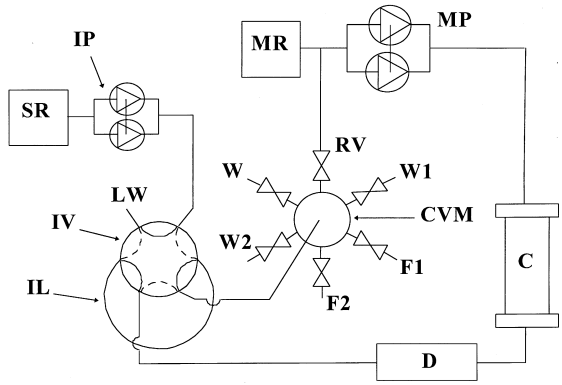

Fig. 4. Schematic diagram of a CLRPIPI system. MR, mobile phase reservoir; MP, mobile phase pump; C, column; D, detector; CVM, collection valve manifold; W1, Waste 1 fraction collection valve; F1, Fraction 1 collection valve; F2, Fraction 2 collection valve; W2, Waste 2 collection valve; W, waste valve; RV, recycle valve; SR, sample reservoir; IP, injection pump; IV, injection valve; IL, injection loop; LW, injection valve waste port.

sample go to waste through the waste port (LW). During this initial filling of the injection loop, the mobile phase pump (MP) draws mobile phase from the mobile phase reservoir (MR). The effluent from the column and detector follows the dotted-line flow path through the injection valve and goes to waste through the waste valve (W).

The initial injection of sample is accomplished as follows: Simultaneously, the injection valve is placed in the inject position indicated by the solid-line flow path in Fig. 4, the recycle valve is opened, and the waste valve is closed. The mobile phase circulates in a clockwise direction around the system, as depicted in Fig. 4. The sample in the injection loop is therefore conducted through the recycle valve, through the mobile phase pump and onto the column. When all of the sample originally in the injection loop has been pumped onto the column, the initial injection is complete. The repetitive phase of the process now begins. The following sequence of events occurs for each cycle:

Event 1: (Injection valve to load position, column effluent to waste). When all of the chromatographic profile is on the column, i.e. after the entire profile has been conducted onto the column and before any of the profile elutes from the column, the injection valve is switched to the load position, indicated by

the dotted-line flow path in Fig. 4; the Waste 1 valve (Wl) is opened and any of the other valves (RV, F1, F2, W2, W) that may have been opened are closed. The effluent from the column and detector follows the dotted-line flow path through the injection valve and goes to waste through valve W1. This waste collection step prevents any circulating impurities from contaminating future fractions. During this event, the mobile phase pump is switched on and draws fresh mobile phase from the mobile phase reservoir to make up the mobile phase lost through valve W1.

Event 2: (Loading the injection loop). At the appropriate time the injection loop is filled with sample as described above by switching on the sample injection pump (IP) for the required amount of time. During this event, the effluent from the column and detector continues to go to waste through valve W1.

Event 3: (Detection of the profile's leading edge, collection of Fraction 1). As the chromatographic profile elutes from the column, the detector and control software detect the leading edge of the profile. Collection valve F1 is opened to begin collection of Fraction 1, and valve W1 is closed. During collection of Fraction 1, fresh mobile phase is drawn from the mobile phase reservoir by the mobile phase pump to make up the mobile phase lost during collection of Fraction 1.

Event 4: (End collection of Fraction 1). At the appropriate time, valve F1 closes and the recycle valve (RV) opens. This stops the collection of Fraction 1 and directs the rest of the chromatographic profile through the mobile phase pump and onto the column. No mobile phase is drawn from the mobile phase reservoir by the mobile phase pump when the recycle valve is opened, because no mobile phase is lost through any of the closed valves (W1, F1, F2, W2, W).

Event 5: (Injection of sample). At the appropriate time, as the central part of the chromatographic profile elutes from the column, the injection valve (IV) is switched to the inject position, indicated by the solid-line flow path in Fig. 4. Thus, effluent from the column and detector is diverted through the injection loop (IL), and fresh sample is injected into the interior of the chromatographic profile at the appropriate point in the profile. The profile proceeds through the recycle valve (RV), through the mobile phase pump and onto the column.

Event 6: (Collection of Fraction 2). As the chromatographic profile continues to elute from the column and detector, collection valve F2 is opened at the appropriate time to collect Fraction 2 from the trailing edge of the profile. During collection of Fraction 2, fresh mobile phase is drawn from the mobile phase reservoir by the mobile phase pump to make up for the mobile phase lost during collection of Fraction 2. The chromatographic profile that was recycled onto the column in Events 4 and 5 continues to travel down the column under the influence of the mobile phase pump.

Event 7: (Collection of Waste 2). At the appropriate time, valve F2 closes (ending the collection of Fraction 2), and collection valve W2 opens (beginning the collection of Waste 2). This event occurs after most of the trailing edge has been collected as Fraction 2, i.e., this event occurs in the 'tail' of the profile when the profile has returned almost to baseline, as measured by the detector. Thus, this event can be thought of as the collection of a very dilute fraction or of a waste fraction. We shall consider it to be a waste fraction and shall refer to it as Waste 2. The collection of Waste 2 continues until the end of the cycle so that all of the 'tail' of the profile is collected. The chromatographic profile that was recycled onto the column in Events 4 and 5 continues to travel down the column under the influence of the mobile phase pump.

Event 8: (Switching the mobile phase pump off. This event is used only if the relative time method of controlling collection and injection events is used). Event 8 is a novel method of providing extremely reproducible cycles. The cycle time chosen for the process establishes a window in which each cycle occurs. If the selected cycle time is exactly equal to the true cycle time (the time needed for the profile to complete one circuit), the profile will be stable in this window in each succeeding cycle and will not drift to earlier or later times. If the selected cycle

time is greater than the true cycle time, the profile will drift to earlier times in each succeeding cycle. If the selected cycle time is less than the true cycle time, the profile will drift to later times in each succeeding cycle. In using Event 8, the mobile phase pump is switched off near the end of Event 7 (collection of Waste 2) and remains off for the rest of the cycle. Event 8 is a relative time event, that is, Event 8 occurs at a set time after the detection of the profile's leading edge. A cycle time is chosen that is slightly longer than the true cycle time of the profile. The profile will then tend to drift to earlier times in the next cycle. However, this will cause the mobile phase pump to be off for a longer time during Event 8 in the next cycle, thus decreasing the average velocity of the circulating profile. If the profile slows down too much, the profile will tend to drift to later times in the next cycle. This will cause the mobile phase pump to be off for a shorter time during Event 8, thus increasing the average velocity of the circulating profile. Thus, the circulation time of the profile is forced to oscillate slightly about the chosen cycle time. This results in a very reproducible average circulation time for the chromatographic profile; thus, the process is very reproducible from cycle to cycle. This technique also provides an automatic compensation for fluctuations in the average circulation time of the chromatographic profile due to minor variances in temperature, mobile phase strength, pumping speed, etc.

The cycle ends with the completion of Event 7 or 8 (if Event 8 is not used, the absolute time of each control and collection event must be entered into the control software). The sequence of events begins again with Event 1, and the process is repeated multiple times. Eventually, a mass balance develops, i.e., the amount of each component collected in the fractions is equal to the amount of each component injected. The chromatogram then has the appearance of a steady state process, i.e., the shape and size of the chromatogram, as measured by the detector, are virtually identical for each cycle (see Fig. 5). This state could be accurately described as a periodic steady state: The profile expands in size as it travels around the circuit and during injection, whereas the profile contracts during fraction collection. For reasons of brevity, we shall henceforth refer to this state as a steady state. Once the process has reached steady state, the process can be repeated as many times as necessary until the desired amount of sample has been separated.

3. Experimental

3.1. Apparatus

All experiments were performed on a prototype one-column CycloJet™ 140 system (EM Industries, Hawthorne, NY, USA). The mobile phase pump was an ST 140 preparative HPLC pump from EM Industries. Proprietary TurboPrep® software from EM Industries was used to control all pumps and valves. The software ran on a 486 computer from Dell (Austin, TX, USA). The computer was connected to the system by way of an interface from Opto 22 (Temecula, CA, USA). The injection pump was an Eldex model B-100-S-4 metering pump (Eldex Labs., San Carlos, CA, USA). The flow-rate for this pump was set manually with a micrometer.

The six-port injection valve and its air-powered actuator were obtained from Rheodyne (Cotati, CA, USA). The fraction collection valves and the recycle valve were obtained from Mace (Upland, CA, USA). All valves were air-actuated and were controlled via software through the Opto 22 interface.

The injection loop was made of 1/16 in. O.D. polyether ether ketone (PEEK) tubing and had a volume of 5.0 ml (1 in.=2.54 cm). The tubing between the recycle valve and the mobile phase pump was 1/8 in. O.D. PTFE tubing. All other tubing was 1/16 in. O.D. stainless steel tubing.

The detector was obtained from Knauer (Berlin, Germany). It was a variable-wavelength UV HPLC detector equipped with a high-pressure flow cell. For these experiments, the wavelength was set at 295 nm.

The column was obtained from and packed by MODcol (St. Louis, MO, USA). The dimensions of the column were 250×25 mm I.D. The column was packed with approximately 80 g of LiChrospher C_{18}, particle size 12 μm, average pore diameter 100 Å (Merck, Darmstadt, Germany).

3.2. Control of collection and injection events

In the experiments reported here, the relative time

method (Method 2 in Section 2) was used to determine the initiation times of Events 3–8. To detect the leading edge of the profile, a time window was set in which the control software looked for an ascending slope of 20% of the maximum detector signal per minute.

3.3. Chemicals

The mobile phase was methanol–water (90:10, v/v). Both the methanol and the water were of HPLC grade and were obtained from EM Science (Gibbstown, NJ, USA).

The mixture undergoing separation was a solution of methyl and propyl *p*-hydroxybenzoates. The methyl and propyl *p*-hydroxybenzoates were obtained from Sigma–Aldrich (St. Louis, MO, USA). The sample solution was made by dissolving the methyl and propyl *p*-hydroxybenzoate in methanol–water (80:20, v/v). The final concentration of each component was 30 mg/ml. The methyl *p*-hydroxybenzoate was less retained and eluted first, with the propyl *p*-hydroxybenzoate being eluted second.

3.4. Conditions

The nominal flow-rate of the mobile phase pump was set via the software at 20 ml/min.

The flow-rate of the Eldex injection pump was set at 20 ml/min using the micrometer. Each filling of the loop lasted for 0.25 min, delivering a volume of 5.0 ml to the loop for each cycle. Thus, 150 mg each of methyl and propyl *p*-hydroxybenzoate were delivered to the loop and injected for each cycle. All experiments were run at room temperature. Neither the columns nor the mobile phase were thermostated.

3.5. Analysis of fractions

The fractions were analyzed by analytical HPLC. The pump was an Hitachi L-6000 pump. The mobile phase was methanol–water (60:40, v/v) and the flow-rate was 2.0 ml/min. The column (125× 4 mm I.D.) was packed with Lichrospher RP18 (5 μm particle size, 100 Å pore size) and was obtained from Merck. A Rheodyne six-way valve (model 7000L) equipped with a 20-μl loop was used as the injection valve. The detector was a variable wavelength UV detector from Knauer. The wavelength was set at 285 nm for each analysis.

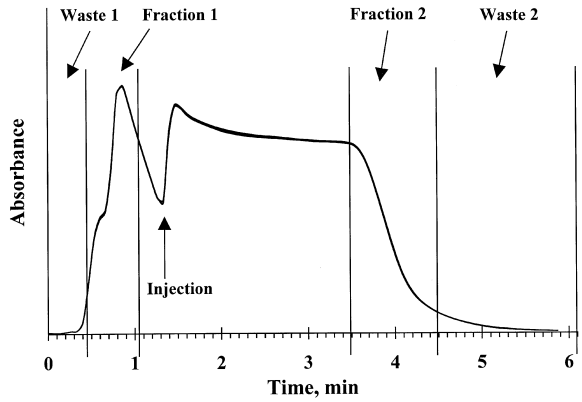

Fig. 5. Final CLRPIPI run. Superimposed CLRPIPI chromatograms for cycles 36–41. Sample, methyl and propyl *p*-hydroxybenzoates, 30 mg each/ml, dissolved in methanol–water (80:20, v/v). Injection volume, 5.0 ml, containing 150 mg of each component. Mobile phase, methanol–water (90:10, v/v). Flow-rate, 20 ml/min. Cycle time, 6.1 min. Event times are shown in Table 1.

The methyl and propyl *p*-hydroxybenzoate peaks were integrated using a Hewlett-Packard 3393A integrator (Hewlett-Packard, Palo Alto, CA, USA). The purities were expressed as area percents. At least three analyses were performed on each fraction, each purity was expressed as an average, and the uncertainties were determined in the 95% confidence interval.

4. Results and discussion

Fig. 5 shows the superimposed chromatograms of cycles 36–41 of a 42-cycle run. The times of all events are shown in Table 1. The cycle time was 6.1 min. Each event was repeated every 6.1 min. For example, 5.0 ml of the sample solution were injected into the interior of the circulating profile every 6.1 min.

Note that, in Fig. 5, the chromatograms from each cycle are virtually identical, indicating that the system is at steady state; 150 mg each of methyl and propyl *p*-hydroxybenzoate were injected for each cycle, and 150 mg of each compound were collected. Of course, this does not imply that the compounds were collected in pure form in each fraction. Rather,

Table 1
Event times for the CLRPIPI process shown in Fig. 5

Event	Description	Time (min)	
		Absolute	Relative
1	Inject valve to load position, valve W opened	0.0	–
2	Load injection loop	0.10	–
3	Collection of Fraction 1, valve F1 opened	0.45	0.0
4	End collection of Fraction 1	1.05	0.60
5	Injection of sample	1.35	0.90
6	Collection of Fraction 2, valve F2 opened	3.75[a]	3.30[a]
7	Collection of Waste 2, valve W2 opened	4.75[a]	4.30[a]
8	Mobile phase pump switched off	5.95	5.50

[a] These are the values entered into the TurboPrep control program. To determine the actual cut points of Fraction 2 and Waste 2 on the chromatogram, subtract 0.25 min. This correction is necessary because, for these events, the volume of the injection loop (5.0 ml) separates the detector and the collection valves. At a flow-rate of 20 ml/min, this represents a lag time of 0.25 min between passage of the cut point through the detector and its arrival at the collection valve manifold.

all one can tell from the identical appearance of the chromatograms is that the total amount of each compound collected was equal to the total injected. However, because methyl *p*-hydroxybenzoate is less retained than propyl *p*-hydroxybenzoate, one would expect that Fraction 1 would be enriched in methyl *p*-hydroxybenzoate, and that Fraction 2 would be enriched in propyl *p*-hydroxybenzoate.

Several cycles were needed for steady state to develop. This is illustrated in Fig. 6 where the superimposed chromatograms of cycles 3, 4, 7, 10, and 41 are shown. Judging by the shapes of each chromatogram, the early part of the profile (0–1 min in Fig. 6) appears to have reached steady state by cycle 3. The rest of the profile, however, took considerably longer to reach steady state. It is estimated that approximately eighteen cycles were needed for the entire profile to reach steady state.

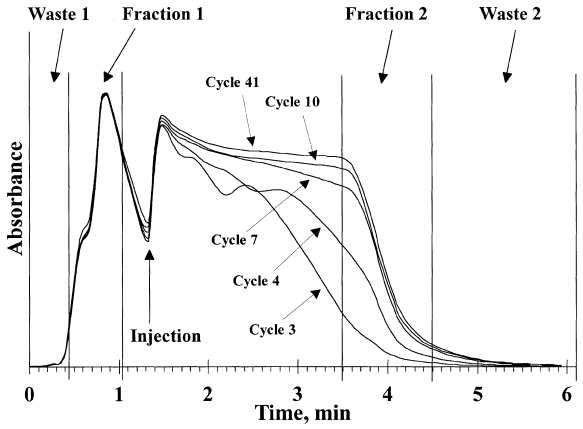

Fig. 6. Final CLRPIPI run. Superimposed CLRPIPI chromatograms for cycles 3, 4, 7, 10, 41, illustrating the evolution towards steady state. Conditions are the same as for Fig. 5.

Fractions 1 and 2 were analyzed by analytical HPLC, as described in Section 3. The analytical results for Fraction 1 are shown in Fig. 7. Four bottles were used to collect Fraction 1. The first bottle was used to collect Fraction 1 from cycles 1–11; the second bottle, cycles 12–22; the third bottle, cycles 23–32 and the fourth bottle, cycles 33–42. The purity of Fraction 1, expressed as area percent, was very high, being 99.8% within experimental error for all cycles.

The analytical results for Fraction 2 are shown in Fig. 8. Six bottles were used to collect Fraction 2. The first bottle was used to collect Fraction 2 from cycles 1–7; the second bottle, cycles 8–14; the third bottle, cycles 15–21; the fourth bottle, cycles 22–28; the fifth bottle, cycles 29–35 and the sixth bottle, cycles 36–42. The purity of Fraction 2, expressed as area percent, was about 98.6% for cycles 1–7. The purity of Fraction 2 from all subsequent cycles (cycles 8–42) was greater than 99.6 area.% within experimental error. Thus, the steady state purity (cycles 18–42) of Fraction 2 is very high, greater than 99.6 area.% within experimental error. Each purity value in Figs. 7 and 8 is an average of at least three measurements, and each uncertainty is expressed in the 95% confidence interval.

Event 8, the turning off of the mobile phase pump near the end of the Waste 2 fraction, was used in this

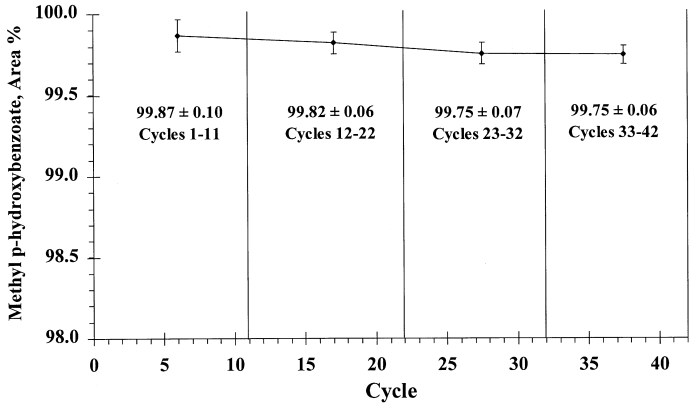

Fig. 7. Final CLRPIPI run, analysis of first fraction.

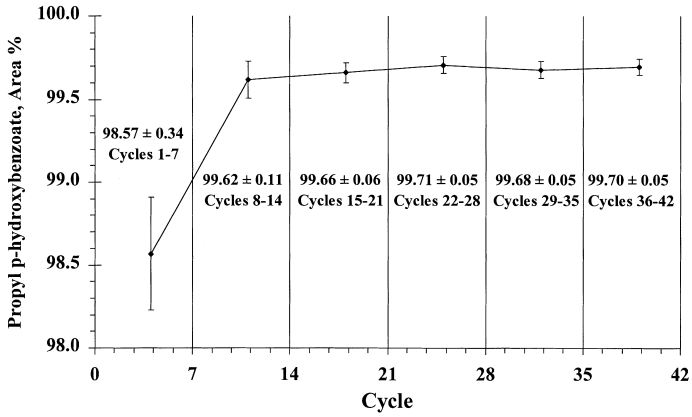

Fig. 8. Final CLRPIPI run, analysis of second fraction.

run. Table 2 illustrates the excellent reproducibility of the occurrence of the leading edge of the chromatographic profile for each cycle when Event 8 is used. The detection of the leading edge varied from 26 to 28 s over the course of the 42 cycles, with the most probable value being 27 s. This resulted in

Table 2
Detection times of the profile's leading edge

Cycle	Time (s)	Cycle	Time (s)	Cycle	Time (s)	Cycle	Time (s)
3	27	13	27	23	27	33	28
4	27	14	27	24	27	34	28
5	27	15	27	25	27	35	27
6	27	16	27	26	27	36	27
7	27	17	27	27	27	37	27
8	27	18	27	28	27	38	27
9	27	19	27	29	27	39	27
10	27	20	28	30	28	40	27
11	26	21	28	31	28	41	27
12	27	22	27	32	28	42	27

excellent consistency of all collection and injection event times from cycle to cycle, and in the excellent reproducibility of the chromatograms shown in Fig. 5.

In the CLRPIPI process, fresh sample is injected periodically (but not continuously) into the interior of the circulating chromatographic profile. As with SMB, fractions are collected at either end of the profile. It is, therefore, a binary separation technique. Although more than two fractions can easily be collected, the process is still binary because the fractions are taken only from the front and rear portions of the profile.

By analogy to SMB, there should be some advantages, relative to conventional and closed-loop recycling batch chromatography, of injecting into the interior of the profile using the CLRPIPI technique. Among these are the following:

1. Only a small amount of resolution of the peaks is necessary. Thus, relatively inexpensive stationary phases could be used.
2. Because high resolution is not needed, high flowrates can be used. This could significantly increase productivity.
3. Because high resolution is not needed, strong mobile phases can be used. This could also significantly increase productivity.
4. Injections occur much more often than with conventional techniques. This has the potential for increasing productivity.

5. Conclusions

Using a simple two-component sample, the feasibility of the CLRPIPI process has been demonstrated. The CLRPIPI process reaches a steady state, as shown in Fig. 5. The process can be made to produce fractions of high purity, as shown in Figs. 7 and 8. Experimental and theoretical work on real-world separations (especially chiral) needs to be done now to determine the usefulness of this technique in large scale separations. Specifically, how does the CLRPIPI process compare to batch HPLC and to SMB as regards production rates, solvent usage and labor costs?

Appendix A

Method development

Methods development for the CLRPIPI method is easy and intuitive, as will be illustrated here for the method shown in Fig. 5. Refer again to Fig. 2, the simple recycling experiment and recall that 150 mg each of methyl and propyl *p*-hydroxybenzoate were injected. Each peak traverses the column at a constant velocity; thus, each peak makes one circuit in its own characteristic cycle time. The midpoint between the peaks also travels at a constant velocity. (In fact the midpoint velocity equals the average velocity of the two peaks). It turns out that the cycle time with which the midpoint makes a circuit will be close to the cycle time of the CLRPIPI process and will therefore be a good initial guess for the CLRPIPI cycle time. Table 3 shows the results of the recycling experiment and gives the initial estimate of the CLRPIPI cycle time as 6.2 min.

The next step is to get an estimate of the size of the chromatographic profile that will develop in the CLRPIPI process. Since we begin collecting significantly sized fractions from the front and back of the profile in the second cycle, our initial estimate is that the size of the CLRPIPI profile will be similar to the second cycle in Fig. 2.

Finally, we must estimate where on the profile the various collection and injection events should occur. We thus need an estimate of the concentration profile of each component. In the CLRPIPI process, during the first cycle, 150 mg of each component is injected near the midpoint of the two peaks. This second injected sample will broaden and separate as it

Table 3
Estimation of the cycle time using closed-loop recycling results (see Fig. 2)

Cycle	Retention time (min)			Midpoint cycle time (min)
	Methyl[a]	Propyl[b]	Midpoint	
1	5.13	5.72	5.43	–
2	10.97	12.27	11.62	6.19
3	16.84	18.79	17.82	6.20

[a]Methyl *p*-hydroxybenzoate peak.
[b]Propyl *p*-hydroxybenzoate peak.

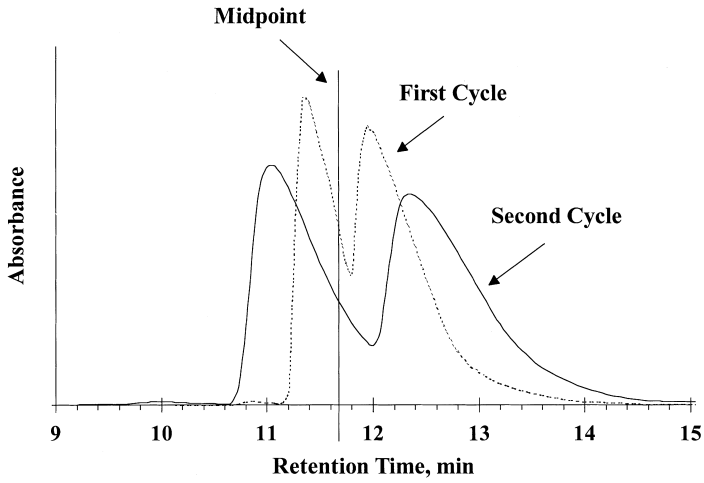

Fig. 9. Method development. Superimposition of first cycle's profile onto the second cycle's profile from Fig. 2.

traverses the column. When it elutes from the column, it will look like the first cycle's profile in Fig. 2, except that it will be physically in the middle of the second cycle profile of the sample that was initially injected. This can be simulated by using a spreadsheet to move the first cycle's profile in Fig. 2 to the right by 6.2 min, so that the midpoints of the first and second cycles' profiles coincide. This is shown in Fig. 9.

Using the retention times shown in Fig. 9, we would estimate that we would start collecting the first fraction near 10.9 min (0.0 min in relative time), end collection of Fraction 1 near the top of the second methyl peak, near 11.3 min (0.40 min in relative time), and inject at the midpoint, near 11.6 min (0.7 min in relative time). The methyl *p*-hydroxybenzoate peaks appear to be tailing significantly into the propyl *p*-hydroxybenzoate peaks. Thus, we will start collecting Fraction 2 near 14.0 min (3.1 min in relative time) and start collecting

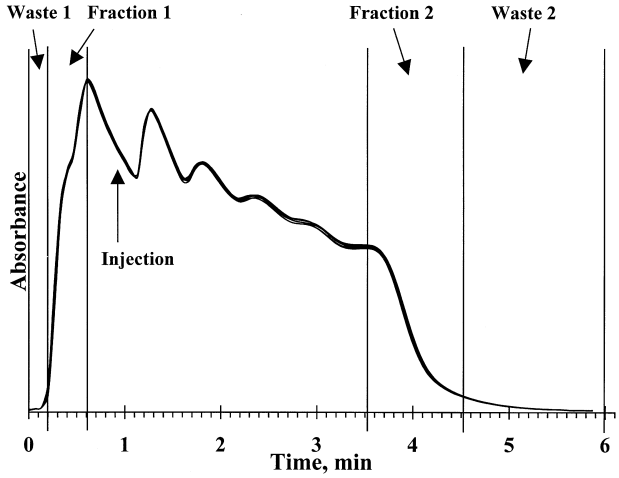

Fig. 10. Methods development CLRPIPI run. Superimposed CLRPIPI chromatograms for cycles 30–35. Conditions were the same as those for Fig. 5. Cycle time, 6.0 min. Event times are given in the text of Appendix A.

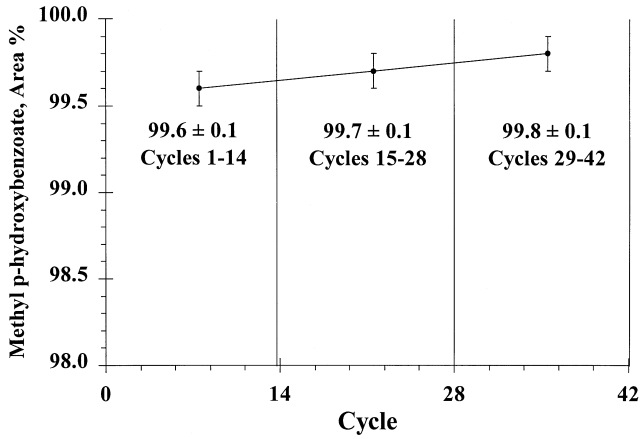

Fig. 11. Methods development CLRPIPI run, analysis of first fraction.

Waste 2 near 15.0 min (4.1 min in relative time). To cut Fraction 2 and Waste 2 at the correct points on the profile, allowance must be made for the volume of the injection loop that separates the detector and collection valves (the injection valve is in the inject position during these two events). The injection loop has a volume of 5.0 ml and, at a flow-rate of 20 ml/min, this represents a lag of 0.25 min between the time the cut point passes the detector and the time it reaches the collection valve. Therefore, in order to start the collection of Fraction 2 at 3.1 min (relative time) on the profile, 3.35 min (relative time) must actually be entered into the program to compensate for the 0.25 min lag. Similarly, 4.35 min (relative time) must be entered into the program to effect a cut time of 4.1 min (relative time) on the profile for Waste 2. Finally, for Event 8, a relative time of 5.5 min was used.

These event times where used in the initial CLRPIPI method and the results are shown in Fig. 10 for cycles 30–35. Given the excellent reproducibility of these chromatograms, a steady state appears to have developed.

Fractions 1 and 2 were analyzed as described in Section 3 and the results are shown in Fig. 11 for Fraction 1 and in Fig. 12 for Fraction 2. The purity of Fraction 1, expressed as area percent, was very high, being 99.7% within experimental error for all cycles.

The purity of Fraction 2 in the early cycles (1–7)

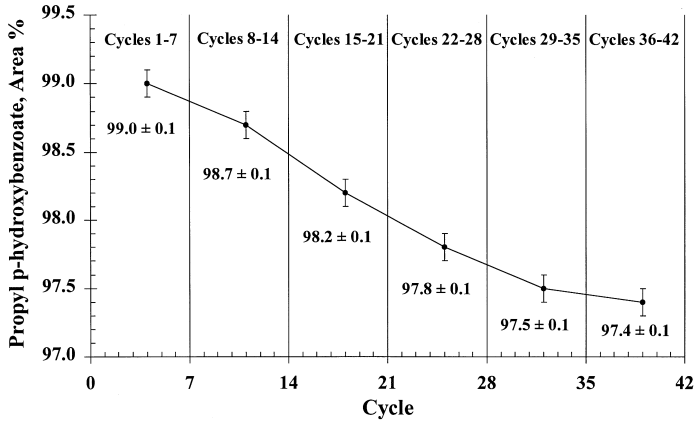

Fig. 12. Methods development CLRPIPI run, analysis of second fraction.

was high, at 99.0%. However, the purity of Fraction 2 decreased with cycle number until cycles 36–42, where it had reached an average value of 97.4 area.%. (Again all purity values in Figs. 11 and 12 are averages of at least three measurements, and the uncertainties are expressed in the 95% confidence interval).

Based on the data in Fig. 12, the process appears to have reached steady state near cycle 36. However, Fraction 2 was not pure. Although 150 mg of methyl *p*-hydroxybenzoate was collected for each cycle, some of it was collected in Fraction 2.

To increase the purity of Fraction 2, more methyl *p*-hydroxybenzoate must be collected in Fraction 1. This would also increase the recovery of methyl *p*-hydroxybenzoate in Fraction 1. To accomplish this, the collection time of Fraction 1 was increased from 0.4 to 0.6 min. This allowed more methyl *p*-hydroxybenzoate to be collected in Fraction 1. The injection point was moved to the right by a distance of 0.2 to 0.9 min (relative time). This allowed more room for the resolution of the methyl *p*-hydroxybenzoate component to develop, thus making it easier to collect more methyl *p*-hydroxybenzoate in Fraction 1. This strategy was successful as the results of Figs. 5, 7 and 8 demonstrate.

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