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Flip–flop elution concept in preparative liquid chromatography

HENRI COLIN* and PIERRE HILAIREAU
Prochrom, BP 9, 54250 Champigneulles (France)
and
MICHEL MARTIN
Ecole Supérieure de Physique et Chimie Industrielles, Laboratoire de Physique et Mécanique des Milieux Hétérogènes (URA CNRS 857), 10 Rue Vauquelin, 75231 Paris Cedex 05 (France)

ABSTRACT

A new concept is developed for improving the cycle time and the sample throughput in preparative liquid chromatography. In this flip–flop elution procedure, the direction of the mobile phase flow is reversed just after the compound of interest has been collected. The next sample injection is made at the other end of the column to the previous injection as the flow direction has been changed after a certain delay time. This time is determined in such a way that the compound of interest begins to elute (and can be collected) just after the end of the backflush peak of strongly retained components from the previous injection. In this way, no time is lost in separating and eluting the most strongly retained impurities. General expressions for the delay time and the cycle time (time between two consecutive injections) are given for diluted (Gaussian) as well as severely overloaded peaks. Similar expressions are also given for an optimized version of the normal elution procedure in which the direction of the flow of mobile phase remains unchanged. It is shown that the flip–flop operation gives a shorter cycle time than the optimized normal elution process when the time of the end of collection of the compound of interest is less than half the retention time of the last compound in the chromatogram. An experimental demonstration of the flip–flop elution concept is shown. It is emphasized that this concept can be extended to other separation techniques and to analytical separations where quantitative information is searched for only a fraction of the number of sample components.

INTRODUCTION

Recent years have seen rapid developments in high-performance preparative liquid chromatography (HPPLC), at both the theoretical and practical levels. HPPLC is now recognized as a powerful tool for industrial purifications, particularly in the pharmaceutical field. Whereas preparative liquid chromatography (PLC) was considered only few years ago to be a low-performance separation method based on the use of low-quality packing materials (large particle size and size distribution), the situation has changed dramatically recently and the present trend is to use high-quality packing materials with an average particle size in the range 10–20 μm and a narrow size distribution [1]. High efficiencies (more than 30 000 plates/m) can now be obtained

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routinely in large-size preparative columns (up to 45 cm I.D.). At the same time, important theoretical developments have occurred and the phenomena associated with intense overloading in non-linear chromatography are much better understood [2–5]. Economical aspects have also received much more attention in the recent past [6–8] and it has been demonstrated that when the purification conditions are properly optimized, the purification cost using HPPLC can be low enough to make this technique usable for products other than high added value materials.

Very high degrees of purity can be obtained by HPPLC. This, however, requires several conditions to be achieved at the same time. First, it is necessary to use high-quality solvents, otherwise solvent impurities would be concentrated in the purified product. Second, it is also necessary to use packing materials that do not release unwanted chemicals. Finally, contamination of collected material(s) by co-elution with the product(s) of interest of impurities coming from a previous injection must be avoided. This is a very important point, and potential users of HPPLC are often concerned by the fact that it is difficult to be sure that the column is clean before a new injection is made. Dealing with very strongly retained (late-eluted) compounds is thus an important issue. Before discussing the problems associated with such compounds, it is necessary to recall that their elution peaks are very broad and difficult to detect. In the best case, they only produce small baseline disturbances that can easily be ascribed to detector instability. It can also happen (not only with strongly retained products) that the detector is not sensitive to a particular compound and then its peak is not visible on the chromatogram.

There are several possibilities for handling late-eluted peaks, as follows. The most effective, but not necessarily the most convenient and economical, way is to unpack the column after the product of interest has been collected and repack the column with fresh material before a new injection is made. Some purifications in the pharmaceutical industry are made this way. It is also possible to continue elution until all the peaks have been eluted. This can require unacceptably large amounts of solvent, however, and, as indicate above, it is difficult (if possible) to know when everything has been eluted. It must be noted that the next injection can be made before the end of the actual run, provided that the peak of interest is not contaminated by co-eluted impurities. This optimized normal elution procedure is discussed later in this paper.

Another alternative is to find solvent conditions such that the compound of interest is the most retained. This is actually more a theoretical than a practical possibility, particularly since the optimization of the solvent conditions in preparative chromatography is primarily aimed at achieving a high selectivity between the peak of interest and its immediate neighbours.

Gradient elution can be used to clean the column after the product of interest has been eluted. It is an expensive approach, however, as it takes time and requires large amounts of solvent, not only for the cleaning step but also to regenerate the column before the next injection is made. At least three to five column volumes of solvent are typically required for this regeneration step. It must also be mentioned that the process of solvent regeneration (this is the best way to decrease HPPLC purification costs) is more complicated and expensive in gradient elution than isocratic elution. Last but not least, the purification process becomes more complicated, which is not desirable for industrial production conditions in which case as simple procedures as possible should be used.

A precolumn can be used to trap strongly retained impurities. This is a good strategy, but with some limitations. First, it requires an additional piece of hardware and thus increases the cost of the equipment. Second, it creates an additional source of band broadening. Third, the volume of the precolumn is necessarily much smaller than that of the column, and the precolumn is usually oversaturated with unpredictable retention effects. Fourth, the precolumn has to be regenerated (or changed) at some point.

Another possibility is to backflush the column. It will be seen in this paper that the flip–flop concept is an optimized backflush operation.

Finally, the flip–flop operation can be used.

THEORY

In order to describe the flip–flop concept, it is assumed that the mixture to be purified contains three compounds: the substance of interest (later called the "main peak") and two impurities, one eluted before and one after the main peak. The principle of flip–flop operation is illustrated in Fig. 1. In a first step the solvent flows from left to right and the first injection is made at the column left end (inlet). Elution proceeds and the first impurity is eluted, followed by the main product. Just after the collection of the main peak is completed, the flow direction is changed, the column inlet now being at the right end. The second impurity then starts to be backflushed. After a certain delay time (it is shown later how to calculate it), a second injection is made. The delay time is such that the end of the elution of the backflush peak from the strongly retained impurity of the previous injection corresponds to the beginning of the elution of the main peak of the actual injection. The weakly retained impurity of the actual injection can possibly interfere with the backflush peak of the strongly retained impurity from the previous injection, but this does not matter. The process is then repeated, the injections being made in turn at each end of the column. This mode of operation provides in some respect an answer to one of the frequently encountered objectives in preparative chromatography: the optimization of the isolation of the component of interest and the non-separation of the uninteresting components which are often impurities. Indeed, as these components are not to be collected, the optimization of the production throughput requires that no time is lost in separating them.

The critical point with the flip–flop operation is the column bed stability. With columns of regular design, it is often recommended to avoid reversing the flow of solvent (the direction must be what it was during the operation of packing the column), otherwise voids could be created and the column efficiency drastically reduced. This is particularly true with columns of large diameter. A solution to this problem is the technique of dynamic axial compression [9]. In addition to the technical aspect of column stability, the use of flip–flop elution requires the calculation of the delay time between the reversal of the flow direction and the next injection.

A typical preparative chromatogram is shown in Fig. 2. The horizontal lines under the chromatogram are an absolute and a reduced time axis. A reduced time is the ratio of an actual time to the dead time (t_0).

The following assumptions are made: (1) one product, the main component, has to be collected; (2) injection can be made in diluted as well as concentration overload

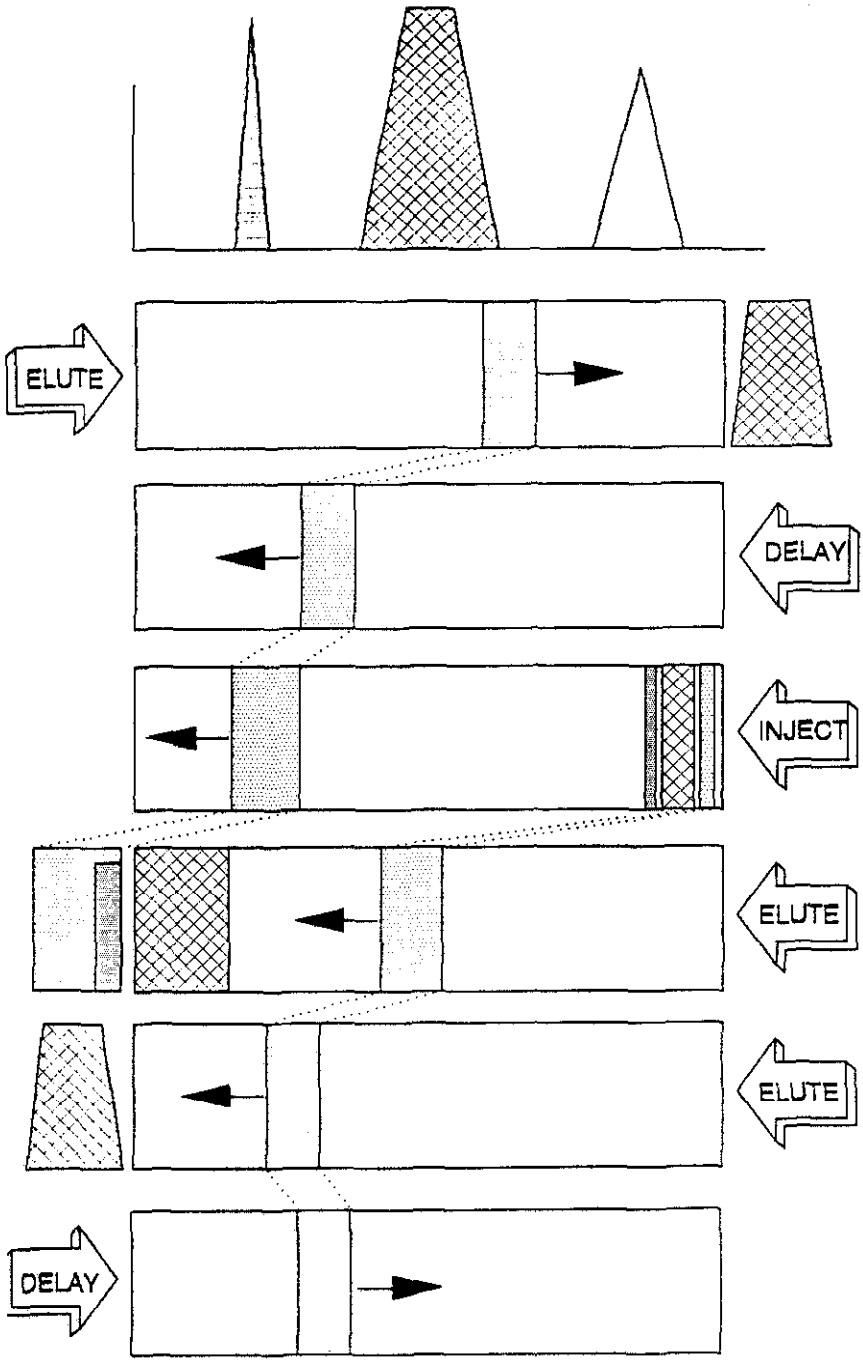

Fig. 1. Principle of flip–flop elution.

conditions; volume overloading can also take place but then in such a way that concentration overload is predominant, as recommended by Knox and Pyper [10]; (3) the possible amount of concentration overloading of the main peak and the start of the collection of this product are determined by the extent of interference with the impurity eluting immediately before this product; (4) whatever the extent of sample overloading, the end of the main peak appears at a constant time equal to the retention time in diluted conditions (*i.e.*, analytical injection) plus column band broadening (expressed in time units); this situation corresponds to a convex isotherm, such as a Langmuir isotherm; (5) no assumpton is made regarding the shape of the main peak (except for point 4).

The reduced times x_i and x_L (see Fig. 2) are given by

$$x_i = t_{Ri}/t_0 = 1 + k'_i \quad (1a)$$

$$x_L = t_{RL}/t_0 = 1 + k'_L \quad (1b)$$

where k' are the capacity factors.

The reduced cycle time is defined as the time between two consecutive injections relative to the time t_0. In flip–flop elution, this reduced time, *RFF*, is defined as

$$RFF = (t_s + DEL)/t_0 \quad (2)$$

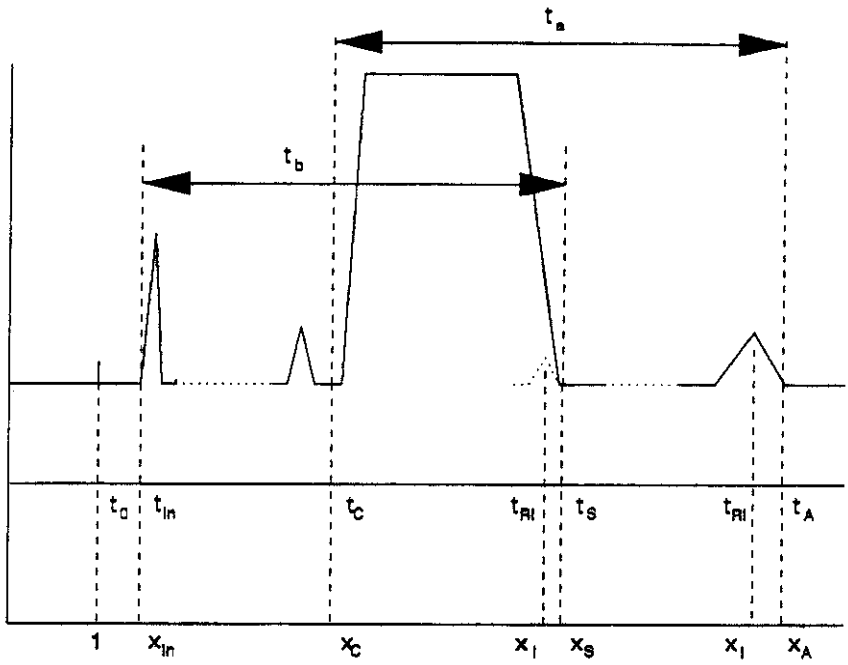

Fig. 2. Typical preparative chromatogram. t_0 = Dead time; t_{in} = retention time of the beginning of the first peak; t_c = time to start collecting the peak of interest; t_{Ri} = retention time of compound i; t_s = time of flow reversal (also end of collection); t_{RL} = retention time of the last compound; t_A = duration of the separation (return to baseline after the last peak). The x_i values correspond to the retention times t_i divided by the dead time t_0.

where t_s is the time elapsed between the injection and the switching of the flow direction and DEL is the delay time. It is recalled that DEL is such that the end of the peak of the most retained impurity from the previous injection (this peak is backflushed and is called in the following the "backflush peak") coincides with the beginning of the collection of the peak of interest in the actual injection.

The time spent in the column by the impurities more retained than the collected component is equal to $2t_s$. In order to calculate the end of the backflush peak, it is necessary to determine the standard deviation of the most retained component (in time units) as it contributes the most to the width of the backflush peak. This can be done using the plate height for this product and writing that this plate height is equal to the variance (in length units) divided by the distance travelled by the product. It is simple to derive the following equation in which the standard deviation (SD) is expressed in time units

$$SD = t_0 \sqrt{2x_L x_s/N} \tag{3}$$

where x_s is equal to t_s/t_0 and N is the plate number corresponding to one full passage through the column by the most retained impurity.

The reduced time corresponding to the end of the backflush peak is then defined as

$$x_{BF} = 2x_s + \lambda_L \sqrt{2x_L x_s/N} \tag{4}$$

where λ_L is a constant related to the peak shape of the last-eluted component and the desired level of purity of the collected peak. λ_L is usually between 2 and 3.

Writing that x_{BF} (associated with the previous injection) is equal to the reduced time of the beginning of the collection of the main peak from the actual injection (*i.e.*, $RFF + x_c$) and combining eqns. 2 and 4, allows the reduced delay time ($RDT = DEL/t_0$) and reduced cycle time to be calculated

$$RDT = x_s - x_c + \lambda_L \sqrt{2x_L x_s/N} \tag{5a}$$

$$RFF = 2x_s - x_c + \lambda_L \sqrt{2x_L x_s/N} \tag{5b}$$

Reduced times x_s, x_L and x_c can be calculated from the capacity factors and appropriate λ values similar to the λ_L parameter introduced in eqn. 4.

In order to evaluate the advantages of flip–flop elution, two cases are examined below. First, it is assumed that the main peak is not significantly overloaded and its shape is close to Gaussian. The total peak width, $x_s - x_c$, is assumed to be four times the standard deviation. Assuming that the column plate number is the same for the main peak and the last peak and taking $\lambda_L = 2$, one can then write eqn. 5a as

$$RDT = [4(1 + k'_i)/\sqrt{N}][1 + \sqrt{0.5(1 + 2/\sqrt{N})(1 + k'_L)/(1 + k'_i)}] \tag{6}$$

Figs. 3 and 4 show how RDT varies with k'_i, k'_L and N. The larger the column efficiency, the smaller is RDT. This is not surprising, as more efficient columns produce narrower peaks. As N is often larger than 2000, it is possible to neglect $2/\sqrt{N}$ compared with 1 in eqn. 6 and then it appears that RDT is inversely proportional to $\sqrt{N}$.

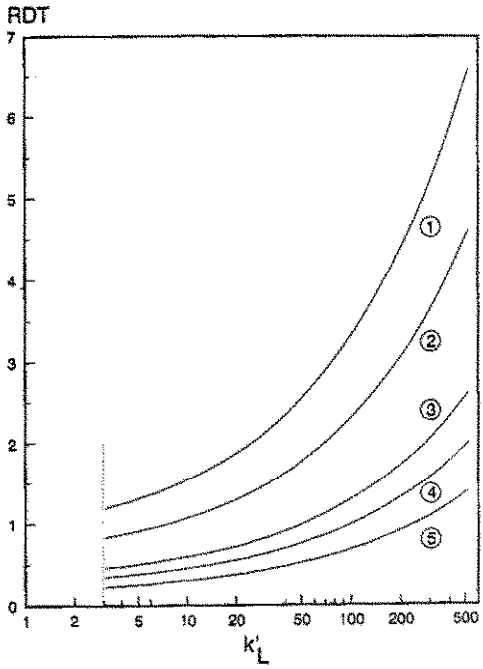

Fig. 3. Effect of the capacity factor of the most retained impurity (k'_L) on the reduced delay time (RDT) for different plate numbers (N). The capacity factor of the main product is assumed to be 3. The main peak is weakly overloaded (see text). N = (1) 500; (2) 1000; (3) 3000; (4) 5000; (5) 10 000.

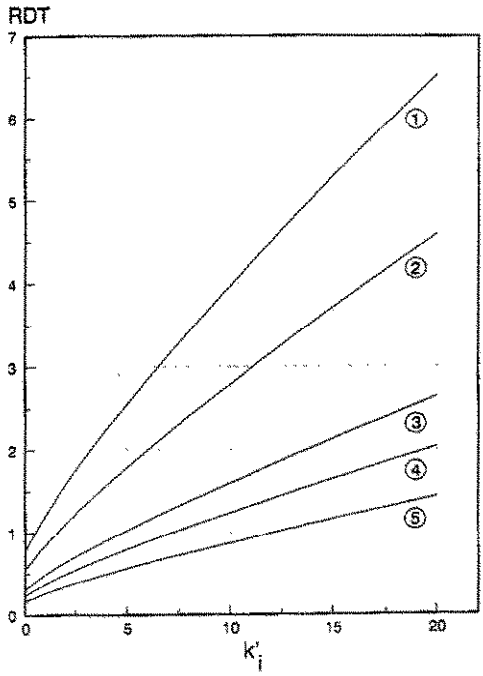

Fig. 4. Effect of the capacity factor of the main product (k'_i) on the reduced delay time (RDT) for different plate numbers (N). The capacity factor of the most retained impurity is assumed to be 20. The main peak is weakly overloaded (see text). N values as in Fig. 3.

For typical values of N and k'_i (3000 and 3, respectively), it can be seen that less than two column volumes of solvent have to be used in order to eliminate a strongly retained impurity with a capacity factor $k'_L = 100$. In such a case, about two times more solvent would be required to do normal backflusing and at least five times more to do gradient elution. If the column efficiency is increased to 5000 plates, then only one column volume of solvent is required to eliminate the same impurity. If the capacity factor of the most strongly retained impurity is more than 100, one can consider that the corresponding compound is almost irreversibly adsorbed (in the particular mobile phase selected) and a guard column can probably be used to eliminate the product.

The effect of k'_i on RDT is shown in Fig. 4 ($k'_L = 20$). The curves indicate that RDT increases almost linearly with k'_i, the rate of increase being smaller with larger column efficiency. When $k'_i = 5$, RDT increases from less than 1 for $N = 5000$ to 2.5 for $N = 500$. The advantage of a large column efficiency is also clearly seen here.

The previous discussion gives an optimistic view of flip–flop elution as it is assumed that the peak of interest is not overloaded. The most pessimistic situation corresponds to $x_c = 1$ (see eqn. 5a). In this case, the collection of the main peak starts immediately at time t_0. In other words, the main peak is so overloaded that its front is moved to the dead time. A real practical situation would be intermediate between this case and the previous one (no peak distorsion). Assuming that $x_c = 1$, eqn. 5a then becomes

$$RDT = k'_i + [2(1 + k'_i)/\sqrt{N}][1 + \sqrt{2(1 + 2/\sqrt{N})(1 + k'_L)/(1 + k'_i)}] \quad (7)$$

The most significant term on the right-hand side of eqn. 7 is k'_i. Because collection of the main peak starts much earlier than before, it is necessary to wait a longer time before the next injection can be made. The effects of k'_i, k'_L and N on RDT are shown in Figs. 5 and 6. The effect of the column efficiency is weaker than before. As shown in Fig. 6, at constant k'_L (= 20), RDT increases linearly with k'_i, at a rate which almost does not depend on the column plate number. To see a significant effect of N, it is necessary to reach large k'_L values (see Fig. 5).

The flip–flop concept described above corresponds to an improvement of the classical backflushing operation. In the latter, the next injection is made when the backflush peak is completely eluted. Then the cycle time in flip–flop operation is lower than in normal backflushing, the difference between the two reduced cycle times being equal to x_c. It is interesting to compare, on a reduced cycle time basis, flip–flop elution with "normal" (one-way) elution. For the comparison to be meaningful, normal elution has to be optimized in order to reduce the cycle time required for the satisfactory collection of the desired product. This can be done by injecting the next sample before the end of the elution of the last component from the previous injection [11]. The reduced cycle time in optimized normal elution, ROE, is then chosen so that the following two conditions are satisfied: (1) the beginning of the collection of the main peak for the actual injection happens simultaneously with (or just after) the end of the last peak from the previous injection (x_A); the corresponding ROE value is T_a (see Fig. 2); (2) the beginning of the first peak (x_{in}) from the actual injection happens simultaneously with (or just after) the end of the main peak from the previous injection; the corresponding ROE value is T_b.

It must be noted that the problem is actually more complex than described above

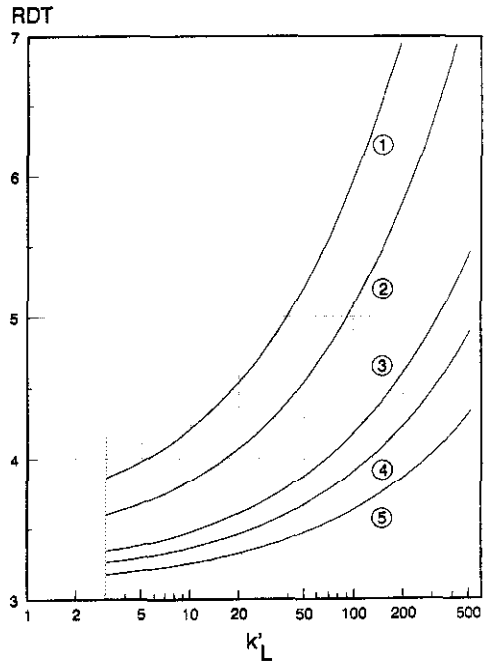

Fig. 5. Effect of the capacity factor of the most retained impurity (k'_t) on the reduced delay time (RDT) for different plate numbers (N). The capacity factor of the main product is assumed to be 3. The main peak is strongly overloaded and begins to elute at the void volume. N values as in Fig. 3.

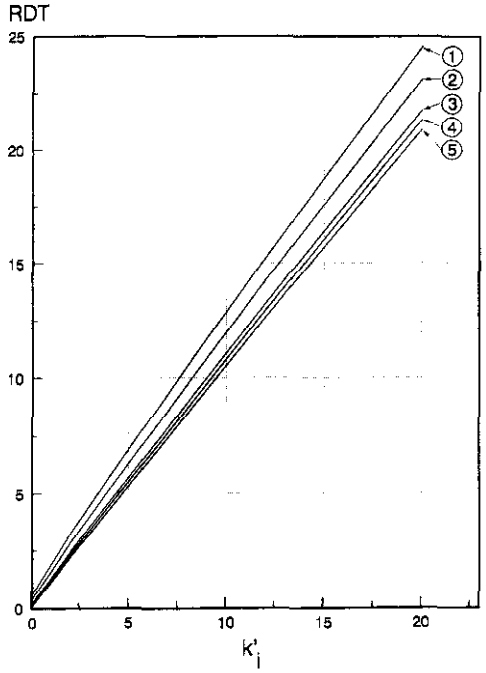

Fig. 6. Effect of the capacity factor of the main product (k'_i) on the reduced delay time (RDT) for different plate numbers (N). The capacity factor of the most retained impurity is assumed to be 20. The main peak is strongly overloaded and begins to elute at the void volume. N values as in Fig. 3.

because, in some particular situations where large gaps between some peaks are present, the injection sequence can be optimized so that the main peak is eluted in a window between adjacent impurities, involving more than two consecutive injections. Such cases are too specific, however, and cannot be included in the frame of a general discussion.

The value of *ROE* to be selected is equal to sup (T_a, T_b). Conditions 1 and 2 described above correspond to

$$T_a + x_c > x_A \tag{8a}$$
$$T_b + x_{in} > x_s \tag{8b}$$

In the following, optimized normal and flip–flop elution are compared. Two situations must be discussed depending on the relative values of T_a and T_b. It is first assumed that T_a is larger than T_b. This is equivalent to

$$x_s < x_A - x_c + x_{in} \tag{9}$$

Flip–flop elution will be better (shorter cycle time) than optimized elution when *RFF* < *ROE*, that is, according to eqns. 5b and 8a, when

$$2x_s - x_c + \lambda_L \sqrt{2x_L x_s/N} < x_A - x_c \tag{10}$$

or when

$$\sqrt{x_s} < 0.5[-\lambda_L \sqrt{x_L/2N} + \sqrt{\lambda_L^2 x_L/2N + 2x_A}] \tag{11}$$

Noting that x_A is equal to $x_L(1 + \lambda_L/\sqrt{N})$, condition 10 becomes after rearrangement

$$x_s < x_L/2 \qquad \text{or} \qquad t_s < t_{R,L}/2 \tag{12}$$

The condition has a very simple form: flip–flop elution is preferable to optimized normal elution when the switch time (end of collection of the main product) is less than half the retention time of the most retained impurity.

It can be shown that if T_b is less than T_a, *RFF* is always larger than *ROE*, and conversely that if x_s is less than $x_L/2$, then T_a is larger than T_b. Accordingly, the condition $x_s < x_L/2$ is necessary and sufficient for *RFF* to be lower than *ROE*.

If, as it has been assumed, the end of the main peak is not modified by overloading, then condition 12 can be rewritten as

$$k_i' < [0.5(1 + k_L')/(1 + \lambda_s/\sqrt{N_i})] - 1 \tag{13}$$

where N_i is the column efficiency for the main peak and λ_s a parameter which depends on the degree of interference accepted with the impurity eluted immediately after the main peak. This parameter is usually between 2 and 3.

Before discussing condition 13, the following comments should be made: the general condition described by relation 12 does not assume that the column efficiency

is the same for all peaks in the sample; in the previous discussion, the peak eluted just before the compound of interest does not play any role and, accordingly, condition 12 is valid even if the beginning of the collection of the main product does not correspond to the end of the peak of impurity eluted just before; and the general treatment developed above indicates that there is no need to make the assumption that only the main peak is overloaded, as other peaks can also be overloaded. Condition 12 is thus very general. It is the same whether the main peak is eluted in overloaded conditions or not.

In order to compare practically flip–flop and optimized elution, it is assumed that λ_s is equal to 2 and the column efficiency is larger than 2000 plates (the term $\lambda_s/\sqrt{N_i}$ can be neglected compared with 1). Under these conditions, relation 13 takes the simple form

$$k'_i < (k'_L - 1)/2 \tag{14}$$

The general conditions expressed by inequalities 12 or 14 for the cases where flip–flop operation is superior to optimized normal elution are in agreement with qualitative expectations. Indeed, backflush operation allows all components dispersed along the column at a given time to be combined in a single peak at one column extremity. Therefore, it is not surprising that flip–flop elution, which is an optimized version of backflushing, is especially useful when the unwanted impurities occupy an important fraction of the column length when the peak of interest is eluted, that is, when the main component elutes in the first part of the chromatogram.

It is interesting to calculate the relative gain in cycle time (relative time gain, *RTG*) when using flip–flop elution compared with optimized normal elution. *RTG* is given by

$$RTG = 1 - (RFF/ROE) \tag{15}$$

RFF is given by eqn. 5b and *ROE* is equal to $T_a = x_A - x_c$ (from eqn. 8a). From eqn. 15, it is simple to derive the ratio of the cycle times (*CTR*) in optimized normal elution to those in flip–flop elution

$$CTR = 1/(1 - RTG) \tag{16}$$

For the sake of simplicity, it is assumed in the following that λ_L is equal to 2 and the main peak is not significantly overloaded. It is then simple to derive the equation giving *RTG*

$$RTG = \frac{K - 2 - (4/\sqrt{N})(1 + \sqrt{K/2})}{K - 1 + 2/\sqrt{N}} \tag{17}$$

where K is defined as

$$K = (1 + 2/\sqrt{N})[(1 + k'_L)/(1 + k'_i)] \tag{18}$$

Some curves are shown in Figs. 7–9. They show the effect of k'_i on *RTG* and *CTR* for different k'_L values, assuming $N = 500$ (Fig. 7), 3000 (Fig. 8) and 10 000 (Fig. 9). The values of *RTG* and *CTR* are close to 0 and 1, respectively, when k'_i is close to $(k'_L - 1)/2$ (see eqn. 15). For larger k'_i values, optimized normal elution is preferable to flip–flop elution. The advantages of flip–flop elution over optimized normal elution clearly appear at large values of k'_L. For instance, when k'_i is 3 and k'_L is 50, the cycle time in flip–flop elution is about nine times shorter than it is in optimized normal elution.

It must be remembered that eqn. 17 and Figs. 7–9 are obtained assuming that the main product is not overloaded and that its width is equal to four times the standard deviation of the peak. In the case of strong overloading conditions for the main product, the advantage of flip–flop operation over optimized normal elution is less than that calculated in eqn. 17 and shown in Figs. 7–9. If RTG_{dil} represents the *RTG* calculated in eqn. 17, it is easy to show that the *RTG* value in overloaded conditions (RTG_{olc}) is given by

$$RTG_{olc} = RTG_{dil} \cdot \frac{x_A - x_i(1 - 2/\sqrt{N})}{x_A - x_c} \tag{19}$$

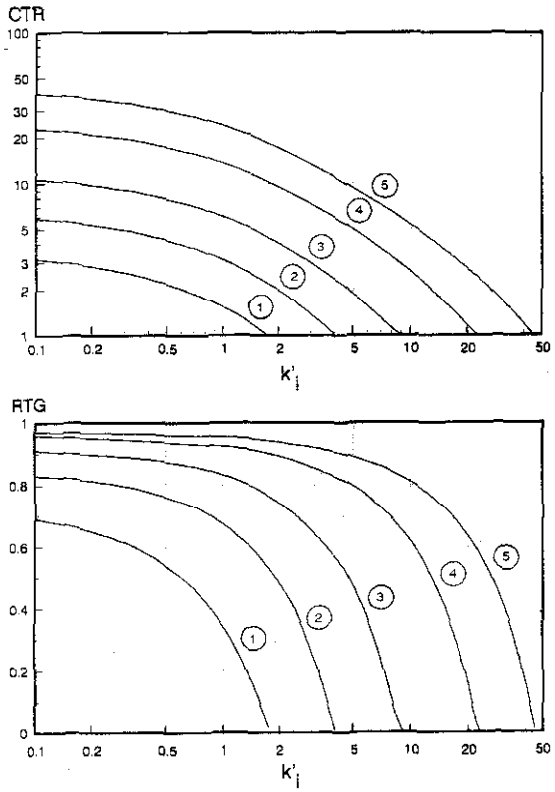

Fig. 7. Effect of the capacity factor of the main product (k'_i) on the reduced time gain (*RTG*) and the cycle time ratio (*CTR*) for different values of the capacity factor of the most strongly retained impurity (k'_L). The number of theoretical plates is $N = 500$. k'_L = (1) 5; (2) 10; (3) 20; (4) 50; (5) 100.

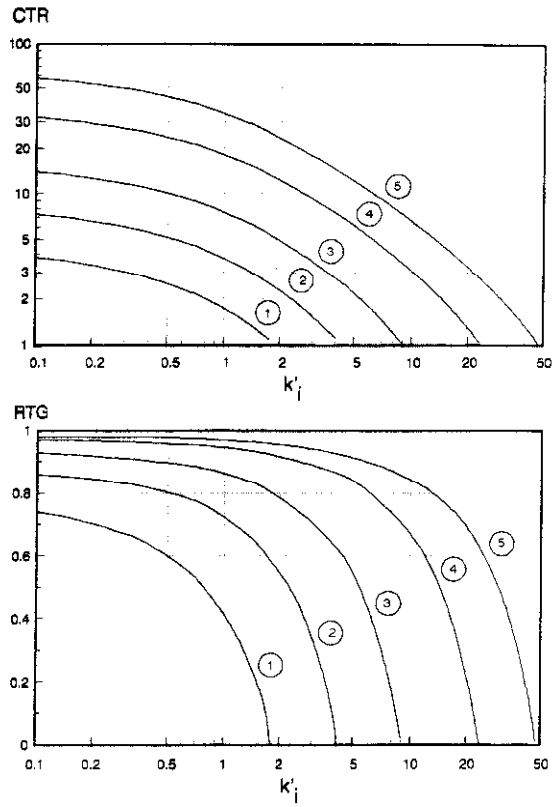

Fig. 8. Same as Fig. 7 but with $N = 3000$.

For instance, in the previous case ($k'_i = 3, k'_L = 50, N = 3000$), if the duration of collection of the main peak ($x_s - x_c$) is five or ten times larger than the width of the diluted peak, the cycle time in flip–flop elution is only 7.9 or 6.6 times shorter than that in optimized normal elution, respectively (instead of 9.4 times for a diluted peak). Nevertheless, this is still a definitive advantage of the flip–flop operation, particularly for medium- to large-scale preparative chromatography where the costs of solvent (proportional to the purification time) and labour are usually the most significant contributions to the cost of purification.

RESULTS AND DISCUSSION

An experimental demonstration of the flip–flop operation is shown in Figs. 10 and 11 for the separation of a natural extract of steroids by reversed-phase chromatography. The experiments were made using an analytical Zorbax C_{18} (10 μm) column (25 × 0.46 cm I.D.) (DuPont, Wilmington, DE, USA). The eluent was acetonitrile–water (50:50, v/v) at a flow-rate of 3 ml/min, with UV detection at 254 nm.

The chromatogram shown in Fig. 10A is for a normal run. The last visible peak is eluted at about 17 min. Actually, some other compounds are likely to be left on the

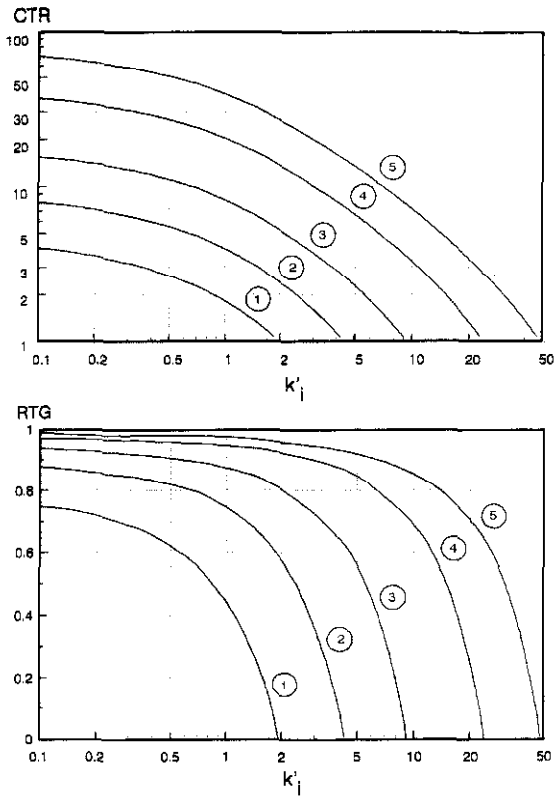

Fig. 9. Same as Fig. 7 but with $N = 10\,000$.

column at that time as continuation of elution over a longer time revealed that the baseline is not perfectly flat and shows some minor disturbances (not detected as peaks by the data processing system). The largest peak (shaded in Fig. 10A) is the compound of interest (about 60% pure based on relative peak areas). It is possible from the chromatogram to calculate the capacity factors of the main product ($k'_i = 3.4$) and the assumed last impurity ($k'_L = 15.8$), and the average column efficiency ($N = 2500$). In order to collect the main peak at the required purity, it is necessary to collect between times 4.0 and 4.8 min. At the latter time, the flow direction is changed and the late impurities are backflushed. This is shown in Fig. 10B and C. Fig. 10B shows the first part of the separation (collection of the main product) and Fig. 10C the backflushing of the late impurities. According to calculations, the end of the backflush peak should appear at 5.2 min (arrow in Fig. 10C) after the reversal of flow. In fact, the time is almost 6.7 min. Several factors can contribute to the difference between the calculated and experimental values. First, it is often observed that the column efficiency decreases with increasing k'. This increases the time when the backflush peak is observed (*e.g.*, see eqn. 3). Another possible explanation is the presence of strongly retained impurities (eluted after 17 min). Although such impurities do not modify the centre of gravity of the backflush peak, they do affect the end of this peak.

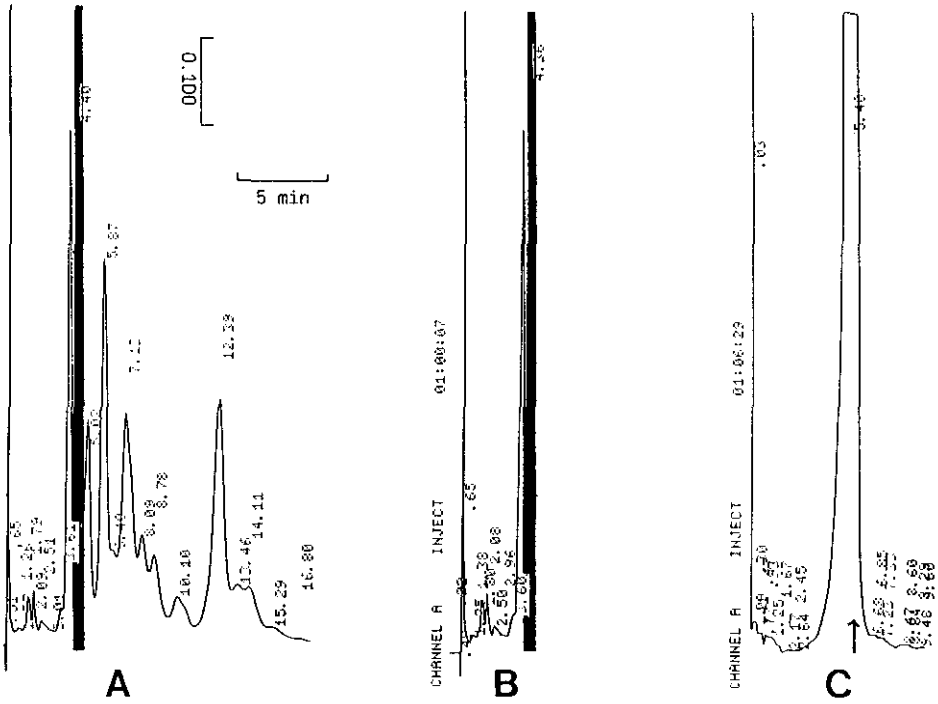

Fig. 10. Separation of a natural extract of steroids on an analytical C_{18} column with acetonitrile–water (50:50) as eluent. The abscissa is the separation time and the ordinate the detector signal. (A) Normal separation. (B) Collection of the main product. At the end of the main peak the flow direction is changed and the late impurities are being backflushed. (C) Backflush peak of the late impurities.

From the experimental value of the time of the end of the backflush peak, it is possible to select the proper parameters for flip–flop elution. A series of five injections is shown in Fig. 11. Since the beginning of collection is at time 4 min and the end of the backflush peak is at time 6.7 min, it is necessary to wait 2.7 min (about 2.7 column volumes) after reversing the flow direction and making the next injection. If normal optimized elution were applied, the cycle time would be about 13 min (total separation time = 17 min and beginning of collection = 4 min), compared with 7.3 min (4.8 + 2.7) in flip–flop elution. The corresponding savings in solvent consumption would be very significant in preparative work. As can be seen in Fig. 10C, the process is very stable, even though this case is difficult because of the large impurity eluted just before the main peak. For the last injection, the normal elution process was resumed and accordingly the end of the chromatogram is similar to that in Fig. 10A.

CONCLUSIONS

Flip–flop elution appears to be a simple yet powerful way to solve the problem of strongly retained components in PLC. When the time of the end of the collection of the main peak is less than half the retention time of the most strongly retained impurity, flip–flop elution gives shorter cycle times (and thus larger production capacities) than

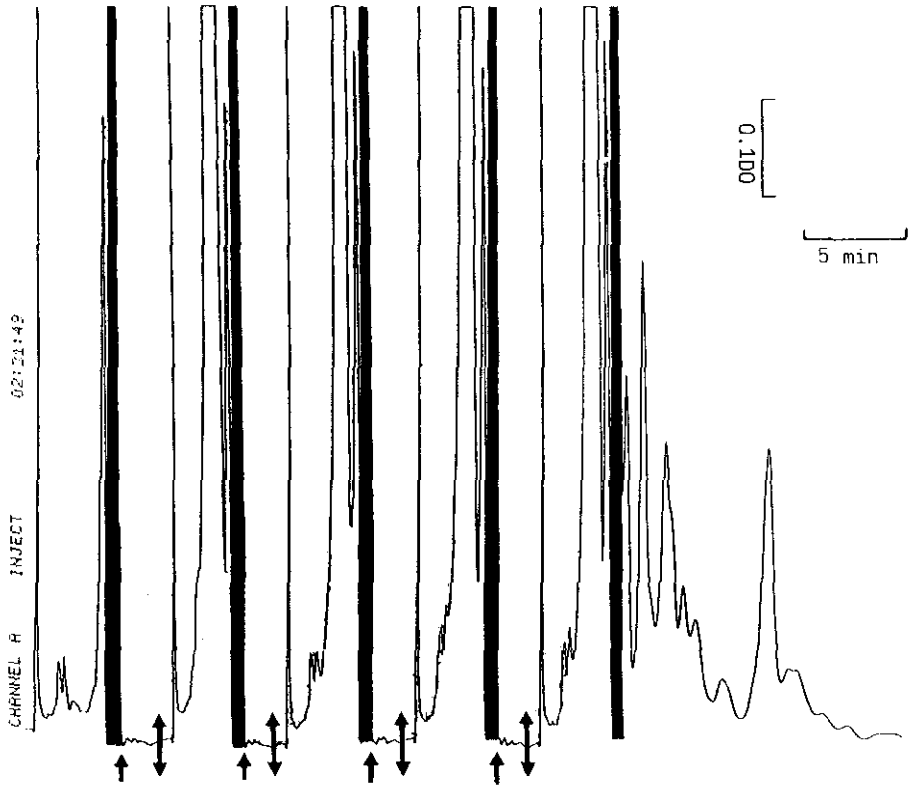

Fig. 11. Same conditions as for Fig. 10. Five injections are made in flip–flop conditions. The single-headed arrows on the baseline indicate when the flow direction is changed. The double-headed arrows indicate when a new injection is made. The abscissa is the separation time and the ordinate the detector signal.

optimized normal elution. In many instances, flip–flop elution is preferable to using a guard column or regenerating the purification column by gradient elution or simple backflushing. The advantages of flip–flop elution are particularly important when economics are considered.

The flip–flop concept has been described above while bearing in mind its application to PLC. It must be realized, however, that this concept is not restricted to PLC but can more generally be applied to gas as well as liquid chromatography, to analytical as well as preparative chromatography and to column chromatography as well as other elution separation techniques (electrophoresis, field-flow fractionation, etc.). Indeed, the flip–flop concept is useful when separation is required for only a fraction of the number of sample components. This is the case in preparative chromatography where generally the collection of one (often the major) component of the sample is sought. This can also be the case in application of analytical separation methods where quantitative information on only one or a few sample components is required (drug or forensic analysis, for instance). Then the mean routine analysis time per sample can be greatly reduced, compared with conventional procedures, by using flip–flop or optimized elution operation, depending on which one is the best.

The equations developed above for the specific case of preparative chromatography can be adapted directly to an analytical objective if the reduced times x_c and x_s correspond to the beginning of the elution of the first component of interest and the end of elution of the last component of interest, respectively. Making the difference between these two reduced times as small as possible, while maintaining a satisfactory resolution between the components of interest constitutes in that case the main challenge for the optimization of the separation conditions. In any case, the criterion expressed by eqn. 12 can be used to select the best of the two optimized processes, flip–flop or optimized normal elution.

REFERENCES

1 M. Verzele, *Anal. Chem.* 62 (1990) 265A.
2 G. Guiochon and S. Ghodbane, *J. Phys. Chem.* 92 (1988) 3682.
3 S. Ghodbane and G. Guiochon, *Anal. Chem.* 61 (1989) 1276.
4 S. Ghodbane and G. Guiochon, *Anal. Chem.* 61 (1989) 1368.
5 G. Guiochon and A. Katti, *Chromatographia*, 24 (1987) 165.
6 W. Skea, in P. R. Brown and R. A. Hartwick (Editors), *High Performance Liquid Chromatography*, Wiley, New York, 1989, p. 479–528.
7 I. Mazsaroff and F. E. Regnier, *J. Liq. Chromatogr.*, 9 (1986) 2583.
8 R. M. N. Nicoud and H. Colin, *LC · GC*, 8 (1990) 24.
9 H. Colin, P. Hilaireau and J. de Tournemire, *LC · GC*, 8 (1990) 302.
10 J. H. Knox and H. Pyper, *J. Chromatogr.*, 363 (1986) 1.
11 G. Mann, presented at the *7th International Symposium on Preparative Chromatography, Ghent, April 8–11, 1990.*