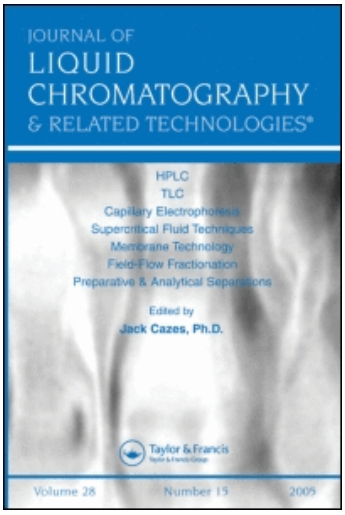

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Using the Liquid Nature of the Stationary Phase: The Elution-Extrusion Method

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Using the Liquid Nature of the Stationary Phase: The Elution-Extrusion Method

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Abstract: Countercurrent chromatography (CCC) is a chromatographic technique with a support-free liquid stationary phase. Taking advantage of the liquid nature of the stationary phase, it is possible to perform unique operations not possible with chromatographic methods based on solid stationary phases. The principle of band broadening inside a chromatographic column is extensively detailed using the random walk model in a hypothetical 200 cell Craig machine. It should be noted that the zone spreading inside the column depends only on the band position, not on the time or number of transfers that were needed to carry the band at its position. Since the whole column content is liquid in CCC, it is possible to extrude it after the bands are separated without waiting for the elution in the mobile phase. It was found that the extrusion step did not disrupt the separated bands too much, provided that the centrifugal field was not modified. The elution-extrusion protocol is given. A mixture of steroid compounds is used to validate the method. An application of the method in the rapid estimation of the hydrophobicity range of microbial extracts in an industrial laboratory is presented.

Keywords: Elution, Extrusion, Liquid stationary phase, Hydrophobicity, Band broadening, Random walk model

INTRODUCTION

Countercurrent chromatography (CCC) is a chromatographic technique with a support-free liquid stationary phase.[1] It starts to be widely used in the

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preparation and purification of natural products form crude extracts. Indeed, the main interest of a liquid stationary phase is its loading capability. Solutes access the volume of the stationary phase rather than just the surface of a solid stationary phase. This surface, the interphase, is overloaded much more rapidly.[2] There are other major advantages of the use of a liquid stationary phase such as, (i) the possibility to use either phase as the stationary phase, (ii) linked to (i), it is the possible to switch the phase role during the separation, this is called the "dual-mode" method.[3] (iii) The choice of possible biphasic liquid systems has practically no limit, since it is possible to mix any number of solvents. (iv) It is possible to perform chemical reactions within the liquid stationary phase allowing high loading displacement chromatography. The chemical reaction can be ion-exchange to separate ions.[4] It can be acid-base reactions; the method was called "pH-zone refining CCC" by its inventor: Yoishiro Ito.[5]

It was recently proposed again to use the liquid character of the stationary phase in CCC to extend the polarity range of separable solutes by extruding the CCC column content after a regular elution.[6] This method was first developed by Conway who called it elution-extrusion CCC.[7] It was used, without specifically mentioning it, in a recent dereplication procedure.[8] In this work, it is pointed out that the elution-extrusion method works because the broadening of the solute bands does not occur inside the column but outside. The general theory already exposed is recalled, simplified, and applied to a real example.

BAND BROADENING IN LIQUID CHROMATOGRAPHY

In any chromatographic separation method, high efficiency is desired since it will produce high resolution factors. Dilution, an unavoidable phenomenon in the chromatographic elution process, is quantified in term of broadening of the width of the injected band at the head of the column.

Giddings first derived the complete theory of solute separation by chromatographic processes. He noted that the solute band broadening is very different inside the column, where the mobile phase and the stationary phase are in equilibrium, and outside the column, where the solutes elute only in the mobile phase.[9] It is worth to recall this theory, because CCC is the only chromatographic technique where it is readily possible to recover the column content since it is all liquids.

Band Broadening Inside a Chromatographic Column

Inside the column, both the stationary phase and the mobile phase are in contact. There is a possible equilibrium of the solute concentration in the two phases. It is very important to understand that the bands broaden inside the column as they move toward the exit. Giddings modeled the broadening

of solute bands using the random walk model,[9] and expressed it through σ, the standard deviation of the peak Gaussian curve as:

$$\sigma = l\sqrt{n} \tag{1}$$

in which l is the fixed step length and n is the number of step taken to move the solute at the position x inside the column of length L.[9]

The position x_i of solute i inside the column depends on its elution volume, V_{ri}, the mobile phase volume needed to push the solute outside the column ($x = L$):

$$V_{ri} = V_m + K_{Di}V_s \tag{2}$$

and

$$x_i = LV_{CM}/V_{ri} \tag{3}$$

V_m, V_s and K_{Di} are respectively the mobile and stationary phase volumes inside the column and the solute distribution ratio also called partition coefficient. V_{CM} is the mobile phase volume that was passed after injection (CM for classical mode[6]). Of course, $V_{CM} < V_{ri}$ so that $x_i < L$, otherwise the solute eluted and is no more inside the column.

The random walk step length, l_i, of solute i depends on the "cell" number of the column that we defined (somewhat arbitrarily) as the column efficiency, N, expressed in plate number. It also depends on the distance, l_e, traveled by the solute due to the elution process and after one transfer:

$$l_e = Lv/V_{ri} \tag{4}$$

in which v is the mobile phase volume introduced in the first cell to produce one more transfer. The random walk step length l_i is the geometric mean between the "cell length", L/N, called "high equivalent to a theoretical plate" and the distance l_e. It is expressed as:

$$l_i = \sqrt{\frac{l_e L}{N}} \tag{5}$$

The number of steps needed by the solute i to reach position x_i is simply:

$$n_i = V_{CM}/v = x_i/l_e \tag{6}$$

The standard deviation, σ, inside the column is expressed by:

$$\sigma = l_i\sqrt{n_i} = L\sqrt{\frac{V_{CM}}{NV_{ri}}} \tag{7}$$

The important point demonstrated by Eq. 7 is that, for any more or less retained solute, the decreased length of the random walk step (Eqs. 4 and 5) exactly compensate the increased number of necessary steps needed to reach a given point inside the column (Eq. 6). This means that the peak

width inside the column depends neither on the solute distribution ratio, K_D, nor on the column phase ratio, V_s/V_m. It depends only on the column efficiency and the peak location inside the column. Of course, the volume of mobile phase needed to move a given solute at the x_i position inside the column depends on its distribution ratio. Figure 1 illustrates these essential points.

Figure 1 represents a hypothetical column of 200 cells (taken as 200 plates in first approximation), volume 66.3 mL, equilibrated with a biphasic liquid system, and a Sf value of 0.7 ($Sf = V_s/V_t$; $V_m = 20$ mL and $V_s = 46.3$ mL). Four solutes are injected and pushed by fresh mobile phase. The concentrations of the solutes in the mobile phase are represented with a positive value in the white area. The corresponding solute concentrations in the liquid stationary phase are represented with a negative value in the shaded area. By definition, in each cell, the ratio of the solute concentration in the stationary phase over that in the mobile phase is the distribution ratio, K_D. Table 1 lists the distribution ratios and includes a fifth compound not shown in Figure 1. A pump delivers $v = 0.1$ mL of fresh mobile phase in the first cell to make one transfer.

Figure 1A shows the column content after 350 transfers ($V_{CM} = 35$ mL of mobile phase). Solute 1, with a K_D value of 0.5, is located near the exit of the column between Cell 138 and Cell 190, with a maximum concentration in Cell 162. Its peak, inside the column, has a width of 25 cells at 50% of the peak height. Solute 2, with a K_D of 4, did not move as much. It is centered on Cell 34, with a peak width of 12 cells. Solutes 3 and 4, with K_D of 21 and 102, respectively, are located in Cells 2 and 1. This means that they are both still at the column entrance (not shown in Figure 1).

Figure 1B shows the column content after 1670 transfers ($V_{CM} = 167$ mL of mobile phase). This particular value was selected so that Solute 3, with a K_D value of 21, is located exactly where Solute 2 was in Figure 1A. Also, Solute 2 moved exactly where Solute 1 was in Figure 1A. Table 1 shows that the peak widths of the two solutes are exactly the same when they are located at the same positions. Any solute passing by Cell 34 has a peak width at half height of 12 cells. In Cell 162, closer to the column exit, the peak width is 25 cells. Figure 1C confirms this essential point with Solutes 3 and 4, respectively, pushed in Cells 162 and 34, after 8050 transfers (850 mL of mobile phase). Again, the peak width is the same when the solute is located in the same cells.

Band Broadening Outside the Chromatographic Column

Position of the solutes inside the column, x_i, and standard deviation, σ, expressed in unit length are not possible after elution of the solutes. Retention volumes and standard deviation expressed in mobile phase volume unit are necessary.

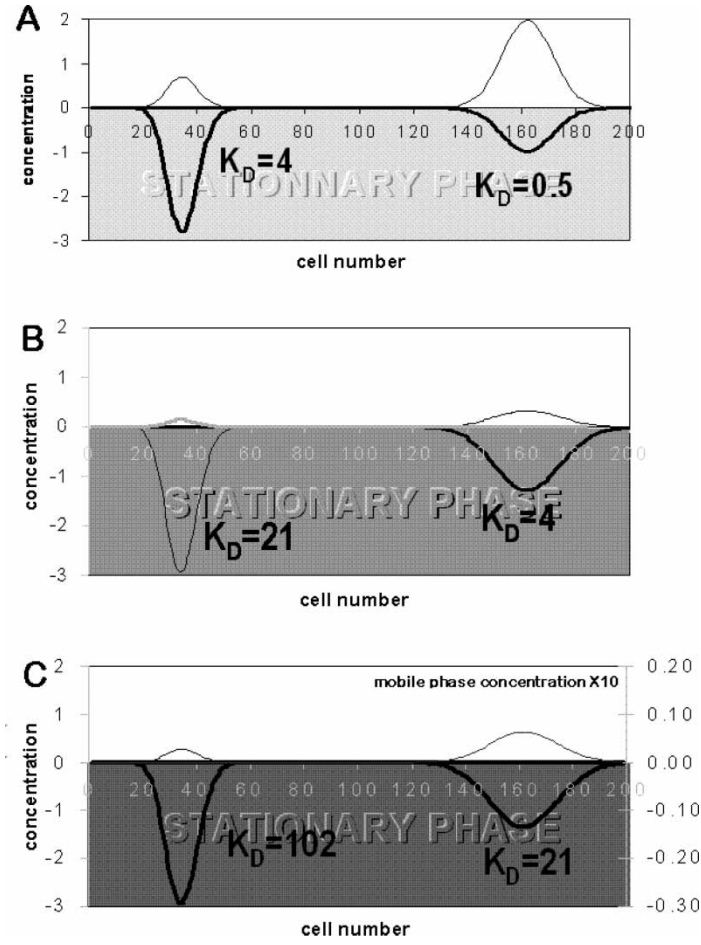


Figure 1. Diagram showing the peak width of four compounds inside a hypothetical 200 plate (cell) column. A, after 350 transfers; B, after 1670 transfers; C, after 8050 transfers. See text and Table 1 for all data.

By definition, the solutes leave the column (elute) when the volume of mobile phase, V_{CM}, introduced in the column inlet is equal to the retention volume V_{ri}. For $V_{CM} = V_{ri}$, the peak maximum is located at the column exit and $x_i = L$ (Eq. 3). As pointed out in the previous section, all solutes located inside the column, at a given position, have the same peak width. This is true at the column exit for part of the solute still in the column. Equation 7 gives at $x_i = L$ or $V_{CM} = V_{ri}$:

$$\sigma_L = \frac{L}{\sqrt{N}} \tag{8}$$

Table 1. Data corresponding to Figs. 1 and 2

Transfers (V)		A 350 (35 mL)		B 1670 (167 mL)		C 8050 (805 mL)		38500[a] (3.85 L)	
Solute	K	Cell	$W_{0.5h}$	Cell	$W_{0.5h}$	Cell	$W_{0.5h}$	Cell	$W_{0.5h}$
1	0.5	162	25	(43.2 mL)	6.1 mL	(43.2 mL)	6.1 mL	(43.2 mL)	6.1 mL
2	4	34	12	162	25	(206 mL)	29 mL	(206 mL)	29 mL
3	21	7[a]	5.5	34	12	162	25	(994 mL)	140 mL
4	102	2[a]	2.5	7[a]	5.5	34	12	162	25
5[a]	485[a]	1[a]	1	2[a]	2.5	7[a]	5.5	34	12

Machine volume (hypothetical machine): $V_C = 66.4$ mL; $V_m = 20$ mL; $V_s = 46.4$ mL. Sf = 0.7. One transfer corresponds to 0.1 mL mobile phase or 1 mL corresponds to 10 transfers. $W_{0.5h}$ = peak width at 50% height (in number of cells inside the column and mL outside). Values in parenthesis correspond to the elution volumes of solutes not shown in Figure 1.

[a]Not shown in Figure 1.

with σ_L expressed in unit of column length. Equation 8 clearly confirms that the peak width at the column exit, σ_L, is the same for all solutes, depending only on the column length and efficiency.

The mobile phase volume needed to elute the solute outside the column depends on the distribution ratio. All solutes travel at different speeds inside the column; this being the foundation of the chromatographic separation process. The mobile phase volume needed to elute the solute I is related to the distance, l_e, it traveled after one transfer. Then, the standard deviation of the solute band at the column exit, σ_L, is related to the standard deviation of the solute band outside the column, σ_i, by:

$$\sigma_i = \frac{\sigma_L}{l_e} v \tag{9}$$

Combining Equations 4 and 8 and 9, it becomes:

$$\sigma_i = \frac{V_{ri}}{\sqrt{N}} \tag{10}$$

Equation 10 can be trivially transformed in the classical equation to evaluate column efficiency:

$$N = \left(\frac{V_{ri}}{\sigma_i}\right)^2 \tag{11}$$

Figure 2 illustrates the dramatic difference between band width inside and outside the column for the same hypothetical 66.4 mL column and solutes previously used for Figure 1 and listed in Table 1. Figure 2A shows the solute locations after 432 transfer or 43.2 mL of mobile phase, the retention volume of Solute 1. The concentration maximum of Solute 1 is exactly at the column exit ($L = 200$ cell). The left part width of the peak at 50% height is given by Eq. 8 and is $\sigma_L = 14$ cells (1.4 mL). Solute 1 is located 66.7% in the mobile phase and 33.3% in the stationary phase. The right part of Solute 1, located only in the mobile phase, has a width of $432/\sqrt{200} = 31$ cells (3.1 mL). The peak of Solute 1 is twice as wide outside the column than inside.

Figures 2B and C show that the band broadening due to peak elution increases dramatically as the solute distribution ratio increases. Figure 2B shows the concentration profile after 2060 transfers. The retention volume of Solute 2 is 206 mL (Table 1). The maximum of the Solute 2 peak is exactly located at the column exit. The left part of the band has exactly the same width previously calculated for Solute 1, i.e., 14 cells or 1.4 mL. But 80% of Solute 2 is located in the stationary phase and only 20% is located in the mobile phase ($K_{D2} = 4$). A larger mobile phase volume is needed to push it out of the column. The right part of Solute 2 band is $2060/\sqrt{200} = 146$ cells (14.6 mL), more than 10 times wider than the left part inside the column. Figure 2C shows the case of Solute 3, $K_{D3} = 21$ and

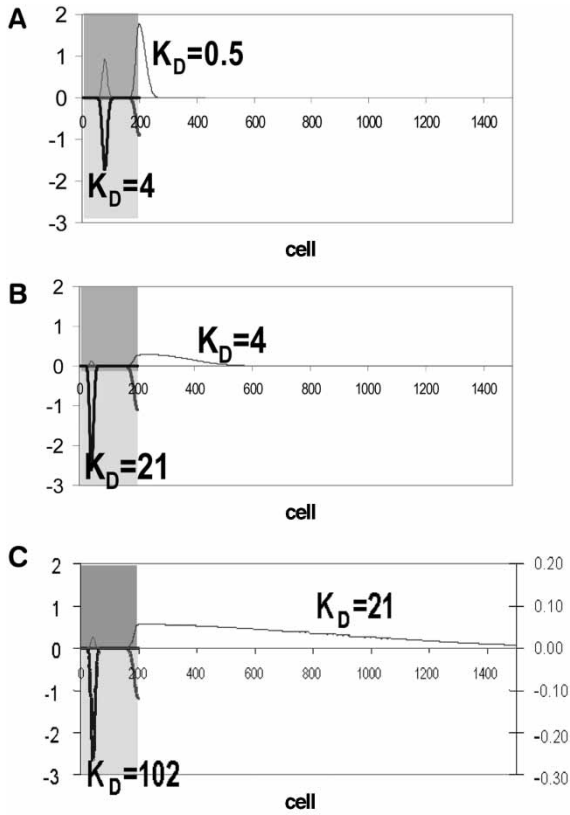


Figure 2. Diagram showing the peak width of four compounds leaving a hypothetical 200 plate (cell) column (shaded area). A, after 432 transfers; B, after 2060 transfers; C, after 9940 transfers; concentration in the mobile phase is ten times higher (right axis) than that in the stationary phase (left axis). See text and Table 1 for all data.

$V_{r3} = 994$ mL or 9940 transfers. The calculation shows that the peak width outside the column is about 50 times wider than that inside the column.

Knowing these facts, it is worth trying to use the unique properties of CCC that allows working with the whole column content, since it is all liquids without any solid support.

THE ELUTION-EXTRUSION METHOD

Procedure

The elution-extrusion method will be performed in four steps: The experiment starts exactly like any classical separation. The solutes with low K_D ratio are eluted in a reasonable time.

After a volume, V_{CM}, of mobile phase has been eluted in Step 1, the liquid that was the stationary phase is introduced in the CCC column without changing anything else. The rotor rotation (centrifugal field), flow rate, and direction are all maintained with the new liquid phase. Two liquid phases elute from the column. An evaporative light scattering detector (ELSD) is able detect non volatile solutes in this situation.

After a volume, V_C, of "stationary" phase has been eluted in the column, its whole content is extruded and the column contains one liquid phase only.

The CCC column can be equilibrated with the mobile phase to be ready for the next run.

In Step 2, the liquid stationary phase is used as a piston extruding the column content. It was found that it is critical to minimize perturbations during the extrusion step to preserve the sharp band inside the column. That is why the centrifugal field (rotor rotation) and flow rate should remain unchanged.[6]

Separation of Sterols

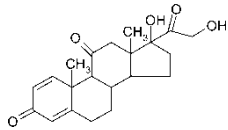
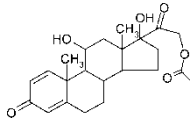
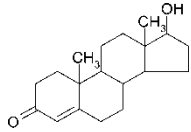
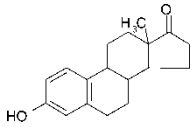
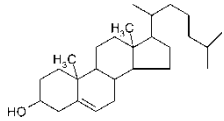
A sample containing the five steroid compounds listed in Table 2 was used as a test mixture to demonstrate the method feasibility. The CCC machine was the model SFCC2000 from Société d'Etudes et Applications des Brevets (SEAB, Villejuif, France), which is a coil planet centrifuge apparatus first designed by Mandava and Ito.[10] This machine was marketed from 1986 to 1992; its distribution is unfortunately discontinued today. It contains three multilayer spools connected in series spinning with a planetary motion around a central axis. The rotor of the chromatograph creates the centrifugal field that maintains the liquid stationary phase. The apparatus was fully described in a previous paper.[11] Each spool was filled with 133 turns of PTFE tubing, 1/16 in. (1.6 mm) internal diameter, 26 m length, coiled in seven layers of 19 turns. The internal volume of one coiled spool was 53 mL. The three coil apparatus had a total internal volume of 158 mL. In this work, only one spool was employed giving a machine volume, $V_C = 52.2$ mL.

The liquid system was a mixture of water/methanol/ethyl acetate/heptane, 6:5:6:5 (v:v:v:v). The mobile phase was the lower aqueous phase used in the head-to-tail direction. The flow rate was 2 mL/min. With a rotor rotation of 700 rpm, 31.2 mL of the organic liquid phase were retained inside the coil (Sf = 60%). The mobile phase volume in the machine was $V_m = 21$ mL.

Baseline Separations Saving Solvents

Figure 3 presents the actual chromatograms obtained with the sterol mixture. Figure 3A shows the separation of the four first sterols in about two hours. Cholesterol was obtained extruding the column content (53 mL) at an

Table 2. Sterols used as test solutes for the EECCC method

Solute	Structure	log $P_{o/w}$	K_D^a	V_r^a (mL)	N^b elution/ (extrusion)
1- Prednisone		1.62	0.116	24.6	200
2- Prednisolone acetate		2.40	0.564	38.6	200
3- Testosterone		3.32	1.44	65.9	200 (500)
4- Estrone		3.13	4.60	164	200 (1900)
5- Cholesterol		8.74	40	1269	(9000)

[a]Liquid System: water/methanol/ethyl acetate/heptane 6/5/6/5 v/v/v/v, aqueous mobile phase, Sf = 0.6, V_m = 21 mL, V_s = 31.2 mL, V_C = 52.2 mL.

[b]Efficiency in plate number. Values in parenthesis are apparent values for Figure 3C since the equation: $5.54(V_r/W_{0.5h})^2$ is not valid in the case of the extrusion process. The apparent value of efficiency for cholesterol in Figure 3A is 55000 plates!

apparent retention volume of 247 mL, instead of 1269 mL (Table 2). This saves 1022 mL of mobile phase (or 8.5 hours of elution time at 2 mL/min). Figure 3B shows the chromatogram obtained when the extrusion procedure was started just after the elution of testosterone (Peak #3) at 80 mL. Estrone and cholesterol eluted in the extrusion mode, baseline separated. The apparent retention volume of cholesterol is 117 mL or 1152 mL lower than its normal elution volume (Table 2). It is even possible to extrude the column content at 25 min (50 mL) just after the elution of prednisolone acetate (Peak #2). The five compounds are still separated at baseline and the chromatogram is done in 45 min (90 mL), saving more than 93% of the time and solvent needed to separate cholesterol the classical way.

Figures 3B and C show a unique feature: the most retained peaks are thinner than the first eluted. This is opposite to what is observed in classical

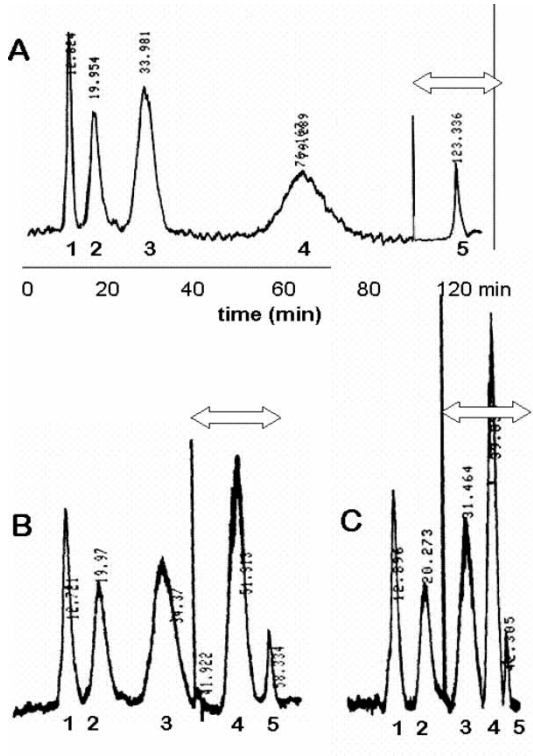


Figure 3. Separation of five steroids compounds by elution-extrusion CCC. 1-prednisone, 2-prednisone acetate, 3-testosterone, 4-estrone, 5-cholesterol. Liquid system: $H_2O/MeOH/EtOAc/C_7H_{16}$ 6/5/6/5 v/v/v/v. Flow rate aqueous phase 2 mL/min, Sf = 0.6, 700 rpm. A-extrusion at 100 min (V_{CM} = 200 mL); B-extrusion at 40 min (V_{CM} = 80 mL); C-extrusion at 25 min (V_{CM} = 50 mL). All three chromatograms have the same time axis; the double arrows show the extrusion step.

isocratic elution where the peak width increases with the retention time (constant efficiency). This confirms the theory exposed previously. The last eluted peaks were located closer to the injection point inside the column. Then, their band was spread less than the one of more advanced peaks inside the column. The later (and broader) peaks were eluted first in the extrusion process. The thinner peaks were eluted last. In the case of Figure 3C, this phenomenon produced an apparent plate count of 500, 1900, and 9000 for testosterone, estrone, and cholesterol, respectively (Table 2). Of course, this plate count is called "apparent" because it is not valid to calculate a plate count when different chromatographic processes are involved.

It is pointed out that continuous detection was possible with the ELS detector that nebulizes the mobile phase, dries it, and looks for remaining

solid solutes. This detector is not sensitive to biphasic volatile liquid systems, but the solutes should be solids. The noisy baseline is due to micro dust particles (or oil droplets) present in the industrial compressed nitrogen used in the nebulizer for economical reasons.

METHOD APPLICATIONS

Solute Distribution Ratio and Peak Position

The elution-extrusion CCC method was first introduced "to extend the hydrophobicity window".[6] From a practical point of view, it means that a solute such as cholesterol, with a K_D value of almost 50 ($V_r > 1200$ mL), can be eluted in a reasonable amount of time and solvent (Figure 3). Indeed, the big advantage of the method is that absolutely no solute remains inside the column after a "stationary" phase volume higher than the column volume has been pumped inside the column.

Trivially, Eq. 2 shows that the solute retention volume, V_r, increases linearly with its distribution ratio, K_D. In a real world sample such as vegetal extracts or biological medium, the solute K_D values can be orders of magnitude apart. It was demonstrated[6] that the peak position of extruded peaks was:

$$V'_{ri} = V_{CM} + V_C(1 - V_{CM}/V_{ri}) \tag{12}$$

In Eq. 12, V'_{ri} is the actual retention volume of Solute i separated using the elution-extrusion method. V_{ri} is the retention volume that Solute i would have in classical elution mode expressed by Eq. 2. From Eq. 2 (classical mode elution) and Eq.12, the expression of K_D can be expressed as a function of the volume, V, of phase eluted. It is the aqueous mobile phase for $V < V_{CM}$ during the classical mode elution. It is the organic "stationary" phase during the extrusion phase, $V > V_{CM}$.

classical mode elution:

$$K_D = \frac{V - V_m}{V_s} \tag{13}$$

extrusion phase:

$$K_D = \frac{1}{V_s}\left[\frac{V_{CM}V_C}{V_C - (V - V_{CM})} - V_m\right] \tag{14}$$

Figure 4 shows the K_D *vs* V plots for two different experiments done with a 175 mL machine. The curve trend would be the same for any device. During the classical mode elution, the K_D value increases linearly with the elution

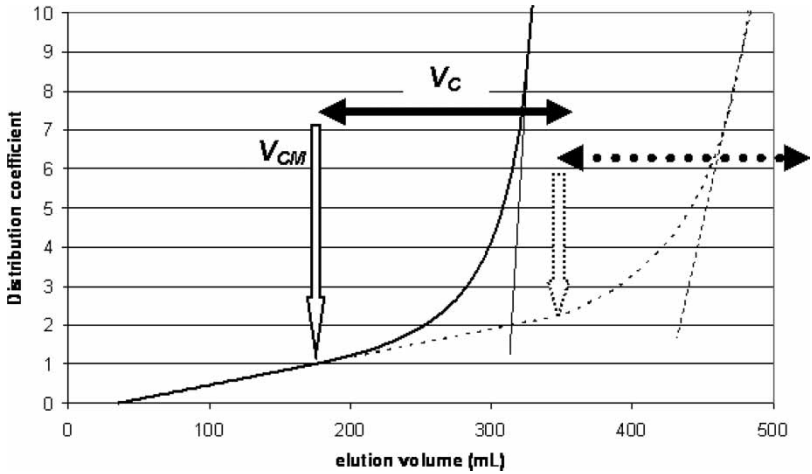


Figure 4. Value of the distribution ratio, K_D, plotted versus the elution volume. Machine volume, $V_C = 175$ mL. Full line, classical mode elution for one column volume up to $V_{CM} = 175\,\text{mL} = V_C$ followed by a necessarily equal volume of extrusion, V_C (double horizontal arrow). Total analysis volume is $2V_C$. Dotted lines, classical mode elution up to $V_{CM} = 350\,\text{mL} = 2V_C$ followed by a volume of extrusion, V_C. Total analysis volume is $3V_C$. The straight lines are the tangents at $K_D = 10$ illustrating the differences between the two dK_D/dV values. The vertical arrow points the starting extrusion volume.

volume with a slope equal to $1/V_s$ (Eq. 13). During the extrusion phase, the slope of the K_D *vs* V curve increases quadratically with the eluted volume V as:

$$\frac{dK_D}{dV} = \frac{V_{CM}V_C}{V_s[V_C - (V - V_{CM})]^2} \tag{15}$$

However, the slope increase is slower for higher values of V_{CM}. As shown by the dotted lines of Figure 4, if the classical mode elution is performed for a longer time, the volume V_{CM} is higher. The extrusion phase necessarily starts at a higher K_D value. The K_D value will also reach infinity for $V = V_{CM} + V_C$ but with a smaller slope. The two tangents at $K_D = 10$ are drawn in Figure 4 to illustrate this point.

This relationship between peak position and distribution ratio are most useful so as to have a rapid idea of the hydrophobicity profile of the solutes contained in a sample. If the classical mode (CM) elution is increased, more solutes will be eluted this way and a slightly better accuracy will be obtained on the K_D values of the extruded compounds. If a rapid view of the hydrophobicity repartition of the compounds in a mixture is desired, it is only necessary to elute one column volume of mobile phase in the classical mode ($V_{CM} = V_C$) and to extrude the remaining compounds by another column volume of the other phase.

Estimation of the Hydrophobicity of a Microbial Extract

The elution-extrusion method was found extremely useful in the fast estimation of the hydrophobicity range of natural and/or biological extracts. A real-world example is briefly presented here. The elution-extrusion CCC method was completed in three hours. A Conway Centrichrom DP100 CCC machine (coil planet centrifuge with two 175 mL coils) was used with the two coils being mounted in parallel. The machine was equilibrated with the system water/methanol/ethyl acetate/heptane, 5/3/5/3 v/v/v/v, using the organic upper phase as the mobile phase pumped in the tail-to-head direction producing a S_f ratio of 80% with $V_m = 35$ mL and $V_s = 140$ mL at 2 mL/min at 800 rpm rotor rotation. One "column" volume of the organic upper phase was used in the classical mode ($V_{CM} = 175$ mL), eluting the components of the injected sample having a K_D value lower than unity in the liquid system used. Another column volume of aqueous phase was used to extrude the "column" content, eluting all remaining solutes. The 2 mL CCC fraction was injected into a HPLC system with a YMC CombiScreen C8 column (4.6 × 50 mm, 5 μm particle). The HPLC elution was done with a acetonitrile/water gradient of mobile phase composition: from water (0.1% v/v phosphoric acid) 60%/acetonitrile 40% to acetonitrile 90% in 10 min, and held at 90% acetonitrile if needed. Most HPLC analyses were done in less than 10 min.

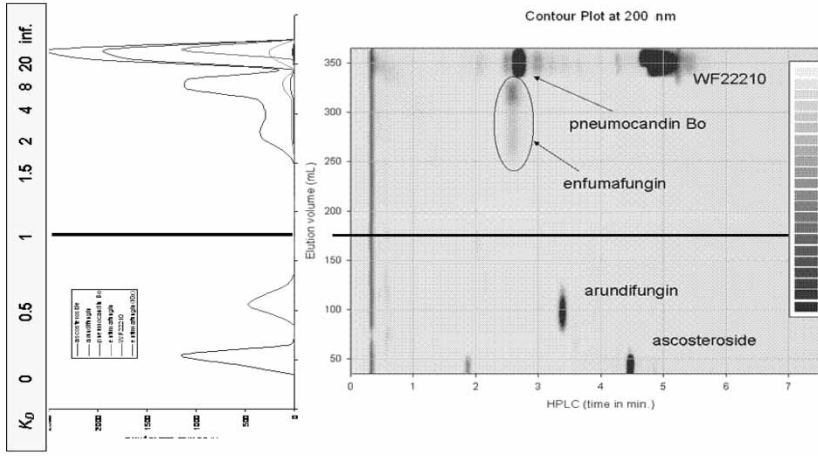


Figure 5. Bi-dimensional CCC-HPLC hydrophobicity evaluation of a mushroom extract. On the left, the elution-extrusion CCC chromatogram, on the right the HPLC chromatograms are displayed horizontally for each mL of CCC eluent. The horizontal line is the limit between the elution mode up to $V_{CM} = V_C = 175$ mL and $K_D = 1$, and the extrusion mode for the same volume. On the far left, the K_D scale is given. It starts at $K_D = 0$ for $V = V_m = 35$ mL, goes through $K_D = 1$ for $V_{CM} = V_C = 175$ mL and end at $V = 2V_C = 350$ mL with $K_D =$ infinity.

Figure 5 shows the study of a microbial extract. The molecular structures will not be given for confidentiality reasons. The CCC elution-extrusion chromatogram is shown on the left after a 90° rotation. The elution mode is at the bottom, the extrusion mode at the top. A proprietary software was able to combine the HPLC chromatograms obtained injecting all 2 mL CCC fractions, and to produce the 2D picture shown on the right of Figure 5. The final large peak obtained at the end of the extrusion step contains at least three components that could be identified by HPLC. The method is now routinely used in the industrial laboratory for the fast dereplication of microbial extracts with antifungal activity.[12]

CONCLUSION

In all chromatographic techniques, the major band broadening of solute peaks occurs when the solutes leave the column. The solute bands are narrow inside the column. CCC is the only chromatographic technique that can use this method of the separation process. The CCC column content is only liquid so that extrusion is possible. It was demonstrated that it is possible to extrude the CCC column content, minimizing mixing. The extruded solute peaks are much narrower than the eluted peaks, saving a significant proportion of mobile phase volume. The elution-extrusion method was found extremely useful to rapidly estimate the hydrophobicity range of a sample.

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