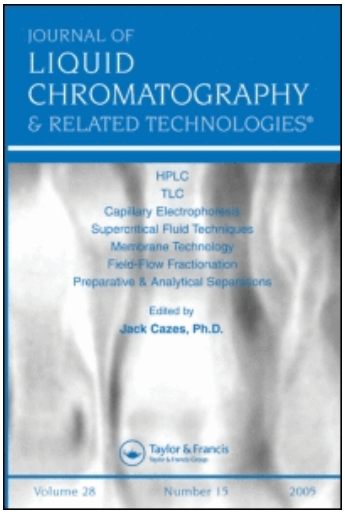

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Chromatographic Models as Tools for Scale-up of Isolation of Natural Products by Semi-preparative HPLC

José Luiz Mazzei[a]; Luiz Antonio d'Avila[a]
[a] Departamento de Processos Orgânicos, Escola de Química, E-204 Centro de Tecnologia, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil

Online publication date: 02 March 2003

To cite this Article Mazzei, José Luiz and d'Avila, Luiz Antonio(2003) 'Chromatographic Models as Tools for Scale-up of Isolation of Natural Products by Semi-preparative HPLC', Journal of Liquid Chromatography & Related Technologies, 26: 2, 177 — 193
To link to this Article: DOI: 10.1081/JLC-120017162
URL: http://dx.doi.org/10.1081/JLC-120017162

MARCEL DEKKER, INC. • 270 MADISON AVENUE • NEW YORK, NY 10016

JOURNAL OF LIQUID CHROMATOGRAPHY & RELATED TECHNOLOGIES®
Vol. 26, No. 2, pp. 177–193, 2003

Chromatographic Models as Tools for Scale-up of Isolation of Natural Products by Semi-preparative HPLC

José Luiz Mazzei* and Luiz Antonio d'Avila

Departamento de Processos Orgânicos, Escola de Química,
Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil

ABSTRACT

Scale-up of high performance liquid chromatography (HPLC) using mathematical models has been found in the literature for separation of binary mixtures or monocomponent materials. This procedure provides graphical representation, in contrast to direct scale-up, which has been usually employed for separation of natural fraction components. In this report, the application of models to the scale-up of isocratic separations, from literature data, of carotenoids, vitamins, ginsenosides, and monoterpenes fractions, in terms of use of larger columns and sample overloads, is discussed. Statistical moment analysis and an ideal rate model were applied using a computer spreadsheet to estimate parameters from analytical data and predict semi-preparative separations. Non-competitive

*Correspondence: José Luiz Mazzei, Departamento de Processos Orgânicos, Escola de Química, E-204 Centro de Tecnologia, Universidade Federal do Rio de Janeiro, 21949-900 Rio de Janeiro, Brazil; E-mail: jmazzei@eq.ufrj.br.

DOI: 10.1081/JLC-120017162 1082-6076 (Print); 1520-572X (Online)

MARCEL DEKKER, INC. • 270 MADISON AVENUE • NEW YORK, NY 10016

effects between the components were assumed, due to the few available data. The predicted chromatograms showed good agreement with the experimentals, demonstrating the applicability of scale-up using models on separation of natural fractions.

Key Words: Semi-preparative HPLC; Separation; Natural products; Simulation; Scale-up; Statistical moment analysis; Ideal rate model.

INTRODUCTION

High performance liquid chromatography (HPLC) is widely used to separate and isolate components from complex mixtures, when high level of purity, yield, and productivity are required. Standards of natural compounds have been obtained at semi-preparative and preparative scales to determine their structures, undertake chemical, pharmacological, and nutritional investigation, and assist in the quality control of herbal medicines, phytomedicines, foodstuffs, and additives. Some carotenoids, vitamins, ginsenosides, monoterpenes, lipids, alkaloids, furocoumarins, sweeteners, polyunsaturated acids, anthocyanins, and other flavonoids are examples of these compounds. Natural drugs for which synthesis is economically unfeasible or unavailable, such as scopolamine, paclitaxel, and derivatives, digitalis glycosides, capsaicin, and vinca alkaloids have also been produced by HPLC at large scale.

Semi-preparative and large scale HPLC separations are derived from the kinetic and thermodynamic conditions established on a small scale, like analytical runs. These conditions are kept unchanged on scale-up, which is calculated by mathematical procedures to reach a well-controlled separation. In direct or linear scale-up,[1–3] a factor is calculated by Eq. (1) from the lengths L and cross-sectional areas A_c of the preparative (P) and analytical (A) columns.

$$\text{Direct scale-up factor} = \frac{L_{(\mathrm{P})}\, A_{c(\mathrm{P})}}{L_{(\mathrm{A})}\, A_{c(\mathrm{A})}} \tag{1}$$

The mobile phase flow rate, column length, and sample quantity have occasionally been changed after scale-up when better separation would be required. However, in these cases, the chromatographic profile at the intended scale could not be easily provided[1] and the retention and separation efficiency could only be predicted by calculation.[2,3]

Mathematical models describing the chromatographic phenomena can also help in HPLC scale-ups, and resulting simulations of chromatographic profile. Three groups have been cited:[4] based on equilibrium theory, plate,

and rate models. Some models have algebraic solutions, like the statistical moment analysis (a plate model) and ideal rate model, which are applied herein. The advantage of these models is the fact that few experiments need be done in the estimation of the parameters, regarding limited quantities of injected sample. Eq. (2) is based on Gaussian distribution and represents the concentration profile[3,5] of a sample component in the mobile phase on statistical moment analysis.

$$C = \left[\frac{C_{\text{inj}}\ V_{\text{inj}}}{F(2\pi\sigma^2)^{1/2}}\right] \exp\left[-\frac{\sigma^2(t - t_R)^2}{2}\right] \tag{2}$$

C_{inj}, V_{inj} and F correspond to the concentration of the component in the injected solution, injection volume, and flow rate, respectively. The injected quantity, represented by zero order moment, only influences the peak height without altering its shape. The retention time t_R and the variance σ^2 of the peak are described by first and second order moments, respectively. Independently of column dimensions, the linear distribution constant K_c and the reduced plate height h are constant parameters, which describe the equilibrium and effective diffusion, respectively, of the sample component in the column. The latter is often quasi-constant for a short range of the mobile phase velocity. The value of K_c is related to the retention factor k and phase ratio β, according to the Eq. (3).

$$K_c = \frac{k}{\beta} \tag{3}$$

K_c, k, h and σ^2 can be estimated from experiments made at small scale using well-known expressions in the literature.[2,3,6] The phase ratio is not determined easily, except for normal chromatography, in which it can be approximately estimated from the interparticle porosity ε.[7] The retention factor, therefore, should be used as an equilibrium parameter instead of the distribution constant.

Statistical moment analysis should only be applied under short injection duration of diluted solutions, and disregarding the influence of extra-column volumes. The resulting peaks are symmetrical, characteristic of linear chromatography. Conditions for linearity are difficult to define, however, Rosset et al.[3] presented criteria that ensure linear chromatography. The injection volume is related to the statistical moments.

$$V_{\text{inj}} \ll 2Ft_R \tag{4}$$

$$V_{\text{inj}} \ll F(12\sigma^2)^{1/2} \tag{5}$$

According to Rosset's approach, linear chromatography is assured in a typical analytical column for samples below 10^{-4} M. When the concentration is about 10^{-2} M, the increased injection duration gradually changes the chromatography from linear to weakly non-linear. In the mean time, the elution profile loses symmetry and can no longer be predicted using the statistical moment analysis. Concentration at the injection greater than 1 M leads to strongly non-linear chromatography, resulting tailing peaks, typical of Langmuir or another convex isotherm. In these conditions, the rise in sample amount injected increases the peak-width so intensely that any effect of axial dispersion can be negligible using the ideal rate model.[7] The hyperbolic shape that represents the rear part of the peak has an asymptote extending until the end retention time t_e, that corresponds to the injection duration plus the retention time, which is obtained in linear conditions.[7] In the case of ideal rate model, the time of the peak maximum $t_{R(\mathrm{INL})}$ is related to the loading factor L_f,[7] expressed by the Eq. (6):

$$L_f = \left\{1 - \left[\frac{t_{\mathrm{R(INL)}} - (V_{\mathrm{inj}}/F) - t_M}{t_R - t_M}\right]^{1/2}\right\}^2 = \frac{C_{\mathrm{inj}}\ V_{\mathrm{inj}}}{q_S(1-\varepsilon)A_c L} \tag{6}$$

The parameter q_S and t_M are the stationary phase saturation capacity and retention time of an unretained compound, respectively. The elution profile of the rear part of the peak is represented by Eq. (7):

$$C = \frac{q_S}{K_c}\left\{\left[\frac{t_R - t_M}{t - (V_{\mathrm{inj}}/F) - t_M}\right]^{1/2} - 1\right\} \tag{7}$$

In strongly non-linear chromatography, displacement effects among sample components become significant and parameters of competitive isotherms should be evaluated.[16]

Recently, computer programmes have been used for the economic and operational optimization of HPLC at large scale.[8–11] Experimental cases of scale-up for peptides and proteins-based samples using models have been published.[12–14] However, for low molecular weight compounds, computer-assisted prediction has only been used for the separation of binary aromatic chemicals and chiral isomers (Table 1). Scale-ups of HPLC using models have not been discussed for separation of any multicomponent mixture of low molecular weight compounds, as typically found in natural fractions and extracts. Direct scale-up of semi-preparative separation of natural compounds,

Table 1. Experimental semi-preparative HPLC found in the literature applying mathematical models for scale-up.

Sample component isolated	Chromatography	Components	Model	Reference
o-Phenyl-phenol	Normal or reversed	One	Statistical moment	[3]
Ethyl and butyl phthalates	Normal	Binary	Statistical moment	[3]
2-Phenyl-ethanol	Reversed	One	Ideal rate	[15]
2-Phenyl-ethanol and benzyl alcohol	Normal	Binary	Non-ideal rate	[8]
Tröger's base (racemate)	Chiral	Binary	Non-ideal rate	[16]
Synthetic racemate of ibuprofen	Chiral	Binary	Non-ideal rate	[10]
Binary mixture	Not indicated	Binary	Non-ideal rate	[9]
α- and β-Isomers of a steroid	Normal	Binary	Non-ideal rate[a]	[1]
α- and β-Isomers of a steroid	Normal	Binary	Non-ideal rate	[11]

[a]Also using direct scale-up.

such as triterpenoid saponins[17] and fractions of omega 3 polyunsaturated fatty acids[18,19] has been found in the literature.

The purpose of the present work is to demonstrate the applicability of mathematical models in the scale-up of semi-preparative separations of natural fractions and extracts, comparing the experimental results found in the literature to those obtained by simulations. The distribution isotherms were assumed non-competitive due to few available experimental data.

EXPERIMENTAL

Selection of Experimental Separations

Reports in HPLC separations by isocratic mode with both analytical and semi-preparative chromatograms at the same thermodynamic conditions were reached in the literature as well as application notes. Four studies on different classes of compounds were selected.

Computational Method

The software Microsoft® Excel-97 was used to prepare electronic spreadsheets, using the equations discussed herein. The spreadsheets estimated the equilibrium and kinetic parameters and simulated the chromatographic profile for both analytical and intended scales. The chromatographic profiles of the components were calculated individually and the final chromatograms were simulated from the sum of them. The calculations were made using IBM-compatible personal computers [Pentium® 233 MHz, 32 Mb RAM or higher configuration].

General Calculations

Column dimensions, flow rate, retention time, and peak-width at half height of each sample component were determined at small scale. The interparticle porosities were estimated from the retention time of unretained component, which was calculated at intended scale, maintaining the value of the interparticle porosity.

The phase ratios were only calculated for normal-phase chromatography. In the analytical chromatograms, each retention time and peak-width were approximately determined. The values of the retention factor, linear distribution constant,

and reduced plate height of each peak were calculated by the spreadsheets and kept constant on the scale-up prediction. The total molar concentration of the injection was estimated using the mass and volume data and assuming the molecular weight of the most abundant compound, since there were no details on the concentration of each sample component. As the concentration of each component was unknown, the intensities of the simulated peaks were adjusted proportionally to those presented in the analytical chromatograms, in order to take detector sensitivity into consideration. It does not influence the parameters calculated by the model based on the statistical moment analysis. The Rosset's linearity criteria was evaluated according to the Eq. (4) and (5).

RESULTS AND DISCUSSION

Analytical (a) and semi-preparative (c) experimental chromatograms for carotenoids, vitamins, ginsenosides, and monoterpenes are shown in the Figs. 1–4, respectively. The conditions are presented in Table 2, including the total molar concentration estimated.

The operational parameters estimated at analytical scale are shown in Table 3. Assuming the first peaks in all cases as unretained sample components, the interparticle porosities were estimated. This assumption could not be valid, because the first peaks can represent a retained or partly excluded compound, but it was applied in this work in order to take advantages of the few available data.

The maximum injection volumes at both scales have satisfied the Rosset's linearity criteria, according to Eq. (4) and (5). The estimated thermodynamic and kinetic parameters calculated by the spreadsheets can be seen in Table 4. The different operational variables changed between the experimented scales were studied individually.

Scale-Up in Linear Chromatography

On Same Column

Figure 1 illustrates the increase in injected mass and volume under the same conditions (as can be seen in Table 2), in order to attain isolation of paprika (*Capsicum annuum*) carotenoids by normal-phase HPLC.[20] β-Carotene was assumed an unretained sample component. Linear chromatography can be predicted in both scales with the concentrations under study (Table 2), because the column volume was approximately fifty-fold a typical analytical column.

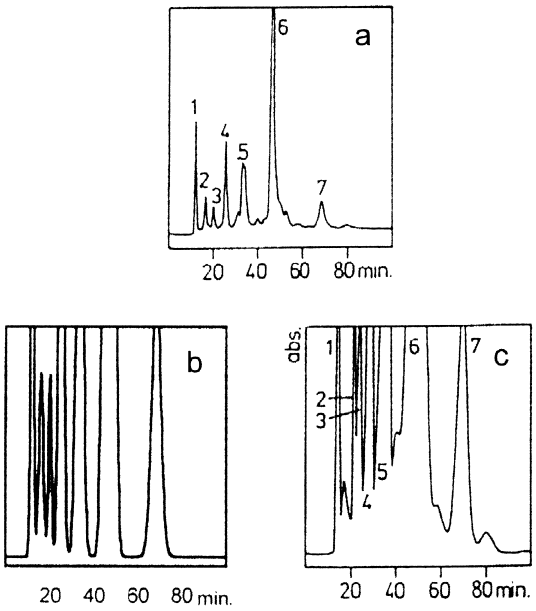

Figure 1. Separation of carotenoids from the paprika fruit by normal-phase HPLC: (a) analytical experiment; (b) semi-preparative chromatogram simulated by the model using statistical moment analysis; (c) semi-preparative experiment for a larger quantity of sample. Conditions and captions in Tables 2 and 4, respectively. *Source*: Figures a and c reprinted from *Chromatographia*, vol. 27, page 326, with permission from the authors.

In order to represent an experimental increase of volume and mass of all components at the injection, these values were changed in the spreadsheet. The semi-preparative chromatogram was predicted from the estimated parameters using Eq. (2). It is represented in Fig. 1b, showing the same range of intensity as in the analytical scale. In other words, it was simulated at the same attenuation. It can be compared to the result obtained experimentally (Fig. 1c). In this scale-up prediction, only data concerning to the zero order moment was modified. Despite its simplicity, the increased intensities of the peaks and the resolution between the sample components on the predicted chromatogram were quite similar to that obtained experimentally. The retention times of cryptoxanthin and cryptoxanthin epoxide were not exactly estimated, as seen in Fig. 1c, peaks 2 and 3, respectively. This exception cannot be explained.

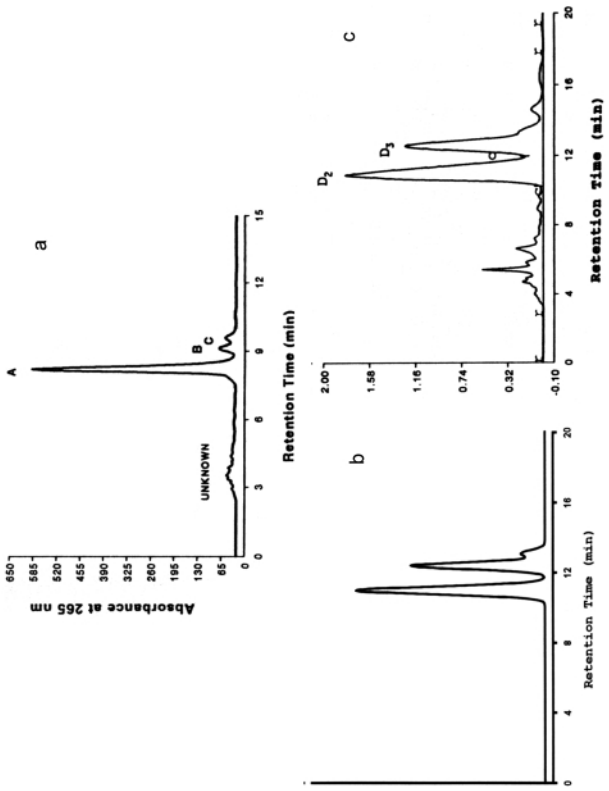

Figure 2. Separation of vitamins D_2 and D_3 from a resin of vitamin D using reversed-phase HPLC: (a) analytical experiment; (b) semi-preparative chromatogram simulated by the model based on statistical moment analysis; (c) semi-preparative experiment. Conditions and captions in Tables 2 and 4, respectively. *Source:* Figures a and c reprinted from Journal of Chromatography, vol. 590, pages 170 and 171, Copyright © 1992, with permission from Elsevier Science.

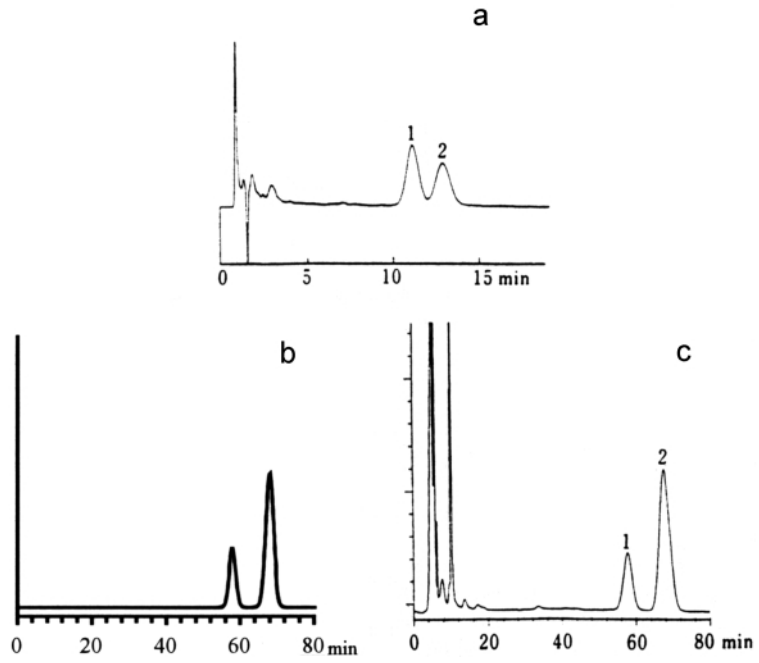

Figure 3. Separation of ginsenosides from *Panax ginseng* root by reversed-phase HPLC: (a) analytical experiment; (b) semi-preparative chromatogram simulated by the model using statistical moment analysis; (c) semi-preparative experiment. Conditions and captions in Tables 2 and 4, respectively. *Source*: Figures a and c reprinted from Chemical & Pharmaceutical Bulletin, vol. 38, page 1630, Copyright © 1990, with permission from The Pharmaceutical Society of Japan.

Columns with Different Length

The same model was applied to the separation of vitamin-D[22] by reversed-phase HPLC (Fig. 2). The selectivity and retention times of the main compounds were accurately represented by the simulated chromatogram (Figs. 2b and c). The relative intensities were corrected due to the different wavelengths selected for detection (Table 2). The assumption of linear chromatography is justified by the exact predicted retention as experimented, in a comparison between Fig. 2c and b, despite the high total molar concentration at the injection (Table 2). The estimation was acceptable for injections of up to 500 mg in a semi-preparative column. The resolution

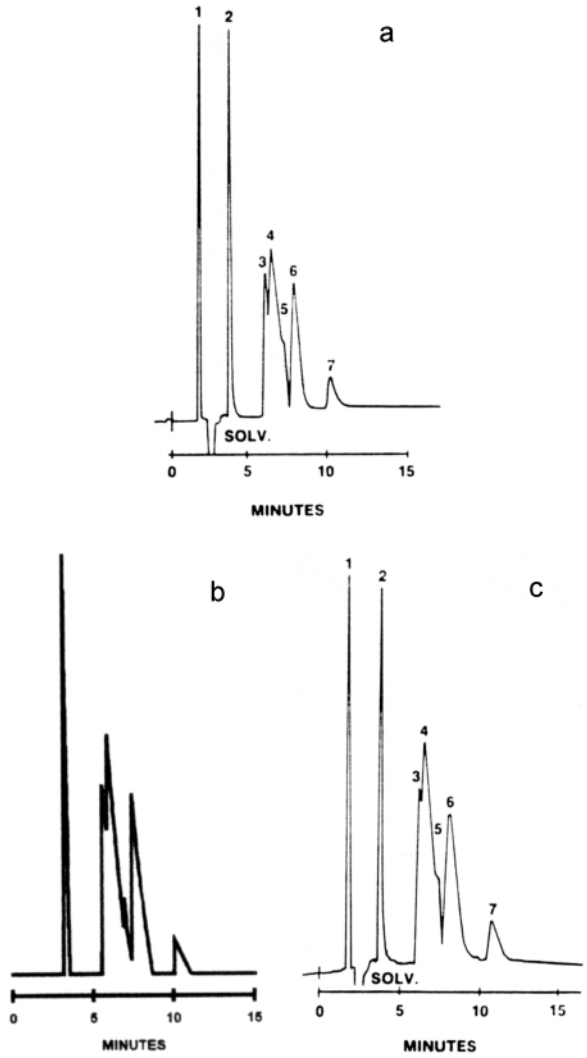

Figure 4. Separation of a mixture consisted of crude oxidation product of (+)-limonene plus carvone (not diluted) by normal-phase HPLC: (a) 4 μL injected; (b) chromatogram simulated by the ideal rate model, not including the first peak (see text); (c) experimental increasing mass and volume, 7 μL injected. Conditions and captions in Tables 2 and 4, respectively. *Source*: Figures a and c reprinted from the Journal of Chromatography, vol. 178, page 577, Copyright © 1979, with permission from Elsevier Science.

Table 2. Conditions for separation of natural substances using HPLC in the cases selected.

	Figure			
	1	2	3	4
Components	Carotenoids	Vitamins	Ginsenosides	Monoterpenes
Stationary phase	Silicagel 60H	Zorbax PRO-10 C_{18}	Modified porous glass–ODS	Partisil 10-PXS silicagel
Mobile phase	Acetone–petroleum ether (30 : 70)	CH_3OH–CH_3CN–hexane (95 : 3 : 2)	CH_3CN–H_2O (16 : 84)	Ethyl acetate–CH_2Cl_2 (2.5 : 97.5)
Particle (μm)	15	10	20	10
Column (mm)	500 × 20	250 × 4.6[a] and 250 × 50.8[b]	150 × 4[a] and 500 × 20[b]	250 × 4.6
Flow rate (mL/min)	5	1[a] and 90[b]	1[a] and 15[b]	2
Injection	1 mg (1 mL)[a] and 40 mg (5 mL)[b]	0.05 mg (20 μL)[a] and 500 mg (2 mL)[b]	Not indicated[a] and 300–600 mg[b]	4 μL[a] and 7 μL[b]
C_{inj} (molar)	1.4×10^{-3}[a] and 0.01[b]	6×10^{-3}[a] and 0.6[b]	Not determined	Not diluted
Detection	435 nm	265 nm[a] and 300 nm[b]	203 nm	Differential refractometer
Reference	[20]	[21]	[22]	[23]

[a]Analytical separation.
[b]Semi-preparative separation.

MARCEL DEKKER, INC. • 270 MADISON AVENUE • NEW YORK, NY 10016

Table 3. Data estimated from the experimental chromatograms for scale-up prediction.

Parameter[a]	Figure 1a	Figure 2a	Figure 3a	Figure 4a
t_M (min)	11.9	2.9	1.1	1.5
ε	0.38	0.7	0.6	0.72
β	1.63	—	—	0.38

[a]t_M, ε and β are the retention time of an unretained compound, interparticle porosity and phase ratio, respectively.

between vitamins D_2 and D_3 obtained in practice (Fig. 2c) was a little bit lower than the obtained by simulated data (Fig. 2b).

Columns with Different Width and Length

Another case studied is the scale-up using a larger column in separating ginsenosides from alcoholic extract of *Panax ginseng* root by reversed-phase HPLC.[21] It is illustrated in Fig. 3. The linearity was not verified because the injection volumes were not given by the original work (Table 2). However, the model using statistical moment analysis was employed because the analytical chromatogram showed symmetrical peaks (Fig. 3a), typical of linear chromatography. The first peak in the Fig. 3a was assumed an unretained sample component. After the relative intensities of the peaks were adjusted to simulate the analytical chromatogram, the flow rate and column dimensions described in Table 2 were altered. The spreadsheets presented the simulated semi-preparative chromatogram (Fig. 3b) based on Eq. (2). It provided an adequate representation of the experimental one (Fig. 3c), with regard to the retention and resolution. Without the model, the retention time and resolution at the semi-preparative scale can be predicted, but the resulting chromatogram could not be provided.

Scale-Up in Non-Linear Chromatography

On Same Column

Figure 4 illustrates the semi-preparative separation of the crude oxidation product of (+)-limonene plus carvone in an analytical column by normal-phase HPLC.[23] One would expect the resulting peaks to be as in non-linear

Table 4. Parameters estimated from analytical chromatograms using the computer spreadsheet.

Figure	Peak[a]	Component	k	K_c	h
1a	1	β-Carotene	—[b]	—[b]	80.8
	2	Cryptoxanthin	0.34	0.21	130
	3	Cryptoxanthin epoxide	0.68	0.41	37.9
	4	Unidentified	1.09	0.67	61.2
	5	Cryptocapsin and zeaxanthin	1.79	1.10	61.0
	6	Capsanthin	2.90	1.78	40.6
	7	Capsorubin	4.72	2.89	25.7
2a	A	Vitamin D_2	1.78	—	10.0
	B	Vitamin D_3	2.14	—	6.00
	C	Tachysterol	2.31	—	6.04
3a	1	Ginsenoside-Rg_1	8.20	—	6.20
	2	Ginsenoside-Re	9.80	—	6.29
4a	1	Limonene	—[b]	—[b]	—[c]
	2	Carvone	1.35	3.50	—
	3	*trans-p*-Mentha-1(7), 8-dien-2-ol	3.73	9.69	—
	4	*cis-p*-Mentha-1(7), 8-dien-2-ol	3.92	10.2	—
	5	*trans*- and *cis*-Carveol	4.16	10.8	—
	6	*trans-p*-Mentha-2, 8-dien-1-ol	4.80	12.5	—
	7	*cis-p*-Mentha-2, 8-dien-1-ol	6.40	16.6	—

[a]In the original figures.
[b]Unretained component.
[c]Infinite efficiency considered by the model.

chromatography because the product was not diluted before injection. In fact, all peaks at small scale (Fig. 4a) presented a profile similar to the triangular and hyperbolic shape, typical of mass overload with Langmuir isotherm. This detail suggests the application of the ideal rate model to predict chromatographic profiles for a larger injection volume.

Limonene was assumed an unretained sample component. The approximate retention times for each peak, which predicted linear conditions, were calculated using the end times, injection volume, and flow rate. Thus, k and K_c,

for each sample component, were calculated (Table 4). They are constant parameters, independent of injected sample quantity. The end time of the peaks numbered 3 and 4 cannot be measured accurately due to the very low resolution (Fig. 4a). The loading factors of the components were calculated using Eq. (6), from the times of the peak maximum presented in Fig. 4a. The concentration of each sample component was calculated approximately from the relative area between the peaks, while maintaining the total concentration. With the data of loading factor and estimated concentration of the injection, the apparent stationary phase saturation capacity was estimated for each peak by applying Eq. (6). The same detector sensitivities were admitted for each component, which is a valid assumption for the responses of compounds with similar molecular weights by a differential refractometer detector.

The chromatographic profile of each peak in Fig. 4a was calculated using the Eq. (7) and the resulting one was obtained from the sum of them. After adjusting the new injection volume value to 7 μL, the chromatogram for the intended scale was simulated using the computer spreadsheet. In the simulated results, the peak representing the unretained sample component is omitted because its loading factor cannot be calculated. As the ideal rate model was applied, the injection volume alters the values for the end time of the peak, loading factor, time of the peak maximum, and consequently, the curve of the rear part of the peak, as can be seen in the Eq. (6) and (7). Though the sample quantity at the new scale was approximately double of that used in the first injection, the simulated chromatograms for both scales were similar. The loss of resolution between the peaks as the quantity injected increased, (Fig. 4b) was accurately represented by the simulated chromatogram (Fig. 4c). This result can be observed particularly for peaks 5, 6, and 7, whose parameters were measured more accurately at small scale. It is interesting to point out that details of the chromatogram obtained with double injection volume, under non-linear chromatography, could not have been predicted without applying this model.

The simulated chromatograms at semi-preparative scale were quite similar to the experimental chromatograms reported in the literature, for both linear and non-linear cases. Good similarity with the experimental chromatograms was obtained even with inaccurate parameters, which were estimated from analytical chromatograms. The results suggest the assumption of non-competitive distribution isotherms as a useful simplification for semi-preparative purposes when the productivity is not the subject of optimization. They also validate the mathematical models as a practical tool for the scale-up of semi-preparative separation of multicomponent mixtures, such as natural fraction and extracts, by HPLC in isocratic mode.

ACKNOWLEDGMENTS

The authors are grateful to Dr. Henri Colin for valuable comments and suggestions. We thank the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES/Brazil) for the fellowship to J.L.M.

REFERENCES

1. Heuer, C.; Hugo, P.; Mann, G.; Seidel-Morgenstern, A. Scale up in preparative chromatography. J. Chromatogr. **1996**, *752* (1–2), 19–29.
2. Johnson, E.L.; Stevenson, R. *Basic Liquid Chromatography*; Varian Associates: Palo Alto, California, 1978; 354 pp.
3. Rosset, R.; Caude, M.; Jardy, A. *Manuel Pratique de Chromatographie en Phase Liquide*, 10th Ed.; Masson: Paris, 1982; 274–324.
4. Gu, T. *Mathematical Modeling and Scale up of Liquid Chromatography.* Springer-Verlag: Berlin, 1995; 124 pp.
5. van Deemter, J.J.; Zuiderweg, F.J.; Klinkenberg, A. Longitudinal diffusion and resistance to mass transfer as cases of nonideality in chromatography. Chem. Eng. Sci. **1956**, *5* (6), 271–289.
6. Recommendations for nomenclature for chromatography. Pure Appl. Chem. **1993**, *65* (4), 819–872.
7. Golshan-Shirazi, S.; Guiochon, G. Analytical solution for the ideal model of chromatography in the case of a langmuir isotherm. Anal. Chem. **1988**, *60* (21), 2364–2374.
8. Jageland, P.; Magnusson, J.; Bryntensson, M. Optimisation of industrial-scale high-performance liquid chromatography applications using a newly developed software. J. Chromatogr. A **1994**, *658* (2), 497–504.
9. Jageland, P.T.; Bryntesson, L.M. Economical aspects and optimization in industrial HPLC. Preptech 94, New Jersey, Mar 22–24, 1994; communication, Eka Chemical, 2000.
10. Jageland, P.T.; Bryntesson, L.M.; Möller, P.L. Preparative chiral HPLC separation. In *Modeling, Optimization and Scale-Up*, Purdue University Chromatographic Workshop '95, West Lafayette, IN, Oct 9–10, 1995; communication, Eka Chemical, 2000.
11. Seidel-Morgenstern, A. Optimization and comparison of different modes of preparative chromatography. Analusis **1998**, *26* (7), M46–M55.
12. Kaltenbrunner, O.; Jungbauer, A.; Yamamoto, S. Prediction of the preparative chromatography performance with a very small column. J. Chromatogr. A **1997**, *760* (1), 41–53.
13. Carrère, H.; Bascoul, A.; Floquet, P.; Wilhelm, A.M.; Delmas, H. Whey proteins extraction by fluidized ion exchange chromatography: simplified

modeling and economical optimization. Chem. Eng. J. **1996**, *64* (3), 307–317.
14. Gu, T.; Zheng, Y. A study of the scale up of reversed-phase liquid chromatography. Sep. Purif. Technol. **1999**, *15* (1), 41–58.
15. Zoubaïr El Fallah, M.; Guiochon, G. Gradient elution chromatography at very high column loading: effect of the deviation from the Langmuir model on the peak profile of a single component. Anal. Chem. **1991**, *63* (20), 2244–2252.
16. Seidel-Morgenstern, A.; Guiochon, G. Modelling of the competitive isotherms and the chromatographic separation of two enantiomers. Chem. Eng. Sci. **1993**, *48* (15), 2787–2797.
17. Apers, S.; Foriers, A.; Sindambiwe, J.B.; Vlietinck, A.; Pieters, L. Separation of a triterpenoid saponin mixture from *Maesa lanceolata*: semi-preparative reversed-phase wide pore high performance liquid chromatography with temperature control. J. Pharm. Biomed. Anal. **1998**, *18* (4–5), 737–743.
18. Robles Medina, A.R.; Gimenez, A.G.; Camacho, F.G. Concentration and purification of stearidonic, eicosapentaenoic, and docosahexaenoic acids from cod-liver oil and the marine microalga *Isochrysis galbana.* J. Am. Oil Chem. Soc. **1995**, *72* (5), 575–583.
19. Grant, J. Multigram isolation of omega-3-fatty acids from fish oil using preparative HPLC. Am. Lab. **1988**, *20* (5), 80, 82, 84–85.
20. Isaksen, M.; Francis, G.W. Preparative HPLC of carotenoids. Chromatographia **1989**, *27* (7–8), 325–327.
21. Letter, W.S. Preparative isolation of vitamin-D2 from previtamin-D2 by recycle high-performance liquid-chromatography. J. Chromatogr. **1992**, *590* (1), 169–173.
22. Kanazawa, H.; Nagata, Y.; Matsushima, Y.; Tomoda, M.; Takai, N. Preparative high-performance liquid chromatography on chemically modified porous glass. Isolation of saponins from ginseng. Chem. Pharm. Bull. **1990**, *38* (6), 1630–1632.
23. Jones, B.B.; Clark B.C., Jr.; Iacobucci, G.A. Semi-preparative high-performance liquid chromatographic separation of a characterized flavor mixture of monoterpenes. J. Chromatogr. **1979**, *178* (2), 575–578.

Received July 24, 2002
Accepted August 27, 2002
Manuscript 5919