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Optimization of Production Rate and Recovered Amount in Linear and Nonlinear Preparative Elution Liquid Chromatography

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Abstract

It is commonly thought that the adaptation of an analytical chromotographic separation to the preparative scale requires an increase in resolution to allow for the greater sample size. We show that this statement is only true for semipreparative chromatography, where the aim is only to optimize the amount recovered at a given purity. If the maximum production rate is desired, however, analytical resolution is no longer the main consideration. Whether the criterion is the amount recovered or the production rate, the analytical selectivity is more important than the capacity factors. This is true for a linear as well as a nonlinear optimization procedure. For recovery ratios near unity, the maximum production rate (corresponding to infinite selectivity) is of the order of 5 D mmol/h for a linear optimization procedure and 50 D mmol/h for a nonlinear optimization procedure, where D is the mobile phase flow rate in liters per hour.

INTRODUCTION

When one seeks to adapt an analytical separation to the preparative scale, it is commonly considered that one must increase the analytical resolution in order to handle the greater sample size. This consideration is valid only for semipreparative chromatography, where the aim is to isolate very pure compounds in amounts ranging from one to several hundred milligrams in a small number of injections and a short period of time.

Increasing the resolution, however, requires modifying the phase system,

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and this generally increases the retention time and lowers the solubility of the compounds to be separated. Thus it is hard to predict whether the production rate (i.e., the amount of sample that can be separated in unit time) will increase or decrease. A compromise must be sought between resolution, solubility, and separation time.

The purpose of this study is to optimize the chromatographic phase system; that is, the analytical capacity factors, selectivity, and resolution, for the purpose of preparative separation. The optimization procedure is defined by the criteria of production rate and amount recovered per injection; the constraints are recovery ratio and impurity ratio.

A theoretical paper was recently published by Hupe and Lauer (*1*) on the topic. However, their approach is limited to linear chromatographic behavior and even to almost Gaussian preparative elution profiles. Furthermore, working under conditions for which the contribution to band broadening of the injected volume and the column itself is about equal is rather arbitrary. Finally, this treatment is intended for the most difficult separations where the optimal preparative working conditions are not very far from the analytical ones.

THEORETICAL

In preparative chromatography the production rate R_h can be defined as the ratio of the purified sample amount recovered per injection, Q_r, to the time period between two consecutive injections or cycle time θ:

$$R_h = Q_r/\theta$$

According to the definition of the recovery ratio T_r ($T_r = Q_r/Q_0$, Q_0 being the injected amount (*2*)), Q_r can be expressed as the product T_rQ_0. So we have

$$R_h = T_r\frac{Q_0}{\theta} \tag{1}$$

For a binary mixture separated into two fractions, the impurity ratios T_{i_1} and T_{i_2} can be defined as (*3*)

$$T_{i_1} = Q_2/Q_{r_1} \qquad T_{i_2} = Q_1/Q_{r_2}$$

where Q_{r_1} and Q_{r_2} are the recovered amounts of each compound; Q_1 is the

amount of the first compound contained in the second fraction and Q_2 is the amount of the second compound contained in the first fraction.

In order to discuss the conditions for maximal production rate, one first has to express the optimal injected amount Q_0 and the cycle time θ for a given chromatographic system (mobile and stationary phases, column dimensions). For convenience, the following study refers to a binary mixture, but it can obviously be extended to a mixture containing more than two compounds.

Optimal Injected Amount

In preparative chromatography the sample amount injected in a given chromatographic system must be optimized according to the linearity or nonlinearity of the preparative chromatographic process (*2, 4*). It is of great practical interest to express the optimal injected amount in terms of the chromatographic parameters of the analytical separation of the sample on the preparative chromatographic system. The linear and nonlinear optimization procedures were described in previous papers (*2–4*).

In linear chromatography the maximal injected volume V_{0_1} leading to a recovery ratio close to 1 is given by (*3*)

$$V_{0_1} = 2(\sigma_1 + \sigma_2)(R_s - 1)$$

where R_s is the analytical resolution and σ_1 and σ_2 are the standard deviations of the two analytical peaks. This relationship is established for R_s larger than 1.3 (*3*). V_{0_1} can also be written in terms of the analytical chromatographic parameters (*5, 6*):

$$V_{0_1} = V_{R2} - V_{R1} - 2(\sigma_1 + \sigma_2) \tag{2}$$

$$= V_m[k'_1(\alpha - 1) - \frac{2}{\sqrt{N}}(2 + k'_1 + \alpha k'_1)] \tag{3}$$

V_{R1} and V_{R2} are the analytical retention volumes of the two compounds, V_m is the mobile phase volume inside the column, k'_1 is the analytical capacity factor of the first eluted solute, α is the analytical selectivity, and N is the average column plate number measured *from an analytical injection.* In many practical cases (*6*) (especially when the separation is not too difficult), the term $(2/\sqrt{N})(2 + k'_1 + \alpha k'_1)$ is small compared to $k'_1(\alpha - 1)$. So V_{0_1} can be approximately written

$$V_{o_1} = V_m k'_1(\alpha - 1)$$

For a pulse-shaped injection of this volume, the maximal concentration C_{0_1} consistent with linear behavior must be determined experimentally. It is reported by several authors (*7–9*) that C_{0_1} is a decreasing function of the analytical capacity factor k' but most often remains within the range 5×10^{-3} to 2×10^{-2} M as k' varies. Thus, in the linear case, the maximal injected amount leading to a recovery ratio close to 1 is

$$Q_{0_1} = C_{0_1} V_{0_1} = C_{0_1} V_m k'_1(\alpha - 1) \tag{4}$$

In nonlinear elution chromatography, Eq. (4) no longer applies. The maximal injected amount in a given preparative chromatographic system can now be predicted using the nonlinear behavior model presented in Refs. *2, 4,* and *9*. This model is based on the experimental characterization of the peak shape obtained at high sample loading in adsorption, reversed-phase, and ion-exchange chromatography.

All these peaks are composed of a very steep, near vertical fronting and a large tailing which joins down the baseline for an invariant elution volume equal to $V_R + V_0 + 2\sigma$ (Fig. 1) (V_0 is the injected volume, and V_R and σ are the constant retention volume and standard deviation of the analytical peak recorded in the same chromatographic conditions). We have shown (*4*) that the eluted concentration for this volume remains within 2 to 4% of the apex concentration. As the injected amount is increased, the fronting and the apex of the nonlinear elution profiles move toward shorter retentions. Two more basic properties of these profiles appear by taking the injection ends as the common abscissa origin (i.e., by doing a V_0 translation): (a) the elution profiles obtained from various (volume V_0, concentration C_0) injection couples representing a constant loading Q_0 ($Q_0 = C_0 V_0$) are perfectly superposable; (b) the elution profiles obtained from different injection loadings Q_0 are such that their tailing links up an exponential envelop curve (Fig. 1):

$$C(v) = C_m \exp\left[-\frac{v - V_0 - V_m}{\tau}\right] \tag{5}$$

where V_m is the column hold-up volume and C_m and τ are the two model parameters that can be determined by fitting an exponential curve to the experimental elution profiles. The available experimental results (*9*) showed that C_m depends only on the analytical capacity factor k' of the compound and varies from 0.2 to 0.8 M when k' increases. τ is related to the column

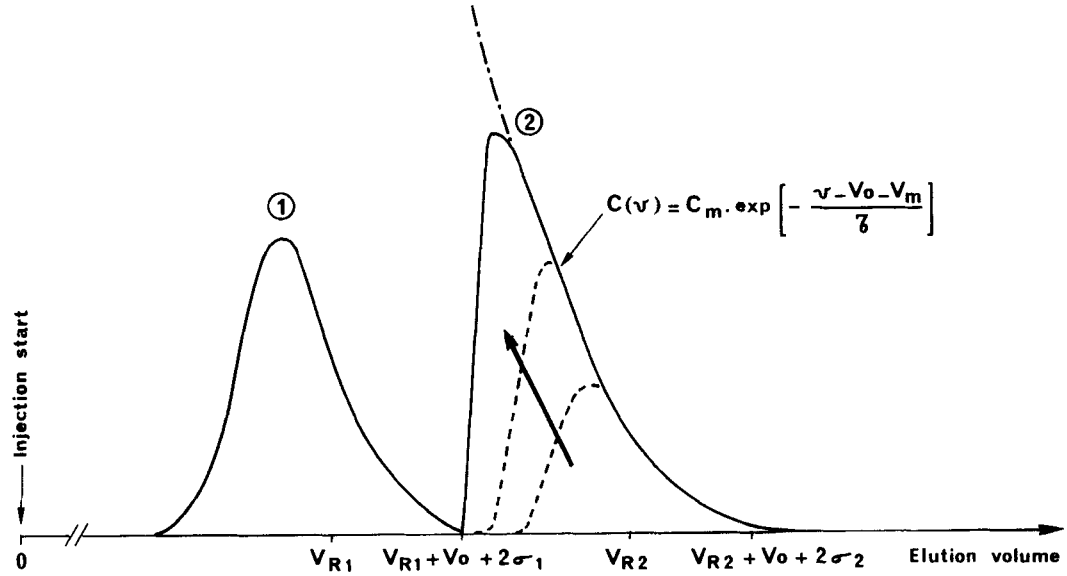

FIG. 1. Assessment of the maximal injected amount $Q_{0_{2n1}}$ of the second eluted compound for a recovery ratio close to 1 in the case of a nonlinear elution process. (--) Exponential envelope $C(v) = C_m \exp - [v - V_0 V_m/\tau]$ of the nonlinear profiles of Compound 2. (——) Nonlinear elution profiles obtained from a given $Q_{0_{2n1}}$ injection of Compound 2 (see Eq. 9). The dashed lines and the arrow show how the elution profile of Compound 2 changes with an increase in the injected amount of Compound 2.

dimensions and the solute retention, and is about equal to $0.2V_R$ (V_R still being the solute analytical retention volume in the same chromatographic conditions). Further experiments showed that this nonlinear model is valid if the injected volume V_0 meets the condition $V_0 < \tau$. Beyond this limit the elution profile broadens drastically and exhibits a plateau in its upper part, which is of no interest for preparative chromatographic separations.

This model enables one to assess the maximal injected amount $Q_{0_{2n1}}$ of the second eluted compound, giving a recovery ratio close to 1 in the case of a nonlinear elution process (it must be noted that the model does not allow one to assess the maximal injected amount of the first eluted compound). The elution volume $V_{\max 2}$ of the second compound peak apex is then determined by the elution end of the first compound (Fig. 1):

$$V_{\max 2} = V_{R1} + V_0 + 2\sigma_1 \tag{6}$$

where V_{R1} and σ_1 are the analytical retention volume and the standard deviation of the first eluted compound. Therefore $Q_{0_{2n1}}$ is equal to the area under the exponential envelope, limited to the abscissa $V_{\max 2}$. By using a well-known property of the exponential curves, one obtains

$$Q_{0_{2n1}} = C_{\max 2}\tau \tag{7}$$

with

$$C_{\max 2} = C_m \exp\left[-\frac{V_{\max 2} - V_0 - V_m}{\tau}\right] \tag{8}$$

Substituting Eq. (6) into Eq. (8) and Eq. (8) into Eq. (7) leads to the final expression for $Q_{0_{2n1}}$:

$$Q_{0_{2n1}} = C_m\tau \exp\left[-\frac{V_{R1} + 2\sigma_1 - V_m}{\tau}\right] \tag{9}$$

with the following condition for the upper limit of the nonlinear injected volume $V_{0_{n1}}$:

$$V_{0_{n1}} < \tau \tag{10}$$

In practice, for a given $Q_{0_{2n1}}$ the injection volume for nonlinear conditions should be made as small as possible so as to produce the sharpest separation.

Relationship (9) can also be expressed in terms of the classical analytical chromatographic parameters α and k'_1:

$$\tau = 0.2V_{R2} = 0.2V_m(1 + \alpha k'_1)$$
$$V_{R1} - V_m = V_m k'_1$$

If σ_1 is negligible compared to V_{R1}, the following simple relationship is derived:

$$Q_{0_{2n1}} = 0.2V_m C_m(1 + \alpha k'_1) \exp\left[-\frac{5k'_1}{1 + \alpha k'_1}\right] \qquad (11)$$

Cycle Time

The cycle time θ was previously introduced by Conder et al. (*10*), Kraak et al. (*11*), and Coq et al. (*6*) for linear preparative chromatography. The more commonly accepted definition is

$$\theta = \frac{V_{R2} + V_0 + 2\sigma_2}{D} \qquad (12)$$

where V_{R2} is the analytical retention volume of the second eluted solute and D is the mobile phase flow-rate. This definition refers to what Conder called "slow cycling" (*10*).

The authors of this paper also pointed out from the experimental results cited above (*2, 4, 9*) that this definition still retains its validity for nonlinear preparative chromatography.

In linear preparative chromatography the cycle time can be expressed by substituting the V_{0_1} expression (Eq. 2) into Eq. (12):

$$\theta = \frac{2V_{R2} - V_{R1} - 2\sigma_1}{D}$$

The term $2\sigma_1$ is generally small compared to $2V_{R2} - V_{R1}$ and can reasonably be neglected. In doing so, the evaluation of θ becomes slightly pessimistic, but a simple expression relating θ to the analytical chromatographic parameters can be found:

$$\theta = \frac{V_m}{D}[1 + k'_1(2\alpha - 1)] \qquad (13)$$

In the nonlinear optimization procedure, the injected volume, $V_{0_{n1}}$, is

shorter than the linear one, V_{0_1}, so that, rigorously, the nonliner cycle time is a little shorter than the linear cycle time given by Eq. (13). However, since a rough estimate of the production rate would be very useful for prediction purposes, we kept using Eq. (13) in the linear and nonlinear cases as well.

Production Rate

Relationships (1), (4), (9), and (13) can now be used to derive the production rate R_h expressions in the case of a recovery ratio equal to 1. For a linear optimization procedure:

$$R_{h\,1} = DC_{0_1} \frac{k'_{1(\alpha-1)}}{1 + k'_1(2\alpha - 1)} \tag{14}$$

For a nonlinear optimization procedure:

$$R_{h\,n1} = 0.2DC_m \frac{1 + \alpha k'_1}{1 + k'_1(2\alpha - 1)} \exp\left[-\frac{5k'_1}{1 + \alpha k'_1} \right] \tag{15}$$

At this point it is worth noting that the expression of the linear production rate $R_{h\,1}$, suitable for most difficult separations (R_s near 1.3), should be derived from Eq. (3) directly:

$$R_{h\,1} = \frac{DC_{0_1}}{1 + k'_1(2\alpha - 1)} \left[k'_1(\alpha - 1) - \frac{2}{\sqrt{N}}(2 + k'_1 + \alpha k'_1) \right] \tag{16}$$

Equation (16) shows the influence of the analytical efficiency (measured by N) of the preparative column, which was recently discussed by Hupe and Lauer (*1*). It indicates that when the mobile and stationary phase system cannot be further improved, the factors affecting N (column length, particle diameter, and mobile phase flow rate) must be chosen so as to render the term $2/\sqrt{N}(2 + k'_1 + \alpha k'_1)$ small enough compared to $k'_1(\alpha - 1)$.

It appears from Eqs. (14) and (15) that the production rate mainly depends on the following two-variable functions:

$$f_1(\alpha, k'_1) = \frac{k'_1(\alpha - 1)}{1 + k'_1(2\alpha - 1)}$$

$$f_{n1}(\alpha, k'_1) = \frac{1 + \alpha k'_1}{1 + k'_1(2\alpha - 1)} \exp\left[-\frac{5k'_1}{1 + \alpha k'_1} \right]$$

A few typical values of these two-variable functions are listed in Table 1. This study shows that the effect of k'_1 and α on the production rate is different for the linear and nonlinear optimization procedures. $f_1(\alpha, k'_1)$ increases quickly with α and slowly with k'_1, whereas $f_{n1}(\alpha, k'_1)$ increases more slowly with α and decreases slightly with k'_1. Besides, it was mentioned above that the parameters C_{0_1} and C_m are slightly decreasing functions of the capacity ratios. Finally, one can conclude that the linear and nonlinear production

TABLE Ia

$$f_1(\alpha, k'_1) = \frac{k'_1(\alpha - 1)}{1 + k'_1(2\alpha - 1)}$$

	k'_1					
α	1	2	5	10		∞
1.1	0.045	0.06	0.07	0.08		0.08
1.5	0.17	0.20	0.23	0.24		0.25
2	0.25	0.29	0.31	0.32	$\frac{\alpha - 1}{2\alpha - 1}$	0.33
5	0.40	0.42	0.43	0.44		0.44
∞	0.50	0.50	0.50	0.50		0.50

TABLE Ib

$$f_{n1}(\alpha, k'_1) = \frac{1 + \alpha k'_1}{1 + k'_1(2\alpha - 1)} \exp\left[-\frac{5k'_1}{1 + \alpha k'_1} \right]$$

	k'_1					
α	1	2	5	10		∞
1.1	0.09	0.04	0.02	0.01		0.01
1.5	0.11	0.07	0.04	0.03		0.03
2	0.14	0.10	0.07	0.06	$\frac{\alpha}{2\alpha - 1} \cdot e^{-5/\alpha}$	0.05
5	0.26	0.23	0.22	0.21		0.20
∞	0.50	0.50	0.50	0.50		0.50

rates are an increasing function of the analytical selectivity α, but it is more difficult to state, from a theoretical point of view only, what the effect of k'_1 is.

In the case of a separation having an infinite selectivity ($\alpha = \infty$), $f_1(\alpha, k'_1)$ and $f_{n1}(\alpha, k'_1)$ are equal to 0.5, whatever the k'_1 value. By respectively taking 10^{-2} and 0.5 M as the most typical values of C_{0_1} and C_m (9), Eqs. (14) and (15) provide the order of magnitude of the maximal production rate that can be reached by preparative elution liquid chromatography. For the linear optimization procedure:

$$R_{h\,1}\ (\mathrm{m}M/\mathrm{h}) = 5D\ (\mathrm{L/h})$$

For the nonlinear optimization procedure:

$$R_{h\,n1}\ (\mathrm{m}M/\mathrm{h}) = 50D(L/\mathrm{h})$$

The nonlinear optimization procedure enables an higher production rate than the linear one. It is preferred whenever the relative ease of the separation and high sample solubility allows injection of a large amount to reach a nonlinear separation process. On the contrary, for the most difficult separations (maximal analytical resolution near to 1–1.3), a linear optimization procedure is preferable.

In this connection, in reversed-phase chromatography, for all the compounds of low and medium polarity, a nonlinear chromatographic process cannot be observed owing to their low solubility in alcohol–water mixtures, and the linear optimization procedure is the only way to proceed.

EXPERIMENTAL

The experiments were performed in reversed phase chromatography with equimolar mixtures of resorcinol and phenol (reagent grade quality from Merck, Darmstadt, G.F.R). The preparative column was made of a 25-cm length, 7.6 mm i.d. stainless steel tubing containing 5 g of Lichroprep R.P.8 (25–40 μm) from Merck. Its hold-up volume is equal to 8 mL. The mobile phases were composed of reagent grade methanol (from Prolabo, Paris, France) and distilled water in various proportions. They were percolated through the column by an Orlita MS 15/7 reciprocating pump (Orlita, Giessen, G.F.R.) at a constant flow rate of 275 mL/h (0.23 cm/s). The injections were made with a Rheodyne 7120 six-way valve (Rheodyne, Berkeley, California) connected to either an Orlita DMP 15/15 reciprocating pump for volumes above 10 mL or homemade sample loops for the

volumes less than 10 mL. For the detection a L.D.C. Spectromonitor II spectrophotometer equipped with 3 mm path-length preparative cells was used. In the overlapping region the column effluent was divided into constant narrow volume fractions in order to determine the actual concentration profiles and to compare with the on-line detector response. Each fraction was quantitatively analyzed by reversed-phase chromatography within less than 4 min. The analytical operating conditions are given in Fig. 2.

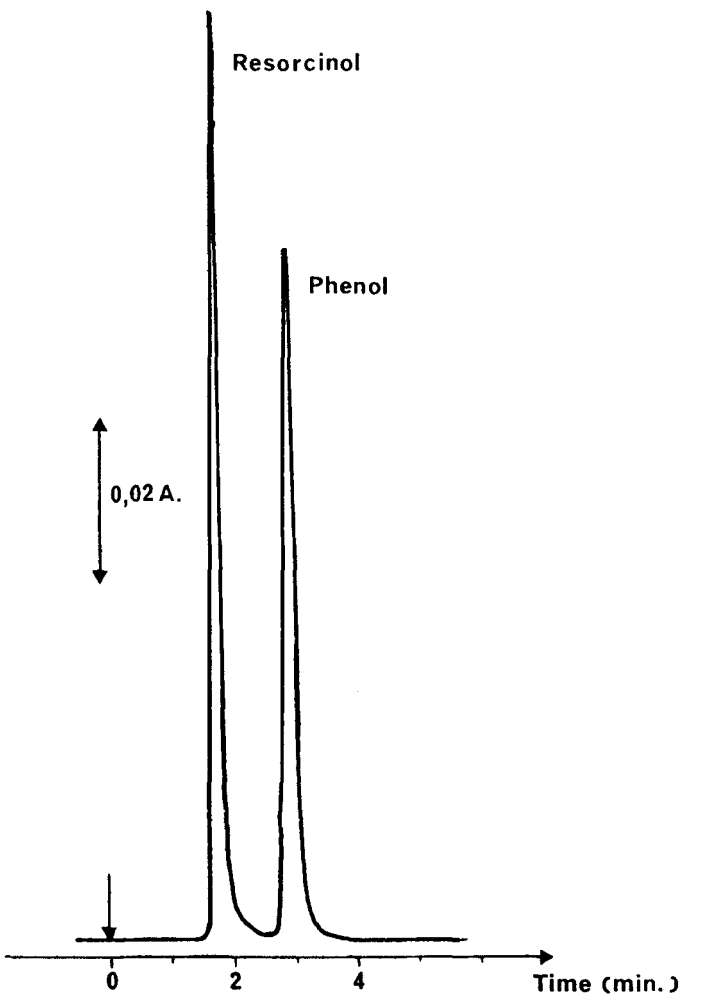

FIG. 2. Analytical chromatogram of an equimolar resorcinol-phenol mixture. 15 cm × 0.48 cm i.d. column. Stationary phase: methanol-water (45:55). Flow rate: 100 mL/h. Injection: $V_0 = 2\mu L$; $C_0 = 0.228\ M$. Spectrophotometric detection at 246 nm.

RESULTS

The effect of the analytical capacity factors, selectivity, and resolution on the amount recovered per injection Q_r and on the production rate R_h (criteria) at constant impurity and recovery ratios (constraints) was studied experimentally. In this study resorcinol–phenol mixtures were used as the test samples. The interest in this separation comes from the possibility of setting the analytical selectivity and resolution in a rather wide range by only modifying the mobile phase composition and keeping constant the stationary phase nature and the mobile phase flow-rate.

Analytical Chromatography

Prior to any preparative injection, it is absolutely necessary to test the analytical performances of a preparative column. For the various mobile phases studied, the characteristics of resorcinol–phenol analytical separations on the preparative column are given in Table 2. As shown, the plate number N remains roughly constant in all the experiments. For what follows, it is worth pointing out that the resolution values 6.5 and 4.6 are afforded by an identical value of the selectivity, $\alpha = 4$.

Preparative Chromatography

For preparative separation it was decided to choose 0.5°/o as the maximal impurity ratios Ti_1 and Ti_2 and 98.5% as the minimal recovery ratios Tr_1 and Tr_2.

TABLE 2
Characteristics of the Analytical Resorcinol (1)–Phenol (2) Separations[a]

Methanol content in the mobile phase (%)	Capacity factor k'		Plate number N		Selectivity α	Resolution R_s
24	(1)	1.3	(1)	710	3.9	6.5
	(2)	5.1	(2)	860		
45	(1)	0.4	(1)	910	4.0	4.6
	(2)	1.6	(2)	1050		
62	(1)	0.18	(1)	850	3.1	2.2
	(2)	0.55	(2)	940		

[a]Operating conditions are given in the Experimental section.

With each mobile phase composition studied, two optimal injections were determined: the first by the linear optimization procedure and the second by the nonlinear one.

In the linear optimization procedure, the maximal injected volume V_{0_1} was calculated from Eq. (2). The results are given in Table 3. The maximal concentration C_1 was determined experimentally by injecting V_{0_1} sample volumes of increasing concentrations until the desired constraint values were attained. As an example for the first mobile phase studied, Table 4 shows how an increase in concentration causes the impurity ratios to increase and the recovery ratios to decrease. Figure 3 represents a linear preparative chromatogram corresponding to an analytical resolution of 4.6 and an analytical selectivity of 4. The full line profile is obtained from the on-line

TABLE 3
Estimation of the Maximal Injected Volume for the Linear (V_{0_1}) and Nonlinear ($V_{0_{n1}}$) Optimization Procedures: (1) Resorcinol, (2) Phenol

Methanol content in the mobile phase (%)	Analytical retention volume (mL)		Analytical standard deviation (mL)		V_{0_1} (mL) from Eq. (2)	τ values for phenol[a]
24	(1)	18.8	(1)	0.71	26.6	
	(2)	50.3	(2)	1.72		(2) 7.8
45	(1)	11.2	(1)	0.37	7.2	
	(2)	20.4	(2)	0.63		(2) 3.2
62	(1)	9.4	(1)	0.32	1.65	
	(2)	12.5	(2)	0.41		(2) 1.9

[a]Upper limit for $V_{0_{n1}}$.

TABLE 4
Experimental Determination of the Maximal Concentration Injected C_{0l} by the Linear Optimization Procedure[a]

V_0 (mL)	C_0 (M)	Q_0 (mM)	Ti_1 (%)	Ti_2 (%)	Tr_1 (%)	Tr_2 (%)
26	10^{-3}	0.026	0	0	100	100
25	5×10^{-3}	0.125	0.05	0.2	99.7	99.6
25.8	10^{-2}	0.26	0.25	0.4	99.1	99.1

[a]Analytical data: see Table 2 (methanol content = 24%) (maximal volume that can be theoretically injected in that case: $V_{0_1} = 26.6$ mL).

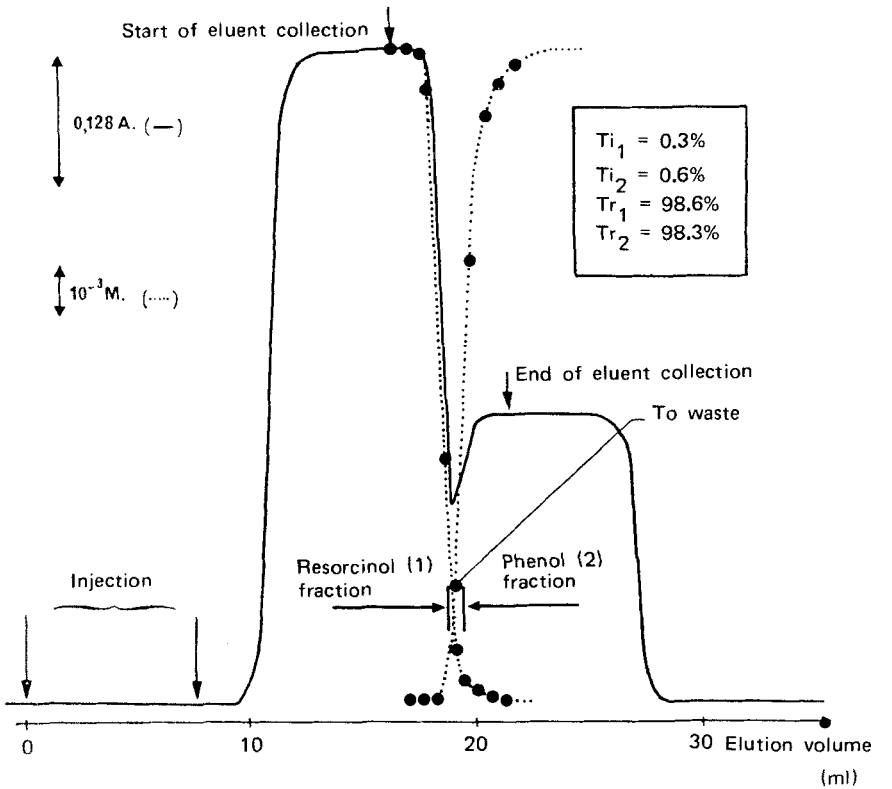

FIG. 3. Linear preparative chromatogram of an equimolar resorcinol-phenol mixture. 25 cm × 0.76 cm i.d. column. Stationary phase: Lichroprep R.P.8 (25–40 μm). Mobile phase: methanol-water (45:55); $D = 275$ mL/h. Injection: $V_0 = 7.75$ mL; $C_0 = 1.26 \times 10^{-2}$ M. The line shows the recorder trace of absorbance at 237 nm; the flat upper portions correspond to the concentration injected ($V_0 > 4\sigma$). The filled circles show concentrations calculated from the quantitative analysis of effluent fractions (see text).

UV detector. The dotted line profile, obtained from quantitative analysis of the effluent fractions collected, gives the actual concentration profiles of the two compounds. In this connection the user is sometimes at a loss as to how to select the fraction volume. We ascertained that a proper order of magnitude is given by the standard deviations σ_1 and σ_2 of the two initial analytical peaks. Here $\sigma_1 = 0.37$ mL and $\sigma_2 = 0.63$ mL. So, a 0.5-mL fraction volume can be chosen here.

The optimal effluent cut-volume between the resorcinol and phenol fractions was adjusted by looking for the best compromise between the impurity and the recovery ratios. As a general rule, it is not possible to shift the effluent cut-volume in order to simultaneously increase the recovery ratio and decrease the impurity ratio. As previously mentioned by Pretorius et al. (*12*), it is often advantageous to divide the effluent not into two, but into three fractions; the third one is a narrow middle fraction consisting of a mixture of the two compounds which can be discarded. This technique was used here and resulted in a substantial decrease in the impurity ratio and a very slight decrease in the recovery ratio.

In the nonlinear optimization procedure, the order of magnitude of the maximal injected amount of phenol is calculated from Eq. (9). To do so we need the values of the model parameters C_m and τ for phenol and all the mobile phase compositions studied. The τ values for phenol are also needed to assess the upper limit for the injected sample volume ($V_{0_{n1}} < \tau$). The couple (C_m, τ) relative to phenol is available from previous experimental work (*9*), but only with a (24:76) methanol–water mobile phase:

$$C_m = 0.8\ M \qquad \tau = 7.8\ \text{mL}$$

Since τ is proportional to V_R and does not depend on the solute nature, its value can be derived for the other mobile phase compositions. The results are given in Table 3 and enable us to choose the injected volume according to condition (10). The maximal phenol amount is thus $Q_{0_{n1}} = 1.34$ mM for that mobile phase composition. However, the experiment showed that a 1.30-mM injection of each compound ($V_0 = 2.6$ mL, $C_0 = 0.5\ M$) leads to low impurity ratios ($T_{i_1} = 0\%$; $T_{i_2} = 0.1\%$) and too high recovery ratios ($Tr_1 = 99.8\%$, $Tr_2 = 99.9\%$). With the prerequisite impurity and recovery ratios, the optimal amount in that case is about twice what was predicted by the model (see Table 5). For the other mobile phases the optimal injections were found by taking into account the results already found. As an example, Fig. 4 shows one of the nonlinear preparative chromatograms. The analytical resolution was 2.2 and the analytical selectivity 3.1. Given that here $\sigma_1 = 0.32$ mL and $\sigma_2 = 0.41$ mL, a 0.25-mL fraction volume can be chosen. The dotted profiles are the actual concentration profiles.

For the three mobile phases studied, the characteristics of the linear and nonlinear optimal injections and the resulting values of the criteria (production rate R_h and amount recovered per injection Q_r) and constraints (impurity and recovery ratios T_i and T_r) are pooled in Table 5. Here the amount recovered per injection Q_r is nearly equal to the injected amount Q_0 since the recovery ratio is due to be at least 98.5%.

TABLE 5
Effect of the Mobile Phase Composition (Rs, α) and the Optimization Procedure (L = linear, N.L. = nonlinear) on the Production Rate

R_s	Optimization	V_0 (mL)	C_0 (M)	Q_0 (or Q_r) (mM)	T_{i_1} (%)	T_{i_2} (%)	T_{r_1} (%)	T_{r_2} (%)	Cycle time (h)	Production rate (mM/h)
$R_s = 6.5$,	L.	25.8	10^{-2}	0.26	0.25	0.4	99.1	99.1	0.29	0.88
$\alpha = 3.9$	N.L.	3.15	8×10^{-1}	2.52	0.2	0.4	99.4	98.8	0.21	12.0
$R_s = 4.6$,	L.	7.15	1.26×10^{-2}	0.09	0.2	0.35	99.4	99.4	0.105	0.85
$\alpha = 4$	N.L.	1.7	6×10^{-1}	1.02	0.1	0.6	99.2	99.1	0.085	12.0
$R_s = 2.2$,	L.	1.35	3.5×10^{-2}	0.05	0.3	1.1	98.5	98.4	0.055	0.87
$\alpha = 3.1$	N.L.	0.6	8×10^{-1}	0.48	0.25	2.2	97.3	97.3	0.05	8.8

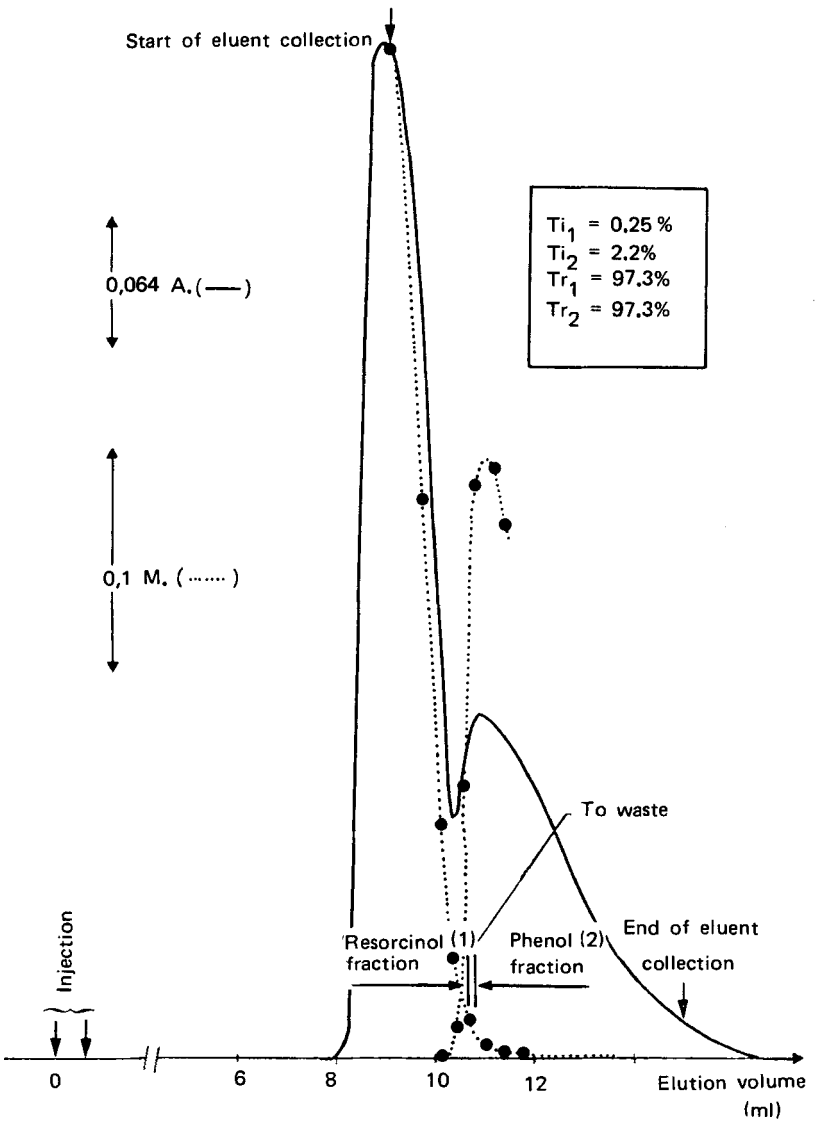

FIG. 4. Nonlinear preparative chromatogram of an equimolar resorcinol–phenol mixture. Operating conditions: see Fig. 3, except mobile phase is methanol-water (62:38) and injections are $V_0 = 0.585$ mL and $C_0 = 0.79$ *M*. The line shows the recorder trace of absorbance at 293 nm; the filled circles show concentrations calculated from quantitative analysis of the effluent fractions.

The cycle-time values were calculated according to Eq. (12). They increase with the injected volume. First of all, as we previously ascertained (*2*), the nonlinear optimization procedure leads to production rates and amounts recovered per injection about 10 times higher than these obtained from a linear optimization procedure.

If we now consider the amount recovered per injection Q_r (or Q_0), Table 5 shows that it increases with the analytical resolution. Thus, if Q_r is the main criterion, the mobile phase has to be selected so as to afford the highest analytical resolution; in this case increasing the selectivity is only one way, among others, to achieve the highest resolution. Furthermore, the number of injections necessary to achieve the required total purified amount is also lessened, which in turn lessens the hazard of peak overlapping between two consecutive injections. Consequently, the number of eluent fractions requiring to be analytically checked is lowered.

If, alternatively, the production-rate criterion is considered, identical values are obtained with identical selectivities ($\alpha = 4$) but different resolutions ($R_s = 6.5$ and 4.6). This result is valid for both the linear and nonlinear optimization procedures. The increase in resolution from 4.6 to 6.5 mainly comes from the increase in the analytical capacity factors. It is thus worthy of note that at constant selectivity the production rate is almost independent on the analytical capacity factors and resolution. It must be kept in mind that the analytical capacity factors also have an effect on the chromatographic system linearity and on the sample solubility in the mobile phase (*8*).

Moreover, when the selectivity decreases from 4 to 3, a simultaneous decrease in the linear and nonlinear production rates can be noticed: for the last two rows of Table 5, four constraints out of eight are not rigorously satisfied. Consequently, at constant impurity (0.5%) and recovery (98.5%) ratios, the linear and nonlinear production rates can be estimated to 0.7 and 7 mM/h, respectively. Finally, if the production rate is the main criterion, the mobile phase has to be selected so as to afford the highest analytical selectivity; resolution is no longer the key parameter.

CONCLUSION

The foregoing results verify the theoretical predictions and allow us to state general rules for selecting the best phase system in preparative chromatography.

The amount recovered per injection and the production rate are both improved by increasing the analytical selectivity, be the optimization linear or nonlinear. It may, however, happen that the mobile phase that gives the best selectivity is a poor solvent for the sample. (It must be emphasized that

capacity factors are in general inversely proportional to solubilities for a given compound in mixed solvents.) In this case a compromise between selectivity and solubility must be found.

The analytical capacity factor optimization is rarely independent of selectivity optimization. However, at constant selectivity, the production rate is independent of the analytical capacity factors, whereas the amount recovered per injection is enhanced by an increase in capacity factors and is directly related to the analytical resolution. This second rule applies both to linear and nonlinear chromatography and means that the proper choice of capacity factor depends on the optimization criterion.

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