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New approach to the computer-assisted selection of mobile phases for high-performance liquid chromatography on the basis of the Snyder theory[☆]

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Abstract

A new approach, based on the Snyder theory, consisting of the computer-assisted selection of suitable mobile phases for separation of non-ionic organic compounds by normal-phase liquid–solid chromatography is detailed. The adsorption properties of the mobile phases and the chromatographed compounds are predicted by taking into account literature data for the composing solvents and functional groups, respectively. The approach was successfully applied to the separation of twenty cis and trans esters by high-performance liquid chromatography (HPLC) using a preliminary characterized adsorbent and three suitable computer-selected mobile phases. © 1999 Elsevier Science B.V. All rights reserved.

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1. Introduction

In normal-phase liquid–solid chromatography such as column chromatography, HPLC and thin-layer chromatography (TLC), the stationary phase or adsorbent does not vary much while the variation of the mobile phases is vast. Thus, the most difficult problem is how to select a suitable mobile phase or phases for a given separation.

As an alternative methodology to the trial and error approach, we applied, over the last ten years, the Snyder theory [1–3] to enable an easier selection of suitable mobile phases and we developed software [4] called LSChrom. This software enables one to characterize mobile phases with the three parameters from the Snyder theory: mobile-phase strength, ϵ , which measures the adsorption energy of the mobile phase per unit of its area under adsorption, localization ability, m , which is connected with the orientation of the mobile phase relative to the adsorption sites and polarity, P' , measuring the ability of the mobile phases for interactions with compounds of different types. According to the Snyder theory, based on the displacement model, mobile phases for a suitable separation should have such values of ϵ

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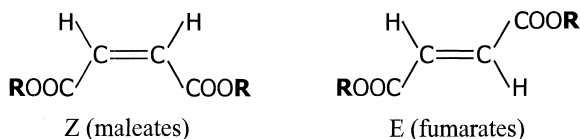
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that will lead to sample retention in the favorable range of $0.2 < k < 10$.

The approach, adopted by us until now (see for instance refs. [5–8]), included the experimental selection of a mobile phase which has a suitable strength, ϵ , and computer selection of other mobile phases having the same or close values of ϵ and different values of m and P' leading to variation of retention and separation.

Recently, one of us (Ch. P.) has incorporated in the LSChrom software another unique aspect of the Snyder theory allowing to predict retention on the basis of solute molecular structure for an adsorbent (silica or alumina) and any value of ϵ . For the given compounds studied, this approach is expected to facilitate the selection of a suitable range of ϵ ensuring retention in the favorable interval ($0.2 < k < 10$). It requires a preliminary characterization (see below) of the adsorbent that will be used.

The present paper shows a first application of the latest version of LSChrom for selection of suitable mobile phases for HPLC of twenty *cis* (*Z*) and *trans* (*E*) esters studied earlier by TLC [5,6].



R = alkyl or cycloalkyl

2. Theory

The Snyder theory describes the adsorption properties of individual functional groups i by their adsorption energy Q_i^o relative to pentane ($\epsilon = 0$) and area under adsorption a_i . The values of Q_i^o and a_i for the functional groups encountered most often in the structure of organic compounds are established [1].

A given compound X is characterized according to an adsorbent with activity $\alpha' = 1$ by its adsorption energy S_X and its molecular area under adsorption A_X . The ratio $\epsilon_X = S_X/A_X$ is the adsorption strength of compound X which is a measure of the adsorption energy of X per unit of its molecular area under

adsorption. The parameter ϵ_X is introduced for the first time by analogy with the mobile-phase strength ϵ .

In a first approximation, S_X and A_X are expressed by the following sums:

$$S_X = \sum_{i=1}^{ng} w_i \cdot Q_i^o \quad (1)$$

$$A_X = \sum_{i=1}^{ng} w_i \cdot a_i \quad (2)$$

where w_i shows how many times a given group i is encountered in the structure of compound X in which ng represents the total number of groups.

The compounds studied by us are stereoisomers which have equal values of S_X and A_X since they have the same functional groups. Thus if we use Eqs. (1) and (2), we do not take into account the stereoisomerism.

Improved precision is obtained if we differentiate between the two isomers and take into account the difference in the adsorption properties of the two isomers and the group localization (g_{loc}) within a given molecule. Only the most strongly adsorbing group k having the greatest value of Q_i^o is adsorbed with localization and the remaining groups are delocalized.

$$S_X = \sum_{i=1}^{ng} w_i \cdot Q_i^o - S_{g_{loc}} + S^{\text{isomer}} \quad (3)$$

$$A_X = \sum_{i=1}^{ng} w_i \cdot a_i + A^{\text{isomer}} \quad (4)$$

where $S_{g_{loc}}$ (expressed by a given product in ref. [1], p. 273) is a correction, calculated on the basis of Snyder's data, for the different localization of the groups and S^{isomer} and A^{isomer} are corrections for the contribution of stereoisomerism to the adsorption energy and area under adsorption, respectively. The Snyder theory is semiempirical and the parameters S^{isomer} and A^{isomer} of each isomer can be found from experimental data.

The thermodynamic description of the displacement model allows to express [1,2] the retention k_X of compound X as a function of mobile-phase strength ϵ taking into account the adsorption properties of X and the adsorbent.

$$k_X(\epsilon) = 10^{k_{\text{shift}} + \alpha'(S_X - \epsilon \cdot A_X)} \quad (5)$$

or

$$k_X(\epsilon) = 10^{k_{\text{shift}} + \alpha'(\epsilon_X - \epsilon)} \quad (5a)$$

where α' and k_{shift} depend only on the adsorbent. The parameter k_{shift} is an abbreviation for the phase ratio $\log [V_a/(V^o/W)]$. The notation “shift” comes from the fact that the phase ratio, along with α' , is a factor leading to variation or shift of the retention of a given compound from one laboratory to another. The two parameters α' and k_{shift} are simultaneously determined (see below) for a given adsorbent by the chromatography of test compounds with known values of S_X with pentane or hexane having $\epsilon=0$.

Eq. (5a) transforms to the following expression if one takes into account the localization ability m and m_X (Δ_X in ref. [2], Fig. 17 and Eq. 41) of the mobile phase and compounds chromatographed, respectively.

$$k_X(\epsilon) = 10^{k_{\text{shift}} + \alpha'(\epsilon_X - \epsilon) - m \cdot m_X} \quad (6)$$

The LSChrom software uses Eq. (5) to select the range of suitable mobile-phase strength $\epsilon_{\text{recommended}}$ prior to selection of m . The retention with a given mobile phase and m can be calculated more precisely, if necessary, by Eq. (6).

3. Experimental

Details on the synthesis and $^1\text{H-NMR}$ spectra of the compounds studied are given in ref. [5].

HPLC was performed with a column 25 cm \times 3.2 mm using LiChrosorb Si 60, 5 μm and three computer selected mobile phases. The flow-rate was 1 ml/min. *p*-Xylene was used to determine t_0 (1.71 min). The column showed a value for the number of theoretical plates N of about 10 000 and this value was input in the software LSChrom. In the case of the mobile phase hexane–ethyl acetate 90:10, refractive indexes were measured by a differential refractometer (Knauer, Berlin, Germany). In all other cases, UV detector Uvikon LCD 725 (Kontron, Zurich, Switzerland) was used at 280 nm. The

experimental values of retention factor k are arithmetic means of two measurements.

The computer selection of the mobile phases was done by the software developed by us, LSChrom [4] Version 2.1 for Windows. A demo version of this software can be downloaded from the Internet at <http://www.members.tripod.com/LSChrom>. Version 2.1 of the software includes some corrections of the procedure [4] for the calculation of the mobile-phase strength ϵ eliminating some of the shortcomings described [9,10]. The most important was an improvement of the analytical expression (ref. [2], Eq. 40) of a given function solving the problem with rare decreases in ϵ with the increase of the amount of the most polar solvent as described [10].

4. Results and discussion

We propose the following approach for the selection of suitable mobile phases for a given separation by normal-phase liquid–solid chromatography. The procedure includes the following steps:

- (1) Preliminary characterization of the adsorbent that will be used.
- (2) Calculation of the adsorption properties of the compounds studied on the basis of their functional groups and corresponding literature data.
- (3) Selection of a range of suitable mobile-phase strengths $\epsilon_{\text{recommended}}$ for a given separation on the basis of an analysis of the calculated retentions of the compounds studied as a function of mobile-phase strength.
- (4) Selection of solvents for a suitable mobile phase and calculation of the amount of each which will ensure ϵ will be in the desired range $\epsilon_{\text{recommended}}$.
- (5) Chromatography of the compounds studied with the selected mobile phase.

Step 1. Any type of adsorbent can be characterized, approximately, by a definite value of selectivity α' . For instance, we have always used $\alpha'=0.57$ for all silicas studied by TLC [5,7,8].

The prediction of $\epsilon_{\text{recommended}}$ by Eq. (5) is only tentative. A more precise characterization of the adsorbent that will be used will give a better prediction. This made us measure α' and k_{shift} . To this end, we chromatographed, with hexane, three

Table 1
Data for S_T [11] and k_T found for the test compounds, T , used

Test solute T	S_T	$\log(k_T)$
<i>p</i> -Xylene	1.77	0.205
Naphthalene	2.02	0.357
Nitrobenzene	4.25	1.446

test compounds T (*p*-xylene, naphthalene and nitrobenzene) having known values [11] of adsorption energy S_X . On the basis of their experimental retention k_X and adsorption energy S_X given in Table 1, we found, as shown in Fig. 1, that $\alpha' = 0.496$ and $k_{\text{shift}} = -0.659$ for LiChrosorb Si 60, 5 μm . We inputted these two values into the LSChrom software.

Step 2. In this step, any compound X studied is decomposed to its functional groups. The user selects the relevant groups from a list included in LSChrom and specifies their number. The corrections $S_{\text{g,loc}}$, S^{isomer} and A^{isomer} are included as relevant groups by the user, and finding their values is described below. The computer calculates the values of adsorption energy S_X and area under adsorption A_X using Eq. (3) and Eq. (4), respectively, using the information entered by the user and the data for Q_i^o and a_i of

Snyder (ref. [1], Table 8-4 and Table 10-2) incorporated in a corresponding file. For the moment, these calculations are only possible for compounds whose functional groups are available in the list offered by the software.

As an example, the decomposition of the isopropyl esters studied (*Z* and *E*) is shown in detail (see also ref. [1], pp. 380–390 and ref. [6]). Both of these two compounds contain one double bond and two ester groups $\text{CO}_2\text{CH}(\text{CH}_3)_2$. Formally, the isopropyl group can be considered as equivalent to one methyl ester group ($\text{Al}-\text{CO}_2\text{CH}_3$), one methylene group ($\text{Al}-\text{CH}_2-\text{Al}$) and one methyl group ($\text{Al}-\text{CH}_3$) where Al=alkyl chain. This decomposition of the compounds into their functional groups allows one to calculate $\sum_{i=1}^{ng} w_i \cdot Q_i^o$ and $\sum_{i=1}^{ng} w_i \cdot a_i$ on the basis of the above-mentioned data of Snyder. We will give an example of one of the calculations for any of the isopropyl esters:

$$\begin{aligned} \sum_{i=1}^{ng} w_i \cdot Q_i^o &= 2 \cdot Q_{\text{C}=(\text{an olefinic carbon atom})}^o + 2 \\ &\quad \cdot Q_{\text{Al}-\text{CO}_2\text{CH}_3}^o + 2 \cdot Q_{\text{Al}-\text{CH}_3}^o + 2 \\ &\quad \cdot Q_{\text{Al}-\text{CH}_2-\text{Al}}^o \\ &= 2 \times 0.25 + 2 \times 5.27 + 2 \times 0.07 + 2 \end{aligned}$$

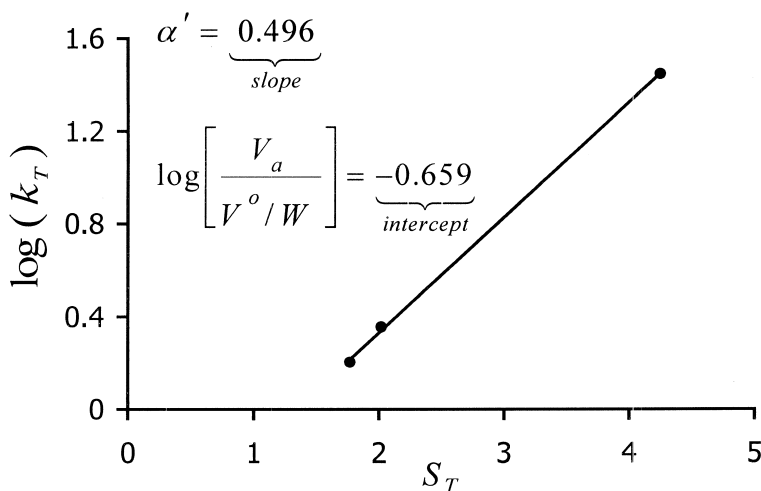


Fig. 1. Characterization of the adsorbent.

$$\times (-0.05) = 11.08$$

For the individual compounds studied, we assumed, on the basis of the data from the application [5] of the Soczewiński method, that the two equal ester groups CO_2R are the localizing groups k showing Q_k^o for silica in the range 4.90–5.22 (ca. 5.0) and the double bond ($Q_i^o = 0.50$) is delocalized. This leads to an approximate absolute value of $S_{g,\text{loc}} = f(Q_k^o) \cdot \sum^{i \neq k} Q_i^o = (2 \times 0.52) \times 0.50 = 0.52$ on the basis of ref. [1], Eq. 10-5 and Table 10-4 at $p = 2$. It is worth noting that the Snyder theory considers that, in general, for two equal groups one of them is localized and the other is delocalized.

The values of the corrections S^{isomer} and A^{isomer} were found in the following way. On the basis of Eq. (5), the linear regression analysis of the experimental retention of the compounds studied, [5] in a logarithmic form as a function of ϵ of the corresponding mobile phases afforded the values of S_X and A_X for each isomer. This allowed one to find the desired corrections using Eqs. (3) and (4). The values

obtained for the Z and E isomers were $S^Z = 0$, $A^Z = 0$, $S^E = -1.36$ and $A^E = -1.1$. The negative values for the E isomers correlates with their weaker retention in TLC [5] with comparison to the Z isomers. The corrections for the stronger retained Z isomers are zero, showing that its experimental retention is predicted by Eqs. (3)–(5) without taking into account corrections owing to the stereoisomerism.

Table 2 shows the values of S_X , A_X and ϵ_X obtained for all the compounds studied. The values of the first two parameters were calculated by LSChrom using Eqs. (3) and (4), literature data for the composing groups and the above mentioned corrections. The values of ϵ_X vary in the range 0.29–0.46.

Step 3. Using the data for α' , k_{shift} , S_X and A_X from Steps 1 and 2, LSChrom predicts using Eq. (5) the retention of the compounds studied as a function of the mobile-phase strength and selects a range of suitable strength $\epsilon_{\text{recommended}}$ of the mobile phases for a given separation. To this end, the software makes a

Table 2
Adsorption properties of the compounds studied

Compound			S_X	A_X	$\epsilon_X = S_X/A_X$
R	Configuration	No.			
$\text{RO}_2\text{C}-\text{CH}=\text{CH}-\text{CO}_2\text{R}$					
CH_3	Z	1	10.52	23.00	0.46
	E	2	9.16	21.90	0.42
CH_2CH_3	Z	3	10.42	24.80	0.42
	E	4	9.06	23.70	0.38
$\text{CH}_2\text{CH}_2\text{CH}_3$	Z	5	10.32	26.60	0.39
	E	6	8.96	25.50	0.35
$\text{CH}(\text{CH}_3)_2$	Z	7	10.56	28.00	0.38
	E	8	9.20	26.90	0.34
$\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_3$	Z	9	10.22	28.40	0.36
	E	10	8.86	27.30	0.32
$\text{CH}(\text{CH}_3)\text{CH}_2\text{CH}_3$	Z	11	10.46	29.80	0.35
	E	12	9.10	28.70	0.32
$\text{CH}_2\text{CH}(\text{CH}_3)_2$	Z	13	10.46	29.80	0.35
	E	14	9.10	28.70	0.32
$\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_3$	Z	15	10.12	30.20	0.34
	E	16	8.76	29.10	0.30
$\text{CH}_2\text{CH}_2\text{CH}(\text{CH}_3)_2$	Z	17	10.36	31.60	0.33
	E	18	9.00	30.50	0.30
C_6H_{11} (cyclohexyl)	Z	19	9.78	30.60	0.32
	E	20	8.42	29.50	0.29

visualized analysis of the calculated retention k and resolution R_s , found on the basis of N and k . LSChrom selects as $\epsilon_{\text{recommended}}$ those values of ϵ for which R_s has maximum values and the retention is more or less in the favorable range. The user can easily find that part of the automatically proposed range of $\epsilon_{\text{recommended}}$ where the retention is only within $0.2 < k < 10$.

The analysis of k and R_s of all compounds studied showed that the two isomeric forms are expected to show proper retentions and to be completely separated by HPLC with the available column and mobile phases having strengths in the range $0.23 \leq \epsilon \leq 0.30$. These values of $\epsilon_{\text{recommended}}$ are up to 0.2 units less than the values of the sample adsorption strength ϵ_x shown in Table 2. If this correlation is kept in general, it could be used to deduce approximately $\epsilon_{\text{recommended}}$ on the basis of ϵ_x .

Step 4. There are plenty of mobile phases having ϵ in the recommended range $0.23 \leq \epsilon \leq 0.30$ for the compounds studied. We selected arbitrarily three such mobile phases. The selection of one of them (hexane–MTBE 92:8) will be given in detail. The mobile-phase strength $\epsilon = 0.263$ is in the recommended range but a question arises as to which mobile phase has such a strength. An answer to this question is given by the list available in LSChrom comprising of about 200 multi-component mobile phases which differ in composition. The computer program calculates, by its reverse calculation mode [4], the amounts of the solvents of any mobile phase that will ensure the desired value of $\epsilon = 0.263$. Thus, the mobile phases included in the list in LSChrom are accompanied by their corresponding values of ϵ , m and P' . They can be sorted by any of these three parameters. We selected from this list the binary mobile phase, hexane–MTBE 92:8, having a strength of $\epsilon = 0.263$ and localization ability $m = 0.71$. Similarly, the mobile phases, hexane–ethyl acetate 90:10 and hexane–diethyl ether 80:20, were selected.

Step 5. We performed HPLC on the compounds studied with the three selected mobile phases having ϵ in the range predicted by LSChrom ($0.23 \leq \epsilon \leq 0.30$) of $\epsilon_{\text{recommended}}$. Table 3 shows the values of ϵ , m and P' of these mobile phases and the corresponding experimental values of the retention factor k and the separation α for the compounds studied.

The most important conclusion based on this data is that the retention k of compounds 1–2 varies from 0.20 to 9.10 covering almost the whole favorable range ($0.2 < k < 10$), i.e. the computer selection of the range of $\epsilon_{\text{recommended}}$ and specific mobile phases is adequate. In addition, any mobile phase leads to separation of all Z – E isomeric pairs studied. The mean value of the separation α is 0.55 versus 0.34 for the case of TLC [5,6].

We would like to point out other features of the data obtained. Similarly to TLC [5,6], any Z isomer has a greater retention than the corresponding E isomer as seen from the positive values of $\log \alpha$ in Table 3. Mobile phases hexane–ethyl acetate 90:10 and hexane–methyl *tert.*-butyl ether (MTBE) 92:8 have equal values of $\epsilon = 0.263$ but different values of m and eventually P' (for the second mobile phase, the value of P' cannot be calculated). This accounts the different retentions found for any compound with these two mobile phases. The second mobile phase has a greater value of m (0.71) and shows a better separation ($\log \alpha = 0.58$) than the first mobile phase ($m = 0.71$ and $\log \alpha = 0.49$). Mobile phase hexane–diethyl ether 80:20 has a somewhat greater value of ϵ (0.293) and the retentions found are usually smaller as it is expected.

5. Conclusions

Independent of the fact that the present successful application of the Snyder theory and software, LSChrom, is related to isomer separation, this software is expected to be of general use for an easier selection of suitable mobile phases for separation of non-ionic compounds by normal-phase HPLC and TLC keeping in mind the following details.

(1) The present version of LSChrom (see the Experimental section) predicts, automatically and roughly, the suitable range of $\epsilon_{\text{recommended}}$ using Eqs. (1), (2) and (5) on the basis of the sample structure using the necessary values of Q_i^o and a_i from ref. [1], Table 8-4 and 10-2.

(2) The user can improve the accuracy of this prediction by incorporating into the software: (a) experimental values of N , k_{shift} , α' , and eventually S^{isomer} and A^{isomer} and, (b) values of $S_{\text{g,loc}}$ derived on

Table 3

Experimental data for the retention factor k and the separation α of the compounds studied with three computer-selected mobile phases

Compound			Retention factor k			
R	Configuration	No.	H-EA 90:10 ^a	H-MTBE 92:8	H-EE 80:20	
RO ₂ C-CH=CH-CO ₂ R			ϵ	0.263	0.263	0.293
			m	0.56	0.71	0.62
			P'	0.53	–	0.64
CH ₃	Z	1	4.68	9.10	3.59	
	E	2	1.74	3.10	1.29	
CH ₂ CH ₃	Z	3	2.40	5.80	2.20	
	E	4	0.93	1.70	0.73	
CH ₂ CH ₂ CH ₃	Z	5	1.37	3.50	1.45	
	E	6	0.53	0.96	0.44	
CH(CH ₃) ₂	Z	7	1.46	3.20	1.37	
	E	8	0.52	0.90	0.42	
CH ₂ CH ₂ CH ₂ CH ₃	Z	9	1.13	2.60	1.13	
	E	10	0.35	0.57	0.27	
CH(CH ₃)CH ₂ CH ₃	Z	11	0.98	2.10	0.94	
	E	12	0.32	0.58	0.27	
CH ₂ CH(CH ₃) ₂	Z	13	1.06	2.20	0.99	
	E	14	0.35	0.56	0.25	
CH ₂ CH ₂ CH ₂ CH ₂ CH ₃	Z	15	0.93	2.10	0.96	
	E	16	0.25	0.48	0.21	
CH ₂ CH ₂ CH(CH ₃) ₂	Z	17	0.90	2.00	0.91	
	E	18	0.23	0.46	0.19	
C ₆ H ₁₁ (cyclohexyl)	Z	19	1.06	2.30	1.06	
	E	20	0.29	0.54	0.26	
			log α			
			H-EA 90:10	H-MTBE 92:8	H-EE 80:20	
1–2			0.43	0.47	0.44	
3–4			0.41	0.53	0.48	
5–6			0.41	0.56	0.52	
7–8			0.45	0.55	0.51	
9–10			0.51	0.66	0.62	
11–12			0.49	0.56	0.54	
13–14			0.48	0.59	0.60	
15–16			0.57	0.64	0.66	
17–18			0.59	0.64	0.68	
19–20			0.56	0.63	0.61	
log α			0.49	0.58	0.57	
			Average log $\alpha = 0.55$			

^a H=hexane, EA=ethyl acetate, MTBE=methyl *tert.*-butyl ether, EE=diethyl ether.

the basis of the data of ref. [1], Table 10-4. In this case the prediction is done by Eqs. (3)–(5) and is described in detail in this paper.

(3) The user can take into account the effect of steric hindrance on Q_i^o (ref. [1], Table 11-3) and

other specific effects summarized in ref. [1] by inputting the corresponding values into the software.

(4) The user is recommended to select and use mobile phases with ϵ values in the range of $\epsilon_{\text{recommended}}$ and different values of m which is a way

to improve the separation of the compounds studied as shown, for instance, in refs. [2] and [5–8].

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