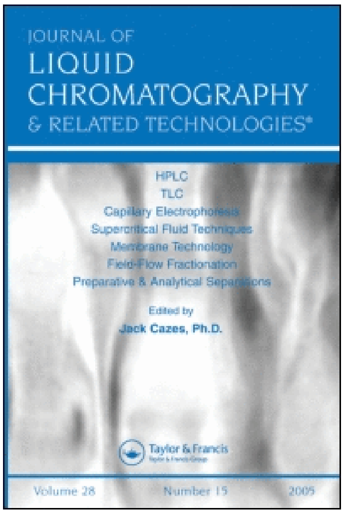

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Automatic Selection of Mobile Phases. IV. Thin-Layer Chromatography on Silica of Simple and Complex Organic Compounds

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Automatic Selection of Mobile Phases. IV. Thin-Layer Chromatography on Silica of Simple and Complex Organic Compounds

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ABSTRACT

The LSChrom software incorporating the Snyder theory was applied to organic compounds with simple and complex structure being *cis* or *trans* olefinic derivatives and substituted tetrahydroisoquinolines, respectively. Lists of recommended mobile phases for thin-layer chromatography (TLC) on silica of the compounds studied were obtained, avoiding any preliminary trial and error experiments. The procedure is based on the structure of any compound, literature data for the adsorption properties of the relevant groups available in the structure, and numerous calculations. The experiments with 12 arbitrarily selected mobile phases from the recommended lists showed, for about 150 measurements, the proper retention of any compound and good separation of the *cis*–*trans*

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isomers. Thus, a new successful application of the both theory and software was achieved.

Key Words: Normal-phase liquid chromatography; Silica; *E*/*Z*-olefins; N-containing heterocycles; Mobile-phase selection; Snyder theory in LSChrom software.

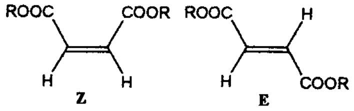
INTRODUCTION

The simplicity, high efficiency in analyzing, simultaneously, a large number of samples, and short analysis time, make thin-layer chromatography (TLC) a widely used separation method, even in the 21st century. The difference in the interactions in the sample-stationary phase and mobile phase–stationary phase is the driving force to achieve separations of many organic compounds by TLC. This method is used daily in laboratories in organic synthesis, natural compounds, medicine, ecology, and industry. The stationary phase is usually polar (silica or alumina) and the mobile phase is non-polar. Consequently, TLC refers to the normal-phase liquid chromatography (NPLC) where separation is governed by adsorption. Nowadays, silica is the first choice for the stationary phase. Thus, the greatest problem in using TLC in specific cases is the selection of suitable mobile phases. The adsorption properties of the mobile phases should match the adsorption properties of the analytes. The correct composition of the mobile phase is usually found by trial and error where many preliminary experiments are done prior to the final analysis.

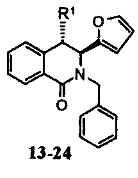
The present series of papers[1–3] offers a new approach for the selection of mobile phases, free from preliminary experiments. Because of this, the approach is considered as automatic selection. It is based on the well-known Snyder theory.[4–6] Its application needs relevant software. The first software of this kind was elaborated by us and called LSChrom.[7] The second, and last, version[8,9] allows for an automatic selection of mobile phases for analysis of organic compounds with known structures. The aim of the papers of this series, is to study the use of the Snyder theory and LSChrom Version (Ver) 2 for proper prediction of suitable mobile phases and, thus, to outline their scope and limitation. Many software products (for instance, see Refs.[10–13]) are used in liquid chromatography. To our knowledge, none performs automatic selection of mobile phases for NPLC.

People using TLC have different qualifications and the majority are not chromatographers. Many of these people are afraid of chromatographic theories or do not have time to comprehend them. LSChrom incorporates some parts of the Snyder theory. Thus, users not familiar with this theory can apply it for solving their specific problems.

This paper describes the application of LSChrom Ver 2 and the Snyder theory, to properly predict suitable mobile phases for TLC on silica of compounds **1–24** from two different groups. Compounds **1–12** are maleates and fumarates having *cis* (*Z*) or *trans* (*E*) configuration, respectively. They are interesting model compounds that were used in our previous TLC[14,15] and HPLC[8] studies. Compounds **13–24** are substituted tetrahydroisoquinolines with complex structures having *trans* configuration. We have used the results of the present study in their preparation[16] to determine the optimum reaction time that is different for any compound. The latter has been obtained in a separate vessel and, thus, separation of compounds **13–24** was not necessary.



1-12



13-24

EXPERIMENTAL

Tables 1–4 summarize the data obtained in this study.

Compounds **1–12** were prepared by esterification of the parent maleic and fumaric acids (see Ref.[14]). Compounds **13–24** were synthesized[16] in analogy to Ref.[17] Their structures and configurations (see Tables 2 and 4) were elucidated by ^{1}H-NMR spectra at 400 MHz. The elemental analysis of any compound was within $\pm 0.40\%$ for the individual elements. Compounds **13–24** have *trans* configuration.

The stationary phases for TLC were two silicas: silica 1 = pre-coated TLC silica 60 F_{254} (Merck, Germany) plates and silica 2 = TLC silica 60 GF_{254} (Merck, Germany). Glass plates, 20 cm × 20 cm, were covered with thin layers (0.2 mm) of silica 2 as described in Ref.[18]. TLC was performed similarly to Ref.[1] with a travelling distance of 8 cm for compounds **1–12** and 18 cm for compounds **13–24**.

The computer program used was LSChrom Ver 2.1 for Windows[9] where calculation procedure 3.1 was made active.

The selected mobile phases by LSChrom are shown in Table 1. These mobile phases are composed of two to four solvents. They were prepared from the relevant solvents by use of analytical reagent grades. The experimental values of R_F (arithmetic means of two measurements), using these mobile

Table 1. Computer selected mobile phases 1–12.

No.	Composition	Vol.%	ε	m	P'
1	Hexane–ethyl acetate	90.84 : 9.16	0.257	0.57	0.49
2	Toluene–ethyl acetate	97.63 : 2.37	0.257	0.09	2.45
3	Toluene–methylene chloride	70.66 : 29.34	0.257	0.05	2.61
4	Toluene–chloroform–*tert*-butyl methyl ether–acetone	84.2 : 15 : 0.4 : 0.4	0.257	0.09	—
5	Benzene–acetonitrile	99.46 : 0.54	0.257	0.02	2.72
6	Cyclohexane–tetrachloromethane–tetrahydrofuran	87.4 : 5 : 7.6	0.257	0.90	0.21
7	Heptane–ethyl acetate	64.3 : 35.7	0.374	0.59	1.70
8	Heptane–acetone	78.5 : 21.5	0.374	0.93	1.25
9	Heptane–isopropanol	85.7 : 14.3	0.374	—	0.73
10	Heptane–isopropanol–ammonia	85.7 : 13.6 : 0.6	~0.374	—	—
11	Tetrachloromethane–acetonitrile	85.4 : 14.6	0.374	1.30	2.21
12	Heptane–ethyl acetate	56.7 : 43.3	0.394	0.59	2.02

phases, are summarized in Table 2, for compounds **1–12**, and in Table 4, for compounds **13–24**.

RESULTS AND DISCUSSION

Retention in liquid chromatography is expressed by R_F, R_M, and retention factor k that are related by the following well-known equation.[4]

$$R_M = \log k = \log\left(\frac{1}{\xi \cdot R_F} - 1\right) \tag{1}$$

where ξ was assumed 1.1 in the present study.[4] Greater values of R_F (faster movement of analyte X) in the chromatographic system correspond to smaller retention R_M or k and vice versa.

Separation α for any Z/E pair of compounds **1–12** was defined as

$$\log \alpha = R_{M(Z)} - R_{M(E)} \tag{2}$$

Table 2. Experimental R_F values of compounds **1–12** with computer selected mobile phases 1–6.

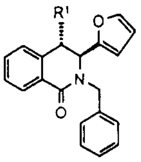
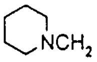
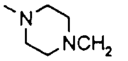
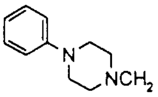
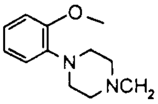
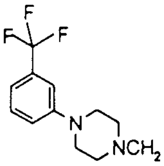
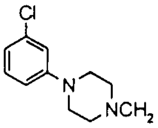
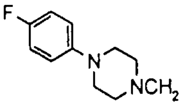
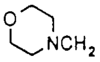
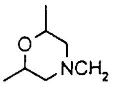
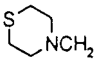
Compound				R_F for a given mobile phase					
R	Configuration	No.		1	2	3	4	5	6
$RO_2C-CH=CH-CO_2R$			ε	0.257	0.257	0.257	0.257	0.257	0.257
			m	0.57	0.09	0.05	0.09	0.02	0.90
			P'	0.49	2.45	2.61	—	2.72	0.21
CH_3	*Z*	**1**		0.21	0.17	0.12	0.17	0.18	0.15
	E	**2**		0.40	0.27	0.20	0.25	0.28	0.30
CH_3CH_2	*Z*	**3**		0.32	0.23	0.13	0.19	0.20	0.24
	E	**4**		0.51	0.32	0.22	0.29	0.31	0.41
$CH_3CH_2CH_2$	*Z*	**5**		0.41	0.27	0.16	0.23	0.26	0.31
	E	**6**		0.59	0.36	0.29	0.38	0.43	0.51
$CH_3CH_2CH_2CH_2$	*Z*	**7**		0.46	0.32	0.21	0.27	0.31	0.34
	E	**8**		0.65	0.43	0.35	0.41	0.49	0.56
$CH_3CH_2CH_2CH_2CH_2$	*Z*	**9**		0.52	0.35	0.25	0.29	0.41	0.39
	E	**10**		0.73	0.48	0.38	0.47	0.57	0.61
C_6H_{11} (cyclohexyl)	*Z*	**11**		0.52	0.33	0.24	0.29	0.33	0.41
	E	**12**		0.75	0.46	0.37	0.46	0.57	0.63
				log α for a given mobile phase					
		1,2		0.42	0.27	0.27	0.22	0.26	0.39
		3,4		0.37	0.21	0.28	0.25	0.26	0.36
		5,6		0.35	0.19	0.34	0.33	0.35	0.40
		7,8		0.39	0.21	0.32	0.29	0.36	0.43
		9,10		0.48	0.25	0.28	0.36	0.31	0.43
		11,12		0.54	0.25	0.28	0.34	0.47	0.43
		Average		0.43	0.23	0.30	0.30	0.34	0.41

Table 3. Structure description by functional groups and adsorption properties of compounds **13–24**.

Group i[a]	Q_i^o	a_i	Number of relevant functional groups in compound											
			13	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**
C=	0.25	1.00	16	16	16	16	22	22	22	22	22	16	16	16
Al–$\mathbf{CH_2}$–Al	−0.05	0.90	3	4	9	8	8	8	8	8	8	8	8	8
Al–$\mathbf{CH_3}$	5.80	10.50	−1	−1	−1		−1	−1	−1	−1	−1	−2		−2
Al–**N** (tert)	9.60	10.30			1	2	2	2	2	2	2	1	1	1
Al–$\mathbf{CONH_2}$	3.61	9.00	1	1	1	1	1	1	1	1	1	1	1	1
Al–$\mathbf{OCH_3}$	0.07	1.60	1	1	1	1	1	1	1	1	1	2	2	1
Al–$\mathbf{SCH_3}$	2.94	7.40												1
Al–$\mathbf{CO_2CH_3}$	5.27	10.50	1											
Al–**OH**	5.60	8.50		1										
Al–**F**	1.54	1.20							3					
Ar–**F**	−0.15	0.40									1			
Ar–**Cl**	−0.20	0.70								1				
Ar–$\mathbf{OCH_3}$	1.83	4.60						1						
ΣQ_i^o			22.26	22.54	22.49	28.41	29.84	31.67	34.46	29.64	29.69	26.08	26.22	25.41
Σa_i			46.90	45.80	52.30	63.50	67.90	72.50	71.50	68.60	68.30	58.80	62.00	57.20
R_F (calc. at $\varepsilon = 0.374$)			0.11	0.05	0.55	0.11	0.14	0.13	0.01	0.24	0.20	0.21	0.52	0.23

[a] Al, alkyl; Ar, aryl.

Table 4. Experimental R_F values of compounds **13–24** with computer selected mobile phases 7–12.

Compound			R_F for a given mobile phase					
R^1	No.		7	8	9	10	11	12
		ε	0.374	0.374	0.374	~0.374	0.374	0.394
		m	0.59	0.93	—	—	1.30	0.59
		P'	1.70	1.25	0.73	—	2.21	2.02
CH_3OOC	**13**		0.49	0.21	0.46	0.48	0.61	0.57
$HO\text{-}CH_2$	**14**		0.34	0.13	0.39	0.38	0.48	0.39
	**15**		0.61	0.38	0.54	0.59	0.74	0.67
	**16**		0.02	0.04	0.03	0.23	0.08	0.02
	**17**		0.57	0.28	0.53	0.55	0.71	0.65
	**18**		0.50	0.24	0.48	0.51	0.68	0.60
	**19**		0.57	0.26	0.52	0.54	0.72	0.65
	**20**		0.57	0.27	0.52	0.55	0.74	0.67
	**21**		0.54	0.25	0.50	0.53	0.69	0.64
	**22**		0.47	0.24	0.46	0.49	0.63	0.58
	**23**		0.56	0.31	0.50	0.54	0.70	0.65
	**24**		0.58	0.30	0.52	0.56	0.77	0.67

The theoretical background of LSChrom has been repeatedly described.[1–3,7,8] We would like to mention only those aspects that will clarify the discussion.

LSChrom Ver 2[9] uses Eqs. (3) and (3a) to predict the retention R_M of analyte X in NPLC (TLC and HPLC):

$$R_M = \log k = R_{M(\text{shift})} + \alpha' \, (S_X - \varepsilon \cdot A_X) \tag{3}$$

$$R_M = \log k = R_{M(\text{shift})} + \alpha' \left(\sum Q_i^o - \varepsilon \cdot \sum a_i \right) \tag{3a}$$

where the right-hand parameters are related to the adsorption properties of the stationary phase, compound X and mobile phase: $R_{M(\text{shift})}$, α'; S_x, A_x; and ε, respectively. Concerning X, its energy of adsorption S_x and area under adsorption A_x are additive. In an initial approximation, they are sums of the contributions Q_i^o or a_i, characteristic for any structural fragment i from the structure of X, as shown in Eq. (3a). The usual structural fragments, incorporated in most organic compounds, are characterized by literature values of Q_i^o and a_i.[4] These data are input in the software.

Until now, LSChrom Ver 2 includes available experimental values of $R_{M\ (\text{shift})}$ and α' for four specific stationary phases: TLC silica of Merck, TLC silica of Riedel-de-Haën, HPLC silica LiChrosorb Si 60 of Merck, and TLC alumina of Merck. The user has to select one of them including the relevant values in the calculations. For any other stationary phase, the values of the two parameters should be established preliminarily.[8]

For any compound that will be analyzed, the user of the software describes its structure, selecting relevant structural fragments from a list, and inputs their number. Thus, LSChrom Ver 2 can calculate S_x and A_x for the relevant compound.

Then, calculation of R_M by Eq. (3a) is done on the basis of the data obtained and successive values of mobile phase strength ε given by the software. Thus, the retention of any compound is predicted in the entire range of ε. An analysis of the retention, of all compounds studied, enables LSChrom Ver 2 to predict and show a recommended value of mobile phase strength ε ($\varepsilon_{\text{recommended}}$). The latter meets two criteria: proper retention in the range $0 < R_F < 1$ and best separation of the poorest separated pair of compounds.

Selection of concrete mobile phases having the recommended value of ε is the most difficult problem solved successfully in LSChrom Ver 2. Thus, a list of about 200 mobile phases is offered. The ratio among the composing solvents is calculated when the desired value of ε is specified. Moreover, the software can calculate ε, localization m, and polarity P' for any specific mobile phase. The last two parameters tune ε.

Automatic Selection of Mobile Phases

Concerning use of LSChrom Ver 2, the procedure includes the following steps:

Selection of a stationary phase from a list offered by the software. Another adsorbent should be first characterized experimentally.[8]

Description of the structure of any compound studied, selecting the necessary structural fragments from a list, and specifying their number.

Starting the calculation of retention and its analysis and registration of $\varepsilon_{\text{recommended}}$.

Selection of specific mobile phases having $\varepsilon_{\text{recommended}}$.

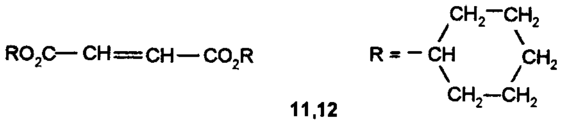
Performance of TLC of the compounds described, on the selected stationary phase with the theoretically found mobile phases.

This procedure was applied to both groups of compounds studied. In general, a good agreement between the calculated values by Eq. (3a), and the experimental values of R_F is not expected. Thus, comparisons between both types of R_F are not included in the following discussion.

Compounds with Simple Structures

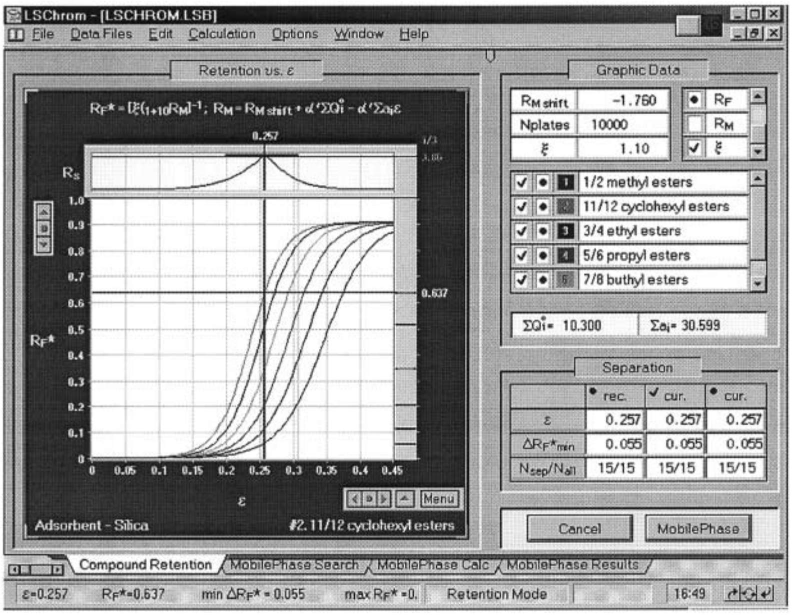
Compounds **1–12** with simple structures were analyzed by LSChrom Ver 2, taking into account silica 1 defined by $R_{M\ (\text{shift})} = -1.76$ and $\alpha' = 0.57$. Differentiation of the *Z* and *E* isomers is not done in the automatic approach of selection of the mobile phases (see below). Thus, the structure of any isomeric pair is described in the same way.

For instance, both *Z*- and *E*-cyclohexyl esters **11** and **12** are expressed by two olefinic carbon atoms (**C**=), twelve aliphatic methylene groups (Al–$\mathbf{CH_2}$–Al, Al = alkyl), two ester groups (Al–$\mathbf{COOCH_3}$) subtracting two Al–$\mathbf{CH_3}$ groups. Note, that the values of Q_i^o and a_i for the **CH** fragment are not known, and this fragment is replaced by $\mathbf{CH_2}$. For compounds **11** and **12**, $S_x = 10.30$ and $A_x = 30.60$.



11,12

In the case of compounds **1–12**, $\varepsilon_{\text{recommended}}$ was 0.257 (see Fig. 1). The software offered a list of numerous mobile phases having that value of ε.



$R_{M\,shift}$	−1.760
Nplates	10000
ξ	1.10

● R_F
☐ R_M
☑ ξ

- ☑ ● 1 1/2 methyl esters
- ☑ ● 2 11/12 cyclohexyl esters
- ☑ ● 3 3/4 ethyl esters
- ☑ ● 4 5/6 propyl esters
- ☑ ● 5 7/8 buthyl esters

$\Sigma Q_i^0 = 10.300$ $\Sigma a_i = 30.599$

	● rec.	✓ cur.	● cur.
ε	0.257	0.257	0.257
$\Delta R_F^*{}_{min}$	0.055	0.055	0.055
N_{sep}/N_{all}	15/15	15/15	15/15

ε=0.257 R_F*=0.637 min ΔR_F^* = 0.055 max R_F^* =0.

Figure 1. Illustration of the mode of LSChrom Ver 2 for calculation and analysis of retention. The value of the recommended mobile phase strength, $\varepsilon_{\text{recommended}}$, is 0.257 for compounds **1–12** on silica 1.

Trying all these mobile phases experimentally is rather time consuming. Thus, we arbitrarily selected six of them (see Table 1, mobile phases 1–6). All these mobile phases have the recommended value of ε and different values of m and P'. They are composed of two to four solvents. Mobile phase 3 contains only weak localizing solvents (toluene and methylene chloride) and has the low value of m (0.05). All other mobile phases have one or more strong localizing solvents such as ethyl acetate, *tert*-butyl methyl ether, acetone, acetonitrile, and tetrahydrofuran. The experimental values of R_F obtained for compounds **1–12** and mobile phases 1–6 are summarized in Table 2, together with the values of log α for separation of the Z/E isomers. Specific chromatograms are shown in Fig. 2. The experimental data obtained, show that retention of any compound with any mobile phase is in the favorable range, above the origin and below the solvent front, namely $0.12 < R_F < 0.75$. The values of R_F with mobile phase 3 of low localization are smaller (0.12–0.37) than those with the other mobile phases.

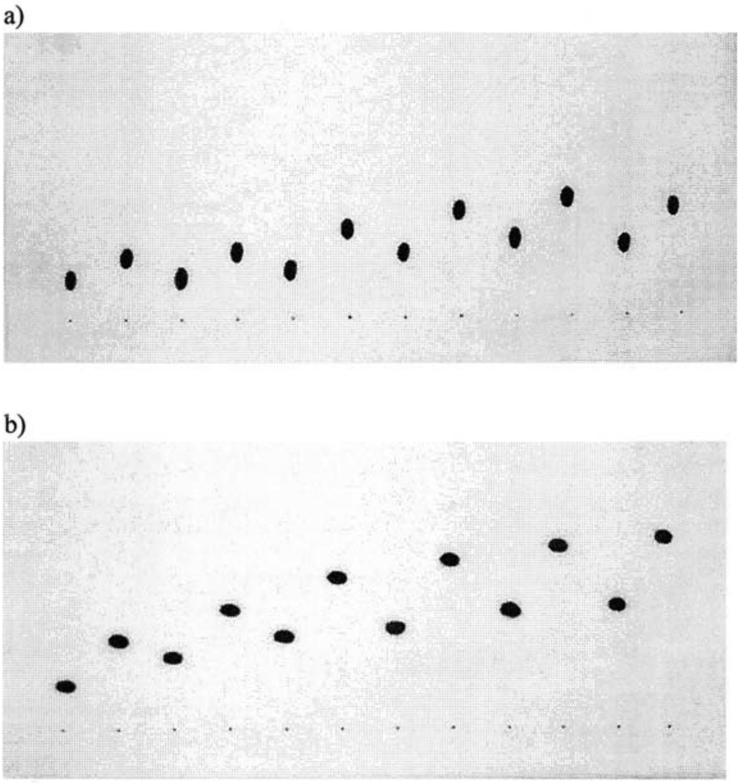
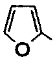


Figure 2. Illustration of the TLC chromatograms of compounds **1–12** on silica 1 with the theoretically selected mobile phase: (a) 3 and (b) 6.

Let us consider other points of the data obtained. Within compounds **1–10**, group R increases by one methylene group in the order methyl–ethyl–propyl–butyl–pentyl. Thus, a decrease of adsorption and retention is expected in this order on the basis of the negative value (−0.05) of Q_i^o for an alkyl methylene group Al–**CH$_2$**–Al. Such a phenomenon was always established separately for the Z/E isomers on the basis of the increasing values of R_F of compounds **1**, **3**, **5**, **7**, **9** and **2**, **4**, **6**, **8**, **10**, respectively. A good separation ($\log \alpha = 0.23–0.43$) was found for any Z/E pair of compounds **1–12** independently, so that the configuration of the compounds was not taken into account in the selection of the mobile phases. Mobile phases that ensure proper retention of Z/E isomers lead to their separation.[14,15] Moreover, the data of Table 2 show that any Z isomer has a stronger retention (and smaller R_F) than the corresponding E isomer, as seen from the positive values of log α. This is well understood on the basis of the expected models of adsorption.[14] Isomer separation is highly influenced by m and P' of the mobile phase. Best separation, and greatest absolute value of log α (0.43), was established for hexane–ethyl acetate 91 : 9 ($m = 0.57$ and $P' = 0.49$). Poorest separation, log $\alpha = 0.23$, was achieved with toluene–ethyl acetate 98 : 2 ($m = 0.09$ and $P' = 2.45$).

Compounds with Complex Structure

For compounds **13–24** with complex structure, silica 2 ($R_{M\ (shift)} = -1.76$ and $\alpha' = 0.57$, see Ref.[1]) was taken into account. Table 3 shows the expression of the structure of any compound by relevant structural elements and their number. It is worth noting that the furanyl group:



is represented by four aromatic carbon atoms (**C**=), one aliphatic ether group (Al–$\mathbf{OCH_3}$), and subtraction of one aliphatic methyl group (Al–$\mathbf{CH_3}$).

In the case of compounds **13–24**, $\varepsilon_{recommended}$ was 0.374. This value is greater than that in the case of compounds **1–12**, owing to the greater adsorption of compounds **13–24**. Four mobile phases with this value of ε and different values of m and P' were selected from a list offered by LSChrom Ver 2 (see Table 1, mobile phases 7–9 and 11). Additionally, mobile phase 12 with a greater value of ε (0.394) was found. This case will be discussed below. Any mobile phase contains one localizing solvent such as ethyl acetate, acetone, isopropanol, or acetonitrile. Such solvents have high individual ε that makes it possible to obtain mobile phases with $\varepsilon = 0.374$.

The experimental values of R_F, obtained on silica 2 for compounds **13–24** and mobile phases 7–12, are summarized in Table 4. The data show that the retention of all compounds is again in the favorable range ($0.02 < R_F < 0.77$). Thus, in the case of compounds with complex structure, the automatically selected mobile phases are suitable for their TLC. This conclusion and the similar conclusions from Refs.[1–3] are important, since the semi-empirical Snyder theory is elaborated on the basis of studies of model compounds, and it is not evident a priori that the theory will be applicable in cases of compounds with complex structure.

Mobile phase 10 is a modification of mobile phase 9 containing 0.6% ammonia aiming at elimination of chemisorption, especially, in the strongest base **16**. With mobile phase 10, this compound showed a significant increase in R_F (0.23), while the remaining compounds showed only a slight change in R_F.

Mobile phase 12 has a greater value of ε (0.394) than $\varepsilon_{\text{recommended}}$. The values of R_F with this mobile phase are also in the favorable range (0.02–0.67), showing that small changes in $\varepsilon_{\text{recommended}}$ are possible.[14,15] In general, an increase of ε from 0.374 to 0.394 should result in greater R_F of any compound. Owing to the small difference in ε, this was, in general, established when passing from mobile phases 7–10 to mobile phase 12. The exceptions with mobile phase 11 can be rationalized on the basis of its greatest value of P' (2.21) and, thus, greatest ability to solve compounds.

Non-automatic Applications

Compounds **1–12** have been studied previously[14,15] by means of LSChrom Ver 1 that permits non-automatic selection of mobile phases only. The approach uses the following criterion: use mobile phases having greater ε if R_F is small and vice versa. Thus, ε and retention are adjusted more easily than in the trial and error approach. If separation is poor with a specific mobile phase, mobile phases with the same value of ε and different values of m and P' should apply.[14,15,19]

LSChrom Ver 2 also performs the non-automatic approach. The latter, is the only possibility if the compounds studied are of unknown structure or their structure cannot be expressed by the structural elements included in the list offered by the software.

Non-automatic application is the differentiation between any kind of isomers based on corrections included in calculation of S_x and A_x. Such corrections can be derived on the basis of a vast number of experiments (see details in Ref.[8]). In this approach, the experimental R_F values are converted to R_M and then related to Eq. (3). The corresponding straight line of R_M vs. ε affords experimental values of S_x (intercept) and A_x (slope) for a specific compound. LSChrom Ver 2 enables one to include the values found for the corrections in the calculation procedure of retention. This approach could not be applied to the present experimental values of R_F of compounds **1–12**, owing to the fact that all mobile phases used have a constant value (0.257) of ε.

CONCLUSIONS

The application of the Snyder theory and LSChrom Ver 2 software was successful in the automatic prediction of twelve mobile phases. The twelve mobile phases gave good TLC results of the simple compounds **1–12** and the complex compounds **13–24** on two silicas. The predictions are based on the analyte structure. Calculation of suitable mobile phase strength ε and subsequent selection of specific mobile phases having such ε are performed.

The adsorption properties of both of the TLC silicas used were characterized by the same values: $R_{\mathrm{M(shift)}} = -1.76$ and $\alpha' = 0.57$. The values are necessary to perform proper automatic selection of the mobile phase. These values are expected to apply to any other TLC silica. Thus, comparisons between different studies will be possible independently of the possibility for precise characterization of any specific stationary phase.

All Z/E isomers were successfully separated independently of the fact that their configurations were not taken into account in the theoretical predictions. This is due to the proper adjustment of retention and great difference in adsorption energy S_x of both isomers.

The LSChrom Ver 2 software enables any user, even those who are not familiar with the Snyder theory, to apply it for automatic selection of mobile phases for normal-phase TLC and HPLC of non-ionic compounds, provided their structures are known and can be expressed by the available in the software structural fragments. If this is not the case, a non-automatic approach is proposed requiring limited preliminary trial and error experiments. A recent use of the theory and software applicable to liquid chromatography under critical conditions is available.[20]

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