"BABEŞ-BOLYAI" UNIVERSITY, CLUJ-NAPOCA
FACULTY OF CHEMISTRY AND CHEMICAL ENGINEERING

DETERMINATION OF THE LIPOPHILICITY OF SOME FOOD ADDITIVES BY CHROMATOGRAPHIC METHODS

PhD Thesis Abstract

PhD Student:
Dorina Casoni

Scientific Adviser:
Prof. Dr. Teodor Hodişan

Cluj-Napoca
- 2010 -

TABLE OF CONTENTS

Keywords:

Reversed-phase high performance liquid chromatography (RP-HPLC)

Reversed-phase high performance thin-layer chromatography (RP-HPTLC)

Lipophilicity indices

Computed log P

Preservatives

Synthetic food dyes

Principal Components Analysis (PCA)

Quantitative structure–retention/property/activity relationships (QSRR/QSPR/QSAR)

General Introduction

The biological/biochemical activity and the environmental fate of a compound are controlled by many factors one of the most important being its lipophilicity, widely expressed by the logarithm of *n*-octanol/water partition coefficient. It is defined by IUPAC as the affinity of a molecule or a moiety for a lipophilic environment. It is commonly measured by its distribution behavior in a biphasic system, either liquid-liquid normally expressed by so called shake-flask methodology, or liquid-solid systems such as liquid chromatographic techniques. This particular property plays an important role in several ADME (absorption, distribution, metabolism and elimination) aspects, as well as in the pharmacodynamic and toxicological profile of drugs [1].

The success of partition coefficient in quantitative structure–activity relationships (QSAR), quantitative structure–property relationships (QSPR) and quantitative structure–retention relationships (QSRR) is well established [2–4]. The compatibility of experimental and theoretical approaches for the determination of organic compound lipophilicity remains also a focus of scientific interest [5, 6]. Determination of partition coefficient using classical "shake-flask" technique has a series of disadvantages (is very tedious, requires relatively large amounts of pure solutes to be examined, and it is limited to log k_{ow} values between −2 and +4) and has been successfully replaced by chromatographic methods: reverse-phase high-performance liquid chromatography (RP-HPLC) and reverse-phase high-performance thin-layer chromatography (RP-HPTLC). RP-HPLC technique has significant advantages: dynamic process, the consumption of the investigated compounds is minimal, high purity chemicals and additional analytical quantification is not required, only the retention time must be determined [7, 8]. The HPLC advantages have attracted considerable interest and the literature is rich in research articles, which investigate the relationships of chromatographic retention with octanol–water partitioning and the common factors underlying the two processes [9–16]. A lot of lipophilicity studies were based on RP-HPLC octadecyl silica (ODS) stationary phases and good correlation between log k_{ow} and log k_w or isocratic log k values were related [17, 18].

To predict a given physicochemical or biological property, the relationships must be identified between the chemical structure and the desired property. Optimally, these relationships should be described in reliable quantitative terms. To get statistically significant relationships and to avoid chance correlations, one needs relatively large series of property parameters. Chromatography is a unique method wich can yeld a great amount of quantitatively comparable,

precise, and reproductible retention data for large sets of structurally diversified compounds. Therefore, quantitative structure – (chromatographic) retention relationships (QSRR) have been considered a model approach to establish strategy and methods of property predictions. QSRR analysis appears especially attractive from the general chemometric point of view because provide the best testing of the applicability of individual structural parameters for property description. Curently, QSRR studies can be applied to: identify the most useful structural descriptors; predict retention for a new analyte and to identify unknown analytes; gain insight into molecular mechanism of separation operating in a given chromatographic system; quantitatively compare separation properties of individual types of chromatographic columns; evaluate properties, other than chromatographic physicochemical properties of analytes, such as lipophilicity; estimate relative bioactivities within sets of drugs and other xenobiotics [19]. In QSRR studies, a relation is searched between molecular descriptors and retention. The aim of this methodology is to derive a model to describe the chromatographic retention on a given chromatographic system, which then can be used for future retention prediction of new solutes. Thus, when a meaningful and statistical significant model is found, no additional experiments are needed to predict the retention for new solutes.

Although experimental log k_{ow} data exist for more than 18000 organic chemicals [20], this number is very low compared to the total number of compounds for which data are desirable. Hence there has been continuing interest in creating methods of calculating log k_{ow} from structure, some of methods existing in generally available computerized form. These are part of software packages which have many data handling features and other related capabilities.

The aim of this work was to determine the lipophilicity parameters for some synthetic food additives (preservatives and dyes) using reverse phase liquid chromatography and different computational methods, to investigate the lipophilic character of these additives by their chromatographic behavior on different stationary phases and to identify the significant molecular properties contributing to their retention.

The first three chapters deal with aspects of theoretical and experimental methods of lipophilicity parameters determinations.

The last four chapters refer to original contributions on the computational and chromatographic determination of lipophilicity parameters for some of food preservatives and synthetic food dyes compounds.

Chapter 2

Lipophilicity determination by chromatographic methods

2.1 Lipophilicity determination by High Performance Liquid Chromatography

RP-HPLC provides a variety of indices (descriptors) that can be used as lipophilicity estimators. The most popular lipophilicity indices measured by RP-HPLC are derived by the retention time, t_r according to the following formula:

$$\log k = \log k_w - S\varphi \qquad (2.1)$$

where:

$$\log k = \log\left(\frac{t_r - t_o}{t_o}\right) \qquad (2.2)$$

and t_o is the retention time of an unretained compound, usually the solvent front or an inorganic salt. k_w refers to the isocratic k value for 'a virtual pure water eluent' and is usually extrapolated value, S is related to the solvent strength of pure organic modifier as mobile phase and φ is the volume fraction of the organic solvent in the mobile phase [21]. The scale of lipophilicity, based on the isocratic retention factors, has been preferred by some authors since it requires fewer experiments. However, linear extrapolation is generally used to obtained log k_w values as more representative lipophilicity indices, their values being of the same order of magnitude as octanol–water partition coefficient (log K_{ow}). Practically, any algorithm of linearization can be applied: log k versus log φ, log k versus log $1/\varphi$ and $1/k$ versus φ.

Another retention-related parameter has been introduced recently, the isocratic chromatographic hydrophobicity index, φ_0. According to Valkó, the φ_0 value represents the volume fraction of the organic solvent in the mobile phase for which the amount of solute in the mobile phase is equal to that in the stationary phase, i.e. the retention factor is 1 (log $k = 0$), $\varphi_0 = \log k_w/S$ [22,23]. It is also possible to obtain a new lipophilicity scale by applying principal component analysis (PCA) directly to the matrix of retention data (k and/or log k values) resulted for all compounds and combinations of methanol–water. In some cases, the scores (linear combinations of retention indices) corresponding to the first principal component (PC1) appeared to be one of the best solutions for the lipophilicity scale resulted from retention data. In addition, a careful investigation of eigenvalues and eigenvectors (loadings) can offer useful information

concerning the chromatographic behavior of the compounds and the retention mechanism [24-26].

2.2 Lipophilicity determination by Reversed-phase Thin-Layer Chromatography

RP-TLC provides a variety of indices (descriptors) that can be used as lipophilicity estimators. The most popular lipophilicity indices estimated by RP-TLC are derived by the R_F according to the following formula:

$$R_M = \log\left(\frac{1}{R_F} - 1\right) \qquad (2.6)$$

where R_F is the retention factor calculated on the basis of migration distance of compound/migration distance of solvent front. Because R_M value generally, depends linearly on the concentration of the organic modifier in the mobile phase, the value has been frequently extrapolated to zero concentration of organic modifier (R_{M0}):

$$R_M = R_{M0} + bC \qquad (2.7)$$

where C is the volume fraction of the organic solvent in the mobile phase. The slope b, indicating the role of which the solubility of solute increases in mobile phase, has been associated with the specific hydrophobic surface area and is considered an alternative measure of lipophilicity [27]. Many studies suggested that the biological activity cannot be associated only with R_{M0} values, especially when polar interactions may take place. The specific hydrophobic surface area of the compounds plays an important role, fact confirmed by the R_{M0} and b correlation [28].

PART II
ORIGINAL CONTRIBUTIONS

Chapter 4
Determination of the lipophilicity of some food preservatives by chromatographic methods

4.1 Introduction

Preservatives are substances commonly added to various foods and pharmaceutical products in order to prolong their shelf life. They can be found in foods, beverages, pharmaceuticals, and personal care products. More often combinations of preservatives are commonly used to prevent alteration and degradation of the product formulations. For instance, benzoic acid inhibits bacterial development. Sorbic acid is an antifungal preservative against molds and yeasts [29]. Esters of *p*-hydroxybenzoic acid such as methyl, ethyl, propyl and butyl *p*-hydroxy-benzoates, also possess antifungal properties. It is also known that most of preservatives may be harmful to the consumers due to their potency to induce allergic contact dermatitis [30, 31] and also the allergic reactions to foods represent a prominent, actual and increasing problem in clinical medicine [32].

Usually, chromatographic lipophilicity results are compared with lipophilicity indices calculated by different established software. Thus the molecules of studied compounds were drawn into Hyperchem [33] and optimized using the MM+ molecular mechanics force field. The optimized geometries were loaded into the ChemDraw Ultra 8.0 [34] and DRAGON Plus version 5.4 [35] software in order to calculate various lipophilicity descriptors. Some of theoretical lipophilicity indices were obtained by using the internet module (ALOGPS 2.1-vcclab [36]. The experimental values of log K_{ow} (determined by "shake-flask" method) were compiled and compared from different sources [37].

4.2 Determination of the lipophilicity of some food preservatives by High -Performance Liquid Chromatography

4.2.1 Experimental

The chromatography was performed on an Agilent 1100 Series LC system consisting of a vacuum degassing unit, a binary high pressure pump, a standard automatic sample injector, a column thermostat and a diode array detector (DAD). The system was connected to an 1100

MSD mass spectrometer. The chromatographic behavior of the compounds was studied on a endcapped C18 (LiChroCART, Purosphere RP-18e, 3mm×125mm, 5 μm particle size), double endcapped C8 (Zorbax, Eclipse XDBC8, 4.6mm×150mm, 5μm particle size), CN100 (Saulentechnik, Lichrosphere, 4mm×250mm, 5 μm particle size) and endcapped NH_2 (Supelcosil LC-NH_2, 3mm×150mm, 3 μm particle size) HPLC columns. The mobile phase consisted of methanol and water (0.1% formic acid) in different volume fractions. The solutions to be injected (10^{-4} μg/μL) were prepared by dissolving the solutes in methanol and diluted in water. The injection volume was 10 μL. The retention times were measured at 25^0C by the UV-MS detector. The dead time corresponded to the solvent peak were as follows: t_0 (C18) = 0.65 min; t_0 (C8) = 1.60 min; t_0 (CN) = 2.60 min and t_0 (NH_2) = 1.50 min. The detector operated at appropriate wavelength (230–254–366 nm) depending on the compound analyzed. The measurements were carried out at flow rate 1.0 mL/min for RP-C18e, Elicpse XDB-C8, CN100 columns and 0.6 mL/min for NH_2 column. In all cases, five different methanol concentrations were used for the extrapolation to obtain log k_w values.

4.2.2 Results and discussion

The chromatographic results obtained on the four HPLC columns indicated a very good linearity through the concentration of methanol used as organic modifier. The correlation coefficient (r) presented values higher than 0.99 in majority of cases (excepting aminobenzoic acid: r = 0.911 on C18 column and r = 0.970 on NH_2 column and salicylic acid: r = 0.963 on C18 column). The obtained results indicate the highest lipophilicity for butylparaben (log $k_{w(C18)}$ = 3.02; log $k_{w(C8)}$ = 3.49; log $k_{w(CN)}$ = 1.62 and log K_{ow} = 3.57) followed by the other three parabens and tert-butylhydroquinone (log $k_{w(C18)}$ = 2.41; log $k_{w(C8)}$ = 2.57; log $k_{w(CN)}$ = 1.10 and log $K_{ow(estimated)}$ = 2.83), while the less lipophilic compound was 4-aminobenzoic acid (log $k_{w(C18)}$ = 0.10; log $k_{w(C8)}$ = 0.73; log $k_{w(CN)}$ = 0.40 and log K_{ow} = 0.83). The high correlations between the log k_w values determined on the first three columns and log K_{ow}, including also some computed log P values, are well illustrated in Figure 4.2.

The patterns of the chromatographic behavior of the investigated compounds (Figure 4.5) illustrate good regularities of retention factors on C8 and CN columns and these findings might indicate that the same mechanism (lipophilic interactions) is dominant in both cases.

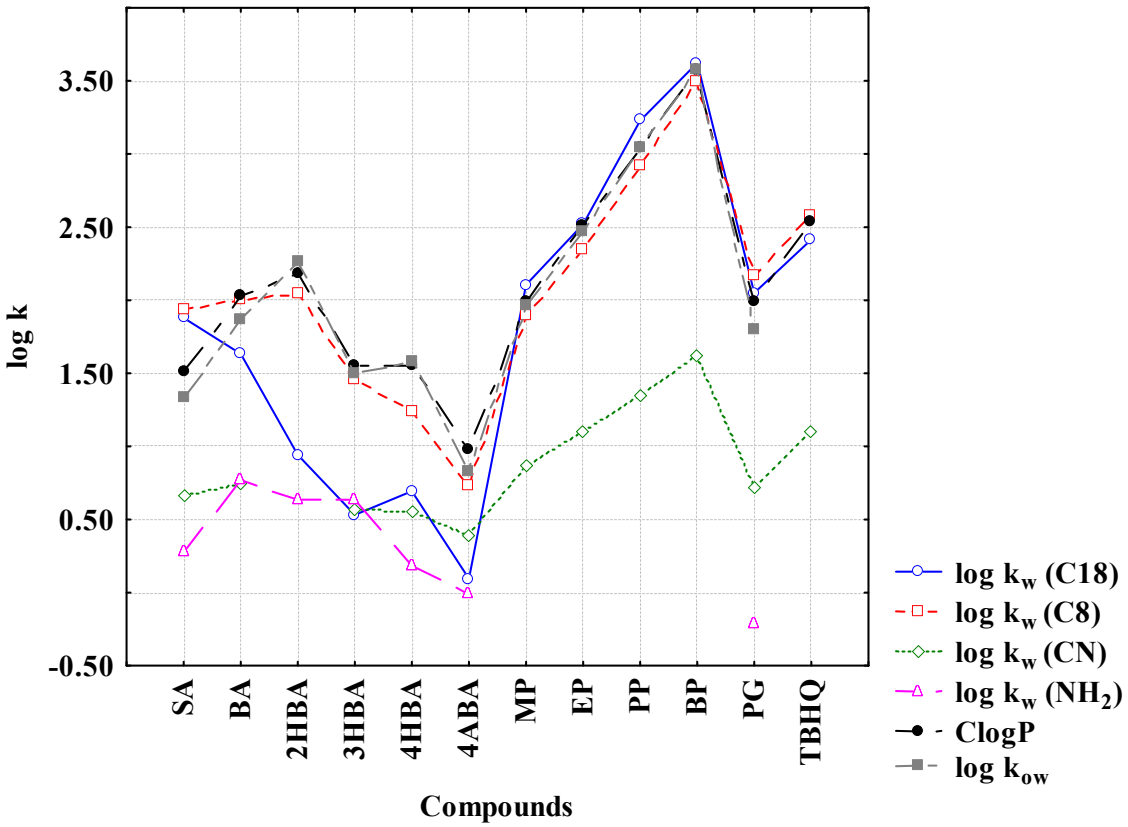


Figure 4.2 The profiles of lipophilicity indices log k_w of the investigated preservatives and some calculated values.

The evidenced irregularities in the case of C18 could be attributed to the "brush" structure of alkyl chains as a function of polarity of mobile phase. At low concentrations of methanol the environment around the bonded moiety is polar and the hydrophobic chains tend to collapse on each other in order to minimize their exposure to the surrounding solvent. As the percent of methanol in mobile phase increases, the medium is less polar and groups are no longer strongly associated with each other. The curious behavior (different from its congeners) of salicylic acid in all cases can be attributed to the well known and documented intramolecular hydrogen bonding.

The quadratic profile of loadings presented in Figure 4.6(a) (k values) and linear profiles Figure 4.6(b) (log k) for the first three columns demonstrate once again a high regular retention behavior in the case of C8 and CN comparing with C18 column.

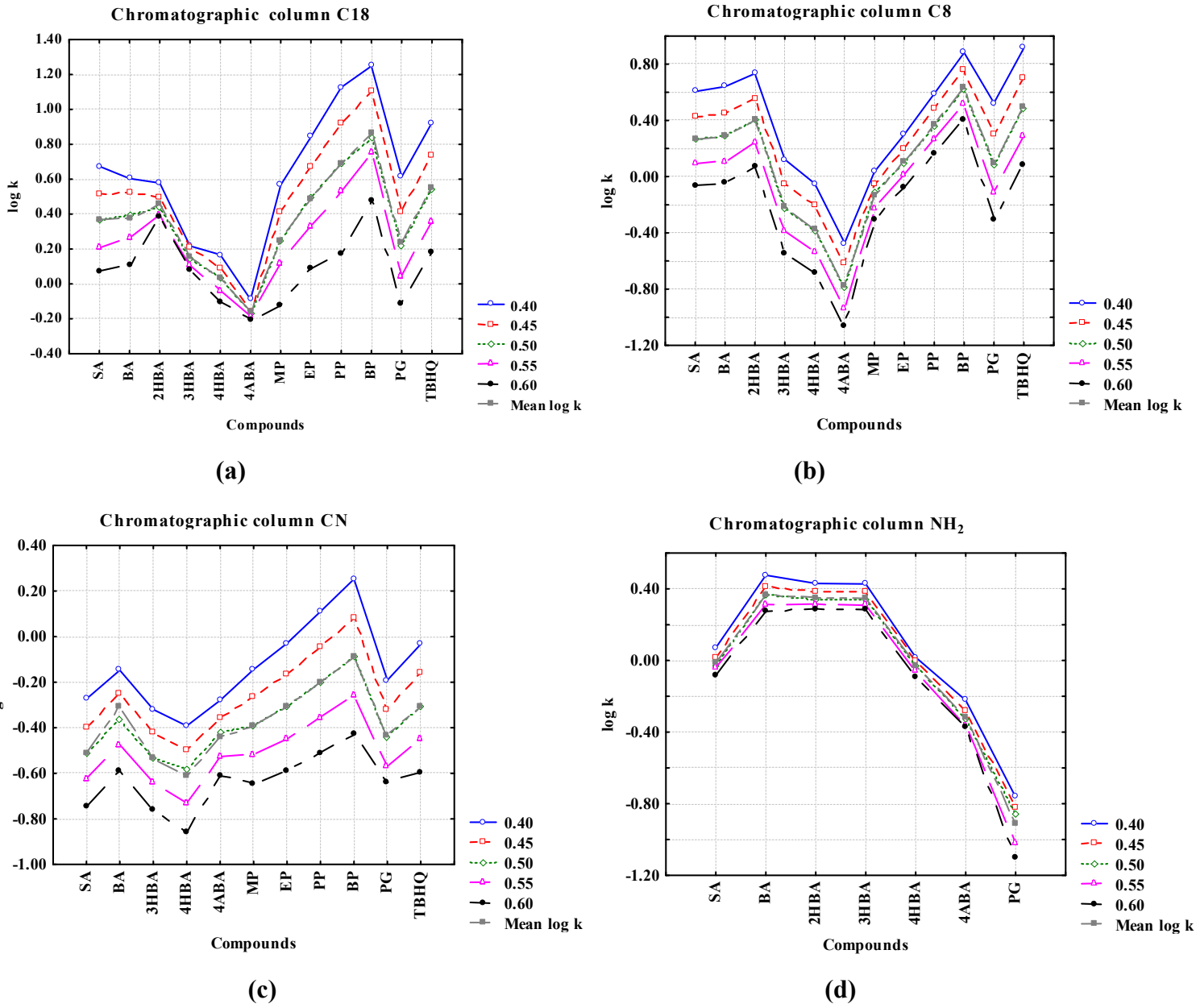
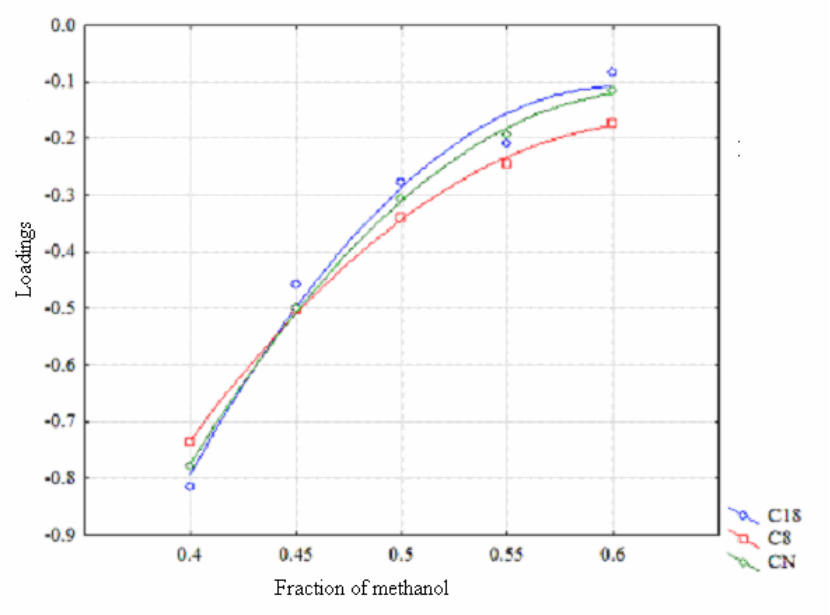
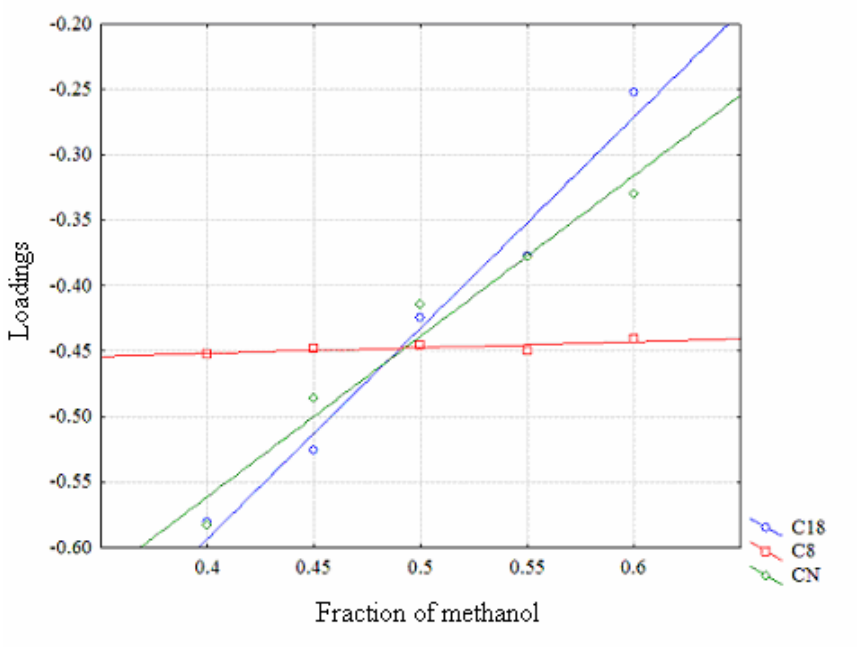


Figure 4.5 Profiles of log *k* for all fraction of methanol: (a) C18; (b) C8; (c) CN, and (d) NH_2.



(**a**)



(**b**)

Figure 4.6 Quadratic loadings profiles of *k* (a) and linear loadings profiles of log *k* (b).

The lipophilicity charts obtained by scatterplots of scores corresponding to log *k* onto the planes described by the first two principal components (Figure 4.7), highlight the congeneric (homologous) series of compounds (parabens) like linear clusters.

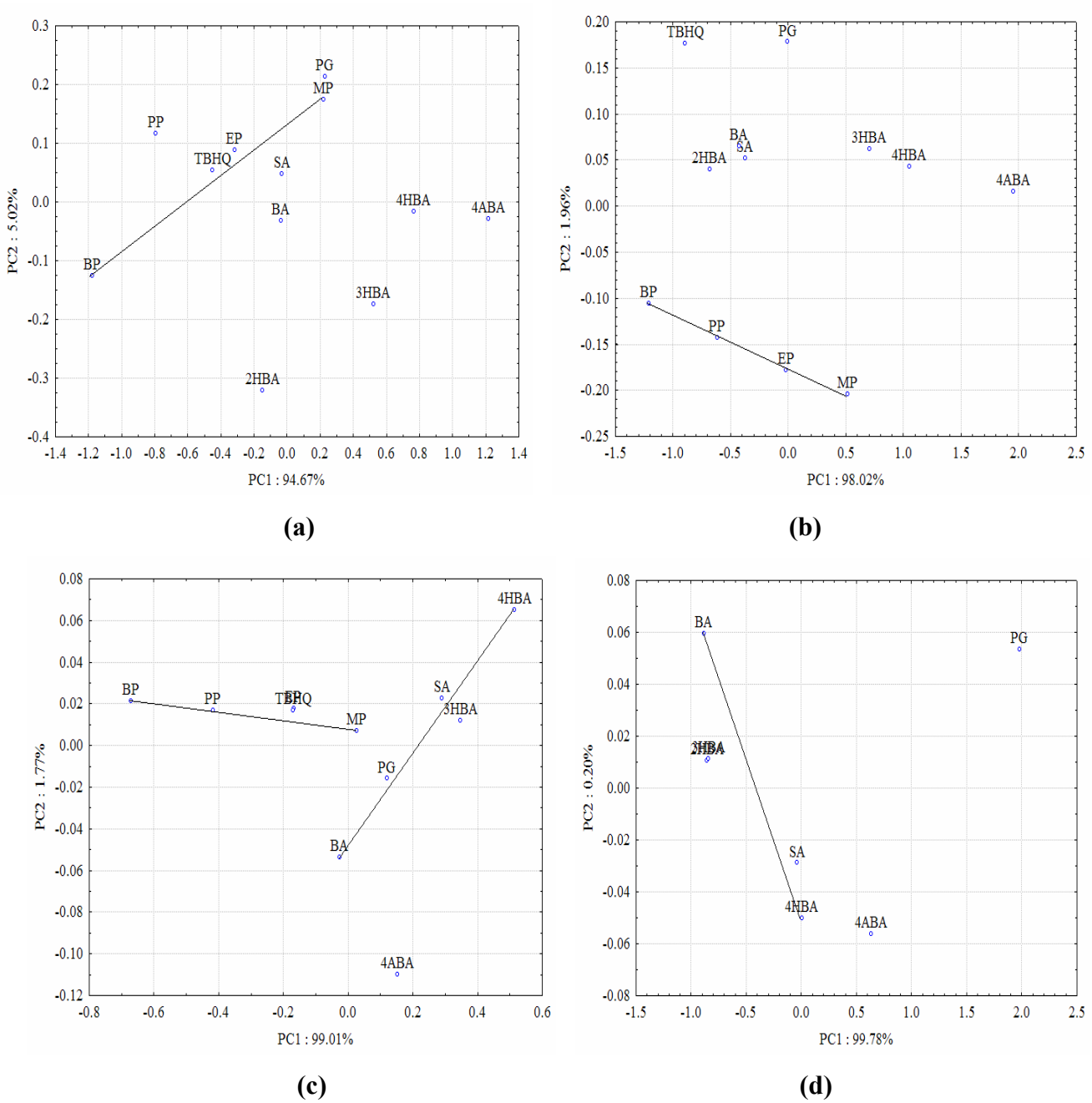


Figure 4.7 Lipophilicity charts corresponding to log k on: (a) C18; (b) C8; (c) CN and (d) NH_2 column.

Statistical data for selected correlations (Tables 4.6) revealed highly significant correlations between the experimental log K_{ow} values and the majority of the calculated log *P* values ($r > 0.90$). The best correlations were obtained for values calculated with XLOGP3 ($r = 1.000$), CLogP ($r = 0.995$), Average logP ($r = 0.985$). Good correlations were also obtained

between experimental log K_{ow} values and some chromatographic indices: log $k_{w(CN)}$ (r = 0.985), $S_{(CN)}$ (r = −0.975), log $k_{w(C8)}$ (r = 0.939), PC1/$k_{(CN)}$ (r = 0.931), PC1/log $k_{(C18)}$ (r = −0.927), mean log $k_{w(C18)}$ (r = 0.925). Comparison of the computed log P values with lipophilicity indices calculated from chromatographic retention time revealed better correlations for the following pairs: $S_{(CN)}$ and ALOGPs (r =−0.992), log $k_{w(CN)}$ and CLogP (r = 0.989), log $k_{w(C8)}$ and AB/LogP (r = 0.971), mean log $k_{(C18)}$ and AB/LogP (r = 0.973), PC1/log $k_{(C18)}$ and AB/LogP (r = 0.970). These direct correlations offer a good opportunity to derive powerful predictive models via Collander-type equation. These models can be used in prediction of different lipophilicity indices.

4.3 Retention behavior of some food preservatives in Thin-Layer Chromatography. Effect of temperature and mobile-phase pH variation

Temperature may have a large effect on the thermodynamics of the retention process, affecting retention factors, selectivity and total analysis time. The effect of temperature on retention is of fundamental importance in gas chromatography but this kind of studies have been reported also in liquid chromatography and thin-layer chromatography.

Numerous authors reported that, in general, retention and selectivity change with temperature in reversed-phase liquid chromatography [38, 39], if the temperature is increased the retention decreases and chromatographic efficiency increases. The increase in efficiency is most often attributed to reduced mobile-phase viscosity.

Near temperature, pH of mobile phase plays an outstanding role in chromatographic retention of analytes with acid/base properties because it can affect the ionization degree of ionizable compounds. In fact, slight variations in the mobile phase pH when it is close to pK_a of the analytes, may cause notable changes in retention times [40]. Although knowledge of pK_a values of compounds in water might give important information about its retention behavior, the pH of mobile phases and pK_a of a solute change when organic modifier is added to the mobile phase.

The objectives of the studies reported in this study was determination of the temperature and mobile-phase pH variation effect on retention behavior of some usually preservatives in both adsorption and partition TLC process.

Table 4.6 Correlation concerning results obtained on C18 and C8 columns (the highest statistical significant values are bold

	log K_{ow}	k (C18)	mean log k (C18)	log k_w (C18)	S (C18)	φ_o C(18)	PC1/k (C18)	PC1/ log k (C18)	mean k (C8)	mean log k C(8)	log k_w C(8)	S C(8)	φ_o (C8)	PC1/ k C(8)	PC1/ log k C(8)
mean $k_{(C18)}$	0.**93**	1.00	**0.93**	0.79	-0.63	-0.55	**-1.00**	**-0.93**	0.87	0.77	**0.94**	-0.88	-0.80	**-0.95**	-0.77
mean log $k_{(C18)}$	**0.92**	**0.93**	1.00	0.84	-0.68	-0.76	**-0.92**	-1.00	0.89	**0.93**	**0.97**	-0.79	**-0.96**	**-0.94**	**-0.93**
log $k_{w\,(C18)}$	0.83	0.79	0.84	1.00	**-0.96**	-0.43	-0.83	-0.87	0.62	0.74	**0.91**	-0.82	-0.86	-0.74	-0.74
$S_{(C18)}$	-0.67	-0.63	-0.68	**-0.96**	1.00	0.25	0.67	0.72	-0.43	-0.60	-0.78	0.72	0.74	0.56	0.60
$\varphi_{o\ (C18)}$	-0.65	-0.55	-0.76	-0.43	0.25	1.00	0.50	0.73	-0.71	-0.83	-0.67	0.41	0.81	0.66	0.83
PC1/$k_{(C18)}$	**-0.92**	-1.00	**-0.92**	-0.83	0.67	0.50	1.00	**0.92**	-0.84	-0.75	**-0.93**	0.89	0.79	**0.93**	0.75
PC1/log $k_{(C18)}$	**-0.93**	**-0.93**	-1.00	-0.87	0.72	0.73	**0.92**	1.00	-0.88	**-0.93**	**-0.98**	0.81	**0.96**	**0.94**	**0.92**
mean $k_{(C8)}$	0.80	0.87	0.89	0.62	-0.43	-0.71	-0.84	-0.88	1.00	**0.91**	0.86	-0.73	-0.81	**-0.98**	**-0.91**
mean log $k_{(C8)}$	0.78	0.77	**0.93**	0.74	-0.60	-0.83	-0.75	**-0.93**	**0.91**	1.00	0.89	-0.68	**-0.95**	**-0.90**	**-1.00**
log $k_{W(C8)}$	**0.94**	**0.94**	**0.97**	**0.91**	-0.78	-0.67	**-0.93**	**-0.98**	0.86	0.89	1.00	**-0.90**	**-0.93**	**-0.93**	-0.89
$S_{(C8)}$	-0.84	-0.88	-0.79	-0.82	0.72	0.41	0.89	0.81	-0.73	-0.68	**-0.90**	1.00	0.70	0.82	0.68
$\varphi_{o(C8)}$	-0.86	-0.80	**-0.96**	-0.86	0.74	0.81	0.79	**0.96**	-0.81	**-0.95**	**-0.93**	0.70	1.00	0.85	**0.95**
PC1/$k_{(C8)}$	-0.88	**-0.95**	**-0.94**	-0.74	0.56	0.66	**0.93**	**0.94**	**-0.98**	**-0.90**	**-0.93**	0.82	0.85	1.00	0.89
PC1/log $k_{(C8)}$	-0.78	-0.77	**-0.93**	-0.74	0.60	0.83	0.75	**0.92**	**-0.91**	**-1.00**	-0.89	0.68	**0.95**	0.89	1.00
CLogP	**0.99**	**0.95**	**0.94**	0.85	-0.69	-0.66	**-0.94**	**-0.94**	0.81	0.80	**0.96**	-0.88	-0.87	-0.89	-0.80
log P^C	**0.93**	**0.93**	0.86	0.86	-0.74	-0.48	**-0.94**	-0.88	0.73	0.70	**0.92**	**-0.91**	-0.77	-0.84	-0.70
log P^V	0.87	**0.93**	**0.92**	0.86	-0.73	-0.56	**-0.93**	**-0.93**	0.82	0.81	**0.96**	**-0.91**	-0.85	**-0.90**	-0.81
log P^B	0.87	**0.90**	0.86	**0.91**	-0.82	-0.46	**-0.92**	-0.88	0.72	0.73	**0.92**	-0.88	-0.80	-0.82	-0.73
MLOGP[1]	**0.91**	0.83	**0.93**	0.79	-0.63	-0.81	-0.81	**-0.93**	0.77	0.87	**0.90**	-0.69	**-0.93**	-0.82	-0.87
ALOGP[1]	**0.91**	**0.93**	0.88	**0.91**	-0.80	-0.49	**-0.94**	**-0.90**	0.76	0.75	**0.96**	**-0.97**	-0.81	-0.86	-0.75
ALOGPs	**0.97**	**0.94**	**0.93**	**0.90**	-0.76	-0.60	**-0.95**	**-0.94**	0.75	0.76	**0.95**	-0.86	-0.87	-0.86	-0.76
AClogP	**0.93**	**0.90**	0.84	**0.92**	-0.83	-0.41	**-0.91**	-0.86	0.69	0.68	**0.93**	**-0.94**	-0.78	-0.80	-0.68
AB/LogP	**0.94**	**0.93**	**0.97**	0.81	-0.64	-0.76	**-0.91**	**-0.97**	0.88	**0.90**	**0.97**	-0.86	**-0.91**	**-0.93**	**-0.90**
miLogP	**0.98**	**0.91**	0.88	0.78	-0.63	-0.60	**-0.91**	-0.88	0.79	0.74	**0.91**	-0.87	-0.80	-0.87	-0.74
ALogP	**0.92**	**0.92**	0.88	**0.92**	-0.83	-0.46	**-0.94**	**-0.90**	0.77	0.75	**0.96**	**-0.95**	-0.81	-0.86	-0.75
MLogP	**0.91**	0.83	**0.94**	0.79	-0.64	-0.81	-0.81	**-0.93**	0.77	0.87	**0.90**	-0.69	**-0.94**	-0.83	-0.87
XLogP2	0.77	0.74	0.81	0.57	-0.39	-0.83	-0.72	-0.80	0.75	0.78	0.82	-0.77	-0.76	-0.77	-0.78
XLogP3	**1.00**	**0.93**	**0.92**	0.83	-0.67	-0.65	**-0.92**	**-0.93**	0.80	0.78	**0.94**	-0.84	-0.86	-0.88	-0.78
AverageLogP	**0.98**	**0.94**	**0.95**	0.88	-0.73	-0.67	**-0.93**	**-0.95**	0.81	0.83	**0.98**	**-0.90**	-0.89	-0.89	-0.83

4.3.1 Experimental

Influence of temperature on chromatographic retention of some usually preservatives was investigated on 20 cm x 20 cm silica gel 60 F_{254} TLC plates (Merck, Darmstadt, Germany). Chromatography was performed in a classic chamber (Camag, Switzerland) previously saturated for 40 min at the development temperature. Standard solutions (2 mg mL^{-1}) of the preservatives were prepared in methanol and 2 μL volumes were spotted on the plates by means of a Linomat 5 semiautomatic sample applicator (CAMAG). Chromatograms were developed at 2±2, 8±2, 17±2, 25±2, 35±2, 45±2 and 55±2 ^{0}C, to a distance of 8 cm using chloroform-acetic acid (99.5%) 8:1 (v/v) as mobile phase. After development, the plates were dried in air at room temperature and examined in UV light at λ = 254 nm in which the compounds were observed as dark spots.

In RP-TLC, influence of both temperature and mobile-phase pH on the chromatographic behavior of preservatives were investigated.

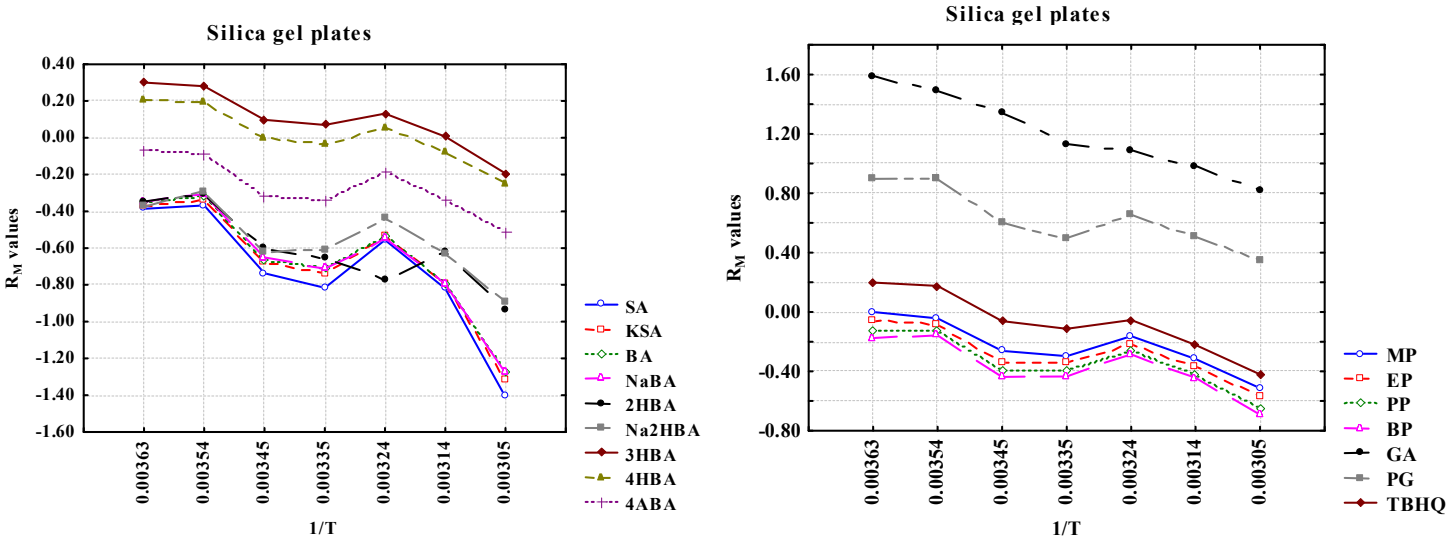
Influence of temperature was investigated on 20 cm x 10 cm TLC plates RP-18W/UV_{254} (Macherey-Nagel, Germany) using methanol-water 2:1 (v/v) as mobile phase. Chromatography was performed in a classic chamber (Camag, Switzerland) previously saturated for 40 min at the development temperature. Standard solutions (2 mg mL^{-1}) of the preservatives were prepared in methanol and 2 μL volumes were spotted on the plates by means of a Linomat 5 semiautomatic sample applicator (CAMAG). Chromatograms were developed at 2±2, 9±2, 17±2, 25±2, 35±2, 45±2 and 55±2 ^{0}C, to a distance of 8 cm. After development the plates were dried in air at room temperature and examined in UV light at λ = 254 nm in which the compounds were observed as dark spots. Each experiment was repeated two times.

Influence of mobile-phase pH was investigated on 10 cm x 10 cm RP-2 F_{254s}, RP-8 F_{254s} and RP-18W F_{254s} HPTLC plates (Merck, Darmstadt, Germany). Chromatography was performed at 25±2 ^{0}C temperature in a classic chamber (Camag, Switzerland) previously saturated with mobile phase for 30 min. Standard solutions (2 mg mL^{-1}) of the preservatives were prepared in methanol and 2 μL volumes were spotted on the plates by means of a Linomat 5 semiautomatic sample applicator (CAMAG). Chromatograms were developed to a distance of 8 cm using mixtures (2:1 v/v) of methanol and buffers of different pH (1.00, 2.00, 4.00, 5.00, 6.00, 6.86, 9.00, 11.00 and 12.00) as mobile phase.

4.3.2 Results and discussion

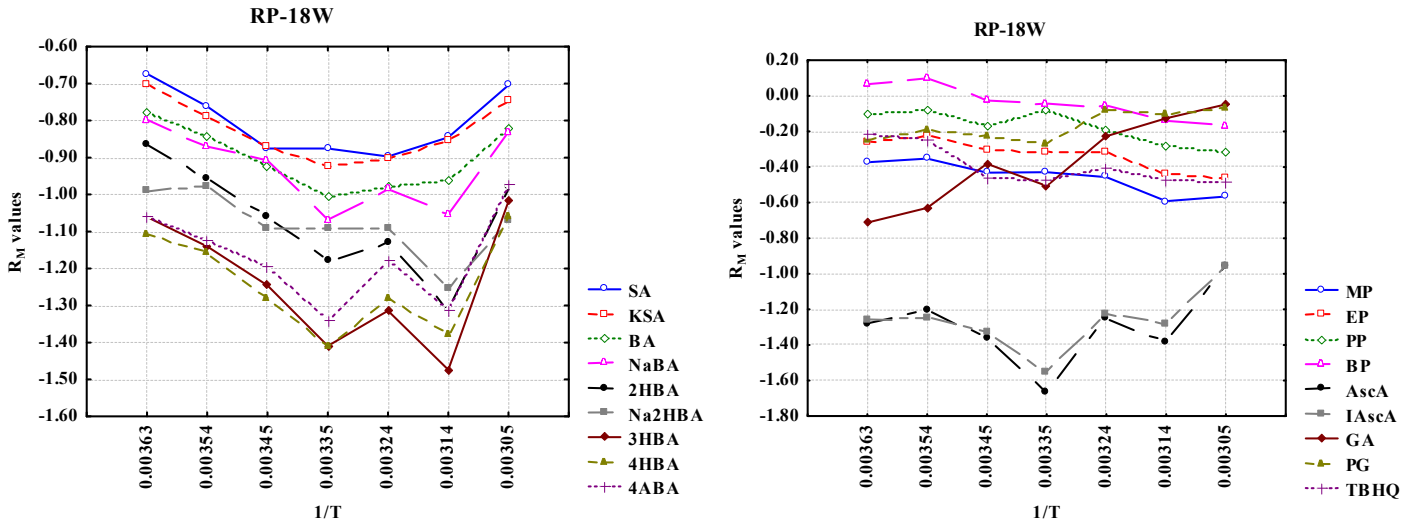
Experimental results showed no regular increased R_F values by increasing of temperature. In range 2^0C-9^0C there are no significant differences in retention of compounds. A linear increasing of R_F values with temperature was observed in NP-TLC for all studied compounds after temperature of 35^0C. A possible explanation of these linearity and non-linearity might be dependence on temperature of vapor pressure of mobile phase components. Good regularities in increasing R_F values were also observed in RP-TLC for some ionic compounds (acids and its salts: SA, KSA, BA, NaBA, 2HBA, Na2HBA) in range of temperature 2^0C-25^0C.

Plots of experimental data (R_M) against 1/T are often linear (Van't Hoff equation) and can be used to predict thermodynamic information about a chromatographic system. Representation of R_M values (determined by NP-TLC Figure 4.9 and by RP-TLC Figure 4.10) showed considerable retention variations characterized by deviations from linearity or by distinct behavior at a particular temperature (like linear Vant'Hoff relationship in a range of temperature) in both, normal and RP-TLC, for acids and its salts compounds (SA, KSA, BA, NaBA, 2HBA and Na2HBA) and for 3HBA, 4HBA and 4ABA in RP-TLC after temperature of 25^0C.



(a) (b)

Figure 4.9 Profiles of experimental data values R_M against 1/T on silica gel plates.



(a) (b)

Figure 4.10 Profiles of experimental data values R_M against 1/T on RP-18W plates.

For these compounds, plots of R_M against 1/T shows good regularities in a range of temperature of 2^0C-25^0C, the retention of compounds having a linear Van't Hoff profile in RP-TLC (Figure 4.11) (r >0.98 for majority of compounds). For the rest of compounds (MP, EP, PP, BP, GA, PG and TBHQ), regular deviations from linearity can be observed in all range of temperature (2^0C-55^0C) in both, normal and RP-TLC. Possible explanations of these deviations can take in consideration properties of the stationary phases, that can be not homogenous throughout the temperature range investigated and properties of mobile phase that might not remain constant in all range of temperature.

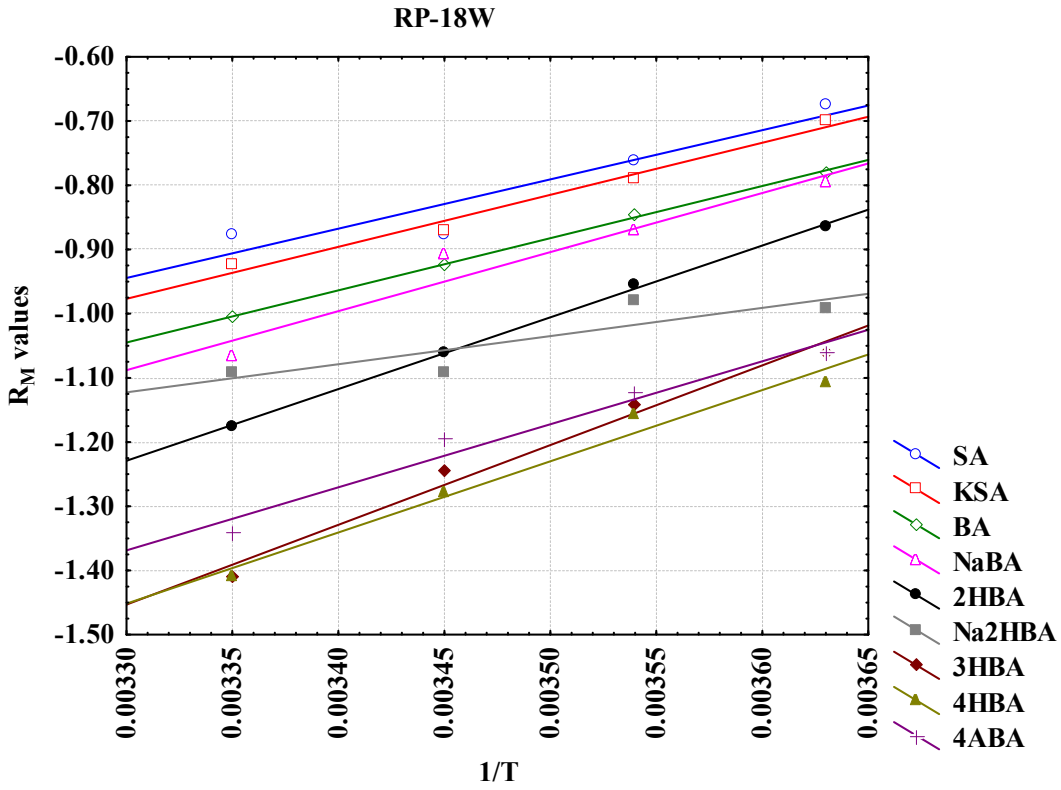


Figure 4.11 Linear dependence of R_M values against 1/T in RP-TLC.

The influence of mobile-phase pH on the retention of preservatives in RP-TLC showed notable change in retentions for Na2HBA and 4ABA for all three types of RP-TLC plates in a range of pH=1 to pH= 4. Possible explanations of these deviations can take in considerations that pK_a values ($pK_{a\ (2HBA)}$= 3 and $pK_{a\ (4ABA)}$= 2.50) of these analytes are closely to mobile phase pH. For a series of compounds such as MP, EP, PP, BP, PG and TBHQ no considerable variation in retentions were observed for all types of stationary phases.

4.4 Determination of the lipophilicity of some food preservatives by Thin-Layer Chromatography and different computation methods

4.4.1 Experimental

The chromatographic behavior of some preservatives was studied on various stationary phases of different polarity: RP-18F254s, RP-18WF254s and CNF254s silica gel bounded plates. Different proportions mixtures of methanol–water were used as mobile phase. The developing distance was 8 cm in all cases.

4.4.2 Results and discussion

The chromatographic behavior of the studied preservatives on the RP-HPTLC plates used in this study is similar and in a very good agreement with their polarity. The patterns are illustrating good regularities of retention factors for all three types of stationary phases (Figure 4.14). These findings might indicate that the same mechanism (lipophilic interactions) is dominant in all cases. The quadratic profile of loadings of R_F values (Figure 4.15a) and linear profiles of loadings of R_M values (Fig. 4.15b) demonstrate once again a high regular retention behavior for all studied compounds.

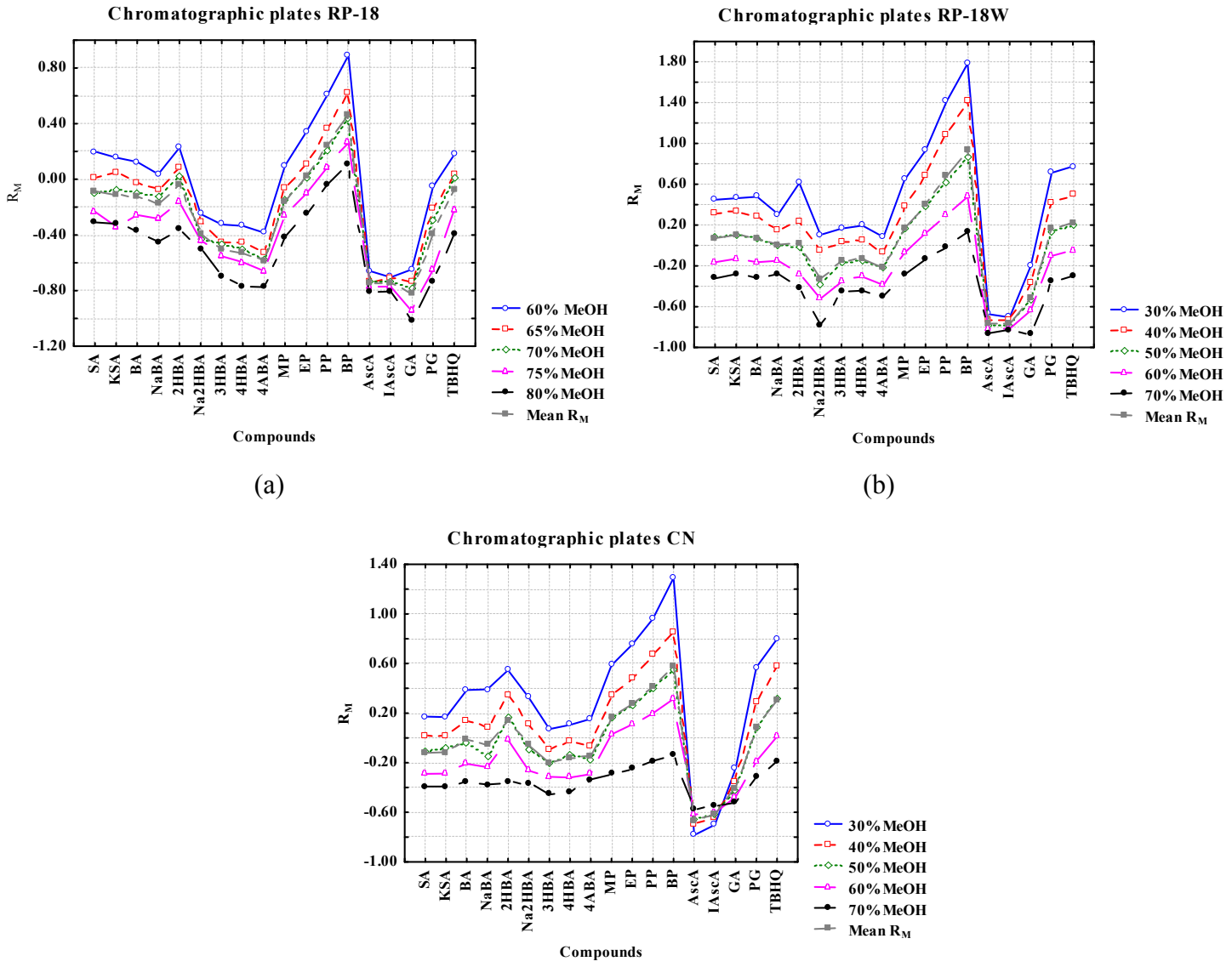
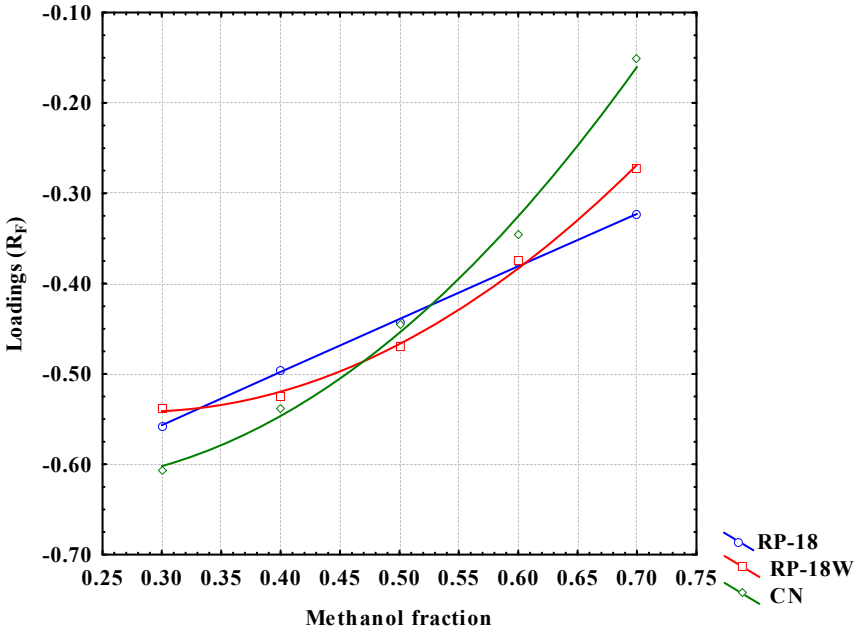
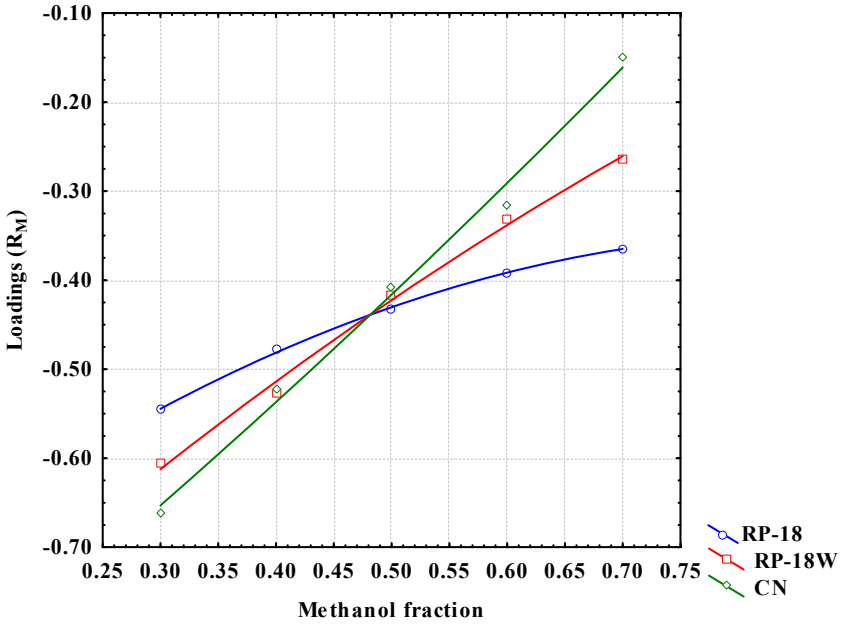


Figure 4.14 The profiles of R_M for all fraction of methanol: (**a**)-RP-18; (**b**)-RP-18W and (**c**)-CN.



(a)



(b)

Figure 4.15 Loadings (eigenvectors) of R_F (a) and of R_M (b) values.

The experimental lipophilicity indices showed high correlations between lipophilicity parameters (R_{M0} and PC1/R_M) determined on the three stationary phases and between them and some computed log P values (Figure 4.16).

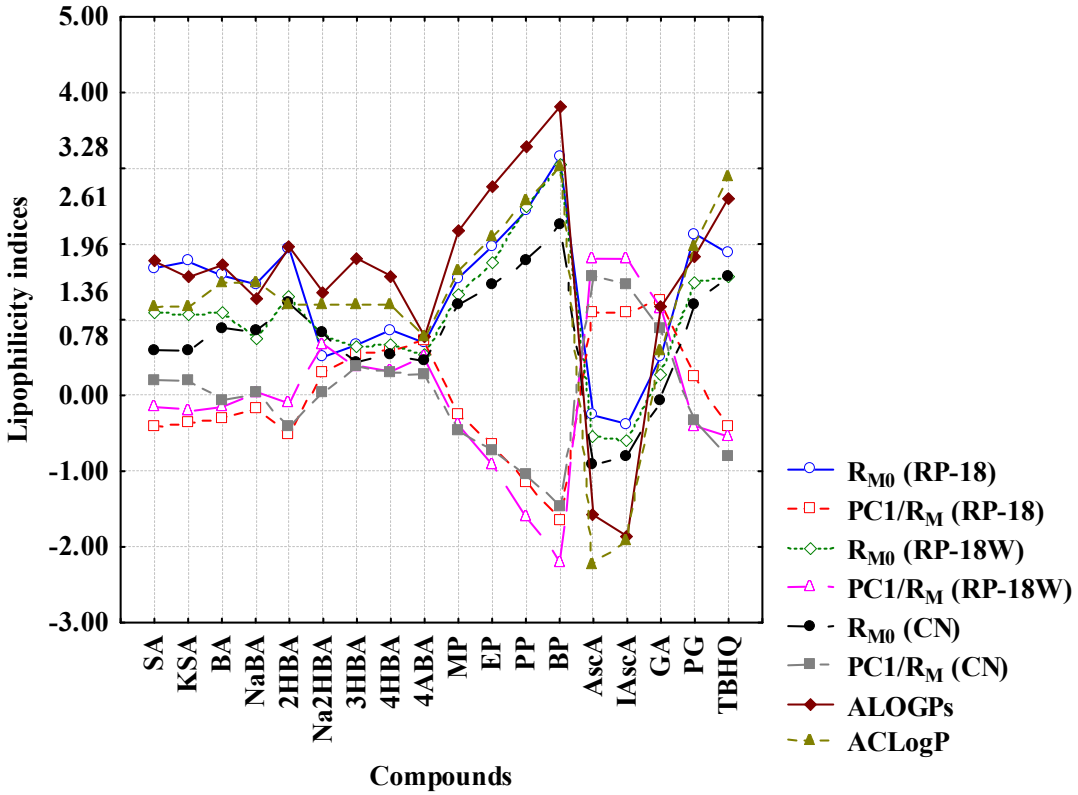


Figure 4.16 The profiles of lipophilicity indices (R_{M0} and $PC1/R_M$) of investigated preservatives.

In order to compare the experimental lipophilicity of investigated compounds estimated by R_{M0}, b, scores corresponding to first principal component of R_F ($PC1/R_F$) and scores corresponding to first principal component of R_M ($PC1/R_M$) with different computed Log P values, a correlation matrix was performed. Highly significant correlations were obtained between different experimental indices of lipophilicity and computed log P values (Table 4.18).

Although in other cases, as one has been mentioned above, the results concerning the scores corresponding to the first principal component (applying PCA directly to the R_F and R_M matrix values) appeared to be one of the best solution for the lipophilicity scale resulted from retention data, in our case, the correlations between scores and computed log P values discussed in this paper were not significantly improved.

Tabelul 4.18 The correlation concerning lipophilicity parameters obtained on different RP-HPTLC plates and some computed log P values.

	R_{M0} RP-18	b RP-18	PC1/R_f RP-18	PC1/R_M RP-18	R_{M0} RP-18W	b RP-18W	PC1/R_f RP-18W	PC1/R_M RP-18W	R_{M0} CN	b-CN	PC1/R_f CN	PC1/R_M CN	Log K_{ow}
R_{M0} RP-18	**1.000**	**-0.966**	**0.919**	**-0.919**	**0.968**	**-0.964**	**0.958**	**-0.958**	**0.962**	**-0.965**	**0.947**	**-0.954**	**0.914**
b-RP18	**-0.966**	**1.000**	-0.786	0.785	**-0.911**	**0.925**	-0.889	0.885	**-0.918**	**0.932**	-0.890	0.897	-0.877
PC1/R_F RP-18	**0.919**	-0.786	**1.000**	**-0.998**	**0.928**	**-0.901**	**0.929**	**-0.937**	0.895	-0.880	**0.901**	**-0.908**	0.843
PC1/R_M RP-18	**-0.919**	0.785	**-0.998**	**1.000**	**-0.927**	0.894	**-0.936**	**0.941**	**-0.901**	0.887	**-0.907**	**0.913**	-0.847
R_{M0} RP-18W	**0.968**	**-0.911**	**0.928**	**-0.927**	**1.000**	**-0.990**	**0.973**	**-0.993**	**0.976**	**-0.970**	**0.959**	**-0.977**	**0.934**
b RP-8W	**-0.964**	**0.925**	**-0.901**	0.894	**-0.990**	**1.000**	**-0.938**	**0.967**	**-0.962**	**0.959**	**-0.939**	**0.960**	**-0.926**
PC1/R_F RP-18W	**0.958**	-0.889	**0.929**	**-0.936**	**0.973**	**-0.938**	**1.000**	**-0.988**	**0.974**	**-0.969**	**0.974**	**-0.976**	**0.931**
PC1/R_M RP-18W	**-0.958**	0.885	**-0.937**	**0.941**	**-0.993**	**0.967**	**-0.988**	**1.000**	**-0.973**	**0.966**	**-0.961**	**0.976**	**-0.926**
R_{M0}-CN	**0.962**	**-0.918**	0.895	**-0.901**	**0.976**	**-0.962**	**0.974**	**-0.973**	**1.000**	**-0.998**	**0.992**	**-0.997**	**0.967**
b-CN	**-0.965**	**0.932**	-0.880	0.887	**-0.970**	**0.959**	**-0.969**	**0.966**	**-0.998**	**1.000**	**-0.985**	**0.990**	**-0.974**
PC1/R_F CN	**0.947**	-0.890	**0.901**	**-0.907**	**0.959**	**-0.939**	**0.974**	**-0.961**	**0.992**	**-0.985**	**1.000**	**-0.996**	**0.945**
PC1/R_M CN	**-0.954**	0.897	**-0.908**	**0.913**	**-0.977**	**0.960**	**-0.976**	**0.976**	**-0.997**	**0.990**	**-0.996**	**1.000**	**-0.954**
CLogP	**0.925**	-0.887	0.851	-0.860	**0.940**	**-0.923**	**0.950**	**-0.940**	**0.975**	**-0.982**	**0.956**	**-0.962**	**0.996**
log P^C	0.831	-0.830	0.711	-0.721	0.841	-0.825	0.861	-0.843	0.891	**-0.909**	0.858	-0.867	**0.960**
log P^V	0.804	-0.817	0.667	-0.676	0.807	-0.798	0.822	-0.803	0.860	-0.882	0.821	-0.831	**0.944**
log P^B	**0.922**	-0.880	0.854	-0.864	**0.935**	**-0.905**	**0.960**	**-0.948**	**0.945**	**-0.952**	**0.920**	-0.931	**0.961**
log P	0.855	-0.845	0.745	-0.756	0.859	-0.837	0.886	-0.865	0.898	**-0.914**	0.867	-0.876	**0.956**
MLOGP[1]	0.871	-0.824	0.816	-0.825	0.867	-0.847	0.899	-0.872	**0.916**	**-0.929**	0.898	-0.898	**0.977**
ALOGP[1]	**0.900**	-0.895	0.779	-0.787	**0.912**	-0.899	**0.918**	**-0.910**	**0.935**	**-0.950**	**0.900**	**-0.913**	**0.974**
ALOGPs	**0.913**	-0.875	0.848	-0.847	**0.947**	**-0.939**	**0.938**	**-0.940**	**0.952**	**-0.956**	**0.926**	**-0.942**	**0.981**
ACLogP	0.889	-0.889	0.763	-0.771	**0.909**	-0.896	**0.917**	**-0.907**	**0.941**	**-0.954**	**0.911**	**-0.922**	**0.975**
AB/LogP	0.895	-0.870	0.803	-0.813	0.888	-0.873	**0.905**	-0.888	**0.931**	**-0.947**	**0.902**	**-0.908**	**0.983**
miLogP	**0.908**	-0.880	0.821	-0.828	**0.926**	**-0.917**	**0.923**	**-0.920**	**0.966**	**-0.976**	**0.941**	**-0.951**	**0.994**
ALOGP	**0.916**	**-0.912**	0.794	-0.799	**0.922**	**-0.911**	**0.926**	**-0.918**	**0.943**	**-0.956**	**0.910**	**-0.923**	**0.973**
MLOGP	0.871	-0.824	0.816	-0.826	0.868	-0.848	**0.900**	-0.873	**0.917**	**-0.930**	0.899	-0.898	**0.977**
KOWWIN	**0.911**	-0.879	0.835	-0.839	**0.924**	**-0.916**	**0.925**	**-0.917**	**0.958**	**-0.966**	**0.935**	**-0.944**	**0.997**
XLOGP2	**0.944**	**-0.943**	0.819	-0.820	**0.914**	**-0.930**	0.891	-0.886	**0.946**	**-0.960**	**0.920**	**-0.925**	**0.963**
XLOGP3	**0.920**	-0.879	0.854	-0.859	**0.941**	**-0.933**	**0.937**	**-0.934**	**0.972**	**-0.978**	**0.951**	**-0.960**	**1.000**

4.7 Determination of the lipophilicity of some preservatives by using impregnated stationary phases.

Parabens, alkyl esters of p-hydroxybenzoic acid, are a class of antimicrobial agents with multiple biological effects. The popular use of parabens in food, cosmetics and pharmaceuticals arises from their low toxicity, inertness, broad spectrum of activity and worldwide regulatory acceptance [30, 31, 41]. The toxicological database for the most commonly used parabens is quite extensive and generally indicates a low degree of systemic toxicity. Several recently published studies, however, have reported adverse effects of propylparaben and butylparaben on the male reproductive system [42, 43]. Literature made the conclusions that the parabens are practically nontoxic, nonmutagenic, nonsensitizing and noncarcinogenic [44].

The purposes of the present study were to investigate the chromatographic behaviour of parabens by RP-HPTLC, to determine retention data on different stationary phases, to correlate these with different computed log P values and with experimental partition coefficients values determined by shake-flask method compiled from the available literature. Also we wanted to find the best vegetable oil that can be used for impregnation of silica gel plates in the purpose to determine lipophilicity of parabens.

4.7.1 Experimental

The chromatographic behavior of the parabens has been investigated on RP-18F_{254S}, RP-18W_{F254S}, CNF_{254S}, Diol $_{F254s}$ and silica gel 60_{F254} plates impregnated with different oils (paraffin, olive, sunflower and corn) using methanol–water mixtures in different volume proportions as mobile phases. The plates were impregnated (after saturation of the chamber with mobile-phase vapours for 15 minutes) by the ascending technique with 10% v/v oil in ethylic ether. The standard solutions of parabens (2 mg/mL) were applied to the plates as spots (2 μL) by means of a Hamilton microsyringe. The development distance was 8 cm for all types of plates. The plates were developed in a saturated chamber by the ascending technique with methanol–water mixtures in different volume proportions. The methanol ranges used in the mobile phases were 50–70% for RP-18W, 60–80% for RP-18, 20–40% for Diol, 30–70% for CN (changed with 10% per step) and 50–65% methanol for oils-impregnated silica gel plates (changed with 5% per step). After development, the plates were dried at room temperature and examined in UV light at 254 nm in which the compounds were observed as dark spots.

4.7.2 Results and discussion

The experimental results showed regular retention behaviour for studied compounds on all RP-HPTLC and different oil-impregnated silica gel plates. The R_M values decreased linearly with the increasing of methanol concentration in the mobile phase in all cases.

The profiles of retention indices (Figure 4.17) showed similar chromatographic behavior of the parabens on the RP-HPTLC and oil-impregnated silicagel plates.

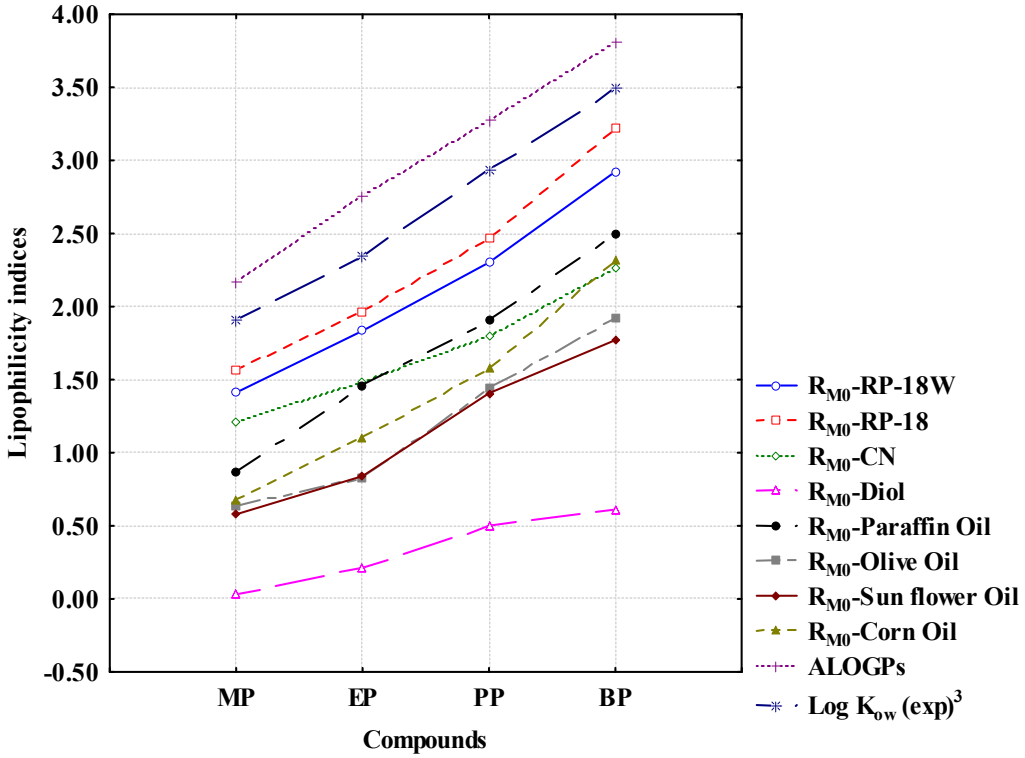


Figure 4.17 The profiles (correlation) of lipophilicity indices (R_{M0}) of the investigated parabens depending by the type of stationary phase.

Linear correlations of log $P(exp)^{a-c}$ (partition coefficients octanol-water determined by shake-flask method, compiled from literature [45]) with chromatographic retention data were observed for all RP-HPTLC and oil impregnated silica gel plates. The correlation coefficients were higher than 0.992 for R_{M0} and higher than 0.996 for PC1/R_M.

In order to compare the chromatographic lipophilicity of investigated compounds estimated by R_{M0}, b and by PC1/R_M, with different computed log P values, a correlation matrix was performed (Tables 4.24 (a).

Table 4.24(a). Correlations between chromatographic lipophilicity indices (R_{M0}) of parabens and experimental and theoretical partition coefficients.

	R_{M0} RP-18W	R_{M0} RP-18	R_{M0} CN	R_{M0} Diol	R_{M0} Paraffin	R_{M0} Olive	R_{M0} Sun fl.	R_{M0} Corn	log k_{ow} $(exp)^1$	log k_{ow} $(exp)^2$	log k_{ow} $(exp)^3$
R_{M0} (RP-18W)	1.000	0.998	0.999	0.973	0.996	0.988	0.989	0.999	0.982	0.996	0.998
B (RP-18W)	-1.000	-0.999	-0.999	-0.971	-0.995	-0.988	-0.988	-0.999	-0.980	-0.995	-0.997
R_{M0} (RP-18)	0.998	1.000	1.000	0.961	0.989	0.989	0.985	1.000	0.974	0.990	0.994
B (RP-18)	-0.994	-0.998	-0.998	-0.945	-0.981	-0.983	-0.975	-0.998	-0.960	-0.980	-0.986
R_{M0}-CN	0.999	1.000	1.000	0.963	0.992	0.987	0.985	1.000	0.975	0.991	0.994
b-CN	-0.996	-0.997	-0.998	-0.948	-0.989	-0.976	-0.973	-0.998	-0.961	-0.985	-0.987
R_{M0}-Diol	0.973	0.961	0.963	1.000	0.979	0.976	0.991	0.964	0.998	0.989	0.986
b-Diol	-0.883	-0.860	-0.863	-0.968	-0.903	-0.899	-0.929	-0.865	-0.953	-0.921	-0.912
R_{M0}-Paraffin	0.996	0.989	0.992	0.979	1.000	0.975	0.984	0.991	0.984	0.998	0.995
b-Paraffin	-0.993	-0.986	-0.989	-0.971	-0.999	-0.966	-0.976	-0.989	-0.976	-0.994	-0.990
R_{M0}-Olive	0.988	0.989	0.987	0.976	0.975	1.000	0.997	0.988	0.987	0.986	0.993
b-Olive	-0.978	-0.978	-0.976	-0.975	-0.963	-0.998	-0.995	-0.977	-0.985	-0.978	-0.986
R_{M0}-Sun flower	0.989	0.985	0.985	0.991	0.984	0.997	1.000	0.985	0.997	0.994	0.996
b-Sun flower	-0.971	-0.964	-0.963	-0.992	-0.965	-0.990	-0.995	-0.965	-0.995	-0.981	-0.984
R_{M0}-Corn	0.999	1.000	1.000	0.964	0.991	0.988	0.985	1.000	0.975	0.992	0.995
b-Corn	-0.999	-0.999	-1.000	-0.959	-0.992	-0.983	-0.981	-1.000	-0.971	-0.990	-0.993
CLogP	0.996	0.989	0.991	0.987	0.999	0.983	0.991	0.991	0.991	1.000	0.998
log P^C	0.996	0.992	0.992	0.989	0.994	0.993	0.997	0.993	0.995	0.999	1.000
log P^V	0.996	0.991	0.992	0.990	0.995	0.992	0.997	0.992	0.995	0.999	1.000
log P^B	0.998	0.996	0.996	0.982	0.992	0.996	0.996	0.996	0.990	0.997	0.999
log P	0.998	0.996	0.996	0.980	0.991	0.996	0.996	0.997	0.989	0.996	0.999
MLOGP[1]	0.994	0.986	0.989	0.988	0.999	0.980	0.990	0.989	0.991	0.999	0.997
ALOGP[1]	0.997	0.993	0.993	0.988	0.993	0.994	0.998	0.993	0.994	0.998	1.000
LOGP(QSAR)	0.986	0.994	0.991	0.932	0.966	0.983	0.970	0.991	0.950	0.968	0.978
ALOGPs	0.994	0.985	0.988	0.986	0.999	0.977	0.987	0.988	0.990	0.999	0.996
AClogP	0.997	0.991	0.992	0.987	0.998	0.986	0.993	0.993	0.992	1.000	0.999
AB/LogP	0.996	0.989	0.991	0.988	0.999	0.983	0.992	0.991	0.992	1.000	0.998
miLogP	0.999	0.997	0.998	0.979	0.995	0.992	0.994	0.998	0.987	0.997	0.999
ALogP	0.997	0.993	0.993	0.987	0.994	0.994	0.997	0.994	0.994	0.998	1.000
MLogP	0.994	0.987	0.989	0.989	0.998	0.981	0.991	0.989	0.992	1.000	0.997
XLOGP2	0.999	0.996	0.998	0.967	0.997	0.979	0.982	0.998	0.976	0.994	0.994
XLOGP3	0.996	0.990	0.991	0.989	0.998	0.986	0.994	0.992	0.993	1.000	0.999
Average LogP	0.996	0.990	0.992	0.989	0.997	0.987	0.994	0.992	0.994	1.000	0.999
log K_{ow} $(exp)^1$	0.982	0.974	0.975	0.998	0.984	0.987	0.997	0.975	1.000	0.993	0.993
log K_{ow} $(exp)^2$	0.996	0.990	0.991	0.989	0.998	0.986	0.994	0.992	0.993	1.000	0.999
log K_{ow} $(exp)^3$	0.998	0.994	0.994	0.986	0.995	0.993	0.996	0.995	0.993	0.999	1.000

Experimental results showed excellent correlations (coefficient correlations higher than 0.99, Table 4.24 a,) between chromatographic R_{M0} values estimated on different stationary phases exceptions being for Diol plates, where the correlations coefficients are lower than 0.98 in some cases. Also, correlation coefficients higher than 0.99 are between R_{M0} values and computed log P values for all types of RP-HPTLC plates and for oil-impregnated silica gel plates.

By using scores of R_M corresponding to the ($PC1/R_M$) as lipophilicity scale, some of

correlation coefficients were improved and are higher than 0.99 for majority of stationary phases. Highly significant correlation coefficients between chromatographic indices of lipophilicity determined on paraffin, olive, sunflower and corn oil-impregnated silica gel plates suggest that these oils are suitable for impregnation of silica gel plates in scope of prediction lipophilicity of parabens and other congeneric compounds. Moreover, the methodology described in this paper can be used for study and comparison of lipophilic character of different vegetable oils or others impregnating materials.

Chapter 5

Modelling of chromatographic lipophilicity of food preservatives

Over the past decade, the quantitative structure-retention/property relationships (QSRR/QSPR) have become a powerful theoretical tool for description and prediction of molecular systems in chromatographic research. It is widely recognized that QSPR equations, derived in a purely empirical fashion from an arbitrary set of descriptors, can give considerable insight into the manner by which chemical structure controls physical and biological properties of compounds. Nowadays, the major goals still are to improve the predictive power and interpretability of the models which can be applied over a wide range of chromatographic systems.

The aim of this study was to identify the significant molecular properties contributing to the preservatives retention and to find an objective manner of quantitative comparison of retention properties of different chemically bonded stationary phases used in liquid chromatography.

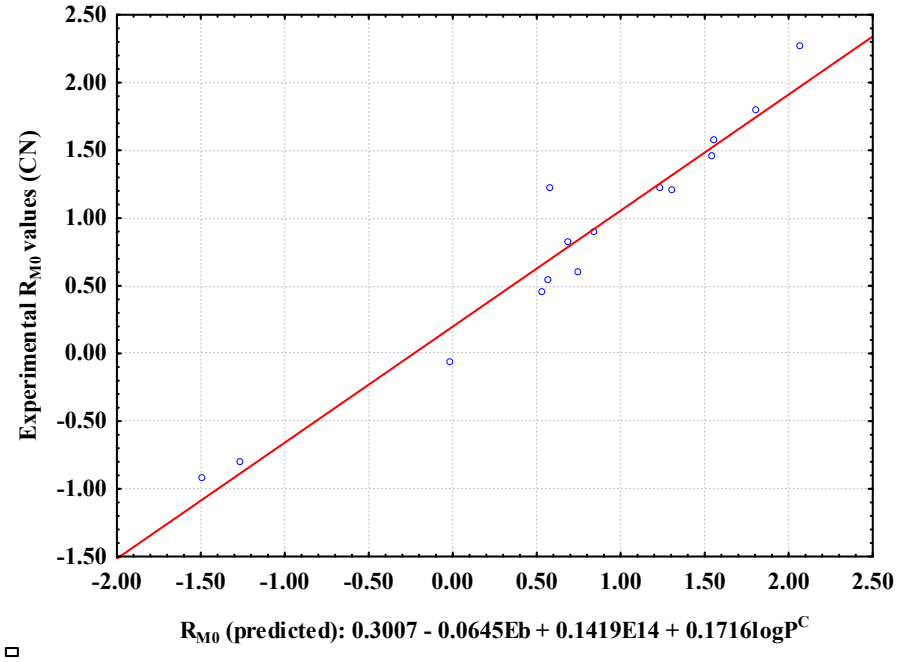
5.3 Results and discussion

An extensive investigation was made for quantitative structure-property (lipophilicity) relationships of studied dyes by using multiple linear regression (MLR) method. Usually in studies applying MLR, a regression analysis is carried out, in order to obtain statistical significant models, taking into account one or a limited number of molecular descriptors. From a variety of potential models with various combinations of descriptors calculated in Dragon software, the statistically significant MLR models (obtained by leave-one-out procedure) containing two or

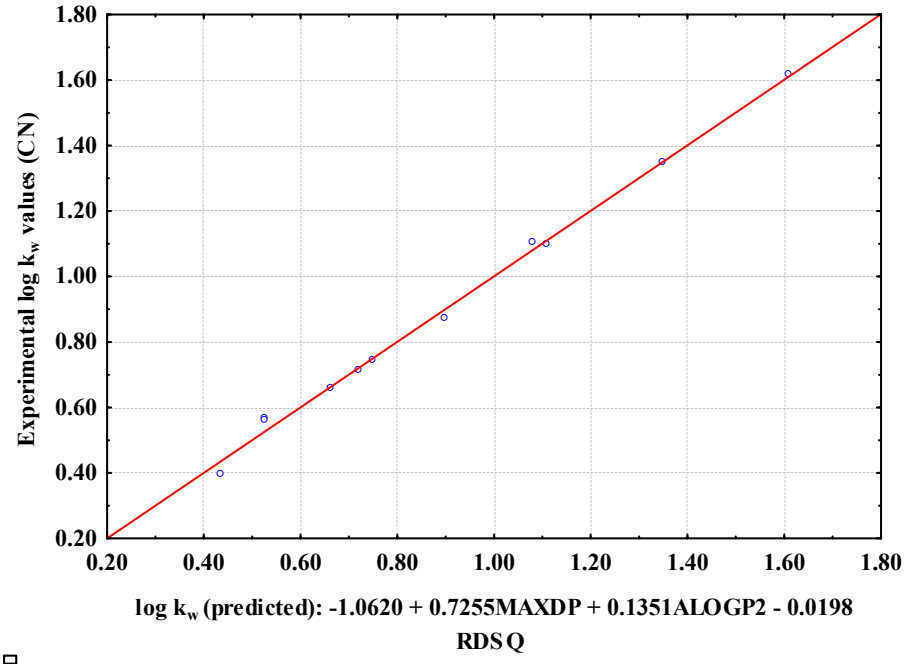
three descriptors were generated by using genetic algorithms (GA). The best predictive models (for log k_w, φ_0 and PC1/log k lipophilicity indices) were chosen by examining the regression statistical parameters Q^2 (leave-one-out crossvalidation coefficient), R^2 (determination coefficient) PRESS (predictive error sum of squares) and s (standard error).

The best predictive HPLC models of preservatives lipophilicity indices were obtained for log k_w and PC1/log k on CN and C8 columns by using descriptors computed in ChemDraw Ultra 8.0 program and those calculated in Dragon 5.4. Most representative descriptors, selected in lipophilicity prediction equations, shows that this property generally depends by thermodynamic parameters (total energy of the molecule (Et) and Gibss energy (G)). Also the best models indicate the topological and geometrical descriptors and the molecular properties like being the most important in preservative lipophilicity prediction.

Applicability of the best models obtained in both TLC and HPLC showed excellent correlations (higher than 0.99 in HPLC and higher than 0.94 in TLC) between chromatographic lipophilicity indices and predicted values of lipophilicity (Figures 5.1).



(a)



(b)

Figure 5.1(a) Plot of predicted vs. experimental lipophilicity indices of preservatives estimated using descriptors calculated by: (a) ChemDraw Ultra 8.0 software and (b) by Dragon software.

Chapter 6

Determination of the antioxidant activity for some food preservatives. Modelling of antioxidant activity using different molecular descriptors

Antioxidants are used in a vide variety of food products, and their activity may vary depending on the temperature, food composition, food structure and availability of oxygen. Radical scavenging is the main mechanism by which antioxidants act in foods. Several methods have been developed in which the antioxidant activity is assessed by the scavenging of synthetic radicals in polar organic solvents. Those used include 2, 2-diphenyl-1-picrylhydrazyl (DPPH) and 2, 2'-azinobis (3-ethylbenzthiazoline-sulphonic acid) (ABTS) radicals. In the DPPH test, the scavenging of DPPH radicals is followed by monitoring the decrease in absorbance at specific wavelengths (515 nm) which occurs due to reduction by the antioxidant or reaction with a radical species. Most papers in which the DPPH method has been used report the scavenging after 15 or 30 min reaction time. The data is commonly reported as EC_{50} or IC_{50}, which is the concentration of antioxidant required for 50% scavenging of DPPH radicals in the specified time period.

The aim of this study was to develop quantitative models for prediction of antioxidant activity of food preservatives and identify the most significant descriptors contributing to this property.

6.3 Modelling of antioxidant activity using different molecular descriptors

A quatitative structure-antioxidant activity relationship (QSAR) study of some food preservatives was performed using multiple linear regression methods. The chemical structures of the preservatives have been characterized by thermodynamic, electronic, topological, geometrical and connectivity indices. From a variety of potential models with various combinations of descriptors calculated in Dragon software, the statistically significant MLR models (obtained by leave-one-out procedure) containing two or three descriptors were generated by using genetic algorithms (GA). The best regression models obtained gave a proper description and a suitable prediction of the antioxidant activity of food preservatives compounds. The statistical parameters showed that both the descriptive and the predictive power of the models are appropriate. The regression coefficient values showed that topological indices play an important role in the description of antioxidant activity of food preservatives.

Applicability of the best models obtained showed excellent correlatins between chromatographic lipophilicity indices and predicted values of lipophilicity (correlation coefficient r = 0.9947) (Figure 6.4).

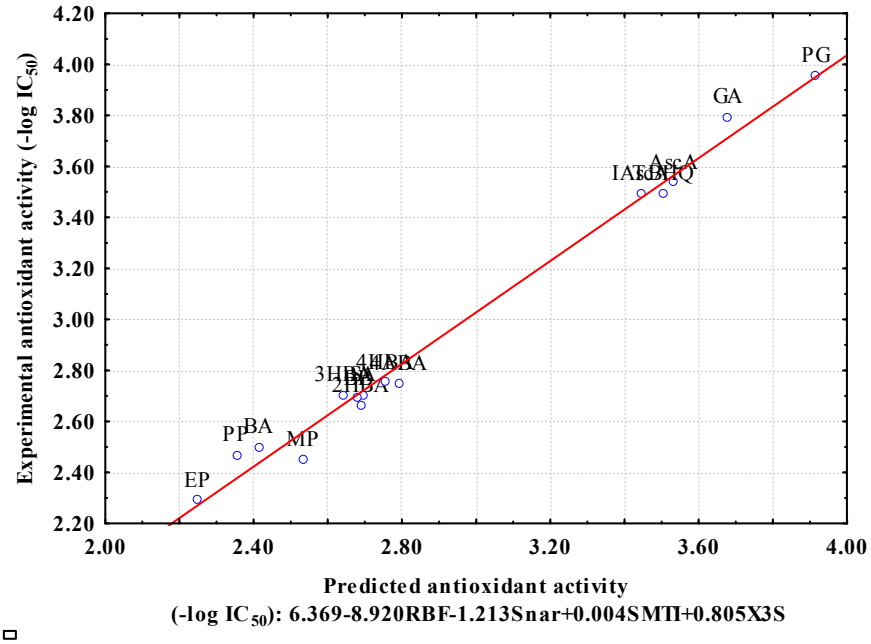


Figure 6.4 Plot of predicted *vs.* experimental antioxidant activity of food preservatives.

Chapter 7

Determination of the lipophilicity of some food synthetic dyes by liquid chromatography and different computation methods

7.1 Introduction

The safety of food dyes has been a matter of concern for several years. Most synthetic dyes have been extensively tested in conventional toxicity studies. However, divergent views have often been expressed on the significance of the same toxicity data [46]. Most of the questions have been associated with the azo colors that some individuals show several allergic reactions, such as urticaria, asthma or rhinitis after their ingestion. The importance of the concept of lipophilicity in the research of pharmacological and toxicological study of different compounds has been recognized since many years [47]. Nowadays, a gradually increasing number of studies use RP-HPLC and RP-TLC for lipophilicity assessment of different classes of

compounds [48-51]. Neverthless, the literature about the lipophilicity of synthetic dyes is still rather scarce.

For dyes considered in this study, a number of partition coefficients (log P, calculated by use of various theoretical procedures) were obtained from different software. All the structures of studied dyes were firstly preoptimized with the Molecular Mechanics Force Field procedure included in Hyperchem version 7.5 [33]. The optimized geometries were loaded in Chem3D Ultra 8.0 and Dragon Plus version 5.4 in order to calculate various lipophilicity descriptors. By using these software, we derived a set of 6 log P values, one of them (Clog P) by using Chem3D Ultra 8.0 and five of the values (HY - hydrophilic factor, MLOGP - Moriguchi's method, MLOGP2 - Squared Moriguchi's method, ALOG P - Ghose-Crippen's method, ALOGP2 - Squared Ghose-Crippen's method) by using Dragon Plus version 5.4 software. Nowadays, there are a large number of internet available modules able to calculate a lot of valuable lipophilicity descriptors. We derived a number of eight lipophilicity descriptors (ALOGPs, AClogP, miLogP, KOWWIN, XLOGP2, XLOGP3, ALogpS, AC logS) by using the Virtual Computational Chemistry Laboratory website (http://www.vcclab.org) and one value ($ClogP^N$) by using the free internet module - New & Improved ClogP calculator (http://intro.bio.umb.edu/111-112/OLLM/111F98/newclogp.html). For the investigated compounds, the experimental partition coefficients values determined by classical "shake-flask" method are missing from literature.

7.2 Lipophilicity of some food synthetic dyes estimated by RP-HPLC method. Modelling of lipophilicity

The aim of this study was to investigate the lipophilic character of some food synthetic dyes by their chromatographic behavior on different stationary phases and to identify the significant molecular properties contributing to their retention. Also we wanted to find an objective manner of quantitative comparison of retention properties of different chemically bonded stationary phases used in RP-HPLC.

7.2.1 Experimental

The lipophilicity of some food synthetic dyes, on four different RP-HPLC columns, was determined based on its retention times. The chromatography was performed on an Agilent 1200 Series LC system consisting of a vacuum degassing unit, a binary high pressure pump, a standard automatic sample injector, a column thermostat and a UV–vis detector (200-600 nm). The chromatographic behavior of the compounds was studied on endcapped C18 (LiChroCART, LiChrospher RP-18e, 4 x 125 mm, 5 μm-particle size), double endcapped C8 (Zorbax, Eclipse XDB-C8, 4.6 × 150 mm, 5 μm-particle size), embedded C16 (Supelco, Discovery Amide C16, 3 x 150 mm, 5 μm-particle size) and CN (Säulentechnik, Lichrosphere CN100, 4 x 250 mm, 5-μm particle size). Due to the large differences of retention behavior of studied compounds, the methanol ranges used in the mobile phase compositions were optimized for each type of column. The mobile phase consisted of ammonium acetate (0.08 mol/L), pH = 6.76) and methanol in proportions varying from 15% to 35% (v/v) and 45% to 65% (v/v) for C18, C8 and C16 columns and from 50% to 70% (v/v) for CN column. The retention times were measured at 25^0C temperature by the UV–vis detector in the visible range, depending on the investigated dye. The wavelengths were as follows: λ=415 nm for quinoline yellow WS; λ=426 for tartrazine; λ=480 for sunset yellow; λ=510 for ponceau 4R; λ=510 for azorubine; λ=520 for erythrosine; λ=520 for amaranth (dye); λ=590 for brilliant blue FCF respective λ=590 for patent blue V. The solutions to be injected (10 μg/mL) were prepared by dissolving the solutes in water. The injection volume was 10 μL in all cases. The measurements were carried out at a flow rate of 1.0 mL/min for C18 and C8 columns and with 0.6 mL/min for C16 and CN column. The dead times corresponding to the solvent peak were as follows: t_0(C18)=0.951 min; t_0(C8)=1.360 min t_0(C16)=1.35 respectively t_0(CN)=4.305 min. In all cases, five different methanol concentrations were used for extrapolation to log k_w values.

Calculation of the molecular descriptors

Studied synthetic dyes were also characterized by 1164 theoretical descriptors calculated using Dragon 5.4 software. The descriptors employed in this study can be arranged in the following groups: *descriptors 2D*: 2D autocorrelations, edge adjacency, Burden eigenvalues, topological and connectivity indices; *descriptors 3D*: RDF, 3D-MORSE, GETAWAY, WHIM, geometrical properties and Randić molecular profiles; *others descriptors*: functional groups, atom-centered fragments, molecular properties, charge descriptors, and constitutional properties.

The computational efforts of descriptors calculation can directly be related to the complexity of the molecular representation on which the calculation is based on. Three-dimensional (3D) descriptors require geometry optimizations prior to the descriptor calculation. In all cases the structures of the dyes were preoptimized with the Molecular Mechanics Force Field (MM+) procedure included in Hyperchem version 7.5 and the resulting geometries were further refined by means of the semi empirical method PM3 (Parametric Method-3) using the Fletcher-Reeves algorithm and a gradient norm limit of 0.009 kcal/Å. Multiple linear regression calculations were performed by the MobyDigs v.1.0 software [52] which selects the most significant variables using genetic algorithms (GA) [53].

7.2.2 Results and discussion

The chromatographic behavior of the investigated synthetic dyes, on the bonded phases used in this study, showed a linear dependence of retention parameters throughout the methanol fraction variance as it is indicated by the regression correlation coefficients higher than 0.99 in all cases. The patterns of chromatographic behavior of the compounds while the methanol fraction has been changed (Figure 7.2) illustrate good regularities on all studied stationary phases. These findings might indicate that the same mechanism is dominant in all cases.

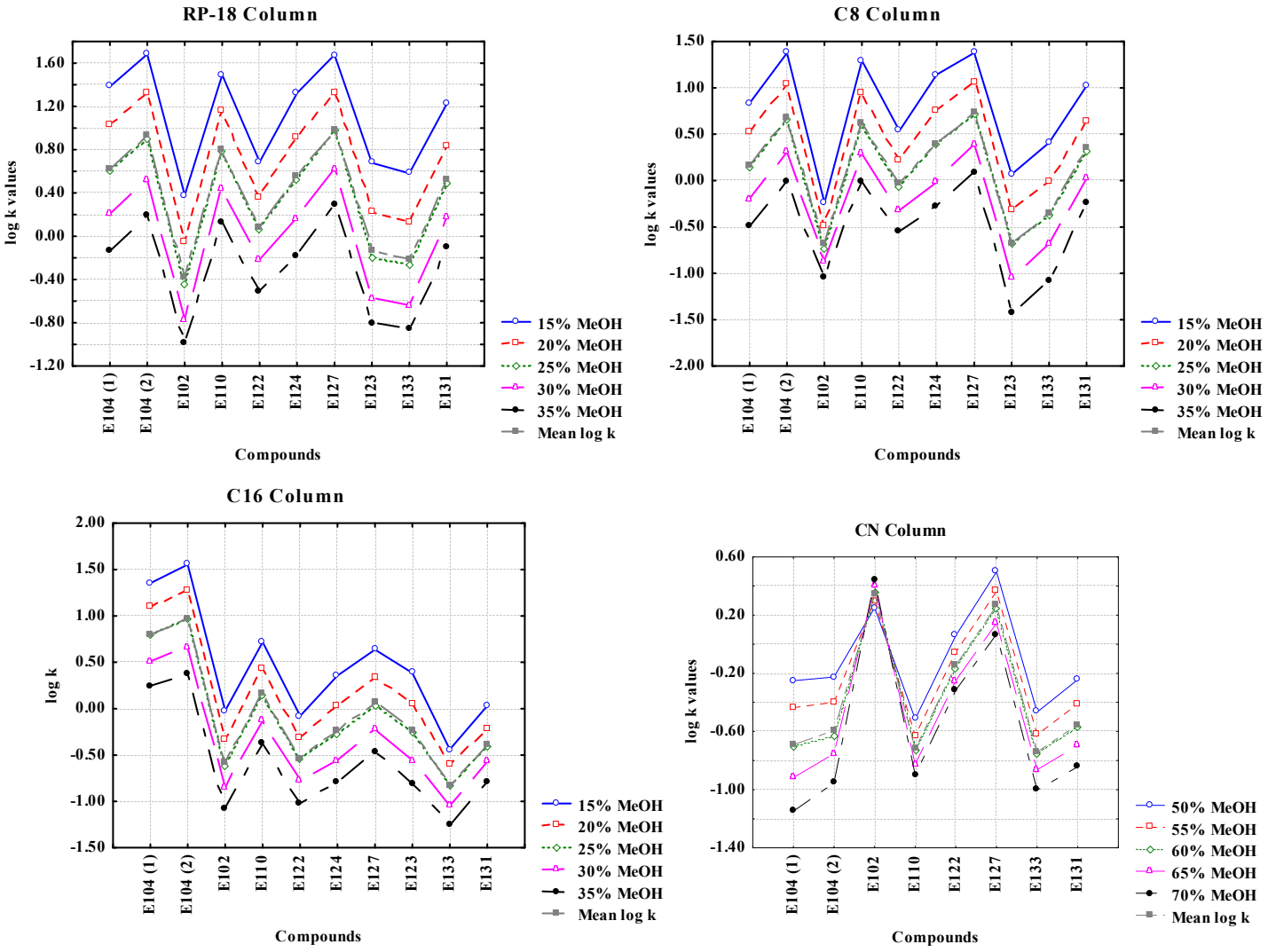


Figure 7.2 Profiles of log k values obtained on studied stationary phases for all fraction of methanol.

The different behavior of tartrazine (E102) in case of CN column can be attributed to the intermediary polarity of this column that could be able to participate in various types of interactions.The quadratic profiles of loadings (Figure 7.4) demonstrate once again a high regular retention behavior of studied dyes on different stationary phases.

The lipophilicity indices obtained showed similar behavior for the compounds investigated, the most lipophilic compound being erythrosine dye and the least lipophilic being tartrazine (exception for CN column). The highest log k_w values were obtained on C18 and C8

columns followed by C16 and respectively CN columns for the majority of compounds.

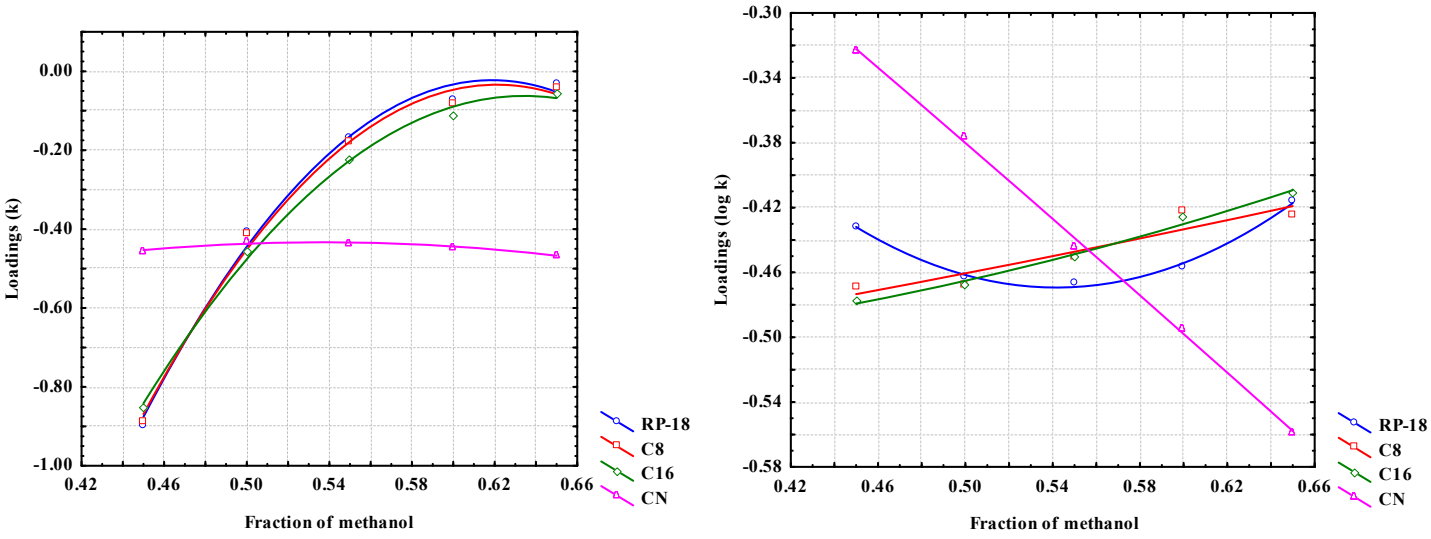


Figure 7.4 Loadings profiles of k values (a) and loadings profiles of log k values (b).

A comparative study has been developed for the chromatographic lipophilicity indices of synthetic dyes and their calculated partition coefficients using different theoretical methods. Among theoretical values of partition coefficient, HY and MLOGP correlate better with log k_w and φ_0 on C18 and C8 stationary phases (Table 7.6). Although the chromatographic behavior of the compounds illustrate good regularities on all studied stationary phases, the lipophilicity indices estimated on C16 and CN columns presented the lowest correlations with majority of the theoretical partition coefficients. These relatively no high correlations, between experimentally and theoretical lipophilicity indices, are probably due to the estimation of log P, which have some limitations for complex structures of compounds. Theoretical lipophilicity values may have a restricted importance because none of the available methods can take into consideration all the effects of molecular conformation these being simplified in many cases. Also the predictions of log P values may be less accurate in the case of molecules containing ionizable groups such as the examined dyes.

Table 7.6 Correlation concerning lipophilicity results obtained on studied columns (the highest statistical significant values are bolded).

	log k_w				**S**				**φ_0**				**PC1/log k**			
	(C18)	**(C8)**	**(C16)**	**(CN)**	**(C18)**	**(C8)**	**(C16)**	**(CN)**	**(C18)**	**(C8)**	**(C16)**	**(CN)**	**(C18)**	**(C8)**	**(C16)**	**(CN)**
log k_w (C18)	1.00	**0.98**	0.82	**0.72**	0.28	-0.28	0.42	-0.54	**-0.98**	**-0.97**	**-0.90**	**-0.78**	-0.46	-0.56	0.01	0.01
log k_w (C8)	**0.98**	1.00	**0.74**	0.68	0.26	-0.40	0.43	-0.59	**-0.95**	**-0.97**	**-0.84**	-0.67	-0.47	-0.59	0.06	0.15
log k_w (C16)	**0.82**	**0.74**	1.00	**0.90**	0.17	-0.21	-0.01	-0.62	**-0.80**	**-0.78**	**-0.96**	**-0.92**	-0.69	-0.64	-0.48	-0.06
log k_w (CN)	0.54	0.53	**0.88**	1.00	-0.40	**-0.70**	-0.40	**-0.90**	-0.43	-0.48	**-0.82**	-0.68	**-0.70**	-0.68	**-0.70**	0.35
S (C18)	0.28	0.26	0.17	-0.23	1.00	0.56	0.48	0.36	-0.47	-0.45	-0.31	-0.19	0.09	-0.09	0.42	-0.39
S (C8)	-0.28	-0.40	-0.21	-0.67	0.56	1.00	0.20	**0.89**	0.15	0.23	0.17	0.20	0.34	0.28	0.24	-0.75
S (C16)	0.42	0.43	-0.01	-0.14	0.48	0.20	1.00	-0.03	-0.47	-0.46	-0.27	0.15	0.42	0.22	0.61	0.21
S (CN)	-0.39	-0.46	-0.65	**-0.90**	0.50	**0.87**	0.28	1.00	0.27	0.36	0.62	0.34	0.63	0.63	0.69	-0.72
φ_0 (C18)	**-0.98**	**-0.95**	**-0.80**	-0.66	-0.47	0.15	-0.47	0.46	1.00	**0.99**	**0.91**	**0.80**	0.41	0.53	-0.09	0.07
φ_0 (C8)	**-0.97**	**-0.97**	**-0.78**	-0.66	-0.45	0.23	-0.46	0.52	**0.99**	1.00	**0.88**	**0.75**	0.45	0.58	-0.07	-0.04
φ_0 (C16)	**-0.90**	**-0.84**	**-0.96**	**-0.91**	-0.31	0.17	-0.27	0.66	**0.91**	**0.88**	1.00	**0.92**	0.54	0.55	0.28	-0.00
φ_0 (CN)	**-0.77**	-0.66	**-0.90**	-0.68	-0.15	0.21	0.17	0.34	**0.77**	**0.73**	**0.92**	1.00	0.48	0.48	0.22	0.34
PC1/k (C18)	-0.54	-0.50	**-0.73**	-0.55	0.10	0.25	0.40	0.37	0.46	0.47	0.57	0.54	**0.95**	**0.90**	0.65	0.04
PC1/k (C8)	-0.53	-0.53	-0.59	-0.39	-0.03	0.24	0.34	0.26	0.48	0.51	0.47	0.44	**0.92**	**0.93**	0.46	0.05
PC1/k (C16)	0.05	0.11	-0.37	-0.54	0.48	0.18	0.31	0.59	-0.14	-0.13	0.26	0.10	0.44	0.27	**0.89**	-0.42
PC1/k (CN)	0.04	0.20	0.07	0.39	-0.27	-0.73	0.09	**-0.74**	0.02	-0.10	-0.12	0.26	-0.24	-0.28	-0.34	**0.97**
PC1/log k (C18)	-0.46	-0.47	-0.69	-0.62	0.09	0.34	0.42	0.53	0.41	0.45	0.54	0.49	1.00	**0.96**	**0.73**	-0.16
PC1/log k (C8)	-0.56	-0.59	-0.64	-0.59	-0.09	0.28	0.22	0.52	0.53	0.58	0.55	0.50	**0.96**	1.00	0.53	-0.17
PC1/log k (C16)	0.01	0.06	-0.48	-0.54	0.42	0.24	0.61	0.52	-0.09	-0.07	0.28	0.21	**0.73**	0.53	1.00	-0.29
PC1/log k (CN)	-0.01	0.14	0.02	0.35	-0.45	**-0.77**	0.04	**-0.72**	0.11	-0.01	-0.04	0.34	-0.25	-0.26	-0.38	1.00
ClogP	**0.71**	0.65	**0.78**	0.46	0.43	-0.01	-0.13	-0.10	**-0.75**	**-0.72**	**-0.72**	**-0.82**	-0.59	-0.61	-0.12	-0.46
Clog P^N	0.58	0.59	0.31	0.12	0.32	-0.17	0.10	0.12	-0.60	-0.58	-0.34	-0.53	-0.09	-0.17	0.47	-0.44
HY	**-0.94**	**-0.90**	**-0.84**	**-0.80**	-0.25	0.23	-0.44	**0.70**	**0.91**	**0.90**	**0.93**	**0.72**	0.48	0.54	0.16	-0.20
MLOGP	**0.97**	**0.96**	**0.70**	0.58	0.23	-0.32	0.48	-0.45	**-0.93**	**-0.92**	**-0.80**	-0.65	-0.31	-0.41	0.17	0.01
ALOGP	**0.80**	**0.81**	0.44	0.13	0.59	0.06	0.54	0.05	**-0.85**	**-0.84**	-0.57	-0.49	-0.18	-0.38	0.47	-0.32
ALOGPs	**0.70**	0.60	**0.74**	0.37	0.49	0.19	0.01	0.07	**-0.75**	-0.69	**-0.71**	**-0.83**	-0.42	-0.45	0.01	-0.65
AC logP	0.59	0.69	0.12	0.22	0.14	-0.54	0.50	-0.30	-0.57	-0.61	-0.28	-0.23	0.12	-0.02	0.58	0.21
milogP	**0.74**	0.68	**0.80**	0.52	0.34	-0.10	-0.14	-0.15	**-0.76**	**-0.73**	**-0.74**	**-0.85**	-0.59	-0.60	-0.13	-0.44
KOWWIN	**0.70**	**0.79**	0.57	0.60	0.30	-0.60	0.15	-0.62	**-0.72**	**-0.78**	-0.63	-0.54	-0.45	-0.53	0.00	0.30
XLOGP2	**0.82**	**0.82**	0.47	0.34	0.23	-0.32	0.41	-0.19	**-0.79**	**-0.78**	-0.57	-0.54	-0.07	-0.18	0.43	-0.15
XLOGP3	**0.77**	**0.80**	0.37	0.35	0.18	-0.42	0.50	-0.29	**-0.74**	**-0.74**	-0.51	-0.45	0.04	-0.07	0.49	0.02
ALOGpS	**-0.79**	**-0.84**	-0.38	-0.41	-0.03	0.51	-0.60	0.48	**0.72**	**0.74**	0.54	0.32	0.02	0.14	-0.36	-0.29
AClogS	**-0.82**	**-0.80**	**-0.80**	-0.64	-0.30	0.29	0.02	0.33	**0.83**	**0.82**	**0.78**	**0.89**	0.52	0.55	0.04	0.28

An extensive investigation was made for quantitative structure-property (lipophilicity) relationships of studied dyes by using multiple linear regression (MLR) method. From a variety of potential models with various combinations of descriptors calculated in Dragon software, the statistically significant MLR models (obtained by leave-one-out procedure) containing two or three descriptors were generated by using genetic algorithms (GA). The best predictive models were chosen by examining the regression statistical parameters Q^2 (leave-one-out crossvalidation coefficient), R^2 (determination coefficient), PRESS (predictive error sum of squares) and s (standard error). Since the model size statistically is limited by the number of solutes, only two variable models were carried out in case of CN column. Most of the regression coefficients are statistically significant and all equations obtained can be acceptable from statistical point of view (regression and prediction). The most important descriptors in these models were accounting for two (2D) and three-dimensional (3D) aspects of the molecular structure but also some complex descriptors (topological, constitutional, conectivity indices and molecular properties) appear to be important for lipophilicity of food dyes. The most representative descriptors can be classified as RDF (Radial Distribution Function), GETAWAY (autocorrelation), 3D-MoRSE signal, Burden Eigenvalues and edge adjacency descriptors. The selected RDF descriptors are related to the atomic van der Waals volumes and atomic polarizabilities. The GETAWAY descriptors are related to the atomic Sanderson electronegativies and atomic van der Waals volumes. Also 2D descriptors (Burden eigenvalues and edge adjacency) shows that atomic polarizabilities, atomic Sanderson electronegativities, atomic van der Waals volumes and edge degrees are the most important properties responsible for dyes retention.

Applicability of the best models obtained showed excellent correlatins between chromatographic lipophilicity indices and predicted values of lipophilicity (Figure 7.6).

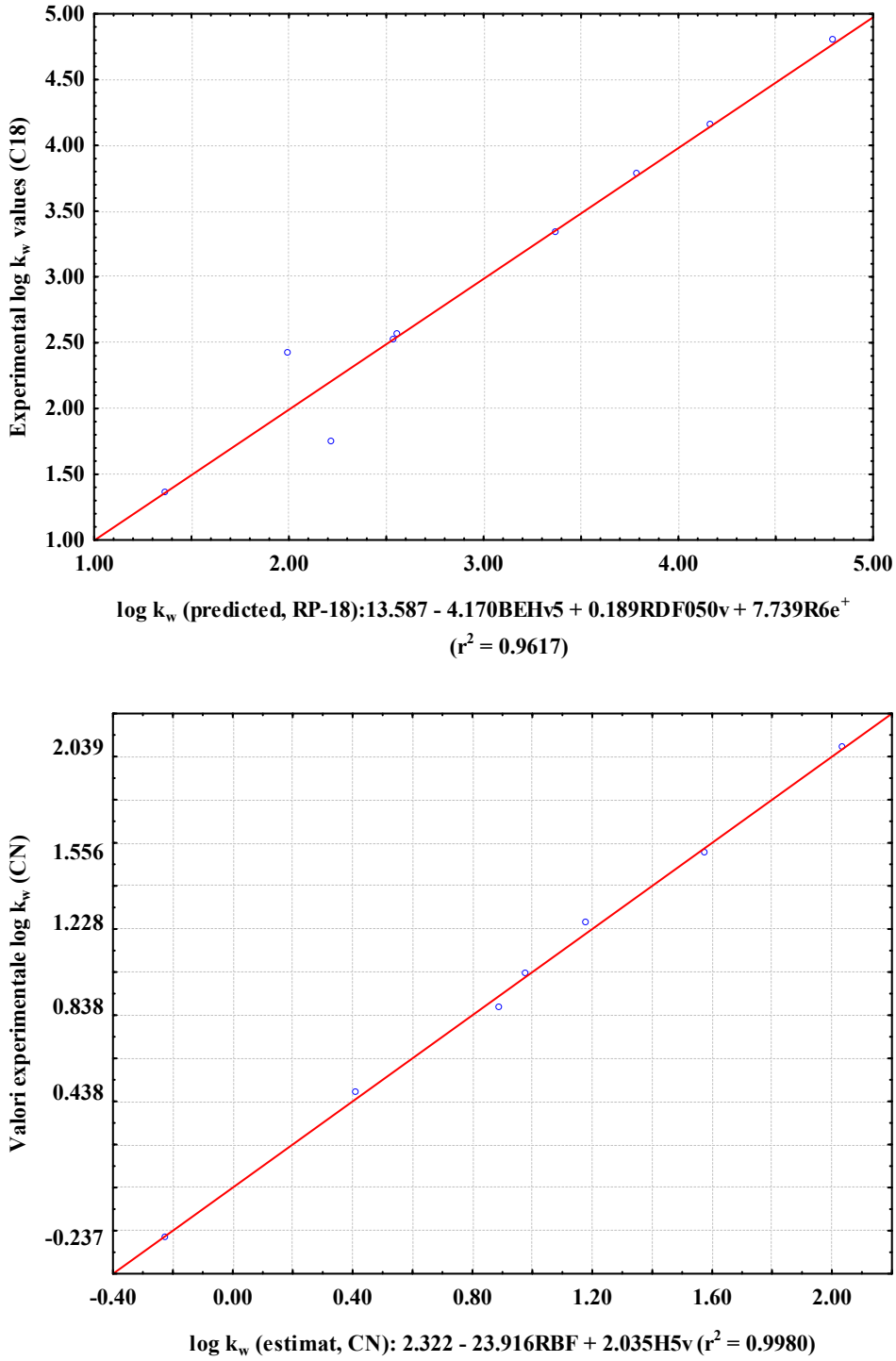


Figure 7.6 Plot of predicted vs. experimental lipophilicity indices of preservatives estimated using descriptors calculated by Dragon 5.4 software.

7.3 Determination of the lipophilicity of some food synthetic dyes by thin-layer chromatography

Due to the large variety of chromatographic plates, thin layer chromatography is considered an successful alternative for lipofilicity determination of different classes of compounds [54].

Because of their commercial importance many analytical procedures has been established and used for quality control of dyes and for evaluation of their impact on human health. The most relevant internationally agreed testing methods used by government, industry and independent laboratories, to assess the safety of chemical products, takes in consideration its lipophilicity parameters. Unfortunately, experimental lipophilicity data are not available in literature for the compounds investigated in this study. The classical experimental procedure using shake-flask method seems to be difficult for some of structurally dyes because of the large difference between water solubility and anticipated solubility in octanol.

In the light of the above considerations, we found it interesting to carry out a comparative study concerning the chromatographic lipophilicity of several synthetic dyes on different stationary phases. Because most of the considered compounds are easily ionizing, the purpose of this paper was also the elucidation of retention mechanism on different types of stationary phase and to assess the use of RP-TLC technique to the lipophilicity determination of this kind of compounds.

7.3.1 Experimental

The chromatographic behavior of some synthetic dyes was studied on different stationary phases: RP-18F_{254s} (20cm X 20cm, Merck Darmstadt-Germany), RP-18W/UV_{254} (20cm X 10cm, Macherey-Nagel) and CNF_{254s} (10cm X 10cm, Merck, Darmstadt-Germany). Chromatography was performed in a normal developing chamber (saturated for 15 minutes with solvent vapors) at room temperature (~22 ^{0}C), using different proportion mixtures of methanol-water as mobile phase (from 20% to 60% methanol in steps of 10% for all types of stationary phases). The developing distance was 8 cm in all cases. After development the plates were dried in air at room temperature and the spots of dyes were apparent from their colors.

7.3.2 Results and discussion

The experimental results obtained showed that the retention of the studied dyes regularly increased as the methanol content of the mobile phase was decreased. Linear relationships characterized by high correlation coefficients were obtained between R_M values and volume fraction of methanol. The profiles of retention indices (R_M) (Figure 7.7) illustrated regular changes of the retention of dyes with changing water-methanol ratio.

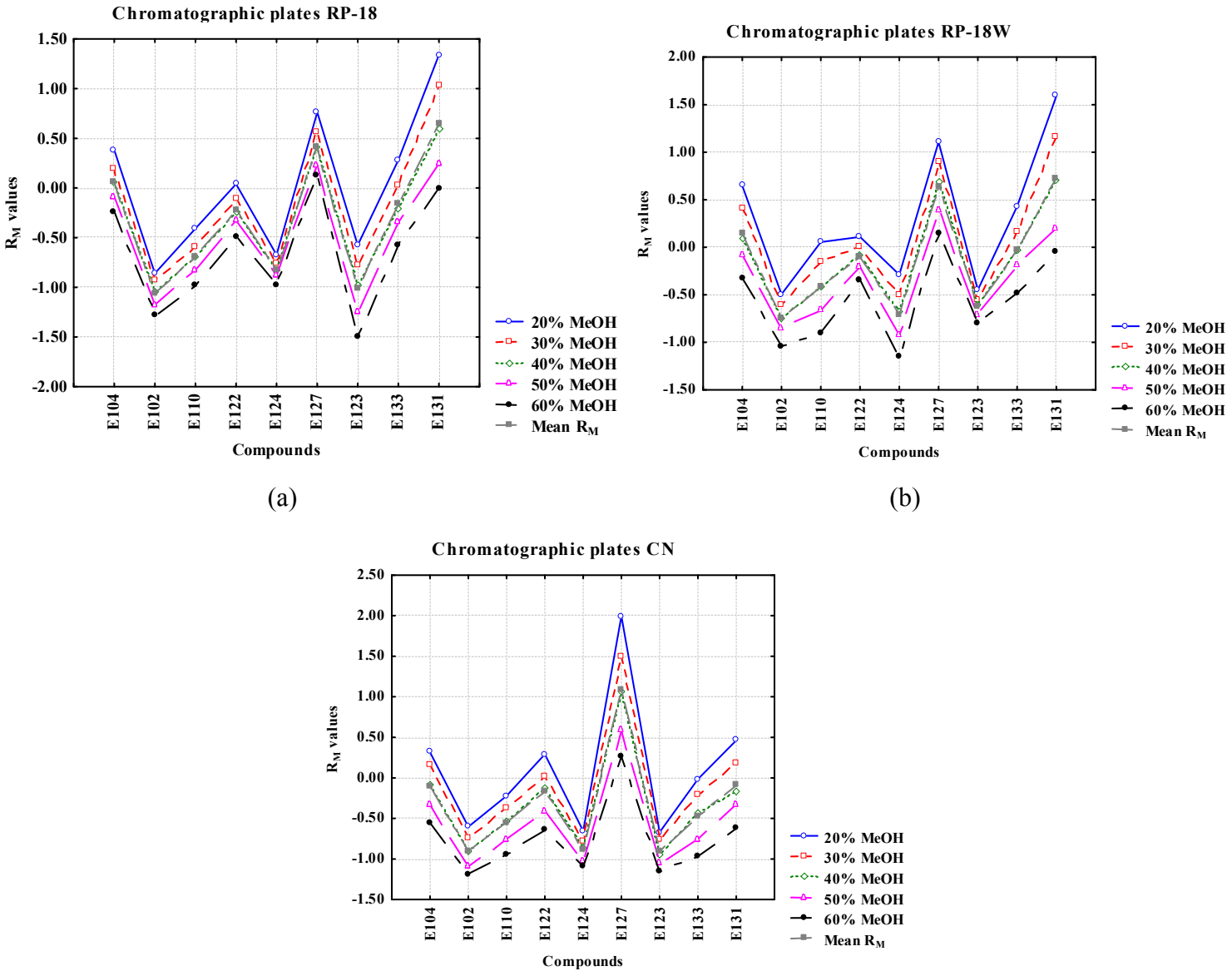
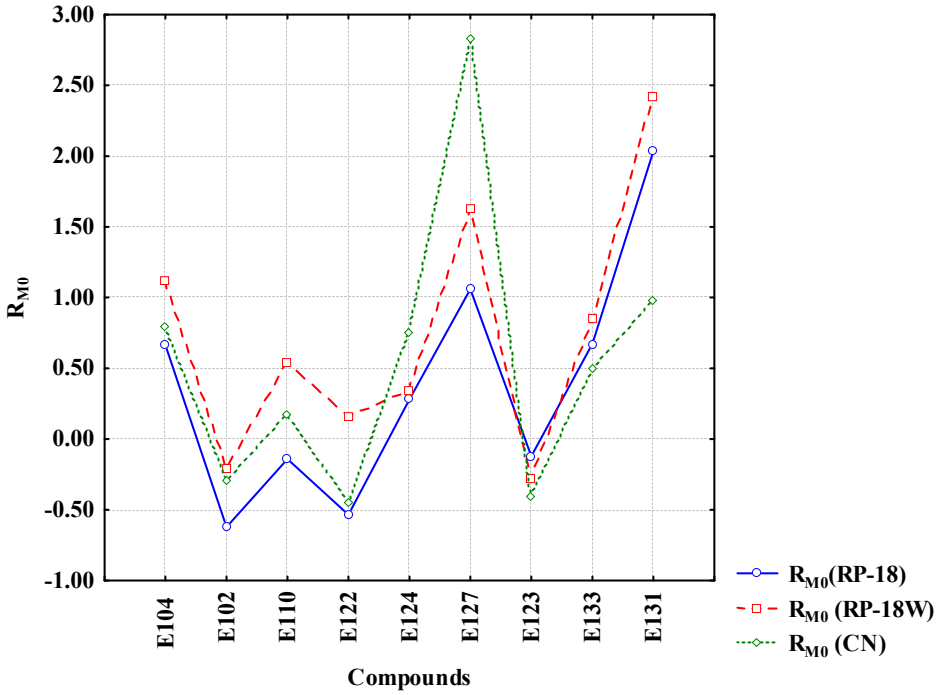


Figure 7.7 The profiles of R_M values for all fraction of methanol on: (a) RP-18; (b) RP-18W and (c) CN stationary phase.

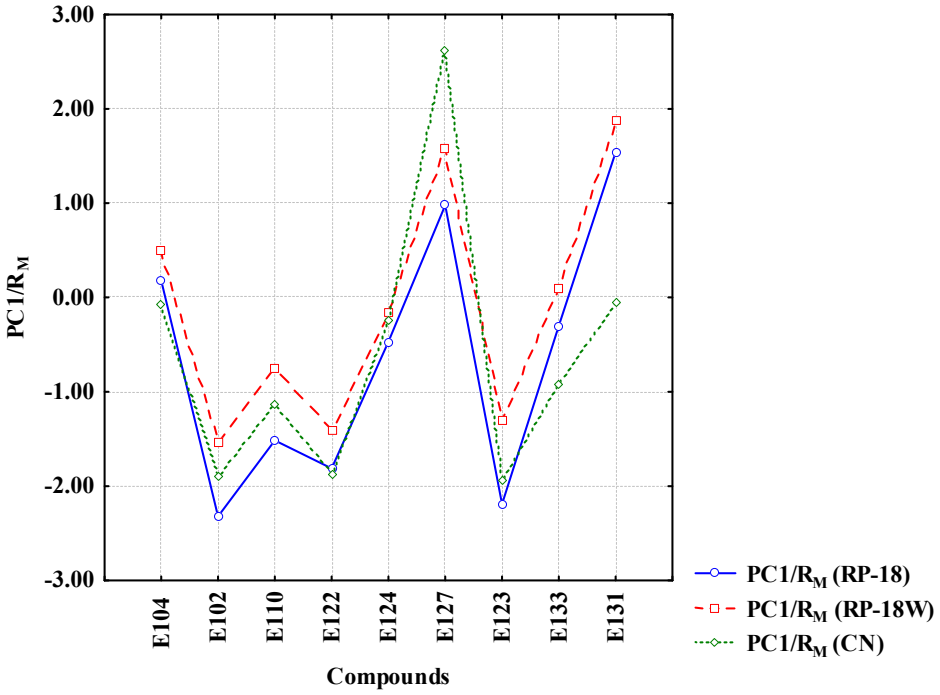
These systematic regularities of retention observed for all three types of stationary phases might indicate that the same mechanism (lipophilic interactions) is dominant in all cases and no secondary mechanisms were highlighted.

The profiles of R_{M0} values and profiles ($PC1/R_M$) of scores obtained by applying PCA directly to the matrix of R_M values (Figure 7.8) evidentiated similarity and differences between the lipophilicity parameters obtained on the three stationary phases. Given the large number of existing stationary phases and their different properties, the problem is the choice of the most suitable stationary phases so that chromatographic results obtained to be comparable with the theoretical calculated values or obtained by established methods. The correlation matrix (Table 7.9) illustrates low compatibilities between chromatographic indices of lipophilicity and the computed Log P values for the investigated dyes. The best correlations were obtained with the values calculated by using topological descriptors (Dragon 5.4 software). The weak correlation may be attributed to the fact that many computer programs do not recognize the potentially ionic character of molecules.

By using $PC1/R_M$ or φ_0 values as estimators for lipophilic character of synthetic dyes, the correlation between these values obtained on the all three stationary phases were significantly improved, correlation coefficient being higher than 0.92 in some cases. These fairly high correlation between φ_0 parameters on three stationary phases may be further evidence that secondary retention mechanisms are absent in all cases.



(a)



(b)

Figure 7.8 The profiles of lipophilicity indices R_{M0} (a) and $PC1/R_M$ (b) of the investigated dyes.

Table 7.9 The correlation concerning the lipophilicity parameters obtained on different RP-TLC plates and some computed log P values. (The highest statistical significant values are bolded).

	media R_M			**R_{M0}**			**b**			**φ_0**			**PC1/R_M**		
	RP-18	RP-18W	CN	RP-18	RP-18W	CN	RP-18	RP-18W	CN	RP-18	RP-18W	CN	RP-18	RP-18W	CN
Media R_M(RP-18)	1.00	**0.99**	**0.81**	**0.83**	**0.93**	0.68	-0.49	**-0.86**	-0.67	-0.65	**-0.84**	-0.64	**0.88**	**0.87**	0.68
Media R_M (RP-18W)	**0.99**	1.00	**0.85**	**0.83**	**0.92**	**0.70**	-0.52	**-0.84**	**-0.70**	-0.65	**-0.80**	-0.62	**0.86**	**0.86**	**0.70**
Media R_M (CN)	**0.81**	**0.85**	1.00	0.53	0.68	**0.83**	-0.09	-0.52	**-0.80**	-0.51	**-0.72**	-0.61	0.67	**0.70**	**0.84**
R_{M0}(RP-18)	**0.83**	**0.83**	0.53	1.00	**0.95**	0.69	**-0.80**	**-0.76**	**-0.71**	**-0.88**	**-0.79**	**-0.77**	**0.95**	**0.96**	0.67
R_{M0} (RP-18W)	**0.93**	**0.92**	0.68	**0.95**	1.00	**0.72**	-0.66	**-0.89**	**-0.73**	**-0.79**	**-0.88**	**-0.78**	**0.95**	**0.96**	**0.71**
R_{M0} (CN)	0.68	**0.70**	**0.83**	0.69	**0.72**	1.00	-0.23	-0.38	**-0.99**	**-0.80**	**-0.83**	**-0.91**	**0.82**	**0.85**	1.00
b (RP-18)	-0.49	-0.52	-0.09	-0.80	-0.66	-0.23	1.00	0.59	0.30	0.63	0.31	0.34	-0.59	-0.63	-0.20
b (RP-18W)	**-0.86**	**-0.84**	-0.52	**-0.76**	**-0.89**	-0.38	0.59	1.00	0.39	0.45	**0.70**	0.46	**-0.73**	**-0.72**	-0.37
b (CN)	-0.67	**-0.70**	**-0.80**	**-0.71**	**-0.73**	**-0.99**	0.30	0.39	1.00	**0.81**	**0.81**	**0.90**	**-0.81**	**-0.85**	**-0.97**
φ_0 (RP-18)	-0.65	-0.65	-0.51	**-0.88**	**-0.79**	**-0.80**	0.63	0.45	**0.81**	1.00	**0.78**	**0.90**	**-0.88**	**-0.91**	**-0.79**
φ_0 (RP-18W)	**-0.84**	**-0.80**	**-0.72**	**-0.79**	**-0.88**	**-0.83**	0.31	**0.70**	**0.81**	**0.78**	1.00	**0.90**	**-0.92**	**-0.90**	**-0.82**
φ_0 (CN)	-0.64	-0.62	-0.61	**-0.77**	**-0.78**	**-0.91**	0.34	0.46	**0.90**	**0.90**	**0.90**	1.00	**-0.88**	**-0.89**	**-0.90**
PC1/R_M (RP-18)	**0.88**	**0.86**	0.67	**0.95**	**0.95**	**0.82**	-0.59	**-0.73**	**-0.81**	**-0.88**	**-0.92**	**-0.88**	1.00	**0.99**	**0.81**
PC1/R_M (RP-18W)	**0.87**	**0.86**	**0.70**	**0.96**	**0.96**	**0.85**	-0.63	**-0.72**	**-0.85**	**-0.91**	**-0.90**	**-0.89**	**0.99**	1.00	**0.84**
PC1/R_M (CN)	0.68	**0.70**	**0.84**	0.67	**0.71**	1.00	-0.20	-0.37	**-0.97**	**-0.79**	**-0.82**	**-0.90**	**0.81**	**0.84**	1.00
log P	-0.41	-0.35	0.14	-0.53	-0.51	0.12	0.60	0.64	-0.10	0.30	0.30	0.19	-0.42	-0.38	0.13
CLog P^{CD}	0.10	0.17	0.59	-0.13	-0.02	0.47	0.36	0.15	-0.46	0.02	-0.13	-0.12	0.01	0.06	0.48
HY	**-0.86**	**-0.88**	**-0.87**	-0.60	**-0.74**	-0.68	0.25	0.68	0.69	0.54	**0.79**	0.57	-0.69	**-0.70**	-0.67
MLOGP	**0.72**	**0.74**	**0.76**	0.57	0.62	0.68	-0.28	-0.48	**-0.73**	-0.53	-0.68	-0.52	0.63	0.64	0.66
ALOGP	0.27	0.29	0.52	0.03	0.15	0.43	0.23	-0.10	-0.47	-0.01	-0.35	-0.20	0.16	0.17	0.40
ALOGPs	0.12	0.19	0.65	-0.19	-0.06	0.50	0.49	0.20	-0.48	0.05	-0.14	-0.14	-0.01	0.03	0.51
AC logP	0.29	0.28	0.15	0.35	0.25	0.18	-0.39	-0.18	-0.26	-0.28	-0.25	-0.12	0.28	0.27	0.14
milogP	0.20	0.27	**0.70**	-0.03	0.06	0.60	0.36	0.14	-0.57	-0.14	-0.25	-0.27	0.13	0.18	0.62
KOWWIN	0.37	0.37	0.44	0.22	0.25	0.30	-0.05	-0.20	-0.27	-0.23	-0.35	-0.16	0.26	0.25	0.32
XLOGP2	0.51	0.55	0.56	0.47	0.44	0.54	-0.36	-0.29	-0.62	-0.41	-0.42	-0.34	0.45	0.48	0.50
XLOGP3	0.49	0.51	0.44	0.47	0.40	0.41	-0.41	-0.28	-0.49	-0.39	-0.38	-0.26	0.43	0.44	0.37
ALOGpS	-0.64	-0.63	-0.43	-0.65	-0.60	-0.44	0.55	0.50	0.52	0.55	0.57	0.42	-0.61	-0.60	-0.40
AClogS	-0.29	-0.33	-0.69	-0.07	-0.11	-0.59	-0.25	-0.09	0.56	0.21	0.30	0.27	-0.21	-0.23	-0.60

Concluding remarks

- Lipophilicity data for two of the most important classes of food additives (preservatives and synthetic dyes) were determined by reversed-phase liquid chromatography with different stationary phases and by using different computation methods.
- Various stationary phases (RP-18, C8, C16 and RP-18W), used in this study, have shown a regular retention behavior for both classes of studied compounds, preservatives and synthetic dyes, in both RP-HPLC and in RP-TLC in all cases.
- Statistical data for preservatives lipophilicity parameters revealed highly significant correlations between the experimental and different computation lipophilicity indices in both RP-HPLC and in RP-TLC in case of all studied stationary phases.
- Thin Layer Chromatography proved to be a suitable technique for estimating preservatives lipophilicity, the results obtained on chromatographic plates RP-18W and CN being comparable with those obtained on chromatographic columns C8 and CN respectively.
- Highly significant correlation coefficients between chromatographic indices of lipophilicity determined on paraffin, olive, sunflower and corn oil-impregnated silica gel plates suggest that any of these oils can be used in impregnation of silica gel plates for prediction lipophilicity of parabens and other congeneric compounds.
- An extensive investigation made for quantitative structure-property (lipophilicity) relationships of studied preservatives, using lipophilicity parameters determined by two chromatographic techniques, revealed statistical significant prediction models for lipophilicity of preservatives compounds.
- The most important descriptors in these models were accounting for descriptors like Gibbs energy and total energy of molecule (calculated by using ChemDraw Ultra 8.0 software) but also topological and geometrical descriptors (calculated by using Dragon 5.4 software) appear to play an important role in the description of lipophilicity of preservatives.
- A quatitative structure-antioxidant activity relationship (QSAR) study of some food preservatives performed using multiple linear regression methods revealed that topological indices (calculated by using Dragon 5.4 software) play an important role in the description of antioxidant activity of food preservatives.

- Statistical data for synthetic dyes lipophilicity parameters revealed no highly significant correlations between the experimental indices estimated on C16 and CN and respectively RP-18, RP-18W and CN plates and different computation lipophilicity indices. These relatively no highly correlations, between experimentally and theoretical lipophilicity indices, are probably due to the estimation of log P, which have some limitations for complex structures of compounds.
- An extensive investigation made for quantitative structure-property (lipophilicity) relationships of studied synthetic dyes, using lipophilicity parameters determined by chromatographic technique, revealed statistical significant prediction models for lipophilicity of dyes and other congeneric compounds.
- The most important descriptors in these models were accounting for two (2D) and three-dimensional (3D) aspects of the molecular structure but also some complex descriptors (topological, constitutional, conectivity indices, molecular properties) appear to play an important role in the description of lipophilicity of food dyes.
- The best predictive models indicated the atomic van der Waals volumes, atomic polarizabilities, atomic Sanderson electronegativity and edge degrees of compounds having the largest influence in the chromatographic mechanism of dyes on all stationary phases.

Selected References

1. B. Testa, P. Crivori, M. Reist, P.A. Carrupt, *Perspect. Drug Discovery Des*. **1** (2000) 179
2. C. Hansch, A. Leo, Exploring QSAR. *Fundamentals and Applications in Chemistry and Biology*, ACS, Washington, *DC* (1995)
3. M. Karelson, *Molecular Descriptors in QSAR/QSPR*, John Wiley&Sons, NewYork (2000)
4. R. Kaliszan, *Structure and Retention in Chromatography: A Chemometric Approach*, Harwood Academic Publishers, Amsterdam (1997)
5. J. Sangster, *Octanol–water Partition Coefficients: Fundamentals and Physical Chemistry, John Wiley & Sons,* New York (1997)
6. R.F. Rekker, R. Mannhold, *Calculation of Drug Lipophilicity: The Hydrophobic Fragmental Constant Approach,* VCH, Weinheim (1992)
7. J.F.K. Huber, C.A.H. Meijers, J.A.R.J. Hulsman, *Anal. Chem*. **44** (1972) 111
8. A. Kaune, M. Knorrenschild, A. Kettrup, *Fresenius J. Anal. Chem*. **352** (1995) 3030
9. X. Liu, H. Tanaka, A. Yamauchi, B. Testa, H. Chuman, *J. Chromatogr. A* **1091** (2005) 51
10. J.G. Dorsey, M.G. Khaledi, *J. Chromatogr. A* **656** (1993) 485
11. R. Kaliszan, *Quant. Struct.-Act. Relat*. **9** (1990) 83
12. A. Bechalany, A. Tsantili-Kakoulidou, N. El Tayar, B. Testa, *J. Chromatogr.* **541** (1991) 221
13. B. Slater, A. McCormack, A. Avdeef, J.E.A. Comer, *J. Pharm. Sci.* **83** (1994) 1280
14. D. Vrakas, C. Giaginis, A. Tsantili-Kakoulidou, *J. Chromatogr. A* **1116** (2006) 158
15. K. Való, *J. Chromatogr. A* **1037** (2004) 299
16. S.E. Lucangioli, C.N. Carducci, V.P. Tripodi, E. Kenndler, *J. Chromatogr. B* **765** (2001) 113
17. T. Brauman, G.Weber, L.H. Grimmen, *J. Chromatogr.* **236** (1983) 329
18. W. J. Lambert, *J. Chromatogr. A* **656** (1993) 469
19. R. Kaliszan*, Chem. Rev.* **107** (2007) 3212
20. J. Sangster, *LOGKOW-a databank of evaluated octanol-water partition coefficients,* Sangster, Research Laboratories, Montreal (1993)
21. C. Giaginis, A. Tsantili-Kakoulidou, *J. Liq. Chromatogr. Relat. Technol.* **31** (2008) 79
22. K. Valkó, P. Segel, *J. Chromatogr.* **631** (1993) 49
23. C. My Du, K. Valkó, C. Bevan, D. Reynolds, *Anal. Chem.* **70** (1997) 4228
24. C. Sarbu, S. Todor, *J. Planar Chromatogr.* **11** (1998) 123
25. C. Sârbu, S. Todor, *J. Chromatogr. A* **822** (1998) 263

26. C. Sârbu, D. Casoni, M. Dărăbanțu, C. Măiereanu, *J. Pharm. Biomed. Anal.* **35** (2004) 213
27. J. Flieger, M. Tatarczak, *J. Planar Chromatogr.* **19** (2006) 386
28. S. Gocan, G. Cimpan, J. Comer, *Lipophilicity measurements by liquid chromatography. In: Grushka E, Grinberg N (eds) Advances in chromatography*. Oxford, UK (2005) 79
29. A. Montafio, A.H. Sinchez, L. Rejano, *Analyst* **120** (1995) 2483
30. M.G. Soni, G.A. Burdock, S.L. Taylor, N.A. Greenberg, *Food Chem. Toxicol*. **39** (2001) 513
31. M.G. Soni, S.L. Taylor, N.A. Greenberg, G.A. Burdock, *Food Chem. Toxicol*. **40** (2002) 1335
32. J. Ring, K. Brockow, H. Behrendt, *J. Chromatogr. B* **756** (2001) 3
33. HyperChem, Release 7.5 for Windows, Molecular Modeling System; Hypercube, Inc. and Autodesk, Inc.
34. Chemical Structure Drawing Standard, ChemDraw Ultra 8.0.3, (2003) http://www.cambridgesoft.com
35. Software DRAGON Plus version 5.4, (2006). http://www.talete.mi.it
36. Virtual Computational Chemistry Laboratory. http://vcclab.or./alogps/start.html
37. The Human Metabolome Project. http://www.metabolomics.ca
38. T.C. Schunkl, M. F. Burke, *J. Chromatogr. A* **656** (1993) 289
39. P.K. Zarzycki, M. Wierzbowska, H. Lamparczyk, *J. Chromatogr. A* **857** (1999) 255
40. S. Espinosa, E. Bosch, M. Roses, *J. Chromatogr. A* **947** (2002) 47
41. S. Oishi, *Toxicol. Ind. Health* **17** (2001) 31
42. C. Lemini, A. Hernandez, R. Jaimez, Y. Franco, M. Avila, A. Castell, *Toxicol. Ind. Health* **20** (2004) 123
43. C. Lemini., R. Jaimez, M. Avila, Y. Franco, F. Larrea, A. E. Lemus, *Toxicol. Ind. Health* **19** (2003) 69
44. A. Scott, M. Masten, *Final Review of Toxicological Literature*, NTP/NIEHS Research Triangle Park, North Carolina (2004) 1
45. T. Angelov, A.Vlasenko, W.Tashkov, *J. Liq. Chromatogr. Relat. Technol.* **31** (2008) 188
46. T. M. Parkinson, J. P. Brown, *Ann. Rev. Nutr*. **1** *(*1981) 175
47. A. Nasal, D. Siluk, R.Kaliszan, *Cur. Med. Chem.* **10** *(*2003) 381
48. C.Giaginis, S.Theocharis, A. Tsantili-Kakoulidou, *J. Chromatogr. A* **1166** *(*2007) 116
49. D. Casoni, A. Kot-Wasik, J. Namieśnik, C. Sâbu, *J. Chromatogr. A* **1216** (2009) 2456

50. D. Casoni, C. Sârbu, *Chromatographia* **70** (2009) 1277

51. D. Casoni, C. Sârbu, *J. Sep. Sci.* **32** (2009) 2377

52. Todeschini, R., *Moby Digs Academic version software for variable subset selection by genetic algorithms,* Rel. 1.0 for Windows, Talete, Milan (2004)

53. A. G. Mercader, P. R. Duchowicz, F. M. Fernández, E. A Castro, *Chemom. Intell. Lab. Syst.* **92** (2008) 138

54. D. Casoni, C. S. Cobzac, C. Sârbu, *Rev. Chim. (Bucureşti)* **61** (2010) 229-234