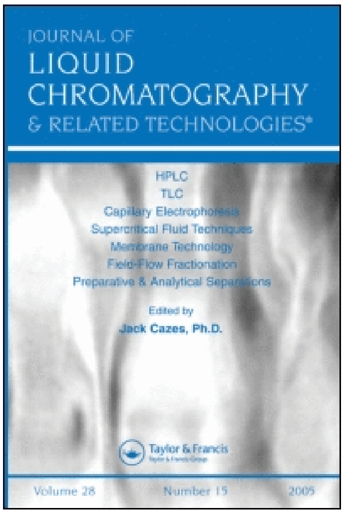

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Identification of New Phthalazine Derivatives by HPTLC-FTIR and Characterization of Their Separation Using Some Molecular Properties

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ABSTRACT

The identification of compounds separated by high performance thin layer chromatography (HPTLC) can be made by coupling the HPTLC with Fourier transform infrared (FTIR) spectroscopy. The quality of HPTLC-FTIR spectra is sufficient for identification of unknown substances, and the information is extremely valuable when examined in reference to the

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spectra of pure compounds, on different portions of the spectrum. If the FTIR spectra are recorded, the identification of compounds that have been separated by HPTLC can be made without using the standards. It is known that the physico-chemical properties of solutes affect their retention parameters. Many molecular parameters have been used in HPTLC studies to find the relationship between chemical structure and chromatographic retention. The correlation between several properties of Phthalazine derivatives such as dipole moment, polarizability, and surface area, and the retention parameters of these compounds, can be realized. The dipole moment was found to be an important descriptor, and this property of the compounds has a major influence on the retention parameter.

Key Words: Phthalazine derivatives; HPTLC; FTIR spectroscopy; FTIR ATR spectra; Molecular properties.

INTRODUCTION

One of the problems in high performance thin layer chromatography (HPTLC) is the identification of separated compounds in the absence of standards. Coupling of two or more analytical techniques in HPTLC has the goal of a rapid and efficient chromatographic separation and subsequent on-line identification of the fraction obtained. Until 1989, the combination HPTLC and ultraviolet-visible (UV-VIS) spectroscopy was the only on-line coupling method available in planar chromatography.[1] The information content of UV-VIS spectra is rather poor and rarely enables unambiguous identification of a substance.

Because IR absorption is observed for nearly all chemical compounds, it is possible to detect and quantify even non-UV absorbing substances on TLC plates, which makes this hyphenated technique universally applicable.[2] Although the ranges are quite well defined, the precise frequency or wavelength at which a specific group absorbs is dependent on its environment within the molecule and on its physical state. The quality of HPTLC-FTIR spectra is sufficient for identification of unknown substances and discrimination between closely related substance.[3] The information is extremely valuable when examined in reference to the spectra of pure compound, on different portions of the spectrum.

Another more important current problem of planar chromatography is the theoretical and experimental elaboration of methods for predicting behavior of compounds in a chromatographic system (predicting R_f values and the succession of compounds in complex mixture analysis). Although, it is known that is impossible to predict chromatographic retention from physical data of pure compounds, there are a series of empirical rules to determine the retention parameters of a given compound.

In recent years, some studies of quantitative structure-retention relationships (QSRR) have been widely accepted in fields of HPLC, GC, and TLC.[4] High performance thin layer chromatography has several advantages: it is rapid and relatively simple, very small amounts of sample are required, and the compounds need not be very pure (because the impurities separate during the chromatographic process). For these reasons, HPTLC has been used in many QSRR studies. Many molecular parameters have been evaluated in HPTLC QSRR studies to find the relationship between chemical structure and chromatographic retention,[5,6] and combinations of several descriptors[7–9] have also been evaluated in these studies.

In solution, the solvent molecules can interact with a certain atom group (*X*) in the same mode even if the type of molecule is R-*X* or *X*-R-*Y*, but to the contrary, the molecular interactions between compounds and solid support depend on the molecule structure. The physico-chemical properties of solutes are affecting the retention parameters. One of the aims of this paper is to demonstrate the influence of three descriptors set—dipole moment, polarizability, and surface area—in solute retention of three series of Phthalazines derivatives, which are different in terms of their radicals.

Phthalazines are a class of compounds possessing many applications, such as pharmaceuticals (antihypertensives, anti-inflammatory, antiviral, etc.), fungicides, protective agents against radiation, and herbicides.[10] The Phthalazine derivatives analyzed in this paper are new synthetic compounds.[11]

EXPERIMENTAL

Materials

Toluene, chloroform, and methanol were commercial solvents (Reactivul, Bucharest). All solvents were of analytical grade. The chromatographic plates (10 × 20 cm) were silica gel 60 F_{254} (Merck, Darmstadt, Germany).

Chromatography

The solutions (0.1%) of the compounds 1–9 (1-*o*-bromophenoxy-4-phenyl-phthalazine; 1-*o*-bromophenoxy-4-tolyl-phthalazine; 1-*o*-bromophenoxy-4-benzyl-phthalazine; 1-*p*-nitrophenoxy-4-phenyl-phthalazine; 1-*p*-nitrophenoxy-4-tolyl-phthalazine; 1-*p*-nitrophenoxy-4-benzyl-phthalazine; 1-[3-(β-diethylaminoethyl)-4-methyl-7-coumarinyloxy]-4-phenyl-phthalazine;

1-[3-(β-diethylaminoethyl)-4-methyl-7-coumarinyloxy]-4-tolyl-phthalazine; 1-[3-(β-diethylaminoethyl)-4-methyl-7-coumarinyloxy]-4-benzyl-phthalazine) were prepared in chloroform. They were separated by HPTLC using toluene–chloroform–methanol (70 : 20 : 1) as the mobile phase, and detection was made in ultra violet (UV) light (254 nm). The plates were developed twice with the same mobile phase at room temperature in a saturated N-chamber, by the ascending technique.

Fourier Transform Infrared Spectroscopy

The Fourier transform infrared (FTIR) spectra of the compounds were recorded in the region 4000–400 cm^{-1} by a Bruker EQUINOX 55 spectrometer, using an Attenuated Total Reflectance (ATR) accessory with a scanning speed of 32 cm^{-1} min^{-1} with the spectral width 2.0 cm^{-1}. Calibration of the spectrometer was made with a reliable, standard, and polystyrene.

RESULTS AND DISCUSSION

With the aim of unambiguous identification of compounds 1–9 (Fig. 1), we investigated their FTIR ATR spectra through a comparison between those: (a) registered on pure crystalline substance; (b) registered on silica gel HPTLC plates; and (c) subtracted spectra, i.e., spectrum (b) minus the spectrum of silica gel. Without the intention of a precise interpretation of the vibrations of the molecules, the FTIR ATR spectra were treated through empirical comparison of the data. For two compounds, 3 and 4, from the Phthalazine derivatives 1–9, examples will be given. The spectra are adequately resolved and of adequate intensity.

We focused our attention on preliminary examinations of the spectra on two important areas, the regions 1600–1300 cm^{-1} and 900–650 cm^{-1}. In the first discussed area on about 1357–1381 cm^{-1}, both compound 3 and 4 showed two strong absorption bands (Figs. 2 and 3), which are present in spectra (a), (b), and (c). They have almost identical absorbency patterns in this region. These bands are too narrow to distinguish the compounds. The strong absorption bands in the 900–650 cm^{-1} region indicates aromatic structures. Aromatics compounds display strong out of plane C—H bending and ring bending absorption bands in this region, which are correlated with the substitution pattern. The exact position of an absorption band within this narrow region reveals several important matches of the spectra and certain identification. In Fig. 2, in this region (900–650 cm^{-1}) more peaks are easily recognized. The corresponding relative intensities of the peaks are obvious in

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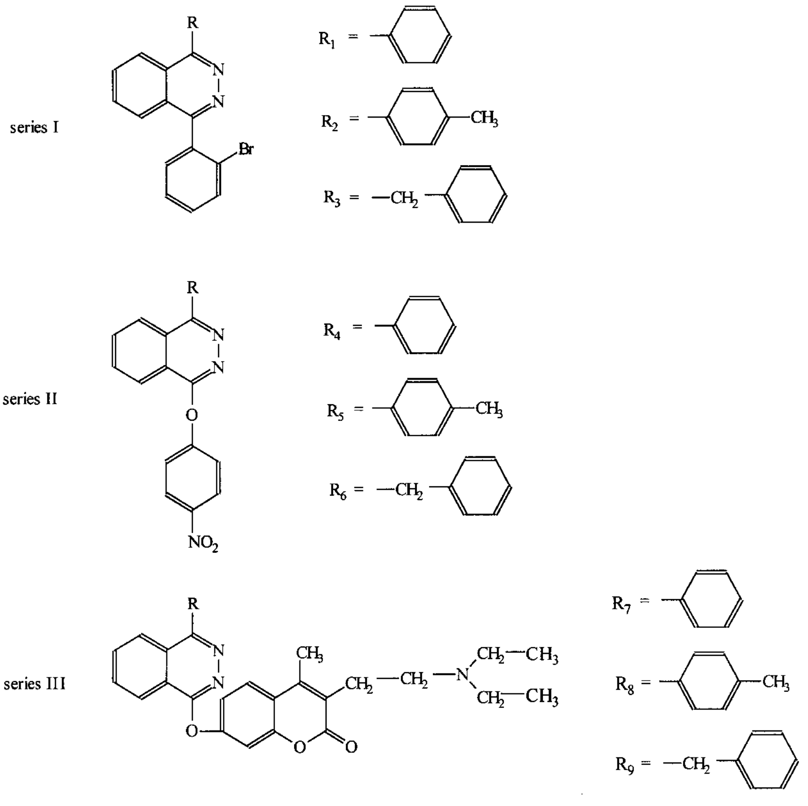

Figure 1. The structures of phthalazine derivatives 1–9.

the spectra. For compound 4 (Fig. 3), the peak at 775 cm^{-1} in the pure compound is present in the subtracted spectra. The peaks at 799 and 829 cm^{-1} are smoothed in the subtracted spectra (c) and are together with the peak at about 803 cm^{-1} from the silica gel in the registered spectra from the plates (b).

A more detailed analysis can reveal other similarities between the spectra even in the intermediate fingerprint region, 1300–900 cm^{-1}, despite the fact that silica gel has a broad absorption band located here. The large surface of the silica gel, with its active OH groups, makes adequate compensation of the sample and reference spectra more difficult. Despite this, the form of this peak and, further, the possibilities of spectra subtraction, can yield some useful details for interpretation. In our case (Fig. 2), the broad hump at about 1180–1200 cm^{-1} is revealed in the compensated spectra, as is even the third band from the triplet (with the highest peak of 1219 cm^{-1}). In the spectra (b)

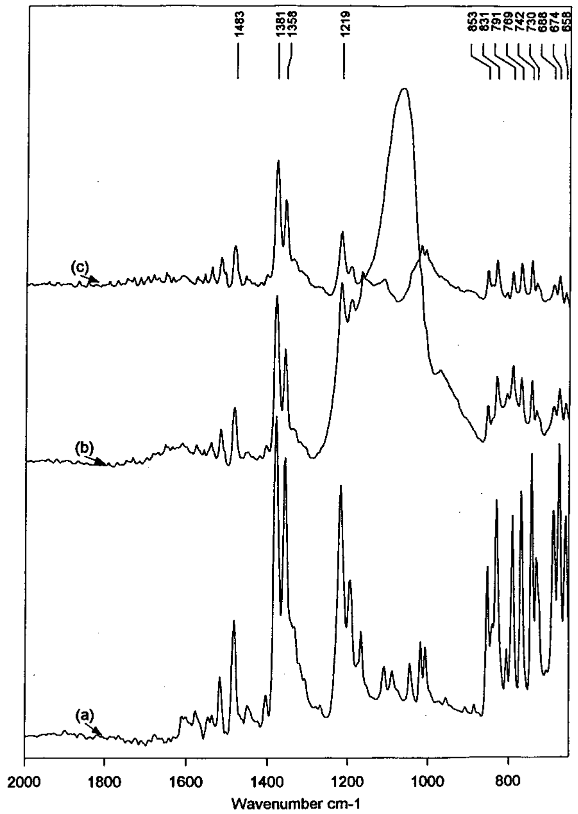

Figure 2. FTIR ATR spectra of compound 3: (a) registered on pure crystalline substance; (b) registered on silicagel HPTLC plates; and (c) subtracted spectrum: spectrum (b) minus spectrum of silica gel.

of substance 4 (Fig. 3), a weak shoulder is revealed after subtraction in (c) of the peak at 1225 cm^{-1}, the highest peak from the multiplex bands at 1220–1230 cm^{-1}. Such weak peaks would be of little value in the more complicated regions of the spectrum, but in some cases they can give extremely useful information during the identification of a compound.

In our opinion, there are enough differences to enable unequivocal identification of a substance member of the group. Shifts in absorption position, and changes in band contours accompanying changes in molecular environment, may also be able to suggest important structural details. Depending on the concentration of the probe, more details can be evident, and the identification

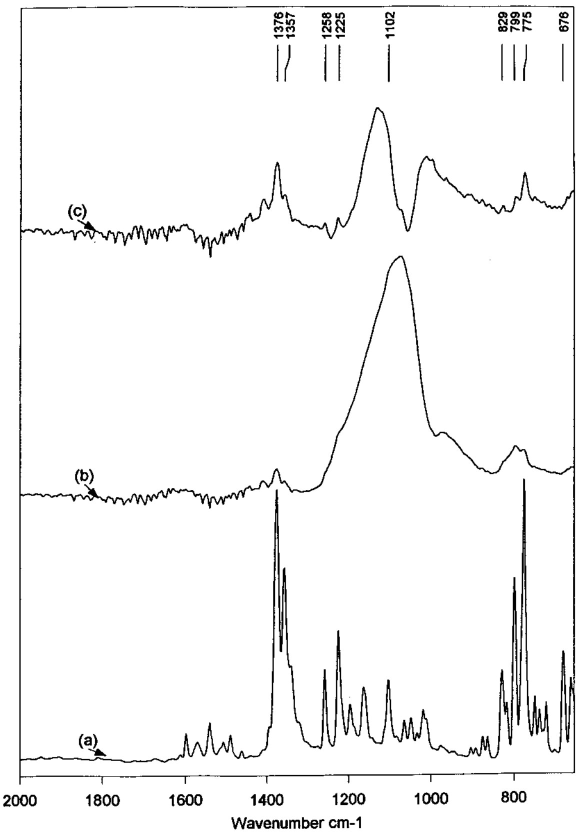

Figure 3. FTIR ATR spectra of compound 4: (a) registered on pure crystalline substance; (b) registered on silica gel HPTLC plates; and (c) subtracted spectrum: spectrum (b) minus spectrum of silica gel.

can be performed without any doubt. Weak bands in the high frequency region resulting from the fundamental absorption of the functional groups can be extremely valuable in the determination of the structure and identification of a substance and should be the subject of further investigation.

Similar compounds may give virtually identical spectra under normal conditions, but differences can be detected with an expanded vertical scale or with a very large sample. By comparison of spectra in which the shapes of peak are important, we should be aware of the substantial differences in the appearance of the spectrum generated by the smoothing of the spectra baselines. Although the ranges are quite definite, the precise frequency or wavelength at which a specific group absorbs, is dependent on its environment within the molecule and on its physical state. The corresponding relative intensities of the signals are evident in the spectra. Shifts in absorption position, and changes in band contours accompanying changes in molecular environment, may also be able to suggest important structural details.

The goal of part two of this paper is the characterization of the HPTLC separation of these new Phthalazine derivatives. The objective of this investigation was to determine the correlation between several solute-related parameters of Phthalazine derivatives and their retention parameters. The properties of the solutes are used as descriptors to predict the retention parameter. The dipole moment, polarizability, and surface area are chosen as descriptors.

The experimentally determined $\mathrm{h}R_\mathrm{f}$ values for each solute are presented in Table 1. The molecular dipole moment, the polarizability, and the surface area are calculated using appropriate computational techniques, and their values are listed in Table 1.

A regression model for predicting the R_f values of individual compounds was constructed. The following model predicts the individual $\mathrm{h}R_\mathrm{f}$ values of Phthalazine derivatives for each series:

$$\mathrm{h}R_\mathrm{f} = a\mu + b\alpha + cA_\mathrm{s}$$

where μ, α, and A_s are the dipole moment, polarizability, and surface area of the compound, and a, b, and c are coefficients. The coefficients are obtained by solving the system of equations for each series. The values of coefficients a, b, and c are presented in Table 2.

Table 1. The experimental $\mathrm{h}R_\mathrm{f}$ values and the descriptor values of compounds.

Series	No.	$\mathrm{h}R_\mathrm{f}$	Dipole moment (D)	Polarizability (Å^3)	Surface area (Å^2)
I	1	66.67	4.112	38.23	401.70
	2	59.44	4.131	40.07	446.44
	3	71.11	4.891	40.07	407.50
II	4	53.33	8.622	38.08	448.58
	5	62.78	8.637	39.92	493.23
	6	67.22	9.068	39.92	461.24
III	7	53.33	7.438	55.70	666.64
	8	61.11	6.161	57.54	660.42
	9	52.78	6.742	57.54	627.27

Table 2. The calculated values of coefficients.

Series	*a*	*b*	*c*
I	−9.0074	7.6965	−0.4743
II	−109.1863	45.1248	−1.6112
III	−6.5000	0.1815	0.1374

The relative influence of each descriptor on the variability of R_f can be quantified by dividing the coefficient for that descriptor by the sum of the absolute values of the coefficients of all descriptors in the model. The relative influence of dipole moment, polarizability, and surface area are 52.44%, 44.81%, and 2.77% for series I; 70.04%, 28.93%, and 1.03% for series II; and 95.33%, 2.67%, and 2.02% for series III, respectively. It can be concluded that the relative influence of descriptors varies with the changing of radicals from the molecular structure. The surface area does not have a significant influence on the retention parameters of compounds. While the dipole moment and polarizability have almost the same influence on the retention parameter for series I, the influence of dipole moment increases for series II and III. Also, for series III, it can be seen that influence of dipole moment is biggest, and the influence of polarizability and surface area can be neglected. It can be concluded that dipole moment was found to be an important descriptor, and this property of the compounds has a major influence on the retention parameter.

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