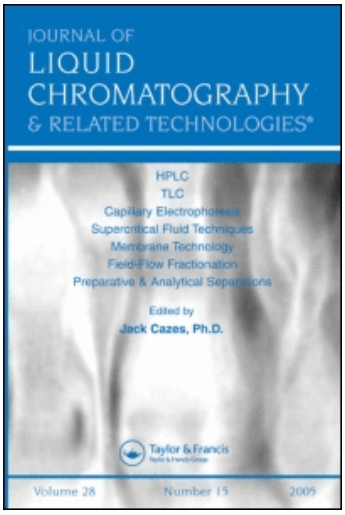

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TLC Determination of Hydrophilicity Parameter of Some Pyridinium Aldoximes

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Abstract: The chemical structures of certain drug candidates give only a minimum of lipophilicity. Investigation of their elution on reversed-phase materials was difficult, even when the water content of the mobile phase was changed. The dominative role

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of lipophilicity, either in the drugs' penetration or in the receptorial drug binding, is considered to be valid solely in the same chemical group of the various drugs.

In the cases of extremely lipophobic (i.e., hydrophilic) compounds, both the determination or calculation of lipophilicity was difficult or impossible. This is the reason that a novel index, the TLC hydrophilicity parameter, should be introduced as a substitute for lipophilicity.

Hydrophilicity can be preferrably determined using a planar stationary phase and an aqueous-organic mobile phase. Numerical data of hydrophilicity on silica stationary phase can be calculated using R_M values, similar to the lipophilicity indices.

Keywords: Pyridinium aldoximes, Hydrophylicity, TLC, Silica, R_M values

INTRODUCTION

Lipophilicity of drugs has a basic importance. If the other conditions (e.g., solubility and also stability in body fluids) are fulfilled, drugs of lipophilic nature are able to penetrate through membranes and to exert definite binding to the proper receptor. Lipophilicity is generally characterized by the octanol/water partition coefficient, a generally accepted physicochemical parameter. Numerical values of lipophilicity are generally given as log P, which mirrors the concentration ratio of the unionized form of the drug in the two unmixable phases.

The classical method to determine lipophilicity is measurement of the octanol—water distribution in a shaken flask. However, this method is time-, solvent- and substance-consuming and became substituted with a wide variety of other methods. Takacs-Novak et al.[1] determined the protonation constants and related them to the octanol/water partition coefficient of various drug candidate compounds. Melander and Horváth[2] used reversed-phase high performance liquid column chromatography (RP-HPLC) to measure the lipophilicity indices. Their results show adequate correspondence of log k to the log P values, determined by distribution methods. Computer assisted calculations (also called *in silico* methods) were also used by several authors; these methods are based on the contributions of the various parts of the organic compound to its total lipophilicity.

This paper deals with a characterization method when neither RP-HPLC nor the determination of protonation can be preferentially used as the compounds are highly ionized through the acceptable pH range.

EXPERIMENTAL

Materials

Solvents such as methanol; ethanol, 96%; isopropanol, n-butanol, n-hexane, and hydrochloric acid were purchased from Reanal Chemical Factory,

Budapest, Hungary in analytically pure quality. Other solvents, such as acetonitrile, acetone, and ammonium hydroxide were obtained from Merck, Darmstadt, Germany, in the best available purity. Water was double-distilled and de-ionized. Paraffin oil, VIII. Ph. Hung. quality, was purchased from a local pharmacy store.

Several pyridinium aldoximes (called K- compounds) were synthesized in the laboratory of Dr. Kuca. The chemical structures of quaternary pyridinium aldoximes, used in this work (K-27 and K-48), are given in Fig. 1. The chemical structures of the other compounds were given in our earlier publications.

Ecdysteroids were isolated from plant sources; their chemical structures are given in our earlier publications.[3–5]

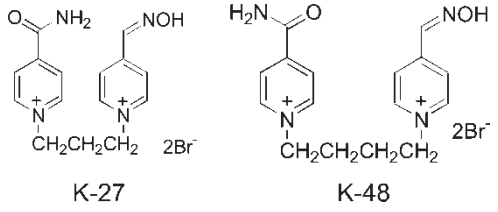
Chromatography

200 mm × 200 mm TLC plates prepared with 0.2 mm layers of silica gel 60 F_{254} (Merck, Darmstadt, Germany) were used. These TLC plates were impregnated by an 18 hours continuous development with 10% paraffin oil in n.hexane.

All-glass TLC chambers of DESAGA (Heidelberg, Germany) were used with glass lids. The sample solutions (10 μL from 5 mg/mL concentration solution) were loaded at a 30 mm distance from the bottom edge of the plates. The development was terminated when the mobile phase front was at the position of 20 mm from the top of the plate. Chambers with unsaturated vapor phase were used.

Hydrophilicity was determined using plain silica gel TLC plates, and the spots were subjected to chromatography using 70%, 80%, and 90% aqueous methanol and 100% methanol (also containing 1% ammonium hydroxide, each) as the mobile phase.

Lipophilicity was determined on paraffin-coated (i.e., non-covalently bound reversed-phase) TLC plates, using 30%, 40%, 50%, 60%, 70%, and 80% aqueous methanol (also containing 1% ammonium hydroxide) as the mobile phase.



K-27 K-48

Figure 1. The chemical structures of K-27 and of K-48.

Detection

The spots were localized under UV light of a DESAGA lamp at 254 nm. Dark spots on fluorescent background were observed, as each one of the investigated substances shows ultraviolet absorbance at 254 nm.

Formulas for the Calculation of R_F and R_M

The R_F and R_M values were calculated using the general relationships, such as:

$$R_F = \frac{\text{Spot distance from the start}}{\text{Front distance from the start}}$$

$$R_M = \log[(1/R_F) - 1]$$

RESULTS

Table 1 gives the linear characteristics of the plots when the various compounds were subjected to normal-phase and reversed-phase chromatography to calculate their hydrophilicity and lipophilicity.

The compounds of hydrophilic characteristics can also be subjected to displacement chromatography (D-FRONT means: the front of displacer). The outcome of the processes is given in Fig. 2. There was no displacement by the displacer front in either water-methanol-hydrochloric acid (Fig. 2a) or water-methanol-hydrochloric acid mobile phase (Fig. 2a) as all compounds [1:K-27, 2:K-48, 3:pralidoxime, 4:obidoxime] remained behind the displacer

Table 1. Characteristics of lines of plots of R_M versus % of organic modifier (methanol)

	Uncoated silica		Silica impregnated with paraffin	
	y°	Slope	y°	Slope
K-27	0.918	−0.031	0.00	0.00
K-48	1.04	−0.034	0.00	0.00
Pralidoxime	0.513	−0.018	0.00	0.00
Obidoxime	0.953	−0.034	0.00	0.00
20-Hydroxyecdysone	−0.5256	−0.007	1,015	−0,0212
Polypodine B	−0.5185	−0.0001		
2-Deoxy-20-hydroxyecdysone	−0.4643	−0.0027	2,390	−0,0382
2-Deoxyecdysone	−0.4833	−0.0021	1,528	−0,0280
20-Hydroxyecdysone-22-acetate	−0.5391	0.001	1,230	−0,0257
Integristerone A	−0.6277	0.014	0,587	−0,0147

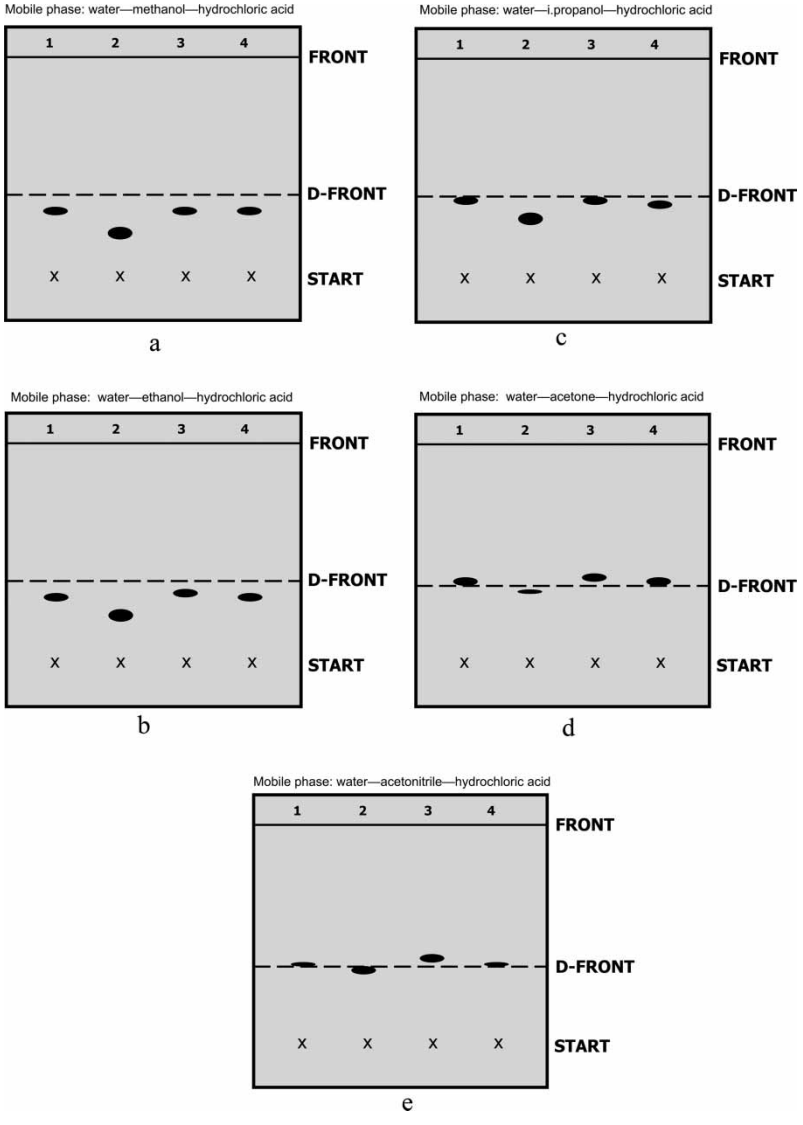


Figure 2. a) TLC of pyridinium aldoximes [1:K-27, 2:K-48, 3:pralidoxime, 4:obidoxime] using TLC silica plates and water-methanol-hydrochloric acid mobile phase; b) TLC of pyridinium aldoximes [1:K-27, 2:K-48, 3:pralidoxime, 4:obidoxime] using TLC silica plates and water-ethanol-hydrochloric acid mobile phase; c) TLC of pyridinium aldoximes [1:K-27, 2:K-48, 3:pralidoxime, 4:obidoxime] using TLC silica plates and water-i-propanol-hydrochloric acid mobile phase; d) TLC of pyridinium aldoximes [1:K-27, 2:K-48, 3:pralidoxime, 4:obidoxime] using TLC silica plates and water-acetone-hydrochloric acid mobile phase; e) TLC of pyridinium aldoximes [1:K-27, 2:K-48, 3:pralidoxime, 4:obidoxime] using TLC silica plates and water-acetonitrile-hydrochloric acid mobile phase.

front. The situation remained the same when methanol was replaced with ethanol (Fig. 2b). Displacement of K-27 and pralidoxime takes place in water-i-propanol-hydrochloric acid mobile phase (Fig. 2c). All the investigated pyridinium aldoximes became displaced in water-acetone-hydrochloric acid (Fig. 2d) and water-acetonitrile-hydrochloric acid (Fig. 2e) mobile phases.

DISCUSSION

Biagi et al.[6] used reversed-phase thin-layer chromatography for the determination of lipophilicity; they found it an easy, fast, time- and substance-saving method. Further advantage of RP-TLC is given by the direct visual observation under daylight or with a UV lamp. The spots at extreme positions (i.e., spots near the start or almost running with the mobile phase front) can be easily located. Also, the use of R_M has facilitated the utilization of the planar method because, instead of closely located R_F values, the R_M values are distributed on a wider scale.

The essential interest of patients (and also that of researchers involved in the development of new drugs) is to have safe drugs. Certain classification of drugs and other xenobiotics differentiates hard drugs, soft drugs, and active metabolites (pro-drugs).

The importance of hard drugs was conceptualized by Ariëns[7] and Ariëns and Simonis.[8] Hard drugs do not yield any metabolites. Their pharmacokinetics are simplified, as they are excreted mainly in their original forms through the kidney and bile. If the hard drug is excreted mainly through the kidney, any difference in the elimination rate between an experimental animal and humans will be primarily dependent upon the renal function of the corresponding species. Lin and Lu[9] mentioned chemical groups for hard drugs, such as bisphosphanates (inhibiting bone resorption) and carboxyalkylpeptides (ACE inhibitor for blood pressure regulation).

A new set of hydrophilicity high-performance liquid chromatography (HPLC) parameters was presented by Parker, Guo, and Hodges.[10] It was found that the HPLC parameters obtained in their study correlated best with antigenicity. The predicted hydrophilicity also correlates with the hydrophilic, accessible, or mobile regions determined by X-ray crystallographic data for several proteins.

Two major shortcomings limit the pharmacological indices that serve to predict the fate of the drugs in the body, including their penetration ability through the various membranes, as well as their receptor binding. Lipophilic compounds can generally penetrate well into the various body compartments, and stronger lipophilic drugs can exert more definite receptor binding than the less lipophilic drugs.

Hydrophilicity parameters promise a wide range of application for solubility. Further utilization of these parameters depends on the future experimental results and theoretical considerations.

Displacement chromatography of K-27, K-48, pralidoxime and obidoxime, shows an otherwise evident phenomenon that migration of the spot is facilitated by the displacer front pushing it forward. If this movement is very limited, the displacer elutes, as it happened.

CONCLUSIONS

Hydrophilicity and the means of its experimental determination may have a basic importance. It could be an important characteristic that substitutes/completes the lipophilicity indices of drugs and drug candidates.

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