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Lipophilicity characterization by reversed-phase liquid chromatography of some furan derivatives

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Abstract

Lipophilicity is one of the properties which influences the partition of a substance in biological media. The present study reports on the chromatographic behaviour of a newly synthesised series of furan derivatives by RP-HPLC and RP-TLC, with methanol–water and acetonitrile–water as mobile phases, in order to establish if the linear relationships between the retention parameters (log k, R_M) and the concentration of organic modifier in the mobile phase, φ, allows the extrapolation procedure. Good correlations between the retention parameters were obtained by RP-HPLC and RP-TLC, and the concentration of organic modifier (methanol, acetonitrile) in the mobile phase was established for the studied furan derivatives. However, for the discussed compounds, acetonitrile has a lower sensitivity to changes in the structures. A good correspondence was obtained between the extrapolated parameters for the methanol–water mobile phase when using RP-HPLC and RP-TLC. However, stronger interactions occur in RP-TLC between the compounds and the residual silanol groups than in RP-HPLC.

Keywords: Lipophilicity; Mobile phase composition; Retention parameters; Furans

1. Introduction

The lipophilicity of a substance is one of the parameters which influences its biological activity. Lipophilicity is usually measured by the partition coefficient of the organic compound between a non-polar phase and water [1]. Fujita et al. has proposed the *n*-octanol–water partition coefficient, $P_{o/w}$, as a measure of a compound's lipophilicity [2]. The determination of the partition coefficient by direct measurement using the shake-flask equilibration method, faces problems such as poor reproducibility, long time for experiments and it needs a reasonable quantity of the pure compound. These difficulties can be overcome by using reversed-phase liquid chromatography [3,4]. The liquid chromatographic method has the advantage of speed of determination, better reproducibility, and the purity of the sample is not a necessary condition. A linear relationship between the retention parameters and the concentration (φ) of organic modifier in the aqueous mobile phase has to be established for a successful chromatographic measurement of lipophilicity. The retention parameter can be log k for RP-HPLC, or $R_M = log\ (1/R_f - 1)$ for RP-TLC [5–9]. The organic modifier in the aqueous mobile phase can be methanol, acetonitrile, tetrahydrofuran for RP-HPLC, and for the RP-TLC experiments also acetone is included. Methanol is the most frequently used organic modifier for the mobile phase, due to its capacity to interact with different

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compound structures and to its water-like structure. The linear relationship between the retention parameter and the organic modifier concentration in the mobile phase is extrapolated to pure water as mobile phase. The extrapolated values, log k_w or R_{Mw}, characterize the partition of the compound between the non-polar hydrocarbon stationary phase and water. For hydrophilic compounds, this value can be measured directly, and it is considered to be related to log P values, as a measure of the lipophilic character of the substance [5–9].

The purpose of the present study is to investigate the chromatographic behavior of a newly synthesized series of furan derivatives by RP-HPLC and RP-TLC, in order to establish if the linear relationships log $k = f(\varphi)$, *or* $R_M = f(\varphi)$ allows the extrapolation procedure. The structures of ten new furan derivatives were related to three substances with pharmaceutical applications, having similar structures: nitrofuran, nitrofurantoine and furazolidone. Taking into account the extrapolated retention parameters, log k_w and R_{Mw}, a lipophilicity scale was obtained, offering information about the 'congenerity' of the studied compounds.

2. Experimental

The structures of the studied furan derivatives are shown in Figs. 1 and 2. With the exception of nitrofurantoine, nitrofurazone and furazolidone, these are newly synthesized compounds in the Faculty of Chemistry and Chemical Engineering (Cluj-Napoca, Romania), and these structures have potential biological activity. The samples were prepared as solutions of 0.1 mg/ml in methanol or in methanol–chloroform (1:1, v/v) for the less soluble substances.

2.1. Reversed-phase high-performance liquid chromatography

The measurements were performed on a LiChrospher C_{18} end-capped column, 250×4.6 mm (Merck, Darmstadt, Germany), by using a Hewlett-Packard 1100 HPLC instrument with a diode-array detection system (Palo Alto, CA, USA). The injection volume was 10 μl in all cases. The void volume was measured with uracil as unretained compound. Uracil was synthesized at the Faculty of Chemistry and Chemical Engineering. The flow-rate was 1 ml/min in all cases, and the optimum detection wavelength was set for each substance according to the maximum absorption in the UV spectra, in the range 250–360 nm.

The measurements were performed in methanol–water and acetonitrile–water, as mobile phases, starting with 90% (v/v) organic modifier and decreasing in 5% (v/v) concentration steps. For the more hydrophilic compounds, the last measurement was carried out with 10% (v/v) organic modifier in the mobile phase (Tables 1 and 2). Each measurement was performed in triplicate and the mean was used in further calculations. Methanol and acetonitrile for measurements were of gradient grade and were obtained from Merck.

2.2. Reversed-phase thin-layer chromatography

Silica gel RP-18 $60F_{254}$ plates, 5×10 cm, (Merck), were used for the measurements by planar chromatography. The samples were applied as spots onto the plates using calibrated micropipettes, 1.5 cm from the bottom edge, and the migration distances were 8 cm in all cases. Each spot was applied in duplicate and the mean R_f value was used for R_M calculation. The plates were developed in normal chambers, previously saturated for 30 min. After development, the plates were dried in a gentle air stream, and were examined in UV light at 254 nm, with a Camag universal lamp (Camag, Muttenz, Switzerland). The migration distances were obtained by scanning the plates with a Shimadzu CS-9000 dual wavelength flying spot scanner (Shimadzu, Kyoto, Japan), at 254 nm, in reflection and zigzag mode.

3. Results and discussion

The furan derivatives shown in Fig. 1 were analyzed by RP-HPLC using methanol–water and acetonitrile–water as mobile phases, and by RP-TLC using methanol–water as mobile phase. Previous investigations have shown that these substances are

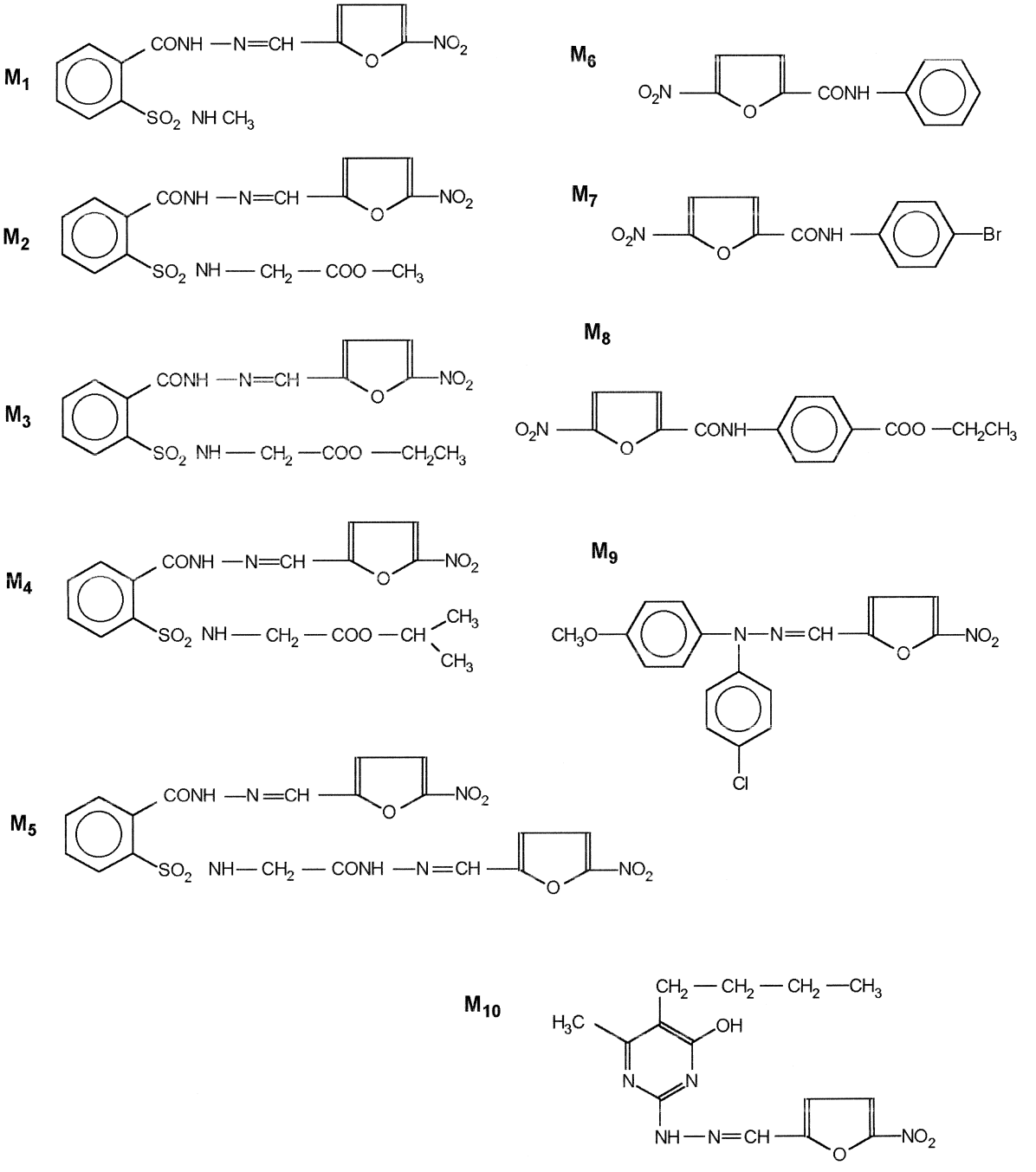
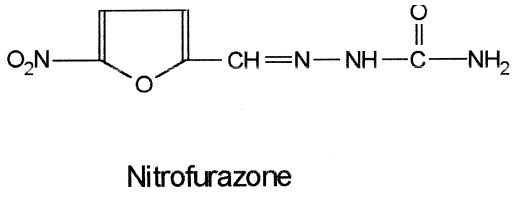
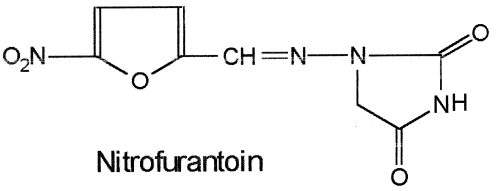


Fig. 1. Structures of the studied new furan derivatives.

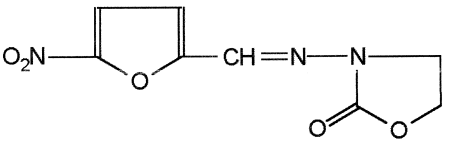
in the neutral form at pH <7.0, so there was no need for buffering the mobile phase. The linear correlations between the retention parameter and the concentration of organic modifier in the mobile phase (Eqs. (1 and 2)) are shown in Tables 1–3 for both techniques



Nitrofurazone



Nitrofurantoin



Furazolidone

Fig. 2. Structures of some well-known pharmaceutical substances, furan derivatives.

$$\log k = a_0 + a_1\varphi, \text{ or } \log k = \log k_w - S\varphi \quad (1)$$

$$R_M = a_0 + a_1\varphi, \text{ or } R_M = R_{Mw} - S\varphi \quad (2)$$

The intercept a_0 models the partition of the compound between the non-polar stationary phase and the aqueous mobile phase, and measures the lipophilicity of the studied substance. Log k_w and R_{Mw} are the retention parameters obtained for pure water as mobile phase by extrapolating the Eqs. (1 and 2). It is assumed that the linearity of the relationships 1 and 2 is maintained even at low concentrations of organic modifier in the mobile phase. However, positive or negative deviations from the linearity were reported in the literature near the extrapolation point [3,5]. For more hydrophilic compounds, the RP-HPLC experiments were carried out even at 10% methanol or acetonitrile, and the good correlation coefficients obtained allow the extrapolation procedure for obtaining the log k_w data. The

Table 1
Linear correlations $\log k = a_0 + a_1\varphi$, obtained by RP-HPLC for the studied furan derivatives; mobile phase: methanol–water; s_{a0}, s_{a1} are the standard errors for the intercept a_0 and for the slope a_1, respectively; s is the fit standard error; r is the correlation coefficient, and $\pm a_0$ *and* $\pm a_1$ are the 95% confidence limits

Compound	Methanol (%)	Log k_w (a_0)	$-S$ (a_1)	r	s_{a0}	s_{a1}	s	$\pm a_0$	$\pm a_1$
M1	80–20	1.803	−0.039	0.9979	0.061	0.001	0.060	0.156	0.003
M2	90–30	2.317	−0.043	0.9974	0.089	0.001	0.074	0.229	0.004
M3	90–30	2.695	−0.045	0.9981	0.080	0.001	0.066	0.205	0.003
M4	90–40	2.954	−0.046	0.9988	0.075	0.001	0.047	0.208	0.003
M5	90–40	3.108	−0.052	0.9960	0.156	0.002	0.097	0.432	0.006
M6	90–30	2.429	−0.035	0.9974	0.060	0.001	0.051	0.142	0.002
M7	90–50	3.558	−0.043	0.9961	0.116	0.002	0.059	0.299	0.004
M8	90–50	3.250	−0.042	0.9945	0.136	0.002	0.069	0.350	0.005
M9	90–55	4.387	−0.053	0.9986	0.099	0.001	0.040	0.274	0.004
M10	90–50	3.273	−0.041	0.9945	0.133	0.002	0.067	0.343	0.005
Nitrofurazone	90–10	1.245	−0.030	0.9974	0.046	0.001	0.063	0.108	0.002
Nitrofurantoin	90–10	1.275	−0.034	0.9929	0.086	0.001	0.118	0.203	0.004
Furazolidone	90–10	1.211	−0.030	0.9932	0.076	0.001	0.104	0.179	0.003

Table 2
Linear correlations $\log k = a_0 + a_1\varphi$, obtained by RP-HPLC for the studied furan derivatives; mobile phase: acetonitrile–water; s_{a0}, s_{a1} are the standard errors for the intercept a_0 and for the slope a_1, respectively; s is the fit standard error; r is the correlation coefficient, and $\pm a_0$ and $\pm a_1$ are the 95% confidence limits

Compound	ACN (%)	Log k_w (a_0)	$-S$ (a_1)	r	s_{a0}	s_{a1}	s	$\pm a_0$	$\pm a_1$
M1	90–10	1.472	−0.032	0.9993	0.026	0.0005	0.036	0.062	0.001
M2	90–20	1.859	−0.035	0.9983	0.050	0.001	0.054	0.123	0.002
M3	90–20	2.097	−0.036	0.9984	0.050	0.001	0.054	0.123	0.002
M4	90–30	2.160	−0.035	0.9994	0.035	0.0005	0.029	0.089	0.001
M5	90–30	2.238	−0.041	0.9989	0.053	0.001	0.045	0.137	0.002
M6	90–30	1.934	−0.030	0.9968	0.068	0.001	0.057	0.176	0.003
M7	90–40	2.365	−0.032	0.9966	0.089	0.001	0.056	0.248	0.004
M8	90–30	2.283	−0.033	0.9979	0.060	0.001	0.050	0.153	0.002
M9	90–50	2.984	−0.038	0.9991	0.066	0.001	0.029	0.210	0.003
M10	90–10	1.120	−0.016	0.9908	0.045	0.001	0.062	0.107	0.002
Nitrofurazone	90–10	0.724	−0.026	0.9987	0.028	0.0005	0.039	0.067	0.001
Nitrofurantoin	90–10	1.055	−0.029	0.9961	0.054	0.001	0.075	0.128	0.002
Furazolidone	90–10	1.055	−0.025	0.9992	0.022	0.0004	0.030	−0.052	0.001

RP-TLC experiments were not performed at very low concentrations of methanol in the mobile phase in order to avoid experimental errors when measuring low R_f values. Another reason is that stronger interactions occur between the studied compounds and the silica gel C_{18} in thin-layer chromatography experiments than in column chromatography, probably due to the free silanol groups (an end-capped column was used in RP-HPLC).

The slope a_1 $(-S)$ is negative in all cases, and it is supposed to be related to the hydrophobic surface of the molecule which interact with the non-polar stationary phase [7]. Examining the data in Tables 1 and 2, it can be observed that the absolute value of the slope a_1 (or the S value) is lower for acetonitrile–water than for methanol–water mobile phase.

It means that for the same differences in the organic modifier concentration (methanol or acetonitrile) a higher difference will be obtained between the corresponding log k values for methanol–water

Table 3
Linear correlations $\log k = a_0 + a_1\varphi$, obtained by RP-TLC for the studied furan derivatives; mobile phase: methanol–water, methanol concentration in the range 95–65%; s_{a0}, s_{a1} are the standard errors for the intrecept a_0 and for the slope a_1, respectively; s is the fit standard error; r is the correlation coefficient, and $\pm a_0$ and $\pm a_1$ are the 95% confidence limits

Compound	R_{Mw} (a_0)	$-S$ (a_1)	r	s_{a0}	s_{a1}	s	$\pm a_0$	$\pm a_1$
M1	1.121	−0.021	0.990	0.109	0.001	0.036	0.281	0.003
M2	1.479	−0.025	0.993	0.104	0.001	0.034	0.268	0.003
M3	1.672	−0.026	0.993	0.115	0.001	0.038	0.295	0.004
M4	1.997	−0.029	0.995	0.106	0.001	0.035	0.273	0.003
M5	1.970	−0.029	0.995	0.107	0.001	0.035	0.276	0.003
M6	1.939	−0.026	0.996	0.088	0.001	0.029	0.225	0.003
M7	3.279	−0.038	0.998	0.090	0.001	0.029	0.231	0.003
M8	2.760	−0.034	0.997	0.097	0.001	0.032	0.250	0.003
M9	4.005	−0.046	0.997	0.128	0.001	0.042	0.330	0.004
M10	2.992	−0.034	0.996	0.110	0.001	0.036	0.283	0.003
Nitrofurazone	0.964	−0.017	0.988	0.096	0.001	0.032	0.247	0.003
Nitrofurantoin	1.010	−0.019	0.996	0.064	0.001	0.021	0.165	0.002
Furazolidone	0.888	−0.014	0.980	0.100	0.001	0.033	0.513	0.003

mobile phase than for acetonitrile–water mobile phase. The plot of Eq. (1) is more steep for methanol–water than for acetonitrile–water mobile phase, so acetonitrile is less sensitive to the changes in the structures of the studied compounds.

Comparing the log k_w values obtained in the RP-HPLC experiments for methanol–water and acetonitrile–water as mobile phase, a poor correlation is obtained (Eq. (3))

$$\log k_{w(ACN)} = 0.336\ (\pm 0.652) + 0.566\ (\pm 0.237) \log k_{w(MeOH)}$$
$$s_{a0} = 0.296,\ s_{a1} = 0.108,\ s = 0.368,\ r = 0.845 \qquad (3)$$

where s_{a0}, s_{a1} are the standard errors for the intercept a_0 and for the slope a_1, respectively; s is the fit standard error; r is the correlation coefficient for 95% confidence limits.

The log k_w values increase from M1 to M5 on a lipophilicity scale, for both types of experiments by RP-HPLC, with methanol–water and acetonitrile–water. This is not the case in the RP-TLC experiments, were the R_{Mw} value for M5 is situated before the corresponding value of M4. The differences in structures and the fragmental constants [10,11] confirm the results obtained by RP-HPLC that the compound M5 is more hydrophobic than M4. This difference in lipophilicity cannot be observed by RP-TLC experiments. The end-capping of silanol groups reduces the unwanted interactions with the compounds, which are stronger in the case of the RP-TLC experiments. The correlation coefficients for the relationship (2) are slightly lower for the RP-TLC experiments, than the correlation coefficients for RP-HPLC (Eq. (1)).

The lipophilicity (log k_w and R_{Mw} values) increases for compounds M6–M8, in the order M6, M8, M7 in all experiments. The differences in structures for these compounds, –Br and –COO–CH_2– CH_3 are sufficiently for obtaining very different retention parameters.

The lower sensitivity of acetonitrile is also evident for nitrofuran, nitrofurantoine and furazolidone. The log k_w value obtained for these drugs in methanol–water and acetonitrile–water in the RP-HPLC experiments show the same value for nitrofurantoine and furazolidone, 1.055, when using acetonitrile–water.

There is a good correlation between the extrapolated parameters for methanol–water mobile phase comparing the RP-HPLC and RP-TLC measurements (Eq. (4))

$$R_{Mw(RP\text{-}TLC)} = -0.440\ (\pm 0.581) + 0.949\ (\pm 0.212) \log k_{w(RP\text{-}HPLC)}$$
$$s_{a0} = 0.264,\ s_{a1} = 0.096,\ s = 0.328,\ r = 0.948 \qquad (4)$$

where s_{a0}, s_{a1} are the standard errors for the intercept a_0 and for the slope a_1, respectively; s is the fit standard error; r is the correlation coefficient for 95% confidence limits.

Eq. (4) shows that the newly synthesised furan derivatives have a similar chromatographic behaviour in RP-HPLC and RP-TLC experiments, when using methanol–water as mobile phase.

The 'congenerity' of substances can be expressed as the linearity between the extrapolated parameter log k_w (or R_{Mw}) and the slope S [12,13] (Eq. (5))

$$\log k_w = b_0 + b_1 S \qquad (5)$$

The linearity of Eq. (5) was checked for the data in Tables 1–3. Relatively poor correlations were obtained for the RP-HPLC experiments, with correlation coefficients $r = 0.845$ for methanol–water as mobile phase (Table 1), and $r = 0.752$ for acetonitrile–water as mobile phase (Table 2). The statistical data were calculated for 95% confidence limits. The correlation was very good for the data in Table 3 (Eq. (6))

$$R_{Mw(RP\text{-}TLC)} = -0.973\ (\pm 0.410) + 108.172\ (\pm 14.200)\ S$$
$$s_{a0} = 0.186,\ s_{a1} = 6.451,\ s = 0.200,\ r = 0.981 \qquad (6)$$

The results can be explained by the differences in retention mechanisms between RP-HPLC and RP-TLC under the described experimental conditions. Also, in the RP-TLC experiments it was not possible to use the same low concentrations of organic modifier in the mobile phase as in RP-HPLC.

Further experiments have to be performed for testing the compounds biological activity, in order to check the relationship with the chromatographic retention parameters.

4. Conclusions

Good correlations between the retention parameters obtained by RP-HPLC and RP-TLC, and the concentration of organic modifier (methanol, acetonitrile) in the mobile phase have been obtained for the studied furan derivatives. However, acetonitrile has lower sensitivity to changes in the compounds structures, and in this case it is not recommended as an organic modifier in the aqueous mobile phase. A good correspondence was obtained between the extrapolated parameters for methanol–water mobile phase comparing the RP-HPLC and RP-TLC measurements. However, stronger interactions between the compounds and the residual silanol groups occur in the RP-TLC experiments than in the RP-HPLC measurements.

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