ISSN 1070-4272, Russian Journal of Applied Chemistry, 2009, Vol. 82, No. 2, pp. 312−316. © Pleiades Publishing, Ltd., 2009.
Original Russian Text © V.G. Berezkin, L.A. Onuchak, E.N. Evtyugina, 2009, published in Zhurnal Prikladnoi Khimii, 2009, Vol. 82, No. 2, pp. 317−321.

ORGANIC SYNTHESIS AND INDUSTRIAL ORGANIC CHEMISTRY

Capillary Thin-Layer Chromatography of Antibacterial Nitrofuran Derivatives

V. G. Berezkin, L. A. Onuchak, and E. N. Evtyugina

Topchiev Institute of Petrochemical Synthesis, Russian Academy of Sciences, Moscow, Russia
Samara State University, State Educational Institution, Samara, Russia

Received January 16, 2008

Abstract—A new version of thin-layer chromatography, capillary thin-layer chromatography, which is similar to traditional planar chromatography in the idea of detection of sorbate bands and to high-performance column chromatography in the implementation of the chromatographic process (in a thin capillary packed with a sorbent), was developed. The chromatographic characteristics of antibacterial nitrofuran derivatives under the conditions of planar and capillary thin-layer chromatography were studied and compared from the viewpoint of performance, reproducibility, and consumption of the sorbent and eluent.

DOI: 10.1134/S107042720902027X

Thin-layer chromatography (TLC) is widely used in medicine, biology, and pharmacy (in drug quality control), and also in various branches of industry and in scientific research [1]. Wide use of TLC is associated with the fact that the method is prompt, sensitive, simple, readily accessible, and cheap. However, the method has also certain drawbacks such as low performance, the necessity of using special chambers saturated with mobile phase vapor, and, as a consequence, poor reproducibility of the chromatographic retention indices [1, 2].

In this study we suggested a new version of TLC, so-called capillary thin-layer chromatography (CTLC), which is similar to traditional planar TLC in the driving force of the liquid phase flow and in the idea of detection of sorbate bands and to high-performance column chromatography in the implementation of the chromatographic process (in a thin capillary packed with a sorbent). In CTLC, the motion and smearing of sorbate bands occur in a very thin sorbent layer (≤0.5 mm) in the capillary space.

The new version of TLC was implemented with colored compounds, antibacterial nitrofuran derivatives, as example. Nitrofuran derivatives are used in medicine for treating infectious diseases [3].

EXPERIMENTAL

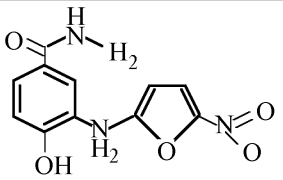
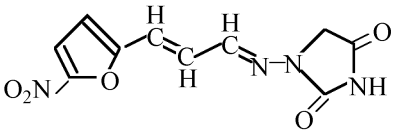
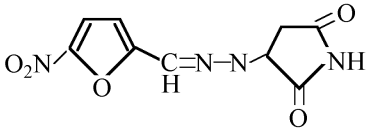
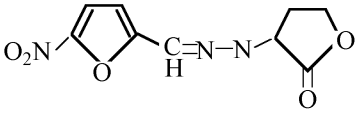
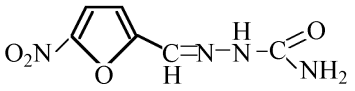
As investigation objects we used drugs based on nitrofuran derivatives: Furagin, Furadonin, Furazolidone, Furacillin, and Ercefuryl. The structural formulas and physicochemical properties of the compounds are given in Table 1.

Samples of substances for the experiments were prepared from drug forms. A tablet was finely divided, and the target substance was extracted with 2 ml of acetonitrile for 0.5 h. The concentrations of the substances extracted from tablets were 0.025 g cm^{-3}.

For traditional ascending TLC, we used Sorbfil PTSKh-AF-V-UF plates (silica gel STKh-1VE as sorbent, dp = 8–12 μm, aluminum support, UV 254 nm) of size 10 × 10 cm, produced by Sorbpolimer Private Company (Krasnodar, Russia). Chromatography was performed in the ascending flow in chambers saturated with eluent vapor. The experiments were performed at room temperature. The eluent path length was 7.5 cm.

For capillary TLC we used capillaries made of melted quartz (without stationary phase on the internal surface), 0.53 mm i.d., produced by Phenomenex (USA) and sold by Akvilon (Moscow). The external polymeric coating of the capillaries was removed by keeping them in acetonitrile

Table 1. Physicochemical properties of the compounds studied [3]

Compound	Structural formula	T_m, °C	M_r	α,* Å^3	μ,* D
Ercefuryl		–	277.24	26.19	6.87
Furagin		–	264.20	23.29	4.77
Furadonin		258–263	238.16	19.81	5.46
Furazolidone		253–259	225.16	19.01	7.73
Furacillin		236–240	198.14	16.83	4.23

* The polarizability α and dipole moment μ of molecules were calculated with HyperChem 7.0 program. The molecular geometries were optimized by PM1 semiempirical method.

for 30 min. For packing the capillary we used the sorbent removed from a TLC plate and ground on a cover glass with a glass rod. A 0.0056-g portion of the finely ground sorbent was introduced into a capillary 5 cm long with intermittent tapping on the surface of the cover glass.

For this study we prepared 20 capillaries with the same packing density. Optical examination showed that the particle size was 5–8 μm with insignificant inclusions of smaller particles (1–2 μm). A 0.2-μl sample was introduced into the starting part of the separating column using a capillary of a smaller diameter (d_c = 0.3 mm). One of the ends of the capillary column packed with the dry sorbent and arranged vertically was immersed in an eluent to a depth of 1–2 mm. No less than three replicate measurements at room temperature were performed. The eluent front length was 4.5 cm. Visually we determined the distances passed by the eluent front and the center of the colored (light yellow) analyte band from the start line as functions of time and calculated the current [$R_f(t)$] and final (R_f) retention factors.

To improve the experimental accuracy, we used a measuring magnifier. The distance passed was measured with an error of 0.5 mm. For better visualization of the eluent front (as eluents we used acetonitrile and acetonitrile–chloroform binary mixtures), we used UV irradiation (254 nm). The separated substances in the mixture were identified using the preliminarily determined R_f values for the individual substances.

After the mixture separation, the plates and capillaries were dried. The chromatographic patterns on a planar layer and in a capillary, obtained by scanning the plates and capillaries with an Epson Perfection 1260 flatbed scanner (China), were processed using the Sorbfil Videodensitometr TLC Quantitative Evalution (Ver. 1.7.0.216) software (Sorbpolimer) for determining R_f and evaluating the separation efficiency (HETP).

Preliminary studies of the chromatographic behavior of nitrofurans by traditional TLC on plates showed that the retention factors of the analytes with acetonitrile as eluent are high and close ($R_f > 0.8$). Apparently, with a polar adsorbent (silica gel), the separation of nitrofurans can be improved by using a binary eluent containing a low-polarity solvent as the second component. As such additive to acetonitrile we used chloroform.

Figure 1 shows the dependences of R_f of the analytes on the volume fraction φ of chloroform in the eluent. It

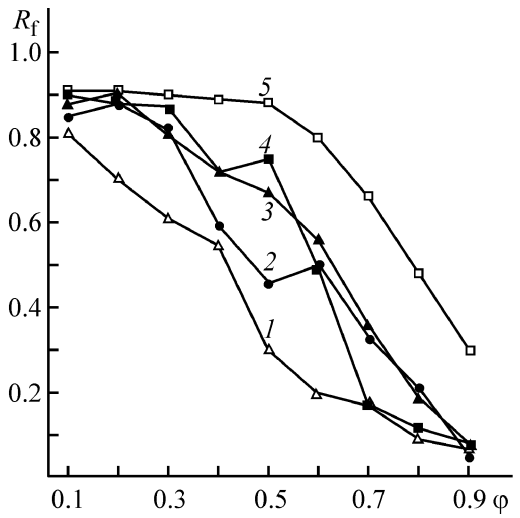


Fig. 1. Retention factor R_f of nitrofuran derivatives as a function of the volume fraction φ of chloroform in the acetonitrile–chloroform binary eluent. Nitrofuran: (*1*) Furacillin, (*2*) Furagin, (*3*) Furadonin, (*4*) Ercefuryl, and (*5*) Furazolidone.

can be seen that, with increasing φ of chloroform and correspondingly with decreasing polarity of the binary mobile phase, the R_f values of nitrofuran tend, on the whole, to decrease, which is due to an increase in the contribution of adsorbate–polar adsorbent interactions to the retention. A complex shape of $R_f(\varphi)$ dependences for certain compounds may be due to nonuniformity of the layer thickness on different plates and to incomplete reproduction of experimental conditions (e.g., air humidity, moisture content of plates, etc.). Figure 1 shows that the R_f values of the examined nitrofurans differ to the greatest extent at φ = 0.5. With the eluent of this composition, R_f increases with an increase in the dipole moment of nitrofuran molecules.

Typical time dependences of the distances passed by the eluent (upwards) $a = a(t)$ and center of the sorbate band $b = b(t)$ on the plate and in the capillary are shown in Fig. 2. The motion of the eluent front under the action of capillary forces in both TLC versions was described by a quadratic equation of the type

$$a^2 = kt + A, \qquad (1)$$

where a^2 is the squared distance from the start line to the front of the mobile phase; k, flow constant (rate coefficient); and t, time of motion of the mobile phase.

Equation (1) differs from the known classical equation in the constant term A. For example, $A = 0$ if the surface of the mobile phase is used as the origin [4].

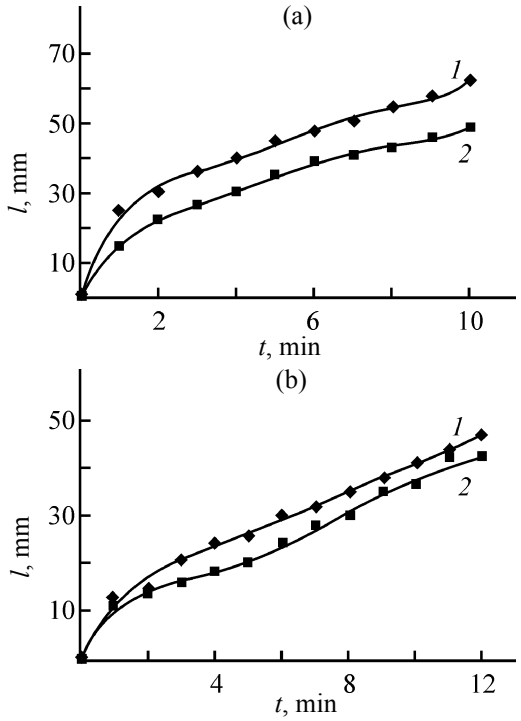


Fig. 2. Distance l passed by the (*1*) eluent and (*2*) center of the colored Furazolidone band in (a) planar and (b) capillary TLC. Sorbent STKh-1VE, eluent acetonitrile–chloroform (1 : 1); the same for Figs. 3 and 4. (*t*) Time.

We found that the motion of the mobile phase front in planar TLC is described by the equation $a^2 = 475.94t - 86.69$ (linear correlation coefficient $R^2 = 0.9804$), and in the capillary version of TLC, by the equation $a^2 = 120.70t + 35.18$ (linear correlation coefficient $R^2 = 0.9982$). The mobile phase front in the new version travels more slowly than in the traditional version of TLC. Slower eluent motion may be due to the presence of an impurity of the fine sorbent fraction in the capillary column.

From the dependences $a = a(t)$ and $b = b(t)$, obtained by the visual method, we determined the current dependences of the R_f values of the analytes. The dependences of R_f of Furazolidone on the time from the start of the experiment, shown in Fig. 3, show that the R_f values for both versions of

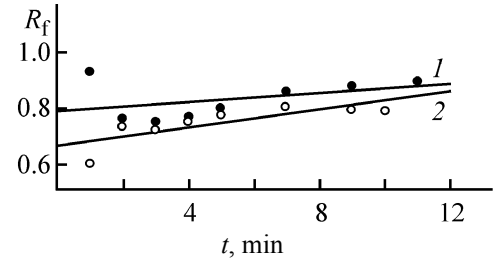


Fig. 3. R_f of Furazolidone as a function of the experiment time t for (*1*) capillary and (*2*) planar TLC.

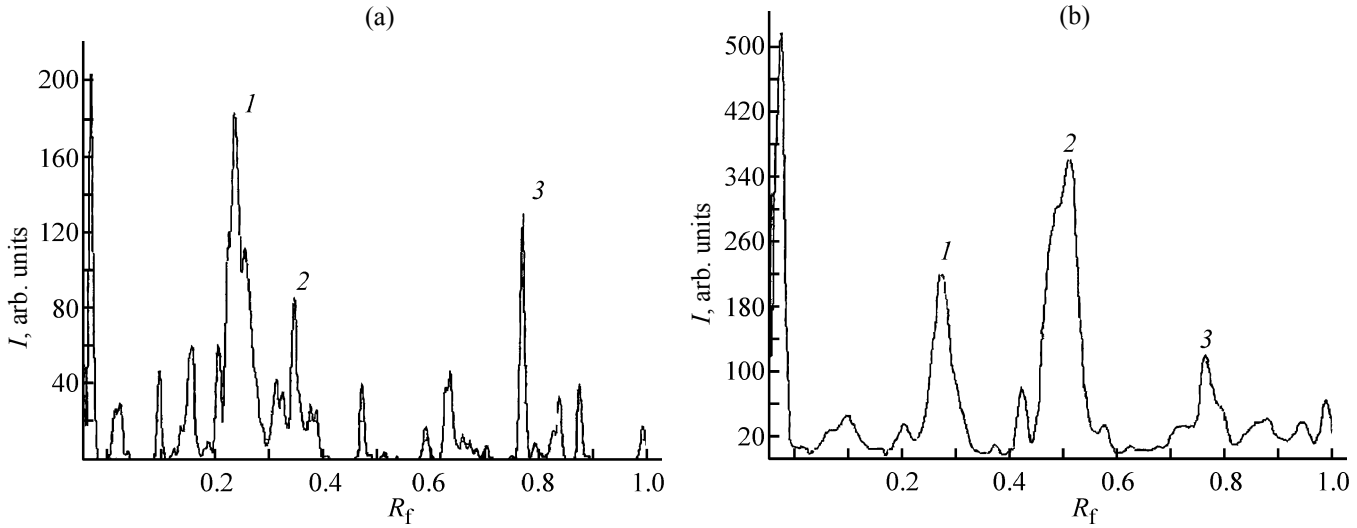


Fig. 4. Chromatogram of a mixture of nitrofuran derivatives: (*1*) Furacillin, (*2*) Furagin, and (*3*) Furazolidone. (*I*) Signal intensity. TLC: (a) capillary and (b) planar.

TLC are close, especially in final steps of the processes.

The final R_f values obtained by both procedures are given in Table 2. To determine R_f, we performed no less than three replicate experiments ($n \geq 3$). We found that, with acetonitrile–chloroform (1 : 1) as eluent, the R_f values for the examined nitrofuran derivatives are in the range from 0.30 for Furacillin to 0.79 for Furazolidone in the case of planar TLC and from 0.32 to 0.89, respectively, for capillary TLC. It can be seen that the relative measurement error ε in capillary TLC is smaller, which is due to the absence of the effect of the gas phase.

In this study we examined the possibility of practical separation of a mixture of three nitrofurans (Furacillin, Furagin, Furazolidone) by capillary and planar TLC with STKh-1VE sorbent [eluent acetonitrile–chloroform, $\varphi(CHCl_3) = 0.5$]. Figures 4a and 4b show the chromatograms (analog curves) obtained by processing the images of a capillary and a plate with the separated components using the Sorbfil Videodensitometr TLC Quantitative Evaluation (Ver 1.7.0.216) software.

The noise in the chromatogram may be due to light reflection from walls of the quartz capillary. The main chromatographic characteristics of planar and capillary TLC are given in Table 3. As can be seen, in capillary TLC for each component of the nitrofuran mixture being separated the number N of theoretical plates increases and, correspondingly, HETP considerably decreases, which is due to weak smearing of the analyte band in the capillary volume ($d = 0.5$ mm) compared to the band smearing on the plate in planar TLC. It should be noted that the performance of column liquid chromatography is principally higher, because in this case, in contrast to planar TLC, there is no fast diffusion of analyte molecules in the surface layer of the eluent wetting the plate surface.

It should be noted that capillary TLC can also be used for separation of colorless substances if the material of capillary walls is transparent for UV radiation (molten quartz, some glasses and polymers).

Table 2. R_f values of nitrofuran derivatives, obtained by planar and capillary ascending TLC. Eluent acetonitrile–chloroform (1 : 1), sorbent STKh-1VE

Nitrofuran	Planar TLC		Capillary TLC	
	$R_f \pm \Delta R_f$	ε, %	$R_f \pm \Delta R_f$	ε, %
Furacillin	0.30 ± 0.11	36.6	0.32 ± 0.07	21.1
Furagin	0.46 ± 0.09	19.6	0.40 ± 0.05	12.5
Furadonin	0.67 ± 0.09	13.4	0.52 ± 0.05	9.6
Ercefuryl	0.75 ± 0.07	9.3	0.60 ± 0.04	6.7
Furazolidone	0.79 ± 0.05	6.3	0.89 ± 0.05	5.6

Table 3. Main chromatographic characteristics of planar and capillary TLC

Nitrofuran	Planar TLC			Capillary TLC		
	R_f	H, μm	N	R_f	H, μm	N
Furacillin	0.30	240	98	0.32	28	480
Furagin	0.46	327	145	0.40	13	1292
Furazolidone	0.79	170	353	0.89	4	9342

CONCLUSIONS

(1) A new version of thin-layer chromatography, capillary thin-layer chromatography, was suggested for separation of colored nitrofuran derivatives.

(2) The advantages of capillary thin-layer chromatography compared to traditional planar chromatography are high performance, lower error of determining the retention factor R_f, minimal consumption of the sorbent and solvent, and no need for using special equipment.

REFERENCES

1. Kirchner, J.G., *Thin-Layer Chromatography*, Perry, E.S., Ed,. New York: Wiley, 1978, 2nd ed.
2. Geiss, F., *Fundamentals of Thin Layer Chromatography: Planar Chromatorgaphy*, Heidelberg: Hüthig, 1987.
3. Mashkovskii, M.D., *Lekarstvennye preparaty* (Drugs), Moscow: Meditsina, 2006.
4. Berezkin, V.G. and Kormishkina, E.V., *Zh. Anal. Khim*., 2006, vol. 61, no. 10, p. 1074.