Capillary action liquid chromatography (caLC) – Comparison of the retention parameters with data obtained using TLC

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Introduction

The simple and effective technique for separating mixtures of substances is thin layer chromatography (TLC), which is widely used in the pharmaceutical industry and in the laboratory of organic synthesis. The principle of separation is a different division of components of the analyzed mixture between the sorbent and the eluent which migrates under the action of capillary forces. The basis of separation is a difference in the rate of migration of the individual substances through layer of adsorbent [1].

In 2009 Bo Zhang and co-workers [2] have published an article on a new technique of chromatography, which associates the rules for a thin-layer chromatography with a column chromatography. This technique is called a capillary action liquid chromatography (caLC). In caLC sorbent was placed in a capillary, which inner diameter is approx. 283-564 μm, and mobile phase migrates by capillary action forces.

An important advantage of caLC is using small amounts of solvents. This is one of the principles of the so-called Green Analytical Chemistry, the application of which is relevant to the protection of the environment [3].

Results and discussion

The chromatographic tests were carried out on TLC plates, and in quartz capillary tubes filled with a sorbent RP-8 (C-8, particle size d_{50} 9.5 – 11.5 μm, Merck). Eluents were mixture: methanol-water with pH modifier (acetate buffer, acetic acid). For this study test phase was selected: methanol-water-acetic acid (4:5:1, v/v) which gave the best separation results. The tested adsorbents were prepared initially, washed by methanol from a surface contamination, and then dried at the temperature of 110°C. Thus prepared sorbent was poured into the capillary column, whisking it by ultrasound, and then secured the ends of the columns by means of glass wool. In our study capillaries with inner diameters 1.4-2.5 mm were used, which is greater than the Zhang's work [2]. The sorbent mass in the capillary was: from 32 mg ($L = 40$ mm, $\phi = 1.4$ mm) to 115 mg ($L = 60$ mm, $\phi = 2.5$ mm). In this paper different ways to develop the chromatograms were compared: ascending, descending and horizontal.

In the tests were used a mixture of 0.05% of methanol solutions of food dyes: allura red AC (E129), patent blue V (E131), brilliant black PN (E151). (Fig. 1.).
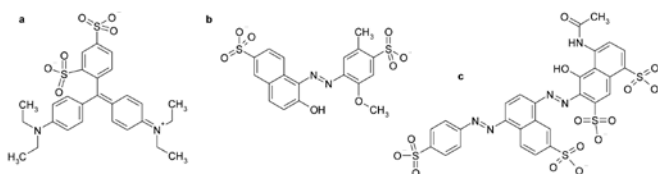

Fig.1. Chemical structure of food dyes: a – patent blue V, b – allura red AC, c – brilliant black PN

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Results obtained during the separation in capillary action liquid chromatography were compared with separation in thin-layer chromatography in the same phase: sorbent RP-8, and the eluent methanol-water-acetic acid (4:5:1, v/v). Chromatograms horizontal drop down in the chamber of "sandwich" DS II (Chromdes Lublin) constructed by Dzido and Soczewiński [4 – 6]. To the caLC analysis was used 0.5 mL of the eluent, and to the TLC approx. 6-8 mL.

Retention parameters were compared with the two chromatographic techniques tested. These parameters were:

retardation factor × 100 (hR_f):

$$hR_f = 100\frac{l}{L} \tag{1}$$

resolution (R_s):

$$R_s = \frac{2\Delta Z_{1,2}}{(w_1 + w_2)} = \frac{2\Delta R_{f1,2} \times L}{(w_1 + w_2)} \tag{2}$$

number of the theoretical plates (N):

$$N = 16\left(\frac{l}{w}\right)^2 = 16\left(\frac{R_f\,L}{w}\right)^2 \tag{3}$$

where:
l – the distance of the zone from the starting line,
L – the distance to develop the chromatogram,
$\Delta Z_{1,2}$ – the distance of adjacent zones,
w_1, w_2 – spot diameter.

The first step of ours investigations were to compare the obtained data retention and caLC capillary with TLC plates in various distance of development (L) (Photo 1). Capillary with an inner diameter 1.4 mm was used, and developing of the chromatograms was followed by an ascending mode.
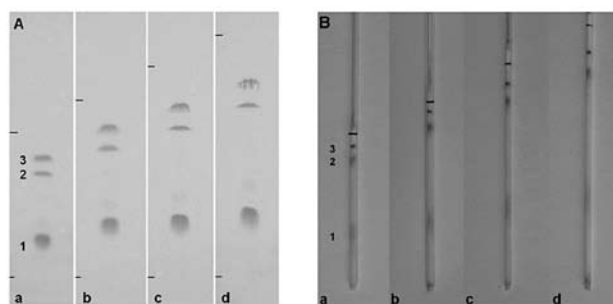

Photo 1. Chromatograms obtained on TLC plates (A) and in caLC columns, diameter 1.4 mm (B), sorbent: RP-8, eluent: methanol-water-acetic acid (4:5:1, v/v). Distance of development (L): a – 40 mm, b – 50 mm, c – 60 mm, d – 70 mm. 1 – patent blue V, 2 – allura red AC, 3 – brilliant black PN

The selected parameters of retention were calculated from obtained chromatograms, and presented in Table 1.

Table 1

Retention parameters obtained from chromatograms in Photo 1

L [mm]	Substance	hR_f		R_s [a]		N		Time of development [min]	
		TLC	caLC	TLC	caLC	TLC	caLC	TLC	caLC
40	(1)	25	30	6.91[1,2]	5.60[1,2]	100	64		
	(2)	72	82	2.29[2,3]	3.50[2,3]	5 980	7 744	17	28
	(3)	82	91			4 356	85 264		
50	(1)	28	35	6.29[1,2]	6.12[1,2]	125	100		
	(2)	72	87	2.67[2,3]	4.00[2,3]	5 184	13 456	24	46
	(3)	84	95			4 515	144 400		
60	(1)	27	33	7.47[1,2]	6.32[1,2]	114	100		
	(2)	73	83	4.00[2,3]	4.00[2,3]	13 767	17 778	32	68
	(3)	83	92			10 000	193 600		
70	(1)	27	31	7.76[1,2]	6.67[1,2]	118	96		
	(2)	74	81	2.67[2,3]	5.50[2,3]	19 228	23 104	42	86
	(3)	83	89			5 980	250 000		

[a] – pairs of separated substances were indicated in parentheses
(1) – patent blue V
(2) – allura red AC
(3) – brilliant black PN

Analyzing the data from the Table 1 can be seen a slightly different value of hR_f, irrespective of the distance of developing, both the TLC plates and caLC capillaries. In the case of capillaries, the presented values of hR_f are slightly larger than separation in TLC. The caLC capillaries have a slightly higher resolution R_s, as for example for the separated pair: allura red – brilliant black. Conversely, for the separated pair: patent blue – allura red, wherein the resolution is higher for thin layer chromatography separation. Considering the number of theoretical plates, which is a measure of the efficiency of the chromatographic system, can be observed that for allura red and brilliant black is definitely better for the analyzes carried out in the caLC capillaries, and amount of theoretical plates reaches up to 250 000/m.

One of the drawback of the caLC technique is the duration of the chromatographic process, which is twice as long in capillaries in compare to separation on TLC plates in the DS II chamber.
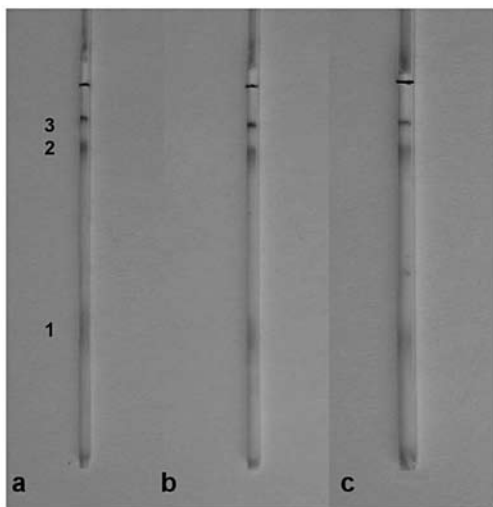

Photo 2. Chromatograms obtained on caLC capillaries, inner diameter: a – 1.4, b – 1.7, c – 2.5 mm, sorbent: RP-8, eluent: methanol-water-acetic acid (4:5:1, v/v). Distance of development – 60 mm, ascending mode. 1 – patent blue V, 2 – allura red AC, 3 – brilliant black PN

The next stage of the study was to verify the effect of the inner diameter of capillaries on data retention dyes studied (Photo 2). Capillary diameters, ϕ = 1.4; 1.7; 2.5 mm were applied, the chromatograms were developed on the distance 60 mm by an ascending mode.

Table 2

Retention parameters obtained from chromatograms in Photo 2

ϕ [mm]	Substance	hR_f	R_s [a]	N	Time of development [min]
1.4	(1)	33	6.32[1,2]	100	
	(2)	83	4.00[2,3]	17 778	68
	(3)	92		193 600	
1.7	(1)	33	5.81[1,2]	95	
	(2)	83	3.33[2,3]	6 400	70
	(3)	92		48 400	
2.5	(1)	30	6.53[1,2]	100	
	(2)	81	3.67[2,3]	6 021	51
	(3)	90		186 624	

[a] – indicated in parentheses pairs separated substances
(1) – patent blue V
(2) – allura red AC
(3) – brilliant black PN

The data presented in Table 2 shows that the diameter of the capillary does not affect the value of hR_f. Also, resolution and efficiency do not show a clear dependence on the diameter of the capillary. Although the development time in the capillary with a diameter of 2.5 mm is the shortest, the other parameters do not change significantly. The highest values of efficiency and resolution were obtained for the capillary with a diameter of 1.4 mm.

The final stage of the study was to examine how retention parameters are influenced by the different way of developing of the chromatograms (Photo 3). Analysis were performed in capillaries with a diameter of 1.4 mm by 60 mm develop, modes: ascending (the capillary tube were placed in the eluent), descending (giving the mobile phase from the top) and horizontal (eluent supplied to the capillary arranged in a horizontal position). Mobile phase power was supplied in all developing methods: this is done by placing a capillary column in vial filled by eluent and secured by silicone septum.
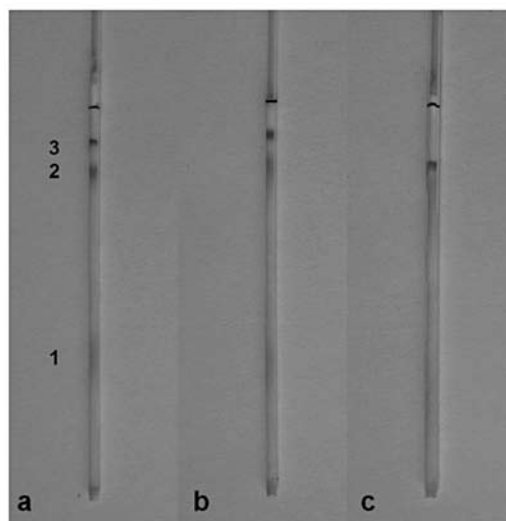

Photo 3. Chromatograms obtained on caLC capillaries inner diameter: 1.4 mm, sorbent: RP-8, eluent: methanol-water-acetic acid (4:5:1, v/v). Distance of development – 60 mm, mode of development: a – ascending, b – descending, c – horizontal, c – horizontal. 1 – patent blue V, 2 – allura red AC, 3 – brilliant black PN

Table 3

Retention parameters obtained from chromatograms in Photo 3

Mode of development	Substance	hR$_f$	R$_s$ [a]	N	Time of development [min]
ascending	(1)	33	6.32[(1,2)]	100	68
	(2)	83	4.00[(2,3)]	17 778	
	(3)	92		193 600	
descending	(1)	31	4.69[(1,2)]	68	79
	(2)	82	2.00[(2,3)]	2 401	
	(3)	91		21 122	
horizontal	(1)	25	4.07[(1,2)]	36	28
	(2)	76	1.54[(2,3)]	1 325	
	(3)	84		18 135	

[a] – indicated in parentheses pairs separated substances
(1) – patent blue V
(2) – allura red AC
(3) – brilliant black PN

Tests have shown that the way of develop the chromatograms has a significant impact on the retention parameters in capillary liquid chromatography (Tab. 3). The highest resolution and efficiency obtained with the development of the chromatogram by ascending mode. This is a process in which the highest resolution is obtained, as evidenced the high values for both R$_s$ first and second pair of the separated dye. The efficiency of the chromatographic system, illustrated by the higher number of theoretical plates is also the best for this process to develop (ascending mode). The least preferred method is horizontal mode. Although the duration of the analysis is the shortest, the retention data: both resolution and efficiency, are the smallest.

Among the analyzed dyes the weakest retention parameters (the lowest number of theoretical plates and the largest width) has patent blue because this dye contains both acidic groups (sulfonic) and basic (amide). The addition of acetic acid to the mobile phase does not withdraw the dissociation of basic groups and the dye has a relatively strong interaction with sorbent in a column in the separation process. Other examined dyes have mainly acidic group and here the addition of acetic acid to the mobile phase can cause blockage of the polar interactions of these substances.

Comparing the efficiency of caLC columns, with the efficiency of micro-HPLC columns (μLC) it can be seen that the columns μLC demonstrate efficiencies of 200 000 theoretical plates (with a length of 250 mm) [7]. Our columns reached efficiencies of 250 000 plates / m at a distance of development 70 mm (Tab. 1 – brilliant black PN). In other analyzes were obtained efficiencies of 190 000 / m (also for brilliant black PN, Tab. 2 and 3).

Such high efficiency obtained for the brilliant black PN, substances with the highest retardation factor (hR$_f$), may be due to a phenomenon demixion that may accompany the substance which migrates almost with the forehead phases.

Summary

Analysis of the retention data obtained during the separation of a mixture of dyes: allura red AC, patent blue V, brilliant black PN in phase: RP-8 – methanol-water-acetic acid (4:5:1, v/v) by thin layer chromatography and by the capillary action liquid chromatography revealed that the efficiency obtained with capillary columns caLC are much higher than on TLC plates and achieve efficiency 250 000 plates / 1m capillary. Comparison the results of retention dates from chromatograms, obtained during the analysis with capillary columns develop in various distances, and the different internal diameters and different methods can conclude that the best results of the analysis

in our tests was obtained in capillary of inner diameter 1.4 mm, and developing of the chromatogram was done over a distance of 60 mm by from the bottom to up mode.

Undoubtedly, the weakness of caLC long time to development the chromatograms, especially in reverse phase systems when the mobile phase are solvents with high viscosity (water-methanol of pH modifier). Much shorter time to development is obtained in analyzing the columns caLC in the classic adsorption phase (for sorbent type Si-60 and the mobile phase n-hexane-ethyl acetate). The time to development was then 40-50 minutes, and was thus comparable with the time to development the TLC with the same sorbents applied to the plate and using the same mobile phase. Undoubtedly, much quicker in μLC chromatographic analysis, the forced flow of the mobile phase, the analysis time is about 15-20 minutes [7].

Thus, there is a simple choice, complicated apparatus and a short time analysis (analysis μLC) or very simple equipment and longer analysis (caLC capillary action chromatography). Separation efficiencies in both cases are comparable.

The technique of capillary liquid chromatography (caLC) may be alternative to thin-layer chromatography (TLC), taking into account both the amount of the sorbent and amount of solvents used for analysis.

Literature

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