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## Journal of Liquid Chromatography & Related Technologies

Publication details, including instructions for authors and subscription information:

<http://www.informaworld.com/smpp/title~content=t713597273>

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Online publication date: 09 February 2003

**To cite this Article** Pyka, A. and Bober, K.(2003) 'Investigation of an Homologous Series of Fatty Acids by TLC. II. Comparison of Separation of Fatty Acids on RP-18 Plates with Different Mobile Phases', *Journal of Liquid Chromatography & Related Technologies*, 26: 16, 2663 – 2671

**To link to this Article:** DOI: 10.1081/JLC-120024536

**URL:** <http://dx.doi.org/10.1081/JLC-120024536>

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JOURNAL OF LIQUID CHROMATOGRAPHY & RELATED TECHNOLOGIES®  
Vol. 26, No. 16, pp. 2663–2671, 2003

## Investigation of an Homologous Series of Fatty Acids by TLC. II. Comparison of Separation of Fatty Acids on RP-18 Plates with Different Mobile Phases

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### ABSTRACT

Fatty acids from octanoic acid to octadecanoic acid have been separated on RP-18 plates with and without a concentrating zone using methanol–water, ethanol–water, and *n*-propanol–water as mobile phases. The cluster analysis for comparison of  $R_M$  parameters of investigated acids for chromatographic conditions used was proposed. Comparison of differences between  $R_F$  values showed that acids from nonanoic to octadecanoic are separated on the longest distance, using as mobile phase methanol–water in a volume ratio of 95 + 5 and on RP-18 plates with and without concentrating zone. Moreover, resolution factor values ( $R_S$ ) calculated suggest that better separations of acids from octanoic to octadecanoic were obtained on RP-18 plates with a concentrating zone and methanol–water (95 + 5, v/v) as mobile phase.

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DOI: 10.1081/JLC-120024536  
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1082-6076 (Print); 1520-572X (Online)  
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*Key Words:* RP-HPTLC; Fatty acids; Resolution factor ( $R_S$ ); Cluster analysis; Different mobile phases.

## INTRODUCTION

There are three basic methods of evaluating fatty acids present in nature: distillation, solubility, and adsorption. For acid characteristics after separation, melting point, boiling point, refractivity, or other physical properties are evaluated.<sup>[1]</sup>

Previously, the higher fatty acids were separated by use of partition thin-layer chromatography on kieselguhr impregnated with paraffin oil or octadecane and using different mobile phases: methanol–water (9 : 1, v/v), ethanol–water (9 : 1, v/v), acetic acid–paraffin oil–water (8 : 1 : 1, v/v), anhydrous acetic acid–methanol–water (17 : 2 : 1, v/v), as well as propionic acid–methanol–water (17 : 2 : 1, v/v).<sup>[2–7]</sup> Currently, free higher fatty acids are also separated on RP-18 plates by use of methanol–water as mobile phase.<sup>[9,10]</sup> Most often investigated are higher saturated fatty acids with the following number of carbon atoms: 12, 14, 16, and 18.<sup>[2,3,8,9]</sup> Moreover, the literature shows that most often investigated are fatty acids of an homologous anseries with double homologous differences.<sup>[2,3]</sup> In our earlier work, we separated acids from pentanoic to tricosanoic on RP-18 plates with and without a concentrating zone, by use of methanol–water (100 : 0, 95 : 5, 90 : 10, v/v) as mobile phases and we stated the relationships between  $R_M$  values and topological indexes.<sup>[10]</sup>

The aim of this work was to compare the separation of acids from octanoic to octadecanoic on plates RP-18 with and without a concentrating zone and with different mobile phases.

## EXPERIMENTAL

Solutions of the acids (octanoic, nonanoic, decanoic, undecanoic, dodecanoic, tridecanoic, tetradecanoic, pentadecanoic, hexadecanoic, heptadecanoic, and octadecanoic) were prepared from chemicals (99% pure) supplied by Larodan Chemicals, Sweden. Methanol, ethanol, and *n*-propanol (analytical grade) were obtained from POCh (Gliwice, Poland).

Partition TLC was performed on 10 × 20 cm glass-backed RP-18 HPTLC plates with and without a concentrating zone (Merck #15498 and 1.05914, respectively). Solutions of the acids, 20 μg acid in 5 μL chloroform, were spotted on the plates and the plates were developed at room temperature in a classical flat-bottomed chamber (Camag, Switzerland) previously saturated for 30 min with the mobile phase. The mobile phases were: methanol–water (90 : 10 v/v),





methanol–water (95 : 5 v/v), 100% methanol, ethanol–water (90 : 10 v/v), ethanol–water (95 : 5 v/v), 100% ethanol, or *n*-propanol–water (90 : 10 v/v), *n*-propanol–water (95 : 5 v/v) and 100% *n*-propanol. The development distance was 7 or 7.5 cm, respectively, for plates with or without a concentrating zone. The substances analyzed were visualized in iodine vapor.  $R_F$  values were measured and converted to  $R_M$  values by use of the formula:

$$R_M = \log\left(\frac{1}{R_F} - 1\right) \quad (1)$$

Resolution factors<sup>[11]</sup> were calculated for all the chromatograms. Resolution ( $R_S$ ) was calculated by use of the formula:

$$R_S = 2 \times \frac{d}{s} \quad (2)$$

where  $d$  is the distance between the centers of two adjacent spots, and  $s$  is the sum of the widths of the two spots in the direction of flow.

## RESULTS AND DISCUSSION

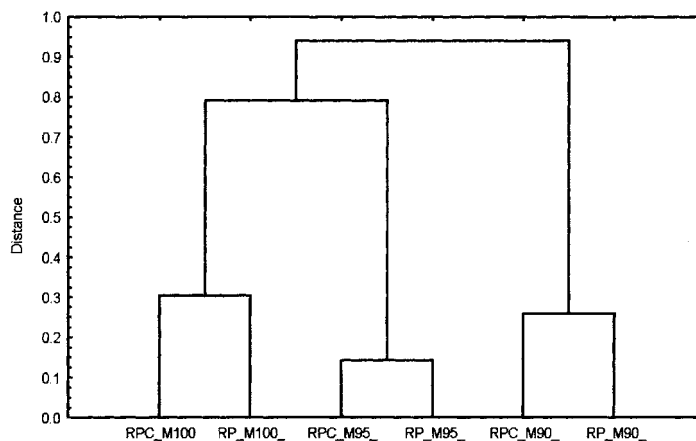
It was stated that acids from octanoic to octadecanoic can be separated on RP-18 plates with and without a concentrating zone, by use of all mobile phases applied. The exception is octanoic acid, which cannot be analyzed on RP-18 plates with a concentrating zone using 100% ethanol as mobile phase.

The comparative cluster analysis of  $R_M$  parameters for evaluation of chromatographic conditions used was proposed. The cluster analysis was made by means of single bond and the Euclidean distance method.

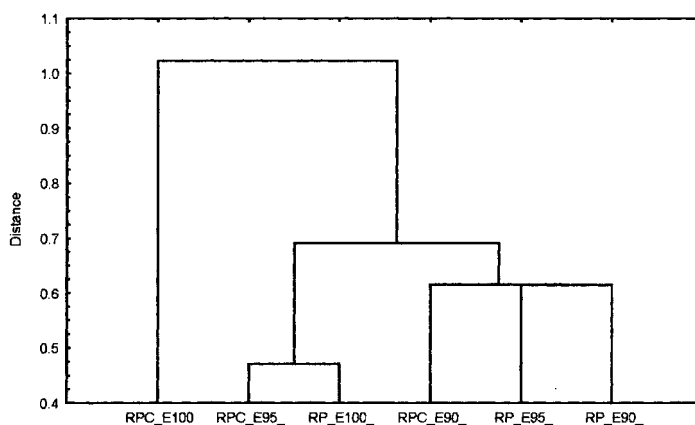
Figure 1 presents a dendrogram of cluster analysis of  $R_M$  parameters for separations on RP-18 plates with and without a concentrating zone, with the application of a methanol–water system in volume ratio of: 90 : 10, 95 : 5, and 100 : 0 as mobile phases. Acids from octanoic to octadecanoic were separated in these chromatographic conditions. The greatest similarity was stated for separations on RP-18 plates with and without a concentrating zone, with application of methanol–water (95 : 5, v/v) as mobile phase, in both cases.

Figure 2 presents a dendrogram of cluster analysis of  $R_M$  parameters for separations on RP-18 plates with and without a concentrating zone, with application of an ethanol–water system in a volume ratio of: 90 : 10, 95 : 5, and 100 : 0 as mobile phases. Acids from nonanoic to octadecanoic were separated in these chromatographic conditions. The greatest similarity was stated for separation on RP-18 plates with ethanol as mobile phase, and for separation on RP-18 plates with a concentrating zone and ethanol–water (95 : 5, v/v) as mobile phase.



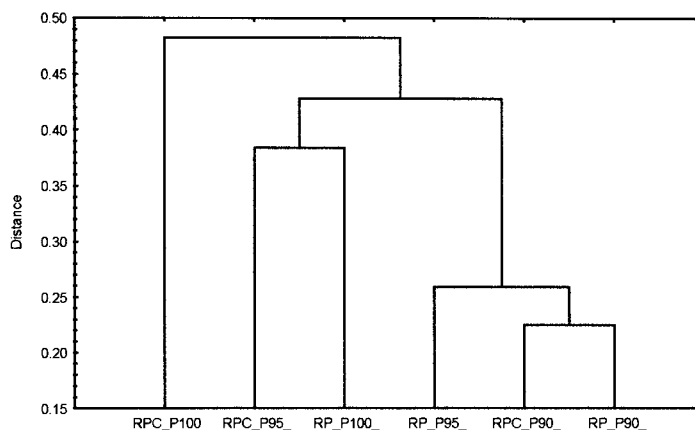


**Figure 1.** Cluster analysis of  $R_M$  parameters of acids from octanoic to octadecanoic, for separations on RP-18 plates with and without a concentrating zone, using methanol–water system as mobile phase. RP\_M100, RP\_M95, and RP\_M90 denote RP-18 plates without a concentrating zone, and mobile phases methanol–water, 100 : 0, 95 : 5, 90 : 10, v/v, respectively; RPC\_M100, RPC\_M95, and RPC\_M90 denote RP-18 plates with a concentrating zone, and mobile phases methanol–water, 100 : 0, 95 : 5, 90 : 10, v/v, respectively.



**Figure 2.** Cluster analysis of  $R_M$  parameters of acids from nonanoic to octadecanoic, for separations on RP-18 plates with and without a concentrating zone using ethanol–water system as mobile phase. RP\_E100, RP\_E95, and RP\_E90 denote RP-18 plates without a concentrating zone and mobile phases ethanol–water, 100 : 0, 95 : 5, 90 : 10, v/v, respectively; RPC\_E100, RPC\_E95, and RPC\_E90 denote RP-18 plates with a concentrating zone and mobile phases ethanol–water, 100 : 0, 95 : 5, 90 : 10, v/v, respectively.





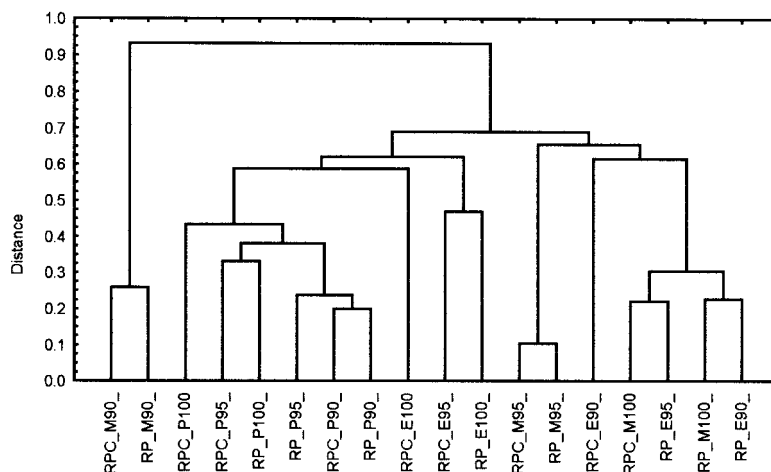
**Figure 3.** Cluster analysis of  $R_M$  parameters of acids from octanoic to octadecanoic for separations on RP-18 plates with and without a concentrating zone using *n*-propanol–water system as the mobile phase. RP\_P100, RP\_P95, and RP\_P90 denote RP-18 plates without a concentrating zone and the mobile phase *n*-propanol–water, 100:0, 95:5, 90:10, v/v, respectively. RPC\_P100, RPC\_P95, and RPC\_P90 denote RP-18 plates with concentrating zone and mobile phase *n*-propanol–water, 100:0, 95:5, 90:10, v/v, respectively.

Figure 3 presents a dendrogram of cluster analysis of  $R_M$  parameters for separations on RP-18 plates with and without a concentrating zone, with application of *n*-propanol–water system in a volume ratio of: 90:10, 95:5, and 100:0 as mobile phases. Acids from octanoic to nonadecanoic were separated in these chromatographic conditions. The greatest similarity was stated for separations on RP-18 plates with and without a concentrating zone, with application in an *n*-propanol–water (90:10, v/v) mobile phase, in both cases.

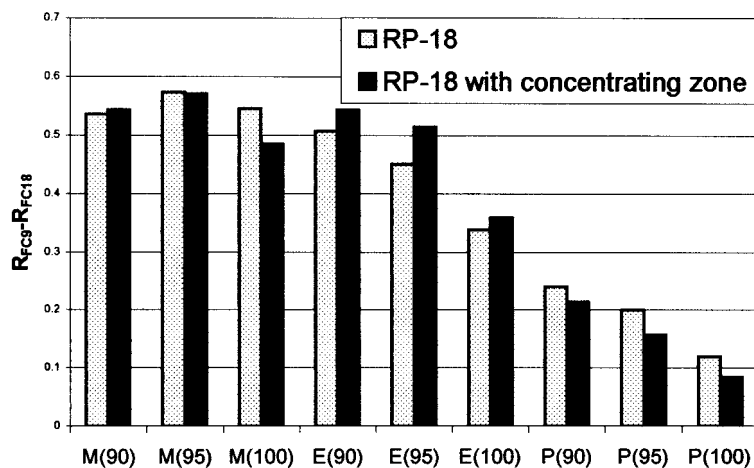
Then, using cluster analysis, all values of the  $R_M$  parameter obtained under all of the chromatographic conditions in which acids from nonanoic to octadecanoic were separated, were compared. This dendrogram is presented in Fig. 4. The greatest similarity for separations on RP-18 plates with and without a concentrating zone, for the mobile phases: methanol–water, ethanol–water, and *n*-propanol–water was obtained for separations with using RP-18 plates with and without a concentrating zone, and methanol–water (95:5, v/v) as mobile phase, in both cases.

A comparison was also made of differences between  $R_F$  values of nonanoic acid and octadecanoic acid (these acids can be separated in all chromatographic conditions), obtained on plates RP-18 with and without a concentrating zone. Acids from nonanoic to octadecanoic were separated on the shortest distance by using *n*-propanol–water as mobile phase regardless of the type of plates (RP-18





**Figure 4.** Cluster analysis of  $R_M$  parameters of acids from nonanoic to octadecanoic, for separations under all chromatographic conditions. Explanations as in Figs. 1, 2, and 3.



**Figure 5.** Comparison of differences between  $R_F$  values of nonanoic and octadecanoic acids, obtained on RP-18 plates with and without a concentrating zone. M(100), M(95), and M(90) denote mobile phases methanol–water, 100 : 0, 95 : 5, 90 : 10, v/v, respectively; E(100), E(95), and E(90) denote mobile phases ethanol–water, 100 : 0, 95 : 5, 90 : 10, v/v, respectively; and P(100), P(95), and P(90) denote mobile phase *n*-propanol–water, 100 : 0, 95 : 5, 90 : 10, v/v, respectively.





with or without a concentrating zone). The acids mentioned above were separated for a relatively long distance by use of the mobile phases: methanol–water and ethanol–water in volume ratio of 90 : 10 and 95 : 5, as well as 100% methanol. The comparison described is presented in Fig. 5. Acids from nonanoic to octadecanoic were separated in the longest distance on RP-18 plates with and without a concentrating zone, with methanol–water (95 : 5, v/v) as the mobile phase.

Table 1 presents  $R_F$ ,  $R_M$  values and resolution values ( $R_S$ ) for acids from octanoic to octadecanoic separated on RP-18 plates with and without a concentrating zone, using methanol–water (95 : 5, v/v).

Figure 6 presents resolution values ( $R_S$ ) for acids from octanoic to octadecanoic separated on RP-18 plates with and without a concentrating zone, and using methanol–water (95 : 5, v/v). Greater  $R_S$  values were obtained for acids separated on RP-18 plates with a concentrating zone. Resolution factors ( $R_S$ ) calculated showed that better separations of acids from octanoic to octadecanoic were obtained on plates with a concentrating zone (Fig. 6), using methanol–water (95 : 5, v/v) as mobile phase.

## CONCLUSION

We stated that cluster analysis can be useful for classification of chromatographic separation conditions of acids from octanoic to octadecanoic.

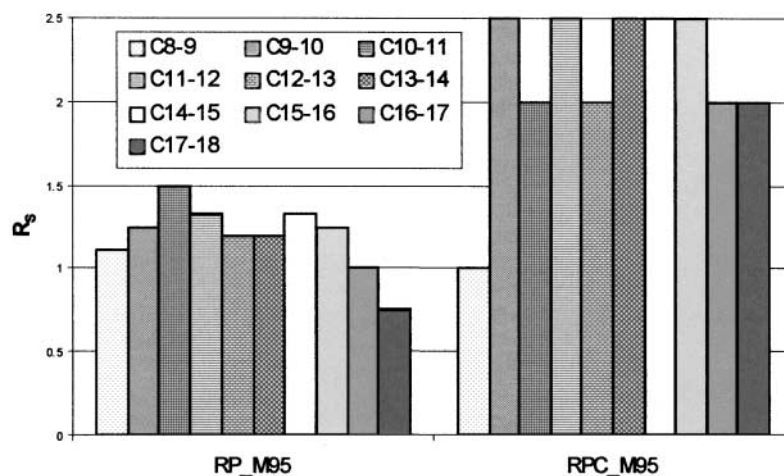
**Table 1.**  $R_F$ ,  $R_M$ , and  $R_S$  values for acids from octanoic to octadecanoic.

Acid	Mobile phase: methanol–water, (95 : 5 v/v)					
	RP-18 plates			RP-18 plates with concentrating zone		
	$R_F$	$R_M$	$R_S$	$R_F$	$R_M$	$R_S$
Octanoic	0.732	−0.436	1.11	0.686	−0.339	1.00
Nonanoic	0.671	−0.310	1.25	0.657	−0.282	2.50
Decanoic	0.610	−0.194	1.50	0.586	−0.151	2.00
Undecanoic	0.536	−0.063	1.33	0.528	−0.049	2.50
Dodecanoic	0.463	0.064	1.20	0.457	0.075	2.00
Tridecanoic	0.390	0.194	1.20	0.400	0.176	2.50
Tetradecanoic	0.305	0.358	1.33	0.328	0.311	2.50
Pentadecanoic	0.244	0.491	1.25	0.257	0.461	2.50
Hexadecanoic	0.183	0.650	1.00	0.186	0.641	2.00
Heptadecanoic	0.122	0.857	0.75	0.128	0.833	2.00
Octadecanoic	0.098	0.964		0.086	1.026	

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**Figure 6.** Resolution factors ( $R_s$ ) calculated for acids from octanoic to octadecanoic obtained on plates RP-18 with and without a concentrating zone using methanol–water (95 + 5, v/v) as mobile phase. RP\_M95 denotes RP-18 plates without a concentrating zone and mobile phase methanol–water (95 : 5, v/v); and RPC\_M95 denotes RP-18 plates with a concentrating zone and the mobile phase methanol–water (95 : 5, v/v).

The best separation of these acids investigated was obtained on RP-18 plates with a concentrating zone, and methanol–water (95 : 5, v/v) as mobile phase. It was confirmed by resolution values calculated for the acids investigated. The chromatographic conditions determined out, using RP-TLC of acids from octanoic to octadecanoic, can be useful for their detection in biological samples and also in pharmaceutical and cosmetic preparations, as well as in environmental samples.

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Received November 10, 2002

Accepted December 10, 2003

Manuscript 6108D

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