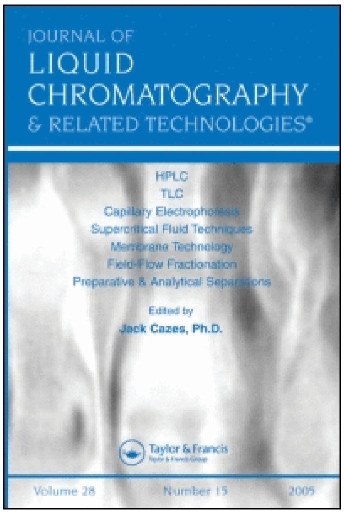

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Influence of the Mobile and Stationary Phases on the Separation of Selected Essential Oil Components and Aroma Substances Investigated by TLC

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Influence of the Mobile and Stationary Phases on the Separation of Selected Essential Oil Components and Aroma Substances Investigated by TLC

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Abstract: Fifteen selected essential oil components and aroma substances were investigated using adsorption thin-layer chromatography on silica gel 60 (E. Merck, #1.05744) and aluminum oxide (E. Merck, #1.05550) plates and the mobile phases benzene–chloroform, benzene–acetone, carbon tetrachloride–acetone, chloroform–acetone, and chlorobenzene–acetone in different volume compositions. The separations were evaluated on the basis of retention parameter R_F and separation parameters values ΔR_F, α, and R_F^{α}. None of the applied chromatographic conditions allowed for the complete separation of all the compounds examined. Aluminium oxide plates and carbon tetrachloride–acetone in volume composition 98:2 were optimal for the separation of the investigated alcohols: (+) borneol (B), geraniol (GR), menthol (M), and linalool (L). Aluminium oxide and carbon tetrachloride–acetone mobile phases in a volume composition of 85:15 were optimal for the separation of the investigated phenols: vanillin (V), guaiacol (G), eugenol (E), and thymol (T). Aluminium oxide and a chlorobenzene–acetone mobile phase in a volume composition of 95:5 was

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optimal for the separation of coumarin (C), cineole (CL), (R)-(−) carvone (CR), cinnamic aldehyde (CA), camphor (CP), (1R)-(−) fenchone (F), and *trans* anethole (A).

Keywords: Adsorption TLC, Silica gel, Aluminium oxide, Essential oil components, Aroma substances, Separation

INTRODUCTION

Essential oil components are active substances present in many medicinal plants. They are a large group of medicinal plants and have a major application in medicine. Various kinds of pharmacological properties are observed among the essential oils, depending on the character of the main components. In general the essential oils act as irritants on the skin and mucous membranes. Some (for example menthol) are aromatic substances and improve the taste and the smell of many drugs. They are also prescription drugs used in medicine, the food industry, and in cosmetics.[1−7] Vanillin, which is not the component of essential oils, is often used as a substance to improve the taste of food.[1,8−10]

Many different compounds make up the composition of one essential oil. For this reason, the separation and detection of the essential oil components and aroma substances is important. Gas chromatography is the main technique for the investigation of essential oils. However, thin-layer chromatography is a less expensive technique than gas chromatography and can be used for preliminary qualitative analysis of essential oils and aroma substances.[11,12]

Our earlier research was concerned with the application of new visualizing reagents and topological indexes for the estimation of the chromatographic separation of selected essential oil components and aroma substances.[13−15]

The aim of this work was to compare the separation of selected essential oil components and aroma substances on silica gel 60 and aluminium oxide as stationary phases using five binary mobile phases in different volume compositions.

EXPERIMENTAL

Chemicals

All solvents (POCh, Gliwice, Poland) and chemicals were of analytical reagent grade.

Sample Preparation

The commercial samples of essential oil components and aroma substances (Sigma-Aldrich, USA): 88 mg geraniol (GR), 100 mg menthol (M), 130.8 mg (+) borneol (B), 118.6 mg linalool (L), 103.7 mg vanillin (V), 100 mg eugenol (E), 100 mg guaiacol (G), 100 mg thymol (T), 100 mg coumarin (C), 158.6 mg cineole (CL), 103.4 mg (R)-(−) carvone (CR), 105 mg cinnamic aldehyde (CA), 141 mg camphor (CP), 142.6 mg (1R)-(-) fenchone (F), and 141.2 mg *trans* anethole (A) were dissolved in 10 mL ethanol.

Thin-Layer Chromatography

Adsorption TLC was performed on plates precoated with layers of silica gel 60 (Merck, #1.05744) and neutral aluminium oxide (Merck, #1.05550). Before the plates were used, they were activated at 120°C for 30 min. A 10 μL microsyringe (Hamilton) was used to apply the standard solutions to the plates. Plates were developed with binary mobile phases: benzene–chloroform (100:0; 80:20; 60:40; 40:60; 20:80; 0:100, v/v), benzene–acetone (100:0; 98:2; 95:5; 90:10; 85:15, v/v), carbon tetrachloride– acetone (100:0; 98:2; 95:5; 90:10; 85:15, v/v), chloroform-acetone (100:0; 98:2; 95:5; 90:10; 85:15, v/v), and chlorobenzene–acetone (100:0; 98:2; 95:5; 90:10; 85:15, v/v). Fifty mL of mobile phase was placed in a chromatographic chamber and the chamber was saturated for 30 min. The development distance was 14 cm at 20°C. The plates were dried at room temperature using a fume cupboard. The spots were visualized using 5% solution of $K_2Cr_2O_7$ in 40% H_2SO_4. Plates 20 cm × 20 cm were sprayed with 10 mL of the visualizing agent.

Separation Parameters

Separation Parameters (ΔR_F)

ΔR_F was calculated according to the formula:

$$\Delta R_F = R_{F1} - R_{F2} \tag{1}$$

where: $R_{F1}$and R_{F2} are the values of two adjacent spots; and $R_{F1} > R_{F2}$

Separation Parameters (α)

The separation factor values (α) were calculated using the equation:[16]

$$\alpha = \frac{1/R_{F1} - 1}{1/R_{F2} - 1} \tag{2}$$

where: R_{F1} and R_{F2} are values of R_F of two neighbouring spots on the chromatogram, and $R_{F1} < F_2$.

Constant of the Pair Separation R_F^{α}[17]

The constant of the pair separation (R_F^{α}) was calculated for the investigated compounds as the ratio of the R_F values of two neighbouring spots on the chromatogram:

$$R_F^{\alpha} = \frac{R_{F1}}{R_{F2}} \tag{3}$$

where: $R_{F1}$and R_{F2} are the values of two adjacent spots; and $R_{F1} > R_{F2}$.

RESULTS AND DISCUSSION

The essential oil components and aroma substances investigated were placed in three groups. Aliphatic alcohols (geraniol, linalool) and monocyclic alcohols (menthol, borneol) were in the first group; phenols (vanillin, eugenol, guaiacol, thymol) were in the second group; and ethers, ketones, aldehydes (coumarin, cineole, (R)-(−) carvone, cinnamic aldehyde, camphor, (1R)-(−) fenchone, and trans anethole) were in the third group (Figure 1). The essential oil components and aroma substances were separated on silica gel and aluminium oxide plates using binary mobile phases: benzene–chloroform, benzene–acetone, carbon tetrachloride–acetone, chloroform-acetone, and chlorobenzene–acetone in different volume compositions. It was demonstrated that the binary mobile phase chloroform–acetone was the least useful of the mobile phases applied. The R_F values of all 15 essential oil components and aroma substances in this mobile phase, in the volume composition of 85:15, were in the range of 0.74 to 0.92 on silica gel plates. However, all of the investigated compounds, except vanillin, migrated on aluminium oxide plates with the solvent front of a mobile phase of chloroform–acetone. From the five remaining binary mobile phases, the mobile phase benzene–chloroform was the least selective in relation to essential oil components and aroma substances studied on silica gel, as well as on aluminium oxide plates.

We considered the mobile phase in respect to the possibility of separation of individual compounds in the three above-mentioned groups. Chemometric characterizations of the adsorbent-binary eluent system for alcohols, phenols, and other essential oil components and aroma substances are shown in Figures 2–4, respectively. Figures 2–4 show only the best selected results, characterizing the best selectivity in relation to separated compounds.

We showed, that on silica gel plates, the alcohols studied were best separated with the mobile phase chloroform–acetone in a volume composition of 90:10, as well as with the mobile phase carbon tetrachloride–acetone in a volume composition of 85:15. However, in these conditions, the pairs of investigated compounds B/GR and GR/M were not well separated, respectively. However, on aluminium oxide plates, the alcohols studied separated the best with carbon tetrachloride–acetone in volume com-

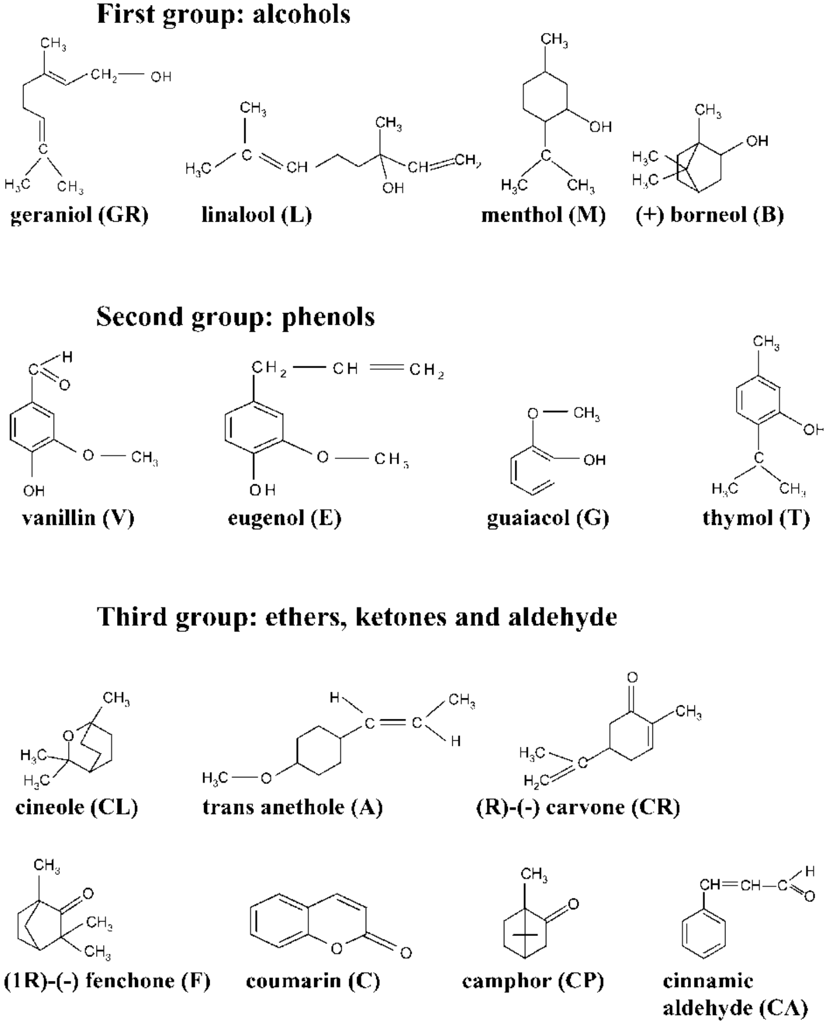

Figure 1. Structures of investigated essential oil components and aroma compounds.

positions 90:10, 95:5, as well as 98:2 as mobile phases (Figure 2). The values of the separation parameter (α), constant of the pair separation ($\mathrm{R}_\mathrm{F}^{\alpha}$) and $\Delta\mathrm{R}_\mathrm{F}$ of two neighbouring spots of investigated alcohols on chromatogram are listed in Table 1. Analysis of data contained in Table 1, indicate that aluminium oxide and the mobile phase carbon tetrachloride–acetone in the volume composition of 98:2 are optimal for the separation of (+) borneol (B), geraniol (GR), menthol (M), and linalool (L).

Phenols: thymol (T), guaiacol (G), eugenol (E), and vanillin (V) were best separated on silica gel plates with the mobile phase benzene–acetone in

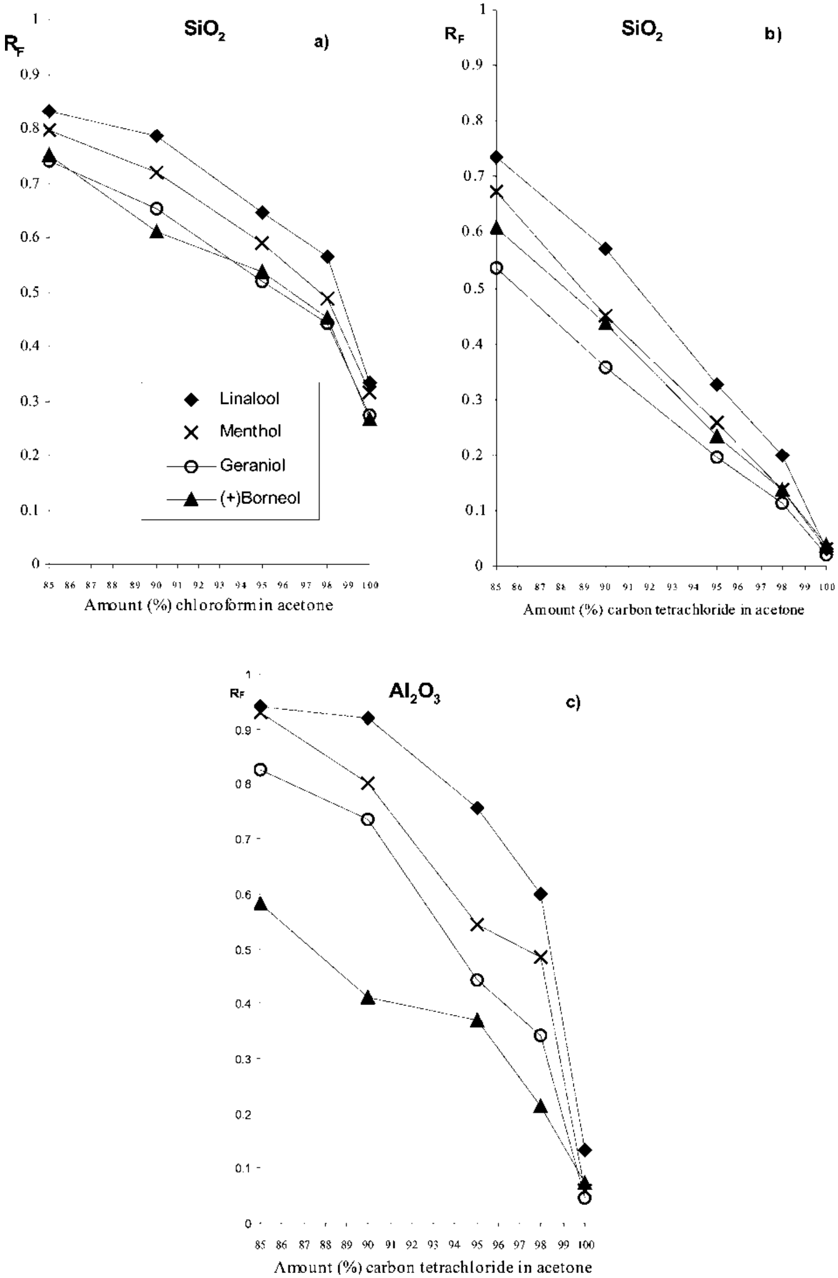

Figure 2. R$_F$ values of the alcohols on (a) silica gel plates with chloroform–acetone as mobile phase, (b) silica gel plates with carbon tetrachloride–acetone as mobile phase, and (c) aluminium oxide plates with carbon tetrachloride–acetone as mobile phase.

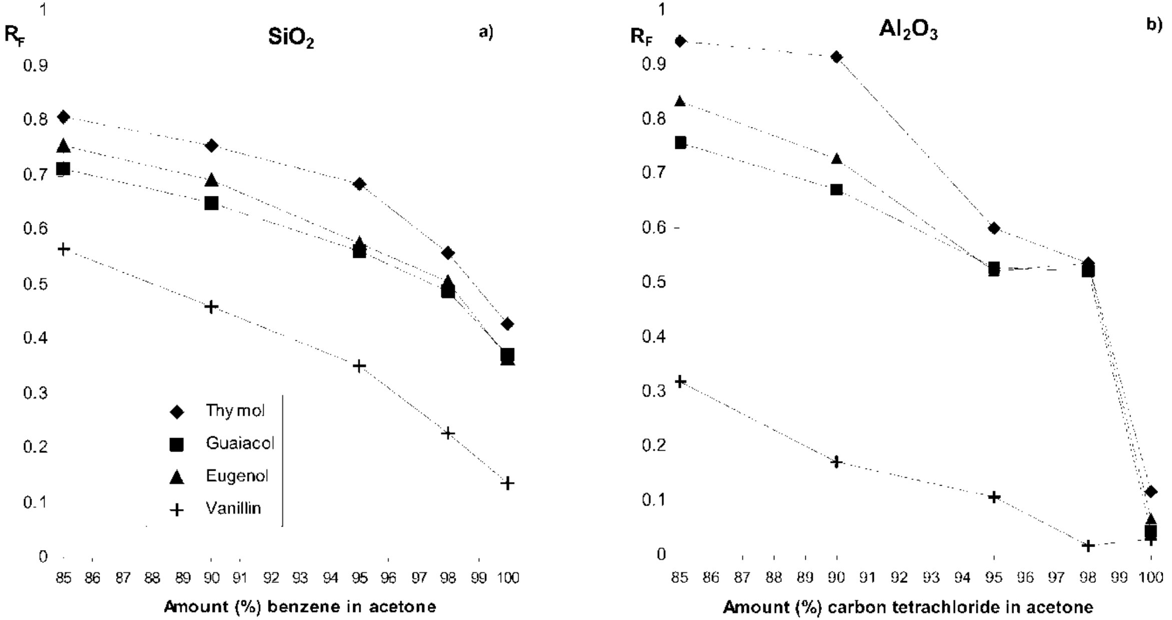

Figure 3. R$_F$ values of the phenols on silica gel plates with (a) benzene–acetone as mobile phase, (b) aluminium oxide plates with carbon tetrachloride–acetone as mobile phase.

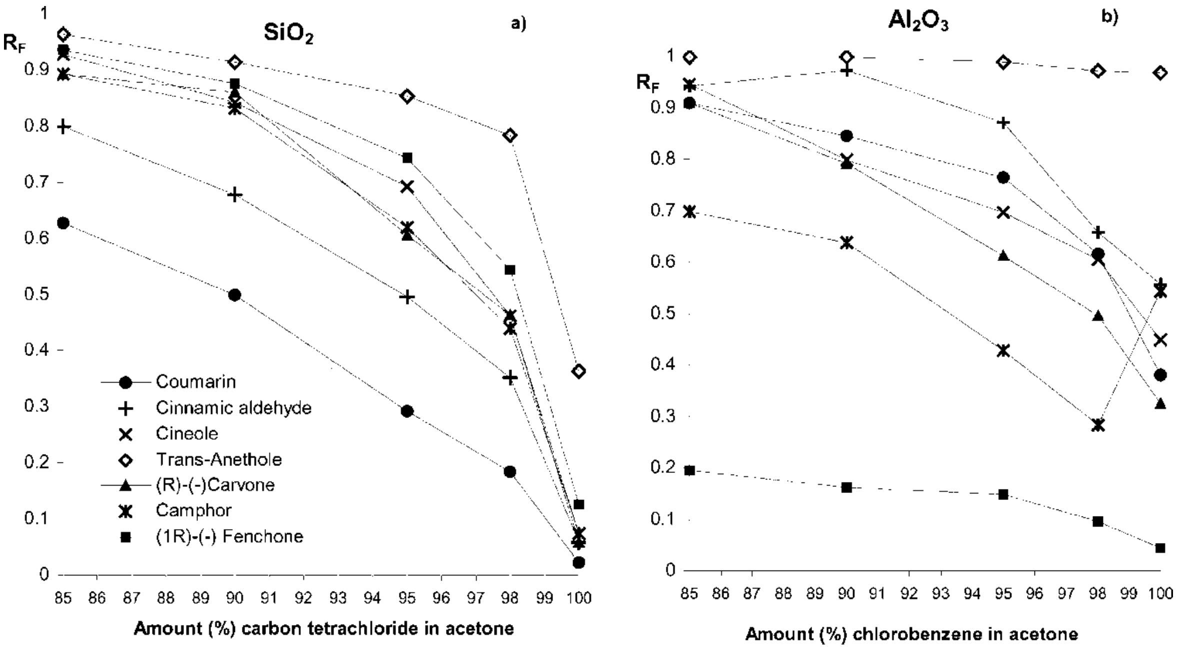

Figure 4. R_F values of the ethers, ketones and aldehyde (a) silica gel plates with (a) carbon tetrachloride–acetone as mobile phase, (b) aluminium oxide plates with chlorobenzene–acetone as mobile phase.

Table 1. The values[a] of separation parameters (α), constant of the pair separation (R_F^α) and ΔR_F of two neighbouring spots of investigated alcohols on chromatogram

Two neighbouring spots	Silica gel						Aluminium oxide								
	Chloroform–acetone			Carbon tetrachloride–acetone			Carbon tetrachloride–acetone								
	90 + 10, v/v			85 + 15, v/v			90 + 10, v/v			95 + 5, v/v			98 + 2, v/v		
	α	R_F^α	ΔR_F	α	R_F^α	ΔR_F	α	R_F^α	ΔR_F	α	R_F^α	ΔR_F	α	R_F^α	ΔR_F
B/GR	1.189	1.065	0.04	1.336	1.132	0.06	3.972	1.786	0.32	1.355	1.197	0.07	1.941	1.628	0.13
GR/M	1.364	1.103	0.07	1.167	1.059	0.03	1.435	1.087	0.06	1.493	1.226	0.10	1.811	1.417	0.14
M/L	1.432	1.093	0.07	1.545	1.145	0.09	2.917	1.151	0.12	2.594	1.390	0.21	1.585	1.234	0.11

[a] Average, n = 5.

Table 2. The values[a] of separation parameters (α), constant of the pair separation (R_F^α) and ΔR_F of two neighbouring spots of investigated phenols on chromatogram

Two neighbouring spots	Silica gel						Aluminium oxide					
	Benzene–acetone						Carbon tetrachloride–acetone					
	90 + 10, v/v			85 + 15, v/v			90 + 10, v/v			85 + 15, v/v		
	α	R_F^α	ΔR_F	α	R_F^α	ΔR_F	α	R_F^α	ΔR_F	α	R_F^α	ΔR_F
V/G	2.178	1.415	0.19	1.897	1.258	0.19	9.908	3.924	0.50	6.637	2.373	0.44
G/E	1.223	1.068	0.04	1.253	1.062	0.04	1.312	1.085	0.06	1.603	1.100	0.09
E/T	1.371	1.091	0.06	1.358	1.069	0.05	3.963	1.255	0.19	3.381	1.133	0.11

[a] Average, n = 5.

Table 3. The values[a] of separation parameters (α), constant of the pair separation (R_F^α) and ΔR_F of two neighbouring spots of investigated ethers, ketones and aldehyde on chromatogram

Two neighbouring spots	Silica gel Carbon tetrachloride-acetone 95 + 5, v/v α	 R_F^α	 ΔR_F	Two neighbouring spots	Aluminium oxide Chlorobenzene-acetone 95 + 5, v/v α	 R_F^α	 ΔR_F
C/CA	2.388	1.699	0.20	F/CP	4.276	2.872	0.28
CA/CR	1.563	1.222	0.11	CP/CR	2.109	1.430	0.18
CR/CP	1.062	1.023	0.01	CR/CL	1.459	1.139	0.08
CP/CL	1.384	1.118	0.07	CL/C	1.406	1.096	0.07
CL/F	1.285	1.074	0.05	C/CA	2.084	1.140	0.12
F/A	2.014	1.148	0.11	CA/A	13.335	1.135	0.12

[a] Average, n = 5.

volume compositions of 90:10 as well as 85:15. However, under these conditions, one pair of substances G/E was poorly separated ($\Delta R_{F(G/E)} = 0.04$). However, on aluminium oxide plates the phenols studied were best separated with carbon tetrachloride–acetone in volume compositions of 85:15 and 90:10 as mobile phases (Figure 3). The values of the separation parameter (α), constant of the pair separation (R_F^α) and ΔR_F of two neighbouring spots of investigated phenols on chromatograms are listed in Table 2. Analysis of data contained in Table 2 indicate that aluminium oxide and the mobile phase carbon tetrachloride–acetone in a volume composition of 85:15 were optimal for the separation of vanillin (V), guaiacol (G), eugenol (E), and thymol (T).

The third group are the ethers, ketones, and aldehyde. We showed that these compounds were best separated on silica gel plates with the mobile phase carbon tetrachloride–acetone in a volume composition of 95:5 and on aluminium oxide plates with the mobile phase chlorobenzene–acetone in a volume composition of 95:5 (Figure 4). However, under these conditions on silica gel, the pair of compounds CR/CP were not separated ($\Delta R_{F(CR/CP)} = 0.01$). The values of separation parameters (α), constant of the pair separation (R_F^α) and ΔR_F of two neighbouring spots of investigated ethers, ketones, and aldehyde on chromatograms are listed in Table 3. Analysis of data contained in Table 3 indicate that aluminium oxide and the mobile phase chlorobenzene–acetone in a volume composition of 95:5 were optimal for separation of coumarin (C), cineole (CL), (R)-(−) carvone (CR), cinnamic aldehyde (CA), camphor (CP), (1R)-(−) fenchone (F), and *trans* anethole (A).

The plots in the obtained figures can be used for recognition of individual essential oil components and aroma substances. The diagrams can also be used for planning of preconcentration of the essential oil components and aroma substances on silica or alumina columns by the SPE method.

Further research is in progress concerning separation of selected essential oil components and aroma substances on silica gel and aluminium oxide using single-component mobile phases and multiple development of chromatographic plates.

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