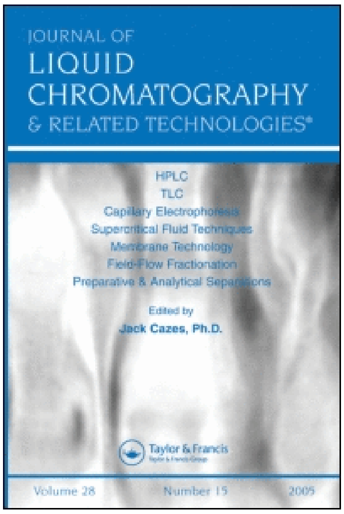

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Influence of Impregnation of a Mixture of Silica Gel and Kieselguhr with Copper (II) Sulphate (VI) on Profile Change of the Spectrodensitograms and the R_F Values of Nicotinic Acid and its Derivatives

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Abstract: Nicotinic acid and its derivatives were separated using adsorption TLC on aluminum plates precoated with a mixture of silica gel 60 and kieselguhr F_{254} non impregnated and impregnated with 2.5% and 5% aqueous solutions of $CuSO_4$. The mixture of an acetone-*n*-hexane in different volume compositions was used as mobile phase. The obtained results of the investigations concerning the evaluation of chromatographic separations were considered in three groups, namely, 1st group: nicotinic acid (1), methyl nicotinate (2), ethyl nicotinate (3), isopropyl nicotinate (4), butyl nicotinate (5), hexyl nicotinate (6), benzyl nicotinate (7); 2nd group: nicotinic acid (1), nicotinamide (8), N-methylnicotinamide (9), N,N-diethylnicotinamide (10); and 3rd group: nicotinic acid (1), nicotinamide (8), 3-pyridinecarbaldehyde (11), 3-pyridinecarbonitrile (12), 3-pyridylmethanol (13), methyl 3-pyridyl ketone (14). Impregnation of the mixture of silica gel and kieselguhr with 2.5% and 5% aqueous solutions of $CuSO_4$ causes a decrease of the R_F values of the 1st, 2nd, and 3rd groups of the compounds in relation to the R_F values of these substances obtained on non impregnated solid support. Impregnation with a water solution of copper (II) sulphate (VI) of the mixture of silica gel and kieselguhr worsens the separation of the 2nd and 3rd groups of the investigated compounds. Improvement of the chromatographic separations is observed only in the case of esters of nicotinic acid (1st group of compounds). It was found that the spectrodensitograms of the studied compounds on impregnated plates and by use of Rhodamine B as visualizing reagent were different than the spectrodensitograms obtained on the plates without use of a visualizing

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reagent. Spectra of the investigated compounds on non impregnated plates and impregnated with aqueous solution of $CuSO_4$ and without the use of a visualizing reagent have two or three absorption bands.

Investigated compounds on the plates impregnated with the aqueous solution of $CuSO_4$ and by use of Rhodamine B as visualizing reagent have one additional absorption band at $\lambda \approx 560$ nm. This fact has analytical significance in the identification of the investigated substances.

Keywords: Nicotinic acid, Nicotinic acid derivatives, NP-TLC, Mixture of silica gel and kieselguhr, Impregnation with $CuSO_4$, Spectrodensitogram

INTRODUCTION

Impregnation is one of the most commonly used modification types with stationary phases.

Depending on the kind of interactions between the reagents and the stationary phases, one can distinguish six basic groups of impregnating agents used in planar chromatography, and namely:[1]

- Non-polar liquids that are able to form a liquid stationary phase for a partition chromatographic RP system that is independent of the matrix used (such as saturated and unsaturated hydrocarbons, silicon oils, and plant oils). These substances can be used to separate nitrophenols, peptides, pesticides, phenols, steroids, and pigments.[1–4]
- Impregnating agents that are able to form complexes. In this category the division can be done on:

  organic compounds, which form ligands with the analyte in a complex compound forming on stationary phase, such as ethylenediaminetetraacetic acid–EDTA. These substances can be used to separate antibiotics, metal ions, and phospholipids.[1,5,6]

  metal ions that act as central atoms. For example, thin-layer plates impregnated with cadmium, copper, zinc, or manganese salts have been used to separate amino acids, aromatic amines, humic acids, peptides, phenolics, bile acids, and sulfonamides.[1,7–11]

  However, thin-layer plates impregnated with silver nitrate can be used to separate fatty acid derivatives, lipids, steroids, α-, and γ-terpinene, as well as α- and β- pinene.[1,12]
- Impregnating agents that are able to form charge transfer complexes (such as caffeine).[1,13]
- Substances that lead to the adjustment of pH values. Acidified carriers (such as perchloric acid)[1,14] are very useful for the separation of aromatic amines, aromatic compounds, and phenolics, whereas sorbents with alkaline pH values (such as sodium hydroxide) can be used for separations of basic compounds and amines.[1,15]

- Impregnating agents that lead to a defined change in the solubility of the analytes in the liquid stationary phase (such as formamide and ammonium sulphate).[1,16,17]
- Cationic and anionic detergents (such as dodecylbenzenesulphonic acid-H-DBS, sodium dioctylsulphosuccinate-Na-DSS).[1,18]

Our previous investigation of bile acids focused on determining the influence of the TLC plates precoated with silica gel 60 F_{254} non impregnated and impregnated with aqueous solutions of $CuSO_4$, $MnSO_4$, $NiSO_4$, and $FeSO_4$ on the separation of bile acids with the use of adsorption TLC.[11]

The aim of this work was a comparison of the separation of nicotinic acid and its derivatives on a mixture of silica gel 60 and kieselguhr F_{254} non impregnated and impregnated with an aqueous solution of $CuSO_4$ using adsorption TLC chromatography.

EXPERIMENTAL

Chemicals

The components of the mobile phase: acetone (POCh, Gliwice, Poland; analytical grade) and *n*-hexane (AnalaR, UK; analytical grade) were used for adsorption TLC analysis. The commercial samples of nicotinic acid (1), methyl nicotinate (2), ethyl nicotinate (3), butyl nicotinate (5), nicotinamide (8), N-methylnicotinamide (9), N,N-dietylnicotinamide (10) (Sigma-Aldrich, Germany), isopropyl nicotinate (4), hexyl nicotinate (6) (Aldrich, Germany), benzyl nicotinate (7) (Fluka, Switzerland), 3-pyridinecarbaldehyde (11), 3-pyridinecarbonitrile (12), 3-pyridylmethanol (13), and methyl 3-pyridyl ketone (14) (E. Merck, Germany) were used as test solutes. The purities of the studied standard samples were at least 97%. Ethanol (POCh, Poland; 96%, analytical grade) was used to prepare the solutions of nicotinic acid and its derivatives. Copper (II) sulphate (VI) 5-hydrate (POCh, Gliwice, Poland; analytical grade) and distilled water were used to prepare the impregnating solution. Rhodamine B (POCh, Gliwice, Poland; analytical grade) and methanol (E. Merck, Germany; for liquid chromatography) were used to prepare the solution of the visualizing reagent.

Impregnation of Stationary Phase

Ready-made TLC aluminium plates (20 × 10 cm) precoated with mixture of silica gel 60 and kieselguhr F_{254} (E. Merck, #1.05567) were impregnated by dipping in impregnant solution for 30 sec. Aqueous solutions of copper (II) sulphate (VI) in concentrations of 2.5% and 5% were used for impregnation. Impregnated plates were dried for 24 h at room temperature (18 ± 1°C).

Thin-Layer Chromatography

TLC was performed on 20 × 10 cm aluminium plates non impregnated and impregnated precoated with a mixture of silica gel 60 and kieselguhr F_{254} (E. Merck, #1.05567). The plates were activated at 120°C for 30 min. Each nicotinic acid derivative (40 mg) was dissolved in 10 mL ethanol. The solution of the standard, 3-pyridinecarbonitrile, was spotted manually using a microcapillary (Camag, Switzerland) onto non impregnated and impregnated plates in 5 μL quantities, and the remaining compounds in 3 μL quantities. The acetone-*n*-hexane in volume compositions of 100:0, 90:10, 80:20, 70:30, 60:40, 50:50, 40:60, 30:70, 20:80, 10:90, and 0:100 was used as mobile phase. The mobile phase (50 mL) was placed in a classical chamber (Camag, Switzerland) and the chamber was saturated with the mobile phase for 20 min. The plates were developed to a distance 7.5 cm at room temperature (18 ± 1°C). The plates were dried for 24 h at room temperature (18 ± 1°C) in a fume cupboard. Detection was carried out by the dipping of impregnated plates in 0.05% methanolic solution of Rhodamine B.

Visualization of Spots by Use of UV Lamp

The spots on non impregnated and impregnated plates were visualized using a UV lamp (Cobrabid, Poland) at $\lambda = 254$ nm.

Spectrodensitometric Analysis

A spectrum scan was then performed with a Camag Scanner TLC 3 operated in the absorbance mode and controlled by winCATS 1.4.1 software. The radiation source was a deuterium lamp emitting a continuous UV spectrum between 190 and 450 nm and a tungsten lamp emitting a spectrum between 370 and 800 nm. Starting wavelength was 200 nm and ending wavelength was 700 nm. The slit dimensions were 8.00 × 0.30 mm, Micro; the optimized optical system was resolution; the scanning speed was 20 nm s^{-1}; the data resolution was 1 nm $step^{-1}$; the measurement type was remission; and the measurement mode was absorption; the optical filter was second order.

Separation Factors

The chromatograms were done in triplicate and each track was scanned three times; the mean R_F values were calculated.

The separation factors, namely: ΔR_F values, selectivity (α),[19] and the constant of the pair separation (R_F^{α})[20] were calculated for all the densitograms.

ΔR_F was calculated according to the formula:

$$\Delta R_{F(1,2)} = R_{F1} - R_{F2} \tag{1}$$

where R_{F1} and R_{F2} are the R_F values of two adjacent peaks on the densitogram; and $R_{F1} > R_{F2}$.

The selectivity (α) was calculated using the equation:

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

where R_{F1} and R_{F2} are the R_F values of two adjacent peaks on the densitogram; and $R_{F1} < R_{F2}$.

The constant of the pair separation (R_F^α) was calculated for the investigated compounds as the ratio of the R_F values of the two adjacent peaks on the densitogram:

$$R^{\alpha}_{F(1,2)} = \frac{R_{F1}}{R_{F2}} \tag{3}$$

where R_{F1} and R_{F2} are the R_F values of two adjacent peaks on the densitogram; and $R_{F1} > R_{F2}$.

Resolution Factor

The resolution of two spots R_s was calculated using the formula:[19]

$$R_s = 2 \times \frac{d}{s} \tag{4}$$

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

RESULTS AND DISCUSSION

The obtained results of the investigations concerning the evaluation of chromatographic separations were considered in three groups:

- 1st group: nicotinic acid (1), methyl nicotinate (2), ethyl nicotinate (3), isopropyl nicotinate (4), butyl nicotinate (5), hexyl nicotinate (6), benzyl nicotinate (7);
- 2nd group: nicotinic acid (1), nicotinamide (8), N-methylnicotinamide (9), N,N-diethylnicotinamide (10);
- 3rd group: nicotinic acid (1), nicotinamide (8), 3-pyridinecarbaldehyde (11), 3-pyridinecarbonitrile (12), 3-pyridylmethanol (13), methyl 3-pyridyl ketone (14).

Comparison of the Separation of 1st Group of the Investigated Compounds

The influence of volume composition of the acetone-*n*-hexane mobile phase on the R_F values of the first group of investigated compounds on a mixture of silica gel and kieselguhr non impregnated and impregnated with 2.5% and 5% aqueous solutions of $CuSO_4$ is presented in Figure 1.

Nicotinic acid practically remained on the start on non impregnated plates using the above mentioned mobile phase in volume compositions of 0:100, 10:90, 20:80, and 30:70. The R_F values of nicotinic acid were in the range from 0.16 to 0.22 in remaining volume compositions of the acetone-*n*-hexane mobile phase. The R_F values of esters of nicotinic acid separated on non impregnated plates increase with an increase in acetone content in the acetone-*n*-hexane mobile phase and reach maximum values in a volume composition of 60:40. The R_F values insignificantly decrease in remaining volume compositions that are 70:30, 80:20, 90:10, 100:0. Separation of nicotinic acid from all its esters occurs when the mixture of the acetone-*n*-hexane is used as mobile phase in all volume compositions (with the exception of 0:100). Separation of all esters of nicotinic acid on non impregnated plates was impossible. On the non impregnated plates they can be separated:

- methyl nicotinate from benzyl nicotinate ($\Delta R_F = 0.08$; $R_F^\alpha = 1.38$; $\alpha = 1.54$; $R_s = 2.20$) and ethyl nicotinate from isopropyl nicotinate ($\Delta R_F = 0.05$; $R_F^\alpha = 1.16$; $\alpha = 1.25$; $R_s = 1.08$) using the above mentioned mobile phase in a volume composition of 10:90;
- benzyl nicotinate from ethyl nicotinate ($\Delta R_F = 0.06$; $R_F^\alpha = 1.15$; $\alpha = 1.28$; $R_s = 1.07$) and ethyl nicotinate from isopropyl nicotinate ($\Delta R_F = 0.05$; $R_F^\alpha = 1.11$; $\alpha = 1.22$; $R_s = 1.07$) using the above mentioned mobile phase in a volume composition of 20:80;
- benzyl nicotinate from ethyl nicotinate ($\Delta R_F = 0.06$; $R_F^\alpha = 1.11$; $\alpha = 1.28$; $R_s = 1.33$) and ethyl nicotinate from isopropyl nicotinate ($\Delta R_F = 0.05$; $R_F^\alpha = 1.08$; $\alpha = 1.24$; $R_s = 1.23$) using the above mentioned mobile phase in a volume composition of 30:70;
- ethyl nicotinate from isopropyl nicotinate ($\Delta R_F = 0.04$; $R_F^\alpha = 1.06$; $\alpha = 1.23$; $R_s = 1.20$) using the above mentioned mobile phase in a volume composition of 40:60; and
- methyl nicotinate from benzyl nicotinate ($\Delta R_F = 0.03$; $R_F^\alpha = 1.03$; $\alpha = 1.34$; $R_s = 1.25$) and ethyl nicotinate from isopropyl nicotinate ($\Delta R_F = 0.03$; $R_F^\alpha = 1.03$; $\alpha = 1.55$; $R_s = 1.25$) using the above mentioned mobile phase in a volume composition of 50:50.

Impregnation of a mixture of silica gel and kieselguhr with 2.5% and 5% aqueous solutions of $CuSO_4$ influences the R_F values of the separated substances, and this effect is greater in the case of impregnation with a 5% aqueous solution of $CuSO_4$. Generally, it should be suggested that impregnation causes the

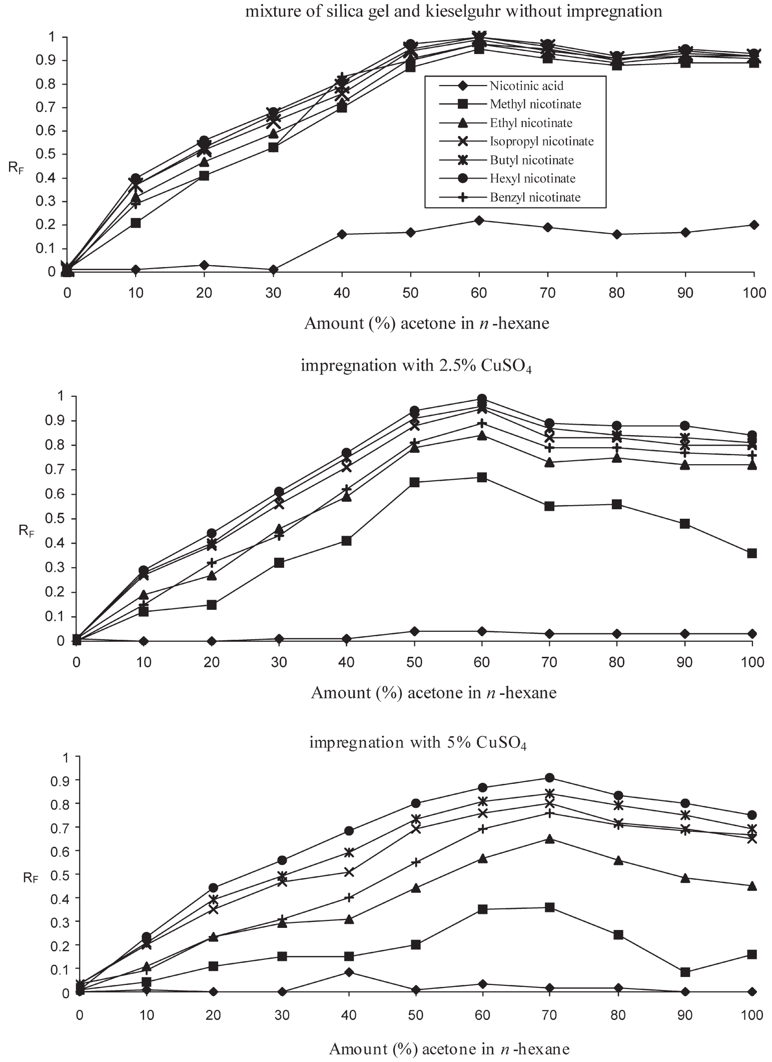


Figure 1. R_F values of the first group of compounds investigated on mixture of silica gel and kieselguhr non impregnated and impregnated with 2.5% and 5% $CuSO_4$ with an acetone-*n*-hexane mobile phase.

decrease of the R_F values of the first group of investigated compounds in relation to the results obtained on non impregnated solid support.

The largest decrease of R_F values on impregnated plates with a 2.5% aqueous solution of $CuSO_4$ in relation to non impregnated plates was observed for methyl nicotinate and ethyl nicotinate. However, a larger decrease of the R_F values was obtained on impregnated plates with a 5%

aqueous solution of $CuSO_4$. Nicotinic acid remains on the start, or is nearly above the start, on impregnated chromatographic plates. It was found that impregnation of the mixture of silica gel and kieselguhr with 2.5% and 5% aqueous solutions of $CuSO_4$ causes an increase of differences in the R_F values between particular esters of nicotinic acid. The detection of the separated substances was carried out using 0.05% methanolic solution of Rhodamine B on impregnated plates and observation in UV at $\lambda = 254$ nm. It was found that the spots of the studied substances under these conditions have a higher width in the direction of the mobile phase flow in the relation to width of the spots obtained on non impregnated plates, in their cases the detection of substances was directly possible in UV at $\lambda = 254$ nm. In spite of big differences in the R_F values between particular esters, $R_s \geq 1$ were not always the values obtained. There is a possibility of separating the largest number of esters on plates impregnated with a 5% aqueous solution of $CuSO_4$:

- methyl nicotinate from ethyl nicotinate ($\Delta R_F = 0.16$; $R_F^\alpha = 2.07$; $\alpha = 2.55$; $R_s = 1.33$), benzyl nicotinate from isopropyl nicotinate ($\Delta R_F = 0.11$; $R_F^\alpha = 1.28$; $\alpha = 1.56$; $R_s = 1.00$) and butyl nicotinate from hexyl nicotinate ($\Delta R_F = 0.09$; $R_F^\alpha = 1.15$; $\alpha = 1.48$; $R_s = 1.12$) using an acetone-*n*-hexane mobile phase in a volume composition of 40:60;
- methyl nicotinate from ethyl nicotinate ($\Delta R_F = 0.24$; $R_F^\alpha = 2.20$; $\alpha = 3.14$; $R_s = 1.71$), ethyl nicotinate from benzyl nicotinate ($\Delta R_F = 0.11$; $R_F^\alpha = 1.25$; $\alpha = 1.56$; $R_s = 1.14$) and benzyl nicotinate from isopropyl nicotinate ($\Delta R_F = 0.14$; $R_F^\alpha = 1.25$; $\alpha = 1.82$; $R_s = 1.38$) using the above-mentioned mobile phase in a volume composition of 50:50.

It was impossible to separate ethyl nicotinate from benzyl nicotinate and isopropyl nicotinate from butyl nicotinate on the plates impregnated with a 5% aqueous solution of $CuSO_4$ and using the above mentioned mobile phase in a volume composition of 40:60. The separation of ethyl nicotinate from benzyl nicotinate, as mentioned above, was only possible by use of an acetone-*n*-hexane mobile phase in a volume composition of 50:50. Separation of isopropyl nicotinate from butyl nicotinate ($\Delta R_F = 0.07$; $R_F^\alpha = 1.10$; $\alpha = 1.46$; $R_s = 1.00$) was only possible on plates impregnated with a 5% aqueous solution of $CuSO_4$ and using an acetone-*n*-hexane mobile phase in a volume composition of 80:20.

Comparison of the Separation of 2nd Group of the Investigated Compounds

The influence of volume composition of the acetone-*n*-hexane mobile phase on the R_F values of the second group of investigated compounds on a mixture of silica gel and kieselguhr non impregnated and impregnated with 2.5% and 5% aqueous solutions of $CuSO_4$ is presented in Figure 2.

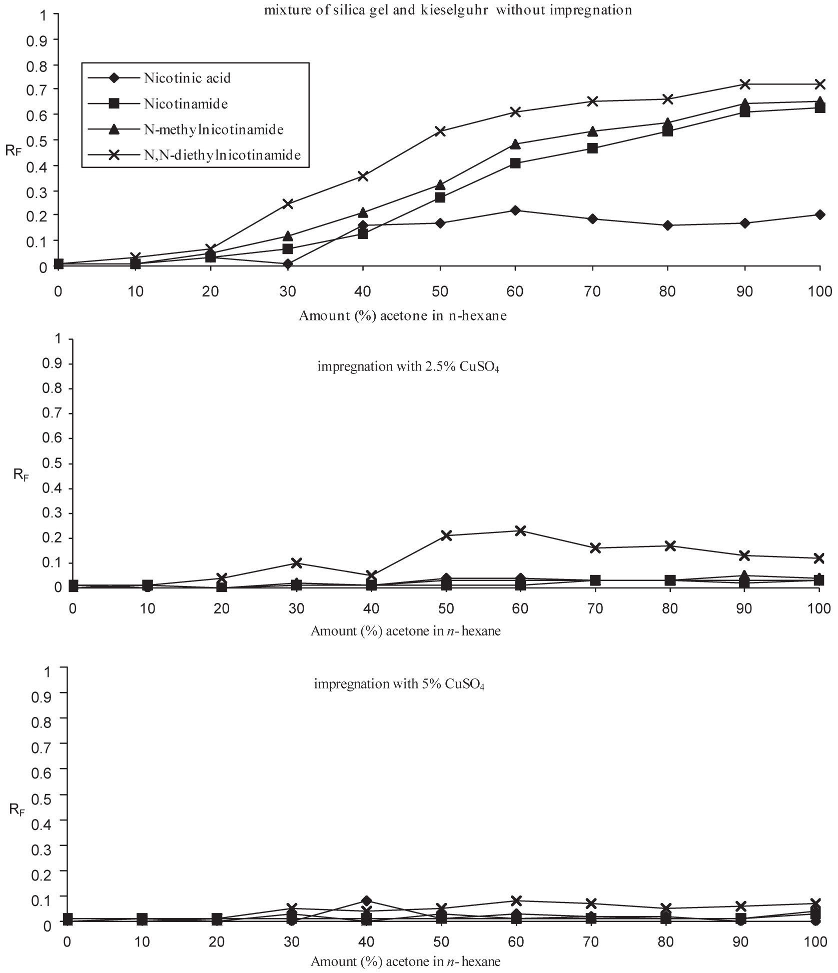


Figure 2. R_F values of the second group of compounds investigated on a mixture of silica gel and kieselguhr, non impregnated and impregnated, with 2.5% and 5% $CuSO_4$ with an acetone-*n*-hexane mobile phase.

There is a possibility of the separation of all compounds of the 2nd group on non impregnated plates precoated with the mixture of silica gel and kieselguhr and using an acetone-*n*-hexane mobile phase in volume compositions of 30:70, 50:50, 60:40, 70:30, and 80:20.

Impregnation of the mixture of silica gel and kieselguhr with 2.5% and 5% aqueous solutions of $CuSO_4$ influence the R_F values of the separated substances. This effect is larger in the case of impregnation with a 5% aqueous solution of $CuSO_4$. Impregnation causes a decrease of the R_F values of the investigated compounds in relation to the results obtained on non impregnated solid support.

There is only possibility of the separation of N,N-diethylnicotinamide from remaining compounds of the 2nd group on plates impregnated with a 2.5% aqueous solution of $CuSO_4$ and using an acetone-*n*-hexane mobile phase in volume compositions of 30:70, 50:50, 60:40, 70:30, 80:20, and 100:0. Impregnation of the mixture of silica gel and kieselguhr with a 5% aqueous solution of $CuSO_4$ causes a very large decrease of the R_F values of the investigated substances ($R_F < 0.1$). Nicotinamide only separates from N,N-diethylnicotinamide ($R_s = 1.00$) and N,N-diethylnicotinamide separates from nicotinic acid ($R_s = 1.00$) on plates impregnated with 5% $CuSO_4$ using the above mentioned mobile phase in a volume composition of 40:60.

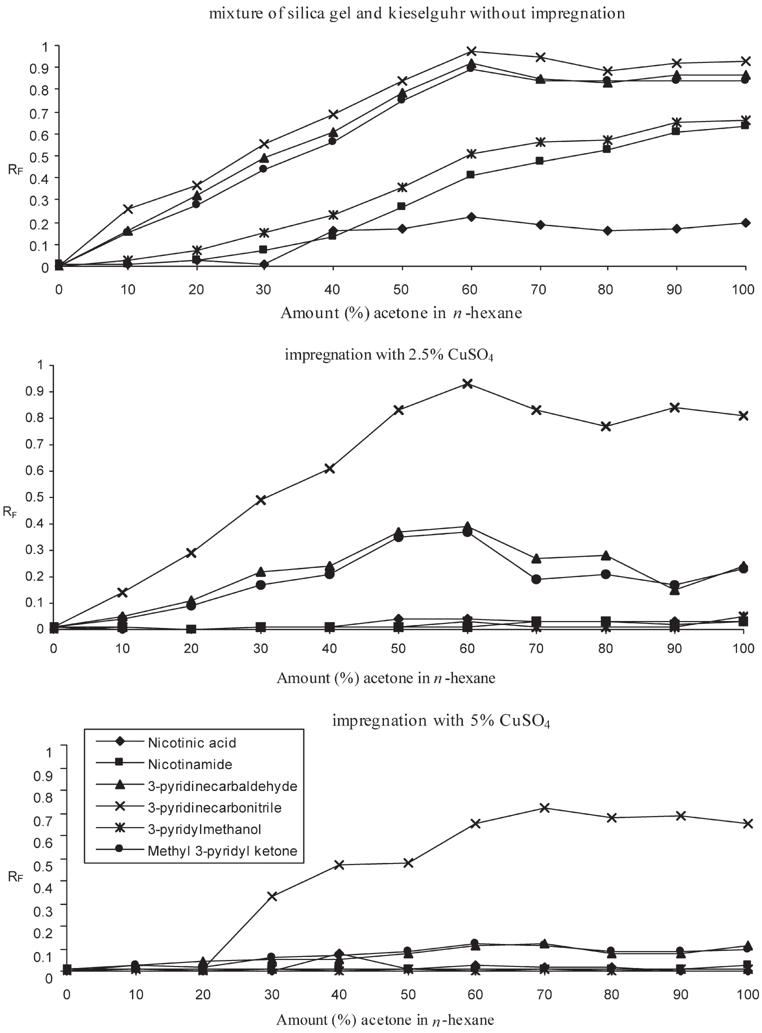
Comparison of the Separation of 3rd Group of the Investigated Compounds

The influence of volume composition of the acetone-*n*-hexane mobile phase on the R_F values of the third group of investigated compounds on a mixture of silica gel and Kieselguhr non impregnated and impregnated with 2.5% and 5% aqueous solutions of $CuSO_4$ is presented in Figure 3.

There is a possibility of the separation of all compounds of the 3rd group on non impregnated plates precoated with the mixture of silica gel and kieselguhr and using the above mentioned mobile phase in a volume composition of 30:70 ($\Delta R_{F(1/8)} = 0.06$, $R_{s(1/8)} = 1.07$; $\Delta R_{F(8/13)} = 0.08$, $R_{s(8/13)} = 1.41$; $\Delta R_{F(13/14)} = 0.29$, $R_{s(13/14)} = 5.50$; $\Delta R_{F(14/11)} = 0.05$, $R_{s(14/11)} = 1.33$; $\Delta R_{F(11/12)} = 0.06$, $R_{s(11/12)} = 1.23$). Impregnation of the mixture of silica gel and kieselguhr with 2.5% and 5% aqueous solutions of $CuSO_4$ causes a decrease of the R_F values of the 3rd group of compounds. The R_F values of nicotinic acid, nicotinamide, and 3-pyridylmethanol separated on plates impregnated with a 2.5% $CuSO_4$ are lower than 0.05. Significant decrease of the R_F values in relation to non impregnated plates is observed for 3-pyridinecarbaldehyde and methyl 3-pyridyl ketone. Impregnation of the plates with a 5% aqueous solution of $CuSO_4$ causes further decrease of the R_F values of the investigated substances. The R_F values of nicotinic acid, nicotinamide, 3-pyridinecarbaldehyde, 3-pyridylmethanol, and methyl 3-pyridyl ketone are lower than 0.12 in all volume compositions of mobile phase. It was found that impregnation of plates with 2.5% and 5% aqueous solutions of $CuSO_4$ worsens the separation of the 3rd group of compounds.

Evaluation of Spectrodensitograms of the Investigated Compounds

In a further part of this work, the spectrodensitometric analysis of the investigated compounds was carried out on the mixture of silica gel and kieselguhr non impregnated and impregnated with a 2.5% aqueous solution of $CuSO_4$ without use of a visualizing reagent, as well as on the mixture of silica gel



mixture of silica gel and kieselguhr without impregnation

impregnation with 2.5% $CuSO_4$

impregnation with 5% $CuSO_4$

Figure 3. R_F values of the third group of compounds investigated on mixture of silica gel and kieselguhr non impregnated and impregnated with 2.5% and 5% $CuSO_4$ with an acetone-*n*-hexane mobile phase.

and kieselguhr impregnated with a 2.5% aqueous solution of $CuSO_4$ and, after detection, using 0.05% methanolic solution of Rhodamine B.

The results of the spectrodensitometric analysis of the investigated compounds on the mixture of silica gel and kieselguhr non impregnated and without use of a visualizing reagent, as well as on the mixture of silica gel and kieselguhr impregnated with a 2.5% aqueous solution of $CuSO_4$ and without use of a visualizing reagent and also using 0.05% methanolic solution of Rhodamine B as visualizing reagent, are presented in Tables 1, 2, and 3.

Table 1. Spectrodensitometric analysis of the investigated compounds after the separation on non impregnated mixture of silica gel 60 and kieselguhr F_{254} (#1.05567) and without use of a visualizing agent

		Remaining absorption bands	
Compound investigated	Band with absorption maximum λ^a_{max} (nm)	λ (nm)	Intensity of signal (AU)
Nicotinic acid	216	262	78.5
Methyl nicotinate	220	263	62.7
Ethyl nicotinate	220	263	65.0
Isopropyl nicotinate	221	263	68.2
Butyl nicotinate	220	263	64.5
Hexyl nicotinate	220	263	64.2
Benzyl nicotinate	219	263	61.1
Nicotinamide	219	262	70.3
N-Methylnicotinamide	220	262	79.5
N,N-Diethylnicotinamide	200	217	94.2
		261	87.2
3-Pyridinecarbaldehyde	232	200	76.1
		271	74.8
3-Pyridinecarbonitrile	218	265	52.5
3-Pyridylmethanol	260	202	79.9
Methyl 3-pyridyl ketone	230	200	72.1
		268	67.9

[a] Intensity of all absorption maximum is equal 95 AU.

Spectrodensitograms of the investigated compounds separated on non impregnated plates and impregnated with a 2.5% aqueous solution of $CuSO_4$ and without use of a visualizing reagent are completely different than spectrodensitograms of the separated substances on the plates impregnated with a 2.5% aqueous solution of $CuSO_4$ and using 0.05% methanolic solution of Rhodamine B as visualizing reagent. Spectra of the investigated compounds obtained on non impregnated plates and without use of a visualizing reagent have two (nicotinic acid, methyl nicotinate, ethyl nicotinate, isopropyl nicotinate, butyl nicotinate, hexyl nicotinate, benzyl nicotinate, nicotinamide, N-methylnicotinamide, 3-pyridinecarbonitrile, 3-pyridylmethanol) or three (N,N-diethylnicotinamide, 3-pyridinecarbaldehyde, methyl 3-pyridyl ketone) absorption bands. Spectra of the studied compounds on plates impregnated with a 2.5% aqueous solution of $CuSO_4$ and without use of a visualizing reagent have two (methyl nicotinate, ethyl nicotinate, isopropyl nicotinate, hexyl nicotinate, 3-pyridylmethanol) or three (nicotinic acid, butyl nicotinate, hexyl nicotinate, nicotinamide, N-methylnicotinamide, N,N-diethylnicotinamide, 3-pyridinecarbaldehyde, 3-pyridinecarbonitrile, methyl 3- pyridyl ketone) absorption bands. However, spectra of the examined compounds on the plates impregnated with

Table 2. Spectrodensitometric analysis of the investigated compounds after separation on mixture of silica gel 60 and kieselguhr F_{254} (#1.05567) impregnated with a 2.5% $CuSO_4$ and without use of a visualizing agent

		Remaining absorption bands	
Compound investigated	Band with absorption maximum λ^a_{max} (nm)	λ (nm)	Intensity of signal (AU)
Nicotinic acid	200	269	47.1
		399	22.7
Methyl nicotinate	217	265	55.5
Ethyl nicotinate	217	265	63.2
Isopropyl nicotinate	219	268	75.3
Butyl nicotinate	200	216	87.8
		272	73.7
Hexyl nicotinate	218	270	72.5
Benzyl nicotinate	200	272	35.3
		307	40.0
Nicotinamide	219	267	83.2
		200	94.0
N-Methylnicotinamide	218	200	91.8
		269	81.5
N,N-Diethylnicotinamide	200	263	66.9
		384	24.4
3-Pyridinecarbaldehyde	200	231	79.0
		271	51.5
3-Pyridinecarbonitrile	200	209	92.4
		271	70.8
3-Pyridylmethanol	267	200	82.9
Methyl 3-pyridyl ketone	200	228	85.7
		268	55.0

[a] Intensity of all absorption maximum is equal 95 AU.

a 2.5% aqueous solution of $CuSO_4$ and using 0.05% methanolic solution of Rhodamine B as visualizing reagent have three or four absorption bands. Generally, it should be suggested, that spectra of the studied compounds obtained on the plates impregnated with a 2.5% aqueous solution of $CuSO_4$ and using 0.05% methanolic solution of Rhodamine B as visualizing reagent have one additional absorption band at $\lambda \approx 560$ nm in relation to spectra of the investigated compounds on the plates without use of a visualizing reagent. This fact has the analytical significance in the identification of the investigated substances.

CONCLUSIONS

It was found that impregnation of the mixture of silica gel and kieselguhr with 2.5% and 5% aqueous solutions of $CuSO_4$ influences separations of nicotinic

Table 3. Spectrodensitometric analysis the investigated compounds after the separation on mixture of silica gel 60 and kieselguhr F_{254} (#1.05567) impregnated with a 2.5% $CuSO_4$ and using 0.05% methanolic solution of Rhodamine B as visualizing agent

		Remaining absorption bands	
Compound investigated	Band with absorption maximum $\lambda^a_{\max}$ (nm)	λ (nm)	Intensity of signal (AU)
Nicotinic acid	265	200	83.7
		218	87.1
		559	65.1
Methyl nicotinate	219	261	82.2
		555	42.1
Ethyl nicotinate	216	262	76.0
		553	48.6
Isopropyl nicotinate	219	264	84.0
		555	50.7
Butyl nicotinate	219	200	86.8
		263	72.2
		555	91.5
Hexyl nicotinate	218	263	80.3
		555	67.7
Benzyl nicotinate	200	214	94.8
		260	79.1
		558	88.2
Nicotinamide	562	217	86.2
		263	88.4
N-Methylnicotinamide	561	200	74.1
		215	72.5
		262	77.8
N,N-Diethylnicotinamide	202	263	92.7
		558	92.1
3-Pyridinecarbaldehyde	200	230	91.7
		270	79.0
		557	87.7
3-Pyridinecarbonitrile	217	264	48.6
		556	68.0
3-Pyridylmethanol	261	200	81.3
		557	89.1
Methyl 3-pyridyl ketone	559	200	92.1
		230	90.4
		267	77.6

[a] Intensity of all absorption maximum is equal 95 AU.

acid and its derivatives in three groups of investigated compounds (1st group: nicotinic acid, methyl nicotinate, ethyl nicotinate, isopropyl nicotinate, butyl nicotinate, hexyl nicotinate, benzyl nicotinate; 2nd group: nicotinic acid, nicotinamide, N-methylnicotinamide, N,N-diethylnicotinamide; 3rd group: nicotinic acid, nicotinamide, 3-pyridinecarbaldehyde, 3-pyridinecarbonitrile, 3-pyridylmethanol, methyl 3-pyridyl ketone).

It was found that on non impregnated mixture of silica gel and kieselguhr in the case of:

- 1st group of compounds the separation of nicotinic acid from its esters; separation of all esters of nicotinic acid was impossible;
- 2nd group of compounds the separation of all substances using an acetone-*n*-hexane mobile phase in volume compositions of 30:70, 50:50, 60:40, 70:30, and 80:20 occurs;
- 3rd group of compounds there is a possibility of the separation of all substances by use of an acetone-*n*-hexane mobile phase in a volume composition of 30:70.

Impregnation of mixture of silica gel and kieselguhr with 2.5% and 5% aqueous solutions of $CuSO_4$ causes a decrease of the R_F values of 1st, 2nd, and 3rd groups of compounds in the relation to the R_F values of these substances obtained on non impregnated solid support.

Impregnation with a water solution of copper (II) sulphate (VI) of the mixture of silica gel and kieselguhr worsens the separations of the 2nd and 3rd groups of the investigated compounds. Improvement of the chromatographic separations is observed only in the case of esters of nicotinic acid (1st group of compounds).

On plates impregnated with a 5% aqueous solution of $CuSO_4$ using an acetone-*n*-hexane mobile phase in a volume composition of:

- 60:40 separates methyl nicotinate from ethyl nicotinate, benzyl nicotinate from isopropyl nicotinate, and butyl nicotinate from hexyl nicotinate;
- 50:50 separates methyl nicotinate from ethyl nicotinate, ethyl nicotinate from benzyl nicotinate, and benzyl nicotinate from isopropyl nicotinate.

However, separation of isopropyl nicotinate from butyl nicotinate was only possible using the above mentioned mobile phase in a volume composition of 80:20.

It might be supposed that the separated substances form complexes with copper.

It was found that the spectrodensitograms of the studied compounds on impregnated plates and by use of Rhodamine B as visualizing reagent are different than the spectrodensitograms obtained on the plates without use of a visualizing reagent. Spectra of the investigated compounds on non impregnated plates and impregnated with an aqueous solution of $CuSO_4$ and without use of a visualizing reagent have two or three absorption bands. Investigated compounds on the plates impregnated with an aqueous solution of $CuSO_4$ and by use of Rhodamine B as visualizing reagent have one additional absorption band at $\lambda \approx 560$ nm. This fact has analytical significance in the identification of the investigated substances.

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