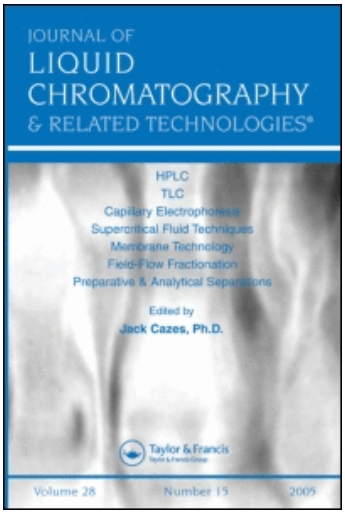

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USE OF TLC AND DENSITOMETRY TO EVALUATE THE CHEMICAL STABILITY OF NICOTINIC ACID AND ITS ESTERS ON SILICA GEL

Wioletta Parys[a]; Alina Pyka[a]
[a] Department of Analytical Chemistry, Faculty of Pharmacy, Medical University of Silesia, Sosnowiec, Poland

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USE OF TLC AND DENSITOMETRY TO EVALUATE THE CHEMICAL STABILITY OF NICOTINIC ACID AND ITS ESTERS ON SILICA GEL

Wioletta Parys and Alina Pyka

Department of Analytical Chemistry, Faculty of Pharmacy, Medical University of Silesia, Sosnowiec, Poland

□ *The chemical stability of nicotinic acid (NA) and its esters, namely: methyl nicotinate (MN), ethyl nicotinate (EN), isopropyl nicotinate (IPN), butyl nicotinate (BN), hexyl nicotinate (HN), and benzyl nicotinate (BNN) heated for 1 to 7 h at 120°C on silica gel was determined. The investigations were made using a normal phase-thin layer chromatography (NP-TLC) method and a Camag densitometer. Heating of nicotinic acid and its esters on silica gel 60 at 120°C for 1 to 7 h caused the formation of the substances as a product of their chemical changes. The most stable was nicotinic acid from all the compounds analyzed during the 1 to 7 h of heating. The most stable substances were isopropyl nicotinate and hexyl nicotinate from all the examined esters. The most unstable were ethyl nicotinate, and methyl nicotinate.*

Keywords chemical stability, densitometry, nicotinic acid, nicotinic acid esters, NP-TLC

INTRODUCTION

The stability of a drug is one of its basic properties. It is understood by the reference to physical, chemical, and biological factors of activity. Each change in the structure and properties of the form of a drug can lead to loss or weakness of pharmacological activity.[1,2] Chemical stability is the most important property of a drug and is easy to estimate. That sort of stability depends on environmental conditions, storage conditions (exposure to light, moisture, temperature, atmospheric gases, presence of bacteria). The chemical decomposition is the result of hydrolysis, oxidation, reduction, photolysis, racemization (isomerization and epimerization), polymerization, and decarbonization reactions.[3]

Chromatographic techniques are widely used for the investigation of the pharmaceutical purity of medical substance; the determination of

Address correspondence to Alina Pyka, Department of Analytical Chemistry, Faculty of Pharmacy, Medical University of Silesia, 4 Jagiellońska Street, PL-41-200 Sosnowiec, Poland. E-mail: apyka@sum.edu.pl

active substance in medicinal preparations and pharmaceutical materials.[4–8]

Nicotinic acid has been used as a vasodilator for many years.[9,10] The flush reaction to nicotinic acid, which occurs rapidly is very characteristic. Esters of the nicotinic acid, such as: methyl nicotinate, ethyl nicotinate, isopropyl nicotinate, butyl nicotinate, hexyl nicotinate and benzyl nicotinate are used in the pharmaceutical industry as ingredients of creams and ointments. These esters cross the skin rapidly and, on enzymatic hydrolysis, release nicotinic acid, which induces skin erythema. Nicotinate esters, which act as prodrugs, enhance the topical penetrationof the active substances.[11–15] Isopropyl nicotinate is used as an analgetic and topical anesthetic agent.[16] Benzyl nicotinate in the presence of liposomes improves skin oxygenation.[17–19] The composition of the liposomes significantly affects the time at which benzyl nicotinate starts to act and, to a lesser extent, the maximum increase of pO_2 in the skin and the effectiveness of the benzyl nicotinate action. However, the size of the liposomes influences both the effectiveness of benzyl nicotinate action and the time at which benzyl nicotinate begins to act.

For many years the Department of Analytical Chemistry in the Faculty of Pharmacy of the Medical Univeristy of Silesia has led investigations of chemical stability of drugs.[20–23] The purpose of this work was to use thin layer chromatography (TLC) with densitometry and spectrodensitometry to investigate the chemical stability of nicotinic acid and its esters heated at 120°C on silica gel.

EXPERIMENTAL

Chemicals

The components of the mobile phases: acetone (Polish Chemical Reagents, Poland; analytical grade), methanol (Merck, Germany; for liquid chromatography), *n*-hexane (AnalaR, UK; analytical grade), and benzene (Research-Evolution Centre of Refinering Industry, Poland) were used for TLC analysis. The commercial samples of nicotinic acid, methyl nicotinate, ethyl nicotinate, and butyl nicotinate (Sigma-Aldrich, Germany), isopropyl nicotinate, and hexyl nicotinate (Aldrich, Germany), and benzyl nicotinate (Fluka, Switzerland) were used as test solutes. The purity of the standard samples was at least 97%.

Preparation of Standard Solutions

Solutions of commercial samples of nicotinic acid and its esters were prepared in ethanol (Polish Chemical Reagents, Poland, 96%, analytical

grade). Nicotinic acid and each of its esters (40 mg) were dissolved in 10 mL of ethanol.

Conditions of Research Done by Thin Layer Chromatography

Preparation of Chromatographic Plates

Before spotting the investigated compounds, the glass plates were precoated by silica gel $60F_{254}$ (E. Merck, # 1.05715); they were prewashed with methanol and dried for 24 h at room temperature ($20 \pm 1°C$). This process permits the removal of impurities from of the layer. The plates were then activated at 120°C for 30 min.

Investigation of Nicotinic Acid and Its Esters Stability on Silica Gel

The above-mentioned compounds were spotted in the amount of 5 μL on activated chromatographic plates. These plates were heated at 120°C during 1, 2, 3, 4, 5, 6, and 7 hours. After this time the standard solutions of nicotinic acid and its esters were spotted on chromatographic plates near the compounds heated at 120°C.

Chromatographic Plate Development

The plates were developed with the following mobile phases:

– methanol – benzene in the volume composition of 50:50 for nicotinic acid;
– acetone – *n*-hexane in the volume composition of 40:60 for methyl nicotinate, ethyl nicotinate, isopropyl nicotinate, butyl nicotinate, hexyl nicotinate, and benzyl nicotinate.

The above-mentioned mobile phases (50 mL) were placed in classical chromatographic chambers (Camag). The chambers were saturated with the mobile phases used for 30 minutes. The plates were developed to a distance 14 cm at room temperature ($18 \pm 1°C$). The plates were then dried for 24 h at room temperature ($18 \pm 1°C$) in a fume cupboard.

Densitometric Investigation of Nicotinic Acid and Its Esters

Densitometric and spectrodensitometric investigations were done using a TLC Scanner 3 (Camag, Switzerland) operated in the absorbance mode and controlled by winCATS 1.4.2 software. The radiation source was a deuterium lamp emitting a continuous UV spectrum between 190 and 450 nm. Densitometric scanning was then performed at multi wavelengths in the

range of 200 to 440 nm, and at wavelength intervals of 20 nm at each step. Densitometric analysis was then performed at the respective absorption maximums ($\lambda_{max} = 263$ nm for nicotinic acid; $\lambda_{max} = 221$ nm for methyl nicotinate, ethyl nicotinate and hexyl nicotinate; $\lambda_{max} = 222$ nm for isopropyl nicotinate, butyl nicotinate; $\lambda_{max} = 220$ nm for benzyl nicotinate). The slit dimensions were 8.00×0.40 mm, Macro; the optimal system was light; the scanning speed was 20 mm s^{-1}; the data resolution was 100 μm $step^{-1}$; the measurement type was remission; and the measurement mode was absorption.

The chromatographic bands obtained on the densitograms were investigated by spectrodensitometric analysis under the following conditions: the slit dimensions were 8.00×0.40 mm, Macro; the optimal system was resolution; the scanning speed was 20 nm s^{-1}; the data resolution was 1 nm $step^{-1}$; the initial wavelength was 200 nm, and final wavelength was 350 nm; the measurement type was remission; and the measurement mode was absorption.

RESULTS AND DISCUSSION

The R_F values, and absorption maxima (λ_{max}) of the chromatographic bands of nicotinic acid and its esters heated for 1 hour and 7 hours at 120°C on silica gel and the standards of investigated substances are presented in Table 1. The nicotinic acid standard had the impurity remaining at the origin of the chromatogram. All standards of nicotinic acid esters investigated had no impurities. Heating of nicotinic acid and its esters on silica gel at 120°C for 1 to 7 h caused the formation of the substances as a product of its chemical changes. The substances as a product of chemical changes, after chromatographic separation, remained at the origin of the chromatograms. The wavelengths of the fundamental absorption bands (λ_{max}) of nicotinic acid and its esters as well as the substances that were the products of their chemical changes are also presented in Table 1. Relationships between the areas (%) of chromatographic bands and heating time (from 0 to 7 h) of the investigated substances are presented in Figure 1. The most stable substance was nicotinic acid from all the compounds analyzed during their heating for 1 to 7 h at 120°C on silica gel. The chromatographic band from the nicotinic acid standard had an $R_F = 0.44$ and an area of 55563.5 AU. The nicotinic acid standard had the impurity remaining at the origin of the chromatogram ($R_F = 0.00$) with an area of 1019 AU. The smallest amount of the substance was a product of the chemical change of nicotinic acid formed during 1 h of heating (an area of 5521.1 AU, that corresponded to 9.08%). However, the largest amount of the substance was a product of the chemical change of nicotinic acid

TABLE 1 R_F Values, and Absorption Maxima (λ_{max}) of the Chromatographic Bands of the Substances Heated on Silica Gel at 120°C for 1 to 7 h and the Standards of the Investigated Substances

			Substances Heated at 120°C on Silica Gel During			
	Standard of Investigated Substances (Non Heated)		1 h		7 h	
	Chromatographic Bands (R_F value, λ_{max} [nm])					
Compound Investigated	R_F	λ_{max} [nm]	R_F	λ_{max} [nm]	R_F	λ_{max} [nm]
Nicotinic acid[a]	0.00 (P)	263	0.00 (P)	264	0.00 (P)	264
	0.44 (NA)	263	0.43 (NA)	263	0.44 (NA)	263
Methyl nicotinate[b]	–	–	0.01 (P)	263	0.01 (P)	263
	0.48 (MN)	221	0.48 (MN)	221	0.50 (MN)	220
Ethyl nicotinate[b]	–	–	0.01 (P)	263	0.01 (P)	263
	0.46 (EN)	221	0.45 (EN)	221	0.48 (EN)	221
Isopropyl nicotinate[b]	–	–	0.01 (P)	263	0.01 (P)	263
	0.51 (IPN)	221	0.49 (IPN)	221	0.50 (IPN)	221
Butyl nicotinate[b]	–	–	0.01 (P)	263	0.01 (P)	264
	0.54 (BN)	222	0.53 (BN)	221	0.55 (BN)	221
Hexyl nicotinate[b]	–	–	0.01 (P)	264	0.00 (P)	264
	0.56 (HN)	221	0.56 (HN)	221	0.58 (HN)	221
Benzyl nicotinate[b]	–	–	0.00 (P)	264	0.01 (P)	264
	0.44 (BNN)	220	0.42 (BNN)	220	0.43 (BNN)	220

[a] Mobile phase: methanol + benzene (50:50, v/v);
[b] Mobile phase: acetone + *n*-hexane (40:60, v/v).

P are symbols of the products of chemical changes of nicotinic acid (NA), methyl nicotinate (MN), ethyl nicotinate (EN), isopropyl nicotinate (IPN), butyl nicotinate (BN), hexyl nicotinate (HN), and benzyl nicotinate (BNN).

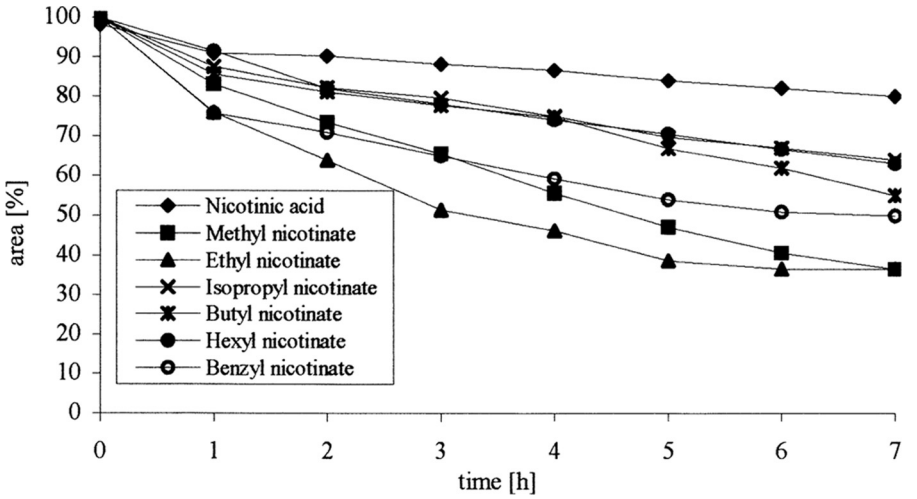

FIGURE 1 Relationship between the areas (%) of the chromatographic bands and heating time (from 0 to 7 h) of nicotinic acid and its esters.

formed during 7 h of heating on silica gel. The area of this chromatographic band was equal to 11072.9 AU, which corresponded to 20.03% of the total spot areas of nicotinic acid and the product of the chemical change of nicotinic acid. The densitograms of the chromatogram background, the standard of nicotinic acid and nicotinic acid heated for 1 to 7 h at 120°C on silica gel are presented in Figure 2.

The most stable substances were isopropyl nicotinate and hexyl nicotinate from all the examined nicotinic acid esters during their heating for 1 to 7 h at 120°C on silica gel. The chromatographic band from the isopropyl nicotinate standard had an $R_F = 0.51$ and an area of 33774.7 AU. Two chromatographic bands at R_F equal to 0.01 and R_F values in the range of 0.47 to 0.50 were observed during its heating at 120°C for 1 to 7 h on silica gel. The substance remaining at the origin was a product of the chemical change of isopropyl nicotinate heated on silica gel and had a fundamental absorption band (λ_{max}) at the wavelength equal to 263 nm. The smallest amount of the substance was a product of the chemical changes of isopropyl nicotinate formed during 1 h of heating (an area of 3800.5 AU, that corresponded to 12.43%). However, the largest amount of the substance

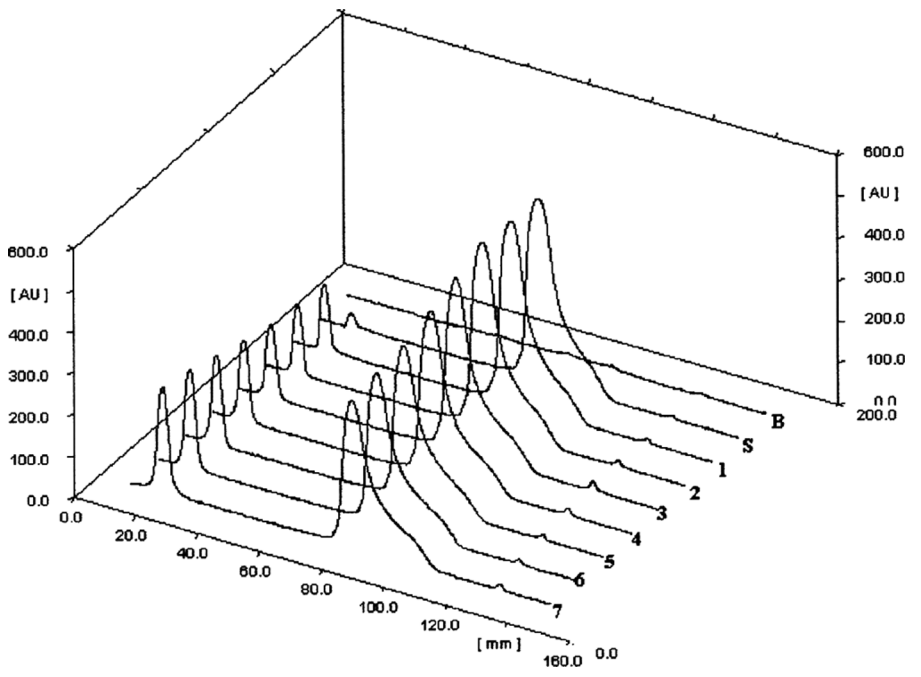

FIGURE 2 The densitograms of the chromatogram background, the standards of nicotinic acid and nicotinic acid heated at 120°C for 1 to 7 h on silica gel; where: B – the chromatogram background; S – the standard of nicotinic acid; 1 – after 1 h of heating; 2 – after 2 h of heating; 3 – after 3 h of heating; 4 – after 4 h of heating; 5 – after 5 h of heating; 6 – after 6 h of heating; 7 – after 7 h of heating.

was a product of the chemical change of isopropyl nicotinate formed during 7 h of heating. The area of this chromatographic band was equal to 9762.6 AU, which corresponded to 36.18% of total spot areas. The chromatographic band from a hexyl nicotinate standard had an $R_F = 0.56$ and an area of 33559.1 AU. Heating of hexyl nicotinate on silica gel at 120°C for 1 to 7 h caused the formation of two chromatographic bands with an R_F equal to 0.00 or 0.01 and R_F values in the range of 0.56 to 0.58. The substance remaining at the origin of the chromatogram was a product of the chemical change of hexyl nicotinate heated on silica gel, and had fundamental absorption band (λ_{max}) at a wavelength equal to 264 nm. The smallest amount of the substance was a product of the chemical change of hexyl nicotinate formed during 1 h of heating (an area of 2885.7 AU, that correspond to 8.53%). However, the largest amount of the substance was a product of chemical changes of hexyl nicotinate formed during 7 h of heating. The area of this chromatographic band is 13763.4 AU, which corresponded to 37.03% of the total spot areas.

Ethyl nicotinate was the most unstable ester of nicotinic acid during its heating for 1 to 7 h at 120°C on silica gel. The chromatographic band from

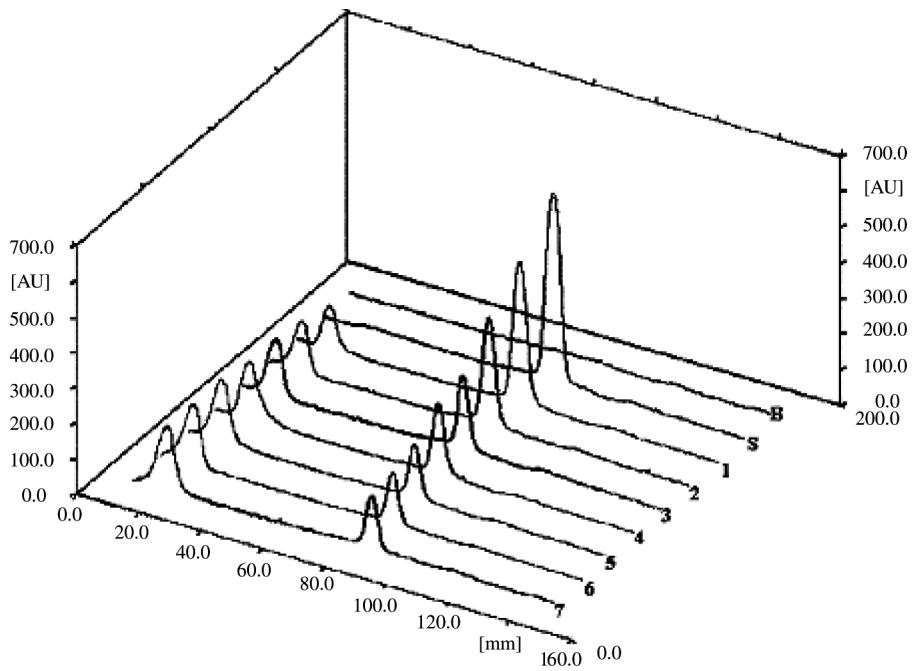

FIGURE 3 The densitograms of the chromatogram background, the standards of ethyl nicotinate and ethyl nicotinate heated at 120°C for 1 to 7 h on silica gel; where: B – the chromatogram background; S – the standard of ethyl nicotinate; 1 – after 1 h of heating; 2 – after 2 h of heating; 3 – after 3 h of heating; 4 – after 4 h of heating; 5 – after 5 h of heating; 6 – after 6 h of heating; 7 – after 7 h of heating.

the ethyl nicotinate standard had an $R_F = 0.46$ and an area of 30974.8 AU. Heating of ethyl nicotinate on silica gel at 120°C for 1 to 7 h caused the formation of two chromatographic bands with an $R_F = 0.01$ and R_F values in the range of 0.45 to 0.48. The substance remaining at the origin of the chromatogram was a product of the chemical change of ethyl nicotinate heated on silica gel and had a fundamental absorption band (λ_{max}) at the wavelength equal to 263 nm. The smallest amount of the substance was a product of chemical change of ethyl nicotinate formed during 1 h of heating (an area of 7042.2 AU, that corresponded to 24.08%). However, the largest amount of the substance was a product of the chemical change of ethyl nicotinate formed during 7 h of heating. The area of this chromatographic band is equal to 12408.1 AU, which corresponded to 63.55% of the total spot areas. The densitograms of the chromatogram background, the ethyl nicotinate standard and ethyl nicotinate heated for 1 to 7 h at 120°C on silica gel are presented in Figure 3. It was also found that methyl nicotinate was the most unstable nicotinic acid ester after 7 h of heating on silica gel (an area of 5403.6 AU, that corresponded to 36.43%).

Consideration all the compounds investigated, which were heated on silica gel at 120°C for 1 to 7 h, the most unstable were ethyl nicotinate, and methyl nicotinate after 7 h of heating.

Further investigations will concern the identification of the products of chemical changes of nicotinic acid and its esters.

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