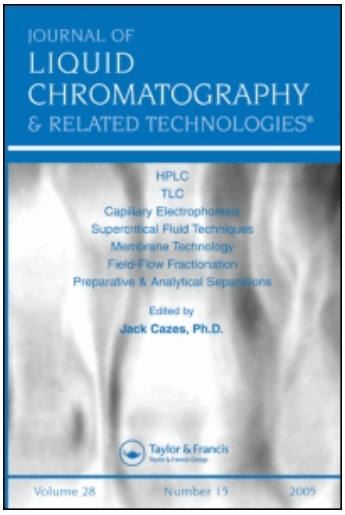

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Densitometric Investigations of Chemical Durability of Pyrocatechol

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Densitometric Investigations of Chemical Durability of Pyrocatechol

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Abstract: The chemical durability of pyrocatechol was investigated on silica gel, as well as in solutions, in relation to different storage conditions. Investigations were made using a NP-TLC method on silica gel and with chloroform–methanol (9:1, v/v) as mobile phase. Densitometric measurements were made with $\lambda = 200$ nm using a Camag densitometer. Chromatographic plates were treated by temperature of 120°C, as well as UV radiation ($\lambda = 254$ nm) during 120 minutes before spotting, and after spotting of pyrocatechol solutions. It was stated that, after 120 minutes at 120°C of exposure of pyrochatechol spotted on silica gel, a decrease of as much as 83% pyrocatechol was observed. However, 15% of pyrocatechol spotting on silica gel decreased after 120 minutes of UV radiation ($\lambda = 254$ nm). The influence of water, solution of physiological salt, and ethanol on the durability of pyrocatechol was also investigated. Pyrocatechol solutions mentioned above were subjected to exposition of visible light, UV radiation ($\lambda = 254$ nm), and temperature (40°C). It was stated that, ethanol has the biggest stabilizing properties in relation to pyrocatechol. Pyrocatechol dissolved in a solution of physiological salt, as well as heated in a temperature of 40°C and irradiated with UV light, undergoes slightly bigger changes in relation to the water solution of pyrocatechol.

Keywords: NP-TLC, Densitometry, Pyrocatechol, Chemical durability

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INTRODUCTION

The durability of the drug is one of its basic properties. It is understood by reference to physical, chemical, and biological factors activity. Each change in structure and properties of the form of the drug can lead to loss, weakness of pharmacological activity, and sometimes even to increase in toxicity.[1,2]

Ability of possible prediction of existing processes of destruction in a particular form of the drug, makes it possible to choose proper parameters during production, which prolong durability of a particular preparation. Chemical, physical, and microbiological durability is important both for the production of the drug and for the patient. Except chemical durability very important is proper, therapeutic activity of drug during whole period of being in turnover. Choice of suitable auxiliary substances has big importance in process of production. Investigations of durability are continued during studying the new form of drug for its first production series. Then the preparation undergoes permanently a quality inspection in settled validity period.[2]

Chemical durability is the most important property of drug and also very easy to estimate. That sort of durability depends on environment conditions, storage conditions (exposure to light, moisture, temperature, atmospheric gases, presence of bacterium). The sort of form of drug also influence on durability. It was shown that chemical reactions rate is the biggest in water solutions, a little bit smaller in suspensions, and the most stable are dry form of drugs. As therapeutic substance as auxiliary substance ingoing to composition of form of drug can be decomposed. Chemical decomposition is the result of hydrolysis, oxidation, reduction, photolysis, racemization (isomerization and epimerization), polimerization, decarboxyzation reactions and so on. Those processes can be prevented by suitable formularization of form of drug, choice of proper package and shutter. The proper conditions for storage given form of drug should be created.[3]

Chromatographic techniques are especially widely used for the investigation of pharmaceutical purity of medical substance, the determination of active substance in medicinal preparations and pharmaceutical materials.[4–11] Many phenol derivatives have definite pharmacological and biological properties.[12–16] Pyrocatechol has antiseptic activity.[17] From many years in Department of Analytical Chemistry in Faculty of Pharmacy of Silesian Academy of Medicine are led investigations of chemical durability of drugs. This work is continuation of research concerning chemical durability of pyrocatechol.[17,18]

The aim of this work was estimation of chemical durability of pyrocatechol on silica gel as well as in solutions in relation to storage conditions.

EXPERIMENTAL

Chemicals

Ethanol, sodium chloride, methanol (POCh, Poland), chloroform (Chempur, Poland), and pyrocatechol (PS PARK, UK) were analytically pure.

Basic Solutions

50 mg of pyrocatechol was dissolved in 25 mL of distilled water, solution of physiological salt and ethanol, respectively, obtaining solutions with concentration of 2 mg/mL.

Exposure of Basic Solutions

From basic solutions mentioned above was taken 5 mL of individual solutions into the flasks and they were subjected to exposure of visible light, UV radiation ($\lambda = 254$ nm) as well as temperature (40°C).

Condition of Research Made by Thin Layer Chromatography

Preparation of Chromatographic Plates

Before spotting pyrocatechol and densitometric analysis, the aluminium plates precoated by silica gel $60F_{254}$ (E. Merck, #1.05554) were developed with methanol-chloroform (1:1, v/v). It is obvious that this process permits removal of impurites from the layer.

Investigation of Pyrocatechol Durability on Silica Gel

Standard ethanol solution of pyrocatechol (5 μL) was spotted onto chromatographic plates precoated by silica gel $60F_{254}$ (E. Merck, #1.05554). Then plates were subjected to exposure of:

a) temperature of 120°C

b) UV radiation ($\lambda = 254$ nm) in room temperature

during 120 minutes. Then, 5 μL of pyrocatechol, as standard, was spotted onto chromatographic plates.

Investigation of Pyrocatechol Solutions Subjected to Exposure

Aluminium plates, precoated with silica gel $60F_{254}$ (E. Merck, #1.05554), were activated during 30 minutes at a temperature of 120°C. Next, pyrocatechol solutions were spotted (2 μL) using a micropipette (Camag, Switzerland) onto chromatographic plates.

Chromatographic Plate Development

Chromatograms were developed in a classical chromatographic chamber (made by Camag) to a height of 14 cm from the start line, using a 50 mL mixture of chloroform-methanol (9:1, v/v) as mobile phase. Chromatographic chambers (before the insertion of plates) were saturated with mobile phase used for 15 minutes.

Densitometric Investigation

Densitometric Investigation of Pyrocatechol

Densitometric investigations were done using a TLC Scanner 3 (Camag, Switzerland). Densitograms of pyrocatechol were done with $\lambda = 200$ nm. This wavelength chosen experimentally (from 200 to 350 nm) as being optimum. Scanning conditions were: wavelength 200 nm; deuterium lamp; measurement type–remission; measurement mode–absorption; slit dimensions 10.00 × 0.40 mm, Macro; scanning speed–20 mm/s; data resolution 100 μm/step; data filtering: Savitsky-Golay 13.

Densitometric Investigation of Solvents

Ethanol (5 μL), distilled water, and a solution of physiological salt were spotted onto an activated chromatographic plate (30 min at 120°C) and the plate was developed using chloroform-methanol (9:1, v/v) as mobile phase. After drying, the plate was subjected to densitometric analysis at $\lambda = 200$ nm on paths adequate for the solvents spotted.

RESULTS AND DISCUSSION

Densitometric Investigation of Solvents for Pyrocatechol

Ethanol, distilled water, and a solution of physiological salt, earlier developed on a plate precoated with silica gel and with chloroform-methanol (9:1, v/v) as mobile phase, were subjected to densitometric investigation. It was stated that solvents used (ethanol, distilled water and solution of physiological

salt) do not contain any impurities possible to be densitometrically detected with $\lambda = 200$ nm. These solvents could serve for investigation of pyrocatechol durability.

Pyrocatechol Durability on Silica Gel

The densitogram of pyrocatechol which, after spotting onto the chromatographic plate, was heated during 120 minutes at a temperature of 120°C, is presented in Figure 1a. The densitogram of pyrocatechol spotted onto a plate after first being activated at a temperature of 120°C during 120 minutes, is presented in Figure 1b.

The following chromatographic bands are seen on the densitogram (Figure 1a): at the start ($R_F = 0.00$), with area 41646 AU, as well as the second with $R_F = 0.64$, from pyrocatechol with an area 7347 AU. The area of the pyrocatechol spot comprises 15%, and the substance at the

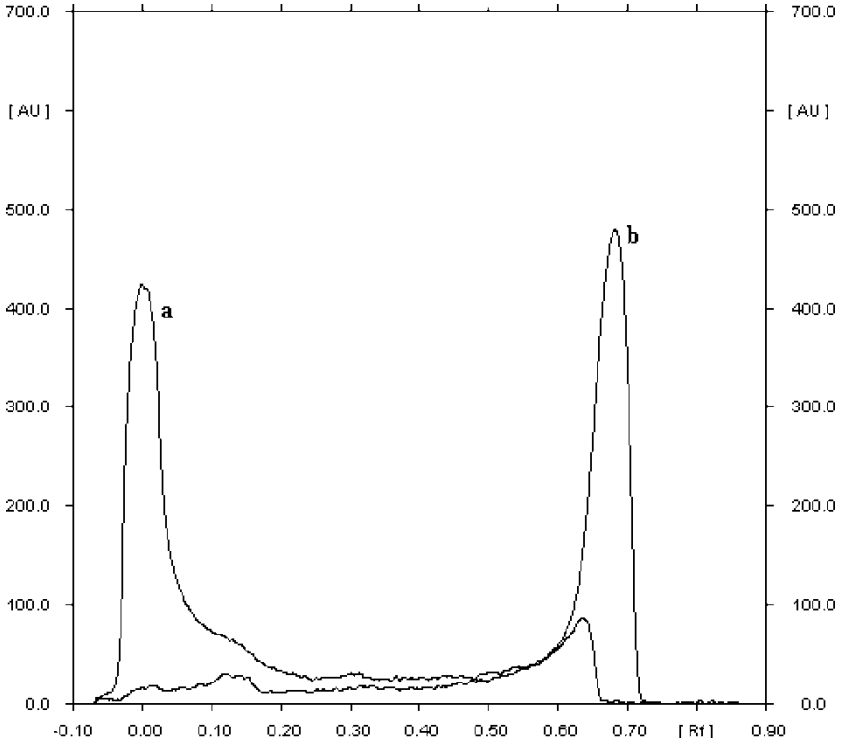

Figure 1. Densitograms of pyrocatechol. (a) after spotting on silica gel, then heated during 120 minutes at a temperature of 120°C. (b) standard (silica gel activated at a temperature of 120°C during 120 min).

start—85% of the total peak area. In the densitogram presented in Figure 1b, the band of pyrocatechol ($R_F = 0.68$) with area 42821 AU is seen. The area of the pyrocatechol spot comprises 100% of the total peak area. The area of pyrocatechol (7437 AU) that was heated on silica gel comprises 17% of the area of the pyrocatechol (42821 AU) investigated as standard. It was stated that, after 120 minutes at 120°C of exposure of pyrochatechol spotted onto silica gel, a decrease of as much as 83% pyrocatechol was observed.

From the heating of pyrocatechol after its spotting onto silica gel apparently causes pyrocatechol oxidation.[19]

The densitogram of pyrocatechol which, after spotting, was subjected to UV radiation with wavelenght $\lambda = 254$ nm during 120 minutes is presented in Figure 2a. The densitogram of standard of pyrocatechol spotted onto silica gel (silica gel before spotting of pyrocatechol was subjected to UV radiation with wavelenght $\lambda = 254$ nm during 120 minutes) is presented in Figure 2b.

The densitogram presented in Figure 2a shows a peak at the start ($R_F = 0.00$), with area 32631 AU (49.21%), as well as a pyrocatechol peak

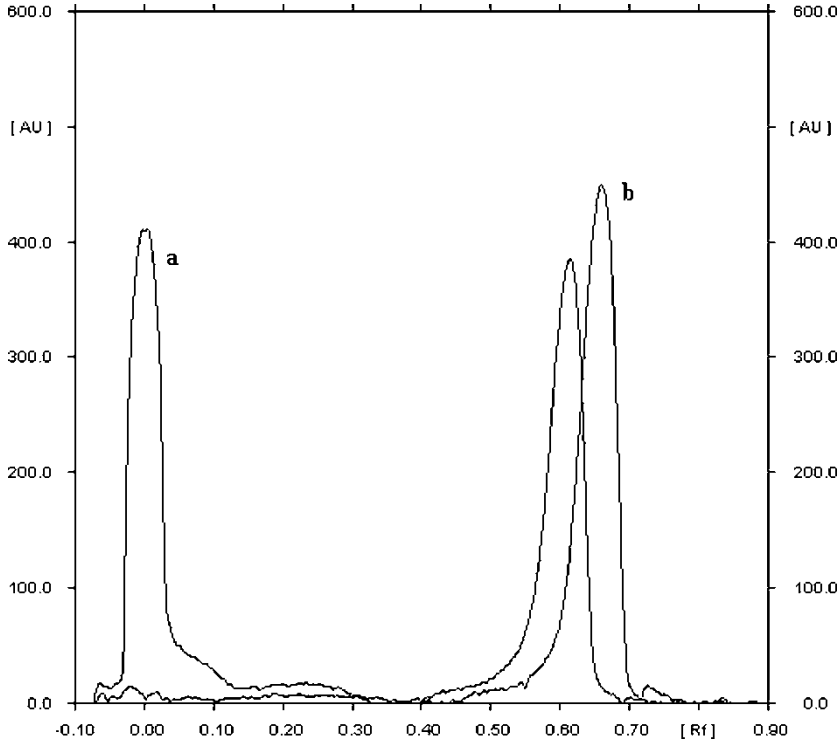

Figure 2. Densitograms of pyrocatechol. (a) after spotting, was subjected to UV radiation at a wavelenght $\lambda = 254$ nm during 120 minutes; (b) standard from plate which was precoated with silica gel (silica gel before spotting of pyrocatechol was subjected to UV radiation with wavelenght $\lambda = 254$ nm during 120 minutes).

with $R_F = 0.61$ and area 33684 AU (50.79%). The densitogram presented in Figure 2b shows the peak derived from pyrocatechol ($R_F = 0.66$) with an area of 39568 AU. The area of the pyrocatechol spot comprises 100% of the total area. The area of pyrocatechol (33684 AU) that was subjected to UV radiation ($\lambda = 254$ nm) represents 85% of the area of pyrocatechol (39568 AU) investigated as a standard. Exposure of pyrocatechol, spotted onto silica gel, with UV radiation, causes pyrocatechol oxidation as well as a change in adsorption in relation to pyrocatechol spotted as a standard. It was stated that, after 120 minutes of exposure of the pyrocatechol spotted onto silica gel with UV radiation at a wavelenght $\lambda = 254$ nm, decreased as much as 15% of the pyrocatechol.

Influence of Ethanol, Distilled Water, and a Solution of Physiological Salt on Pyrocatechol Durability

Changes of colour of the pyrocatechol solutions in ethanol, distilled water, and a solution of physiological salt, exposured to visible light, UV radiation ($\lambda = 254$ nm), and a temperature of 40°C, are presented in Table 1.

Basic solutions were colourless and transparent. Changes of colour indicate pyrocatechol decomposition in these solutions. The most sensitive solution of pyrocatechol, i.e., in the physiological salt, showed changes of colour after the first 24 h in the incubator. Smaller changes appeared in an aqueous solution of pyrocatechol, while the smallest sensitivity was in the ethanol solution of pyrocatechol kept at the same conditions.

Investigated solutions were subjected to chromatographic analysis using NP-TLC on the same day of preparing the standard solutions of pyrocatechol, as well as after finishing their exposure to visible light (160 h), UV radiation (80 h), and temperature of 40°C (380 h). Chromatograms obtained were subjected to densitometric analysis. The results obtained are presented in Table 2.

On the densitogram of the ethanol solution of pyrocatechol, only one peak was observed, with $R_F = 0.65$ from pyrocatechol. However, the pyrocatechol, after dissolving in distilled water (2 h after dissolving), as well as in physiological salt, undergoes oxidation. In the chromatogram obtained from pyrocatechol dissolved in distilled water, the chromatographic band just past the start, with 6.4% of the total area of the chromatographic bands, as well as the band of pyrocatechol with $R_F = 0.79$ and area of 93.6% were seen. A similar situation is in the case of pyrocatechol dissolved in physiological salt. In the densitogram are seen two bands; the first with $R_F = 0.04$ and area of 8% and the second with $R_F = 0.75$ (pyrocatechol) and an area of 92% of the total. This indicates that, in the standard solution of pyrocatechol in distilled water and in the solution of physiological salt certain chemical changes of pyrocatechol occur.

Table 1. Change of colour of pyrocatechol solutions because of the influence visible light, UV radiation ($\lambda = 254$ nm) and increased temperature (40°C)

	Visible light		UV light ($\lambda = 254$ nm)		Increased temperature (40°C)	
Solution investigated	After 70 h	After 160 h	After 35 h	After 80 h	After 165 h	After 380 h
Pyrocatechol in distilled water	Light yellow	Brownish	Light brown	Brown	Light brown	Brown
Pyrocatechol in solution of physiological salt	Light yellow	Brown	Yellow-brown	Light brown	Rusty-brown	Dark brown
Pyrocatechol in ethanol	Yellow	Yellow	Light yellow	Yellow	Yellow	Yellow-brown

Table 2. R_F values and areas (%) of chromatographic bands of solution of pyrocatechol in ethanol, distilled water and solution of physiological salt exposured to visible light (160 h), UV radiation $\lambda = 254$ nm (80 h) as well as temperature of 40°C (380 h)

	Pyrocatechol in ethanol		Pyrocatechol in distilled water		Pyrocatechol in solution of physiological salt	
	R_F	Area (%)	R_F	Area (%)	R_F	Area (%)
Standard			0.05	6.4	0.04	8.0
	0.65	100	0.79	93.6	0.75	92.0
Visible light			0.05	9.5	0.05	9.5
(160 h)	0.66	100	0.75	90.5	0.72	90.5
UV radiation			0.04	12.8	0.04	15.0
($\lambda = 254$ nm) (80 h)	0.70	100.0	0.74	87.2	0.72	85.0
Incubator (380 h)	0.04	7.8	0.05	14.0	0.04	20.8
	0.68	92.2	0.75	86.0	0.71	79.2

Solutions of pyrocatechol in distilled water and physiological salt, due to the influence of visible light, undergo changes. Pyrocatechol dissolved in distilled water, as well as in physiological salt, exposed to visible light activity shows a band over the start with area of 9.5% and a second band from pyrocatechol with an area of 90.5%. However, pyrocatechol dissolved in ethanol and subjected to visible light action does not undergo any changes ($R_F = 0.66$ and are of 100%).

UV radiation at wavelenght $\lambda = 254$ nm causes changes in the nature of pyrocatechol when dissolved in distilled water and physiological salt. In aqueous solution of pyrocatechol, a band near the start ($R_F = 0.04$) with an area of 12.8% and a band from pyrocatechol with $R_F = 0.74$ and an area of 87.2% are observed. In the case of a solution of pyrocatechol in physiological salt, there is also one band near the start with $R_F = 0.04$, identical to the chromatogram of the standard solution, but with a larger area; 15% is observed. The second band, from pyrocatechol, has $R_F = 0.72$ and an area of 85%. This testifies about chemical changes in pyrocatechol solutions mentioned above due to UV radiation. There were no changes in the ethanol solution of pyrocatechol ($R_F = 0.70$ and area of 100%).

Under the influence of a temperature of 40°C, the aqueous solution of pyrocatechol gave one band near the starting point, with the same value ($R_F = 0.05$) as in the chromatogram of the standard solution, but with a larger area (14%) and a band from pyrocatechol with $R_F = 0.75$ and area of 86%. Similarly, pyrocatechol dissolved in a solution of physiological salt and subjected to heating at a temperature of 40°C gives one band with the same value ($R_F = 0.04$) as on the chromatogram of the standard solution

but with a larger area (20.8%), as well as a band from pyrocatechol with $R_F = 0.71$ and an area of 79.2%. Analogous bands were obtained in the case of pyrocatechol dissolved in ethanol and then being heated; the first band with $R_F = 0.04$ and area of 7.8% and a second with $R_F = 0.68$ and area of 92.2% were observed.

The results presented point out that ethanol has the greatest stabilizing properties with respect to pyrocatechol, while the poorest stabilizing properties with respect to pyrocatechol was in a solution of physiological salt.

CONCLUSION

Pyrocatechol, heated during 120 minutes at a temperature of 120°C after its being spotted onto a chromatographic plate precoated with silica gel was decomposed in an amount of 83%. After 120 minutes of UV irradiation with $\lambda = 254$ nm, about 15% of pyrocatechol on the chromatographic plate degraded.

It was stated that ethanol has the greatest stabilizing properties in relation to pyrocatechol. Pyrocatechol dissolved in a solution of physiological salt, as well as being heated at a temperature of 40°C and irradiated with UV light, undergoes a slightly larger change compared to an aqueous solution of pyrocatechol.

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