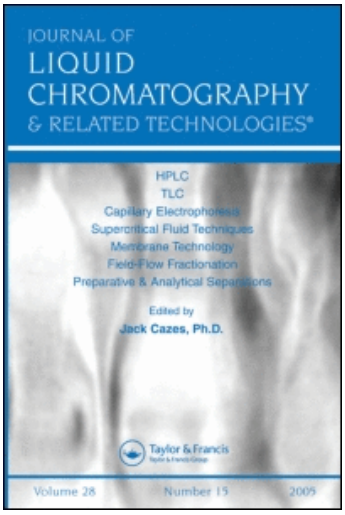

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The Visualizing Agents for Selected Quinolones and Fluoroquinolones

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The Visualizing Agents for Selected Quinolones and Fluoroquinolones

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Abstract: Analysis of compounds having biological activity is very important nowadays. This work shows the possibility of using the visualizing agents such as janus blue, methylene violet, gentian violet, methyl green, cresol red, rodamine B, malachite green, methylene blue, eosin yellowish, and metanil yellow to visualize the selected quinolones and fluoroquinolones after their separation using the TLC method. The visualizing effects were estimated after dipping the chromatographic plates in solutions of the mentioned above visualizing agents and drying at room temperature, at 120°C, or was estimated directly after dipping in solutions of the visualizing agents. The detection limit of quinolones and fluoroquinolones investigated in the conditions of visualization that gave the best visualizing effects was determined. The detection limits for cinoxacin, pipemidic acid, ofloxacin, and pefloxacin were: 0.1 μg, 0.1 μg, 0.5 μg, and 0.75 μg, respectively.

Keywords: Fluoroquinolones, Quinolones, TLC, Visualizing agent

INTRODUCTION

There are many different analytical methods of quinolones analysis, amongst other high performance liquid chromatography (HPLC) methods, liquid chromatography with mass spectrometry (LC-MS) or fluorescence (LC-F) as well as gas chromatography with mass spectrometry

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(GC-MS), planar chromatography, capillary zone electrophoresis, and immunochemical and luminescence methods.[1–8] Polarography, titration, spectrophotometry, and voltammetry also were used.[9–12] Thin layer chromatography is also very popular for quinolones investigation using different stationery and mobile phases.[8,13–24]

There is a necessity for the chromatograms visualization in many cases. The Dragendorf and Forrest and Folin-Ciocalteu agents, as well as iron(III) chloride in hydrochloric acid, iodic agent, and phosphomolybdenic acid in sulfuric(VI) acid were applied for quinolones visualization until now.[15,20] The terbium(III) and europium(III) ions were used as well for visualization of quinolones.[14]

The aim of this work is to investigate new visualizing agents for selected quinolones and fluoroquinolones, as well as to determine the detection limit of quinolones investigated.

EXPERIMENTAL

Solutions of cinoxacin, pipemidic acid, and ofloxacin were prepared from chemicals supplied by Sigma, Germany, whilst pefloxacin was supplied by Chemos, Czech Republic. Solutions of the substances investigated were prepared in 0.2 mol/dm^3 NaOH in the case of cinoxacin and pipemidic acid, as well as in 0.2 mol/dm^3 of HCl in the case of ofloxacin and pefloxacin. The substance of NaOH, HCl as well as methanol, acetonitrile, and acetic acid 99.5% (analytical grade) were obtained from POCh, Poland. The buffer solution was also used during research and was prepared by dissolving appropriate amounts of K_2HPO_4 and KH_2PO_4 (both supplied by POCh, Poland) in distilled water.

Adsorption TLC was performed on three sorts of chromatographic plates, precoated with layers of silica gel 60 F_{254} (Merck, #1.05554), precoated with layers of silica gel 60 (Merck, #1.05553), and precoated with layers of silica gel 60 F_{254} for HPTLC (Merck, #1.05548). Before the plates were used, they were activated at 120°C for 30 min. Solutions of quinolones and fluoroquniolones (50 μg of substance in 10 μL of solvent) were spotted on the plates in the amount of 3 μL using a microsyringe (Hamilton) as well as 2.5; 2; 1.5; 1; 0.8; 0.6; 0.4; 0.2, and 0.1 μL in order to determine the limit of quantification. The plates were developed at room temperature in a classical chromatographic chamber (Camag, Switzerland) previously saturated for 30 min. with the mobile phase.

The mobile phases were: buffer solution (pH = 5.5) – methanol, 40 + 10 (v/v) and acetonitrile–water–acetic acid, 6 + 40 + 4 (v/v/v). The development distance was 7.5 cm.

After the development, the chromatographic plates were dipped in 0.05% solution of vizualizing agents as follows: janus blue, methylene

violet (supplied by Michrom, Great Britain), gentian violet, methyl green (supplied by Fluka, Switzerland), cresol red (supplied by Eurochem BGD, Poland), as well as rodamine B, malachite green, methylene blue, eosin yellowish, and metanil yellow (supplied by POCh, Poland).

The visualizing effect was estimated: directly after dipping in solutions of visualizing agents, after drying at a room temperature during 24 h, and after dipping in solutions of visualizing agents and drying at 120°C during 10 min.

RESULTS AND DISCUSSION

The visualization effects obtained show the usefulness of visualizing agents investigated (janus blue, methylene violet, gentian violet, methyl green, cresol red, rodamine B, malachite green, methylene blue, eosin yellowish, and metanil yellow) to visualize the selected quinolones and fluoroquinolones after their chromatographic separation.

Better visualizing effects were obtained directly after dipping in solutions of visualizing agents in the case of fluoroquinolones on all sorts of plates researched. Negative visualizing effects were obtained for ofloxacin and pefloxacin and rodamine B, gentian violet, and cresol red (except for the separation of ofloxacin on plate 1.05553). In the case of quinolones investigated (cinoxacin and pipemidic acid) only four visualizing agents gave positive effects, i.e., janus blue, methylene blue, eosin yellowish, and methylene violet.

A similar effect was observed in the case of the drying plates at room temperature during 24 h. In the case of ofloxacin and pefloxacin, the spots were not visible after using plates 1.05553 and gentian violet, plates 1.05554 and cresol red (pefloxacin only), and plates 1.05548 and janus blue (pefloxacin only) or gentian violet. The spots coming from quinolones investigated (cinoxacin, pipemidic acid) were visible on plates 1.05553 after dipping in solutions of malachite green, methylene blue, and eosin yellowish, on plates 1.05554 and 1.05548 after dipping in solutions of janus blue, gentian violet, methylene blue, and eosin yellowish.

The visualizing effects of agents researched were also tested after dipping in solutions of visualizing agents and drying at 120°C during 10 min. As previously, the better visualizing effects were obtained in the case of fluoroquinolones investigated. Only gentian violet on plates 1.05548 gave a negative effect. In the case of quinolones investigated only a few of visualizing agents were useful. They were: eosin yellowish (all plates and compounds investigated), metanil yellow (cinoxacin, plates 1.05553 and 1.05554), as well as methylene violet (cinoxacin and all plates investigated).

Almost the all spots on the chromatograms coming from cinoxacin were broadened. Only in the case of plates 1.05548 and eosin yellowish as visualizing agent and for all plates researched and methylene violet were the spots compact.

The spots coming from pipemidic acid mostly were broadened. Only in the case of 1.05548 and eosin yellowish as the visualizing agent and for all plates researched and for methylene violet were the spots compact.

The spots coming from ofloxacin were compact in the case of all of the plates and using janus blue, rodamine B, cresol red, and methyl green as visualizing agents, as well as in the case of plates 1.05548 and malachite green and metanil yellow, plates 1.05553 and 1.05554 using methylene blue and methylene violet as the visualizing agents.

Spots from pefloxacin were compact using janus blue, rodamine B, and all the plates researched, plates 1.05548 and malachite green (after drying at 120°C), plates 1.05553 and 1.05554 and methylene blue and cresol red (after drying at 120°C), as well as using plates 1.00548 and metanil yellow and methyl green and using plates 1.05553 and 1.05554 and methylene violet.

The effort of determination of the detection limit of compounds investigated was also taken. The conditions of visualization that gave the best visualizing effects were chosen. The conditions of separation and visualization as well as values of the detection limit of compounds investigated are presented in Table 1.

In the case of quinolones investigated the smallest amount of compound possible for detection was estimated using three different conditions of separation and visualization, and in the case of fluoroquinolones – six. The limits of detection are as follows:

- cinoxacin – 0.1 μg – on plates 1.05554 using janus blues (directly after dipping in solution of visualizing agent) and on plates 1.05554 using methylene blue (after drying at a room temperature during 24 h).
- pipemidic acid – 0.1 μg – on plates 1.05554 using janus blue (directly after dipping in solution of visualizing agent).
- ofloxacin – 0.5 μg – on plates 1.05554 using cresol red (after drying at 120°C during 10 min.).
- pefloxacin – 0.75 μg – on plates 1.05553 using cresol red (after drying at 120°C during 10 min.).

It was stated that despite that in the case of fluoroquinolones investigated (ofloxacin and pefloxacin) almost all visualizing agents, in all visualizing conditions, can be used for spot identification, the limit of detection for them is lower than in the case of quinolones investigated (cinoxacin and pipemidic acid), for which only a few visualizing agents gave positive visualizing agents.

Table 1. Values of the detection limit of quinolones and fluoroquinolones investigated determined in selected conditons of visualization

Sort of plate	Visualizing agent	Time of visualizing effects estimation	Cinoxacin	Pipemidic acid	Ofloxacin	Pefloxacin
1.05553	Cresol red	After drying at 120°C during 10 min.			0.75 μg	0.75 μg
1.05554	Cresol red	After drying at 120°C during 10 min.			0.5 μg	1 μg
1.05548	Cresol red	After drying at 120°C during 10 min.			0.75 μg	1.5 μg
1.05553	Methyl green	After drying during 24 h at room temperature			1.5 μg	1.5 μg
1.05554	Methyl green	After drying during 24 h at room temperature			1 μg	1 μg
1.05548	Methyl green	After drying during 24 h at room temperature			1.5 μg	1.5 μg
1.05554	Janus blue	Directly after dipping	0.1 μg	0.1 μg		
1.05548	Gentian violet	After drying during 24 h at room temperature	1.5 μg	1.5 μg		
1.05554	Methylene blue	After drying during 24 h at room temperature	0.1 μg	0.5 μg		

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