TLC ANALYSIS OF DRUGS OF THE FLUOROQUINOLONE GROUP

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At present, extensive data are available on the pharmacological properties and clinical administration of drugs belonging to the class of fluoroquinolones [1, 2]. At the same time, there are only a few systematic investigations of their physical and physicochemical properties using modern methods [3 – 5]. Fluoroquinolones are still insufficiently characterized with respect to chromatographic behavior, in particular, in the case of thin-layer chromatography (TLC). TLC is widely used in pharmacy for the identification of drugs and verification of their purity. This method is also frequently used for rapid screening of adulterated drugs. Most of the fluoroquinolones, being highly effective and possessing a very broad spectrum of activity, are potential objects for falsification.

The aim of this study was to develop a procedure based on TLC for the verification of drugs belonging to the class of fluoroquinolones.

MATERIALS AND METHODS

Parent substances: Ciprofloxacin hydrochloride monohydrate, ofloxacin, levofloxacin hemihydrate, pefloxacin methanesulfonate (mesylate) dihydrate, norfloxacin, lomefloxacin hydrochloride, sparfloxacin, and moxifloxacin hydrochloride (below, all substances are indicated according to their international nonpatented names (INN).

Ready-to-use medicinal preparations containing parent substances in various forms: tablets (all fluoroquinolones); injection solutions (ciprofloxacin, ofloxacin, levofloxacin, pefloxacin, and moxifloxacin), drops (ciprofloxacin, ofloxacin, norfloxacin, and lomefloxacin) and ointments (ciprofloxacin, ofloxacin).

Sample Preparation for TLC Analysis

Parent substances. Aliquots (25 mg) of each drug were dissolved with continuous stirring in 25-ml measuring flasks with a small volume of water (for ciprofloxacin, pefloxacin, lomefloxacin, and moxifloxacin) or 95% ethyl alcohol (for ofloxacin, levofloxacin, norfloxacin, and sparfloxacin). Then, each flask was filled with the same solvent to the mark and the content was thoroughly stirred. This yielded test solutions with a drug concentration of 1.0 mg/ml.

Tablets. An accurately weighed amount of crushed tablets (about 50 mg) was placed into a 50-ml measuring flasks and dissolved by vigorously shaking for 5 min in 30 ml of water (for ciprofloxacin, pefloxacin, lomefloxacin, and moxifloxacin) or 95% ethyl alcohol (for ofloxacin, levofloxacin, norfloxacin, and sparfloxacin). Then, the flask was filled with the same solvent to the mark and the content was thoroughly stirred. This solution was filtered through a filter paper (white ribbon grade) into a cone-shaped flask (the first 20-ml fraction was rejected). This yielded test solutions with a drug concentration of about 1.0 mg/ml.

Drops and injection solutions. An appropriate volume of the initial preparation was diluted with the necessary amount of water so as to obtain a test solution with a concentration of about 1.0 mg/ml.

Ointments. The samples (1.0 g) of ointments were placed into cone-shaped measuring flasks with 3 ml of water (for ciprofloxacin) or 95% ethyl alcohol (for ofloxacin) and vigorously shaken for 15 min. The mixture was filtered through filter paper (white ribbon grade) into a tube. This yielded test solutions with a drug concentration of about 1.0 mg/ml.

TLC Conditions

The analyses were performed on 10 × 10 cm Sorbfil plates for high-performance TLC (State Standard TU 26-11-17-89; Sorbpolimer Company, Krasnodar). The plates, coated with silica gel and covered with a phosphor film, were of two types differing by the base material: aluminum (Sorbfil PTSKh-AF-V-UF) or polymer (Sorbfil PTSKh-P-V-UF).

The samples of the test solutions (~2 μl, which corresponded to 2 μg of the parent substance) were applied with the aid of a microsyringe onto the start line. The distances between adjacent spots and between the spots and TLC plate edges must be not less than 14 mm, which allowed 6 samples

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to be tested on each 10 × 10 cm plate. The applied samples were dried in a special heating device (USP-1, Sorbpolimer Company) at a temperature of about 70°C.

Then, the plates with applied samples were run in a glass TLC chamber with dimensions 150 × 20 × 80 mm, which was presaturated with the mobile phase vapor for 20 – 30 min. The plates were run in the ascending mode until the solvent front reached a level of about 8.5 cm. Finally, the plates were dried in air for 5 – 10 min and the spots were revealed by exposure to radiation (254 nm) of a special UV source (UFS 254/365, Sorbpolimer Company).

RESULTS AND DISCUSSION

We have studied the influence of the mobile phase composition and polarity on the mobility of fluoroquinolones and the selectivity of the TLC system. The polarity of eluents was evaluated based on the permittivities of components [6, 7]. Each mobile phase was characterized in terms of the average weighted (effective) permittivity calculated taking into account the content of each solvent in the mixture.

The mobile phases contained the following solvents taken in various ratios (listed in order of increasing polarity): benzene, toluene, diethyl ether, chloroform, ethyl acetate, glacial acetic acid, isoamyl alcohol, butyl alcohol (butanol), isopropyl alcohol, propyl alcohol (propanol), acetone, ethyl alcohol (ethanol), methyl alcohol (methanol), acetonitrile, glycerol, a 25% aqueous ammonia solution, and formamide.

An increase in the content of high-polarity components (25% aqueous ammonia solution, formamide) led to an increase in the mobility of fluoroquinolones but decreased the TLC system selectivity: all spots on the chromatogram had R_f above 0.8 and some occurred on the solvent front. An increase in the content of low-polarity components (ethyl acetate, glacial acetic acid) decreased both the mobility of fluoroquinolones and selectivity of analysis. In the case of low-polarity mobile phases containing benzene, toluene, and chloroform, the spots of fluoroquinolones possess R_f below 0.2 or even remain on the start line.

It was established that optimum mobility and separation of the spots of fluoroquinolones are observed in mobile phases of intermediate polarity (with effective permittivity of the mixture within 20 – 40) containing the following solvents: diethyl ether, ethyl acetate, isoamyl alcohol, butanol, isopropyl alcohol, methanol, acetonitrile, and a 25% aqueous ammonia solution. In these solvents and their mixtures, the R_f values were within 0.2 – 0.8, which, according to now commonly accepted notions is the optimum range [8].

The optimum mobile phase composition was selected taking into account the following facts and assumptions. It was found that the spots exhibit less pronounced blurring in mobile phases containing a 25% aqueous ammonia solution. The optimum content of this component was established at about 20%.

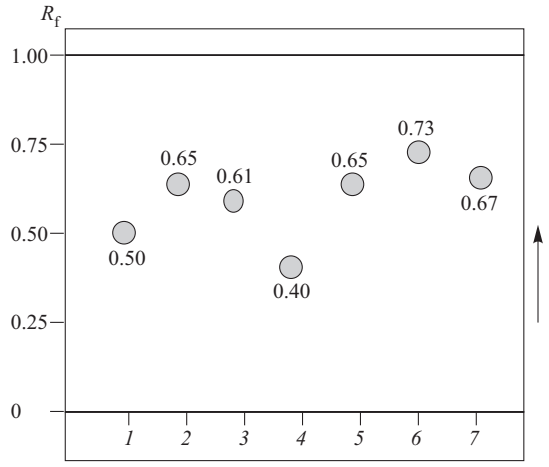

Fig. 1. The typical TLC pattern of fluoroquinolones eluted in a methanol – 25% aqueous ammonia – ethyl acetate – acetonitrile, 1 : 1 : 2 : 1 system (figures at the spots indicate R_f): (*1*) ciprofloxacin; (*2*) ofloxacin and levofloxacin; (*3*) pefloxacin; (*4*) norfloxacin; (*5*) lomefloxacin; (*6*) sparfloxacin; (*7*) moxifloxacin.

In order to increase the TLC system selectivity, it is necessary to use methanol. Similar results can be also obtained using ethanol, but the selectivity is still somewhat lower. The optimum content of methanol is about 20%.

The remaining 60% of the mobile phase has to include two solvents such that they (i) are well miscible with both 25% aqueous ammonia and methanol and (ii) possess significantly different permittivities. The latter circumstance allows the mobile phase polarity to be effectively controlled (by changing the ratio of these components) so as to provide for the optimum mobilities of the fluoroquinolones studied. After additional experiments, we decided that the two remaining components are ethyl acetate and acetonitrile (with permittivities about 6 and 37, respectively).

As a result of this investigation, we have selected the optimum mobile phase composition, which offers a universal base for the TLC analysis of all fluoroquinolones in the group studied: methanol – 25% aqueous ammonia – ethyl acetate – acetonitrile, 1 : 1 : 2 : 1. The effective permittivity calculated for this composition is about 32. The R_f values of the spots of fluoroquinolones fall in the range 0.40 – 0.73. The typical TLC pattern is presented in Fig. 1. It should be noted that, since levofloxacin is the left-rotated isomer of ofloxacin, these compounds are not distinguished by TLC on silica gel.

None of the mobile phases used allowed all fluoroquinolones in the group studied to be effectively separated. Ofloxacin and levofloxacin should be differentiated using other, stereoselective analytical techniques.

It was established that UV irradiation ensures reliable detection of fluoroquinolones applied onto the start line in an amount of 0.5 μg. In order to provide for reliable identification and to obtain clear spots, we applied samples containing about 2 μg of each parent compound.

An analysis of the behavior of samples in the stage of spot detection showed that ofloxacin, moxifloxacin, and sparfloxacin differ in their ability to produce fluorescence under UV irradiation. At a wavelength of 254 nm, most fluoroquinolones are detected as dark spots on the bright (luminescent) background. However, ofloxacin and moxifloxacin excited at this wavelength exhibit a bright violet emission, while sparfloxacin produces a yellowish-green fluorescence. In the case of irradiation at a wavelength of 365 nm, the intensity of fluorescence of the ofloxacin and moxifloxacin spots increased sharply (bright blue emission), while the sparfloxacin spot becomes dark-gray. All the other fluoroquinolones irradiated at 365 nm are detected as dark blue spots, this fluorescence being noncharacteristic and difficult to distinguish on the luminescent plate background.

The proposed method of TLC identification of fluoroquinolones can be included into practical guides on rapid analysis aimed at the verification of parent substance. This technique can be used for the screening of adulterated drugs not containing fluoroquinolones indicated on the package.

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