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High-performance thin-layer chromatography method for monitoring norfloxacin residues on pharmaceutical equipment surfaces

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Abstract

This paper presents a high-performance thin-layer chromatography (HPTLC) method with direct fluorescence measurement for the determination of norfloxacin. The method was validated for the monitoring of norfloxacin residues on stainless steel surfaces at the allowed limit of 10 mg of norfloxacin per square meter. However, it can be adapted for lower amounts of residues owing to the low detection limit of norfloxacin (about 5 ng) and can also be used for other surface materials. Test solutions were analyzed by the new HPTLC method and the known HPLC method for comparison. Accuracy and precision of the new HPTLC method, with a subsequent quantification by densitometer or video system, are comparable with those of the HPLC method. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Pharmaceutical analysis; Norfloxacin; Fluoroquinolones; Antibiotics; Densitometry

1. Introduction

The publication of the ‘‘Guide to Inspection of Validation and Cleaning Processes’’ by the US Food and Drug Administration (FDA) in 1993 [1] has increased the attention to the concepts of cleaning validation in the pharmaceutical industry. The pharmaceutical industry continues to be inspected in the area of cleaning validation [2]. Pharmaceutical manufacturers have to verify that the cleaning procedures for multiple use equipment will remove residues of previous products to an acceptable level. Such residues might have a significant impact on the quality of a pharmaceutical product subsequently produced in the same equipment. Cleaning validation studies should be conducted following a written validation protocol, where a suitable analytical method represents the most important part. Due to the regulatory expectations high-performance liquid chromatography (HPLC) methods are preferred for the residue determination, although there are also other suitable methods (e.g., thin-layer chromatography, TLC [3]), which can be used.

Several HPLC [4–6] and TLC [7–9] methods have already been described for the determination of norfloxacin (Fig. 1) – an antibacterial agent from the group of fluoroquinolones, in different matrices. This paper describes a new high-performance thin-layer chromatography (HPTLC) method with direct fluorescence measurement for the determination of norfloxacin. The method was validated for the monitoring of norfloxacin residues on stainless steel

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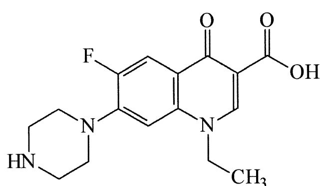

Fig. 1. The structure of norfloxacin.

surfaces at the allowed limit of 10 mg/m^2. Test solutions were analyzed by the new method and the known HPLC method for comparison.

2. Experimental

2.1. Chemicals

All chemicals and solvents, except tetrabutylammoniumhydroxide (for synthesis), were at least of analytical grade. Acetonitrile (LiChrosolv), methanol, chloroform, liquid paraffin and tetrabutylammoniumhydroxide were obtained from Merck (Darmstadt, Germany). NaOH, H_3PO_4 (both analytical-reagent grade) were obtained from Kemika (Zagreb, Croatia). Ammonia (25% solution, analytical-reagent grade) was purchased from Kemična tovarna Podnart (Podnart, Slovenia) and *n*-hexane from Carlo Erba (Milan, Italy).

Water was distilled twice in an all glass apparatus, and deionized using a Milli-Q system (Millipore, Bedford, MA, USA). Norfloxacin was obtained from Sigma–Aldrich (Deisenhofen, Germany).

2.2. Samples

2.2.1. Simulated sample 10 mg/m^2

On a stainless steel surface (35×35 cm) a defined solution of the norfloxacin (1.225 mg in 490 μl of solvent: 0.005 *M* NaOH in water–methanol, 1:1) was spread with a pipette. After air evaporation of solvent, substance remained on the surface as residue. Five samples were prepared.

2.2.2. Preparation of sample test solution

Two bands of cotton (6×6 cm, about 0.5 g) were wet with 0.005 *M* NaOH in water–methanol (1:1) as the extraction solution and used for the quantitative wiping of the marked surface by means of polythene gloves. The cotton bands were placed in an Erlenmeyer flask and shaken with 50 ml of the extraction solution for 30 min. The extract was filtered into a 100-ml volumetric flask. The cotton was additionally rinsed with the extraction solution, which was thereafter filtered and added to the test solution. The volumetric flask was filled up to the mark with the extraction solution.

Three ml of the test solution were pipetted into a 10-ml volumetric flask and filled up to the mark with methanol for the HPTLC determination.

The test solution was 100-fold diluted with 0.085% H_3PO_4 for HPLC determination.

A blank test solution was prepared in the same way as the sample test solution but without norfloxacin.

2.3. Thin-layer chromatography

TLC was performed on 20×10 cm glass-backed silica gel 60 HPTLC plates (Merck article 1.05641, Darmstadt, Germany) prewashed with methanol. All the standards and the samples were applied to the plates by means of the Linomat IV applicator (Camag, Muttenz, Switzerland) equipped with a 100-μl syringe. The band length was 2 mm, the application volume for the standards was 6, 8 and 10 μl of the norfloxacin solution, c=5 μg/ml (30, 40 and 50 ng of norfloxacin) and 10 μl for the samples, the application rate was 14 μl/s. Twenty-five bands per plate were applied 15 mm from the bottom edge, 6 mm apart by using the data-pair technique. The plates were developed in a saturated glass twin-trough chamber (Camag) in a solvent system methanol–chloroform–conc. ammonia (51:34:15, v/v/v), the migration distance being 6 cm. After the separation, the plates were dried in a stream of warm air for about 2 min and then immersed for 4 s into liquid paraffin–*n*-hexane (1:2, v/v) by means of a Camag chromatogram immersion device II.

2.3.1. Scanning and image processing

Quantitative evaluation of the developed HPTLC plates was performed densitometrically using the Camag TLC scanner II equipped with a built-in 12-bit A/D converter, and controlled by an external personal computer via an RS232 interface. The scanner was set to the fluorescence/reflectance

mode, the monochromator bandwidth was 30 nm at the excitation wavelength 313 nm (a Hg source), filter K400, slit dimensions settings of length 6 mm and width 1.2 mm, and a scanning rate of 10 mm/s. The automatic scanning was controlled by the QTLC-pack (KIBK-IFC, 1990) software.

A Camag video documentation system in conjunction with the Reprostar 3 was used for imaging and archiving the thin-layer chromatograms. The objects were captured by means of a highly sensitive video camera – 3×1/2 in. (1 in.=2.54 cm) CCD camera, Model HV-C20 (Hitachi, Denshi, Japan). A special digitizing board (frame grabber) assists rapid processing via the personal computer system. Image acquisition, processing and archiving were controlled via Video Store 2.30, a high-performance documentation software running under Windows 95. All images were taken at λ=366 nm from the distance 56 cm by aperture 1.4 using attachment lens +2 Dpt for optimum adaption to the size of the object. The Camag Video Scan 1.01 program was used for the evaluation of thin-layer chromatograms.

2.4. High-performance liquid chromatography

The liquid chromatograph consisted of a ConstaMetric® 3200 solvent pump (Thermo Separation Products, Riviera Beach, USA), a fluorescence HPLC detector (Shimadzu, RF-530, Kyoto, Japan), and an injector (Rheodyne), equipped with a 10-μl loop. The detector was set at 280 nm and 446 nm for excitation and emission, respectively. Data evaluation was done by the Chrom Jet integrator (Spectra-Physics Analytical, USA).

The separation was performed on a μ-Bondapak C_{18}, 10 μm, 250×4.0 mm column.

The mobile phase used for the separation according to the modified USP method [10] consisted of two solutions, A and B, in the ratio 89:11, v/v. Solution A was 0.025 *M* H_3PO_4, pH was adjusted to 3.0 by a 20% solution of tetrabutylammoniumhydroxide. Solution B was acetonitrile. The mobile phase flow-rate was 1.5 ml/min.

3. Results and discussion

TLC offers a simple and rapid HPTLC method for qualitative and quantitative determination of norfloxacin residues on stainless steel surfaces. Test solutions were analyzed by the new HPTLC method (Figs. 2 and 3) and the known HPLC method (Fig. 4) for comparison. As can be seen in the CCD image (Fig. 2) of the separation of norfloxacin, the new method offers analysis of 25 samples on one HPTLC plate. Nevertheless, even higher sample capacity and lower solvent consumption could be achieved using simultaneous development from both sides of the HPTLC plate in a horizontal developing chamber. This will significantly decrease the volume of mobile phase used per sample, which is one of the most important advantages of the new HPTLC method over the USP HPLC method.

The linearity of the new HPTLC method and the known HPLC method was examined in different concentration range. Comparing the results obtained by HPLC method and the new HPTLC method with densitometric quantification (Figs. 5 and 6), we can conclude that the calibration curves are linear from 10–90 ng of norfloxacin, covering more than required working range (10–60 ng). However, the results obtained by quantification of the same HPTLC plates by two different quantification systems (densitometer and Camag video documentation system) showed some differences. As is evident from Fig. 6, densitometric measurements gave a linear correlation between the peak area and the amount of norfloxacin within the higher concentration range than the measurements obtained by the Camag video documentation system, which gave a polynomial correlation within the same concentration range (Fig. 7).

Examination of the data obtained by the new HPTLC method and by the known HPLC method, presented in Table 1, lead to the conclusion that the difference between mean recoveries is not significant at the 95% level (*t*-test). Also the variance of the HPTLC-CCD camera method is not significantly greater compared to that of the HPLC method (*F*-test, $P=0.05$).This fact verifies, that under optimized conditions, HPTLC with a subsequent quantification by slit-scanning densitometer or video documentation system can produce the results that are comparable with the results obtained by HPLC.

Although the HPTLC method was validated as a limit test for 10 mg of norfloxacin per square meter,

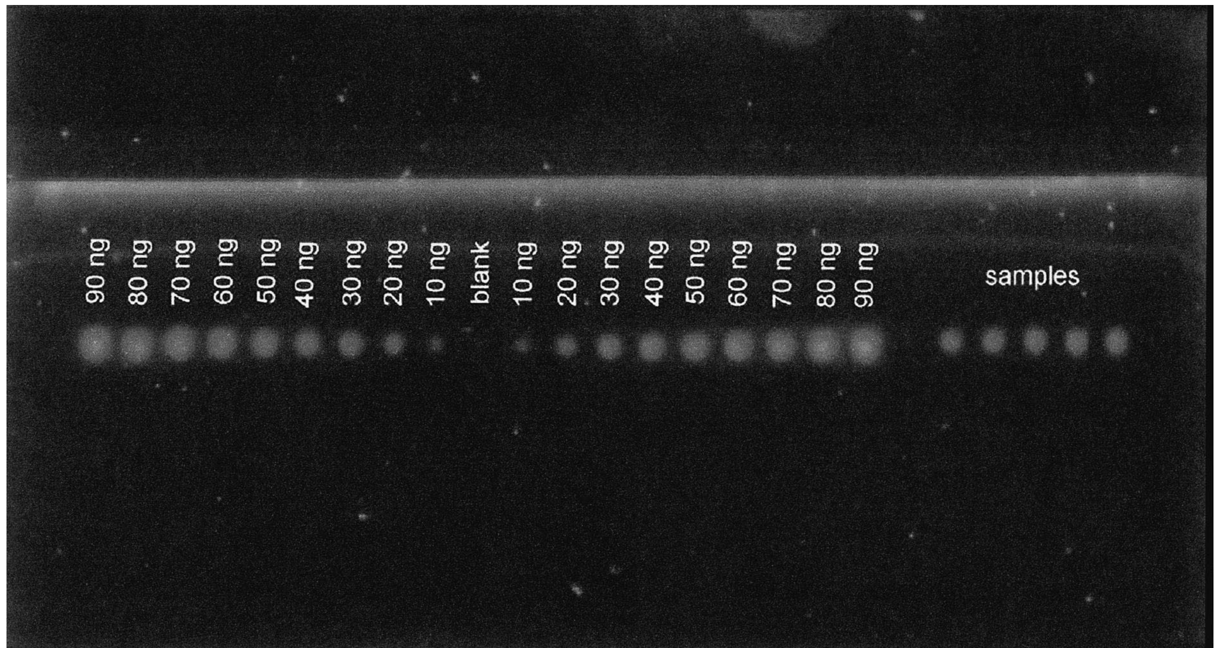

Fig. 2. CCD image of 25 applications of different amounts of norfloxacin on the HPTLC plate.

it can also be adapted for lower amounts of norfloxacin residues owing to the low detection limit of norfloxacin (about 5 ng). Additionally, this method can also be used for other surface materials. The low limit of detection and quantification of norfloxacin enables a simple preparation of test solution.

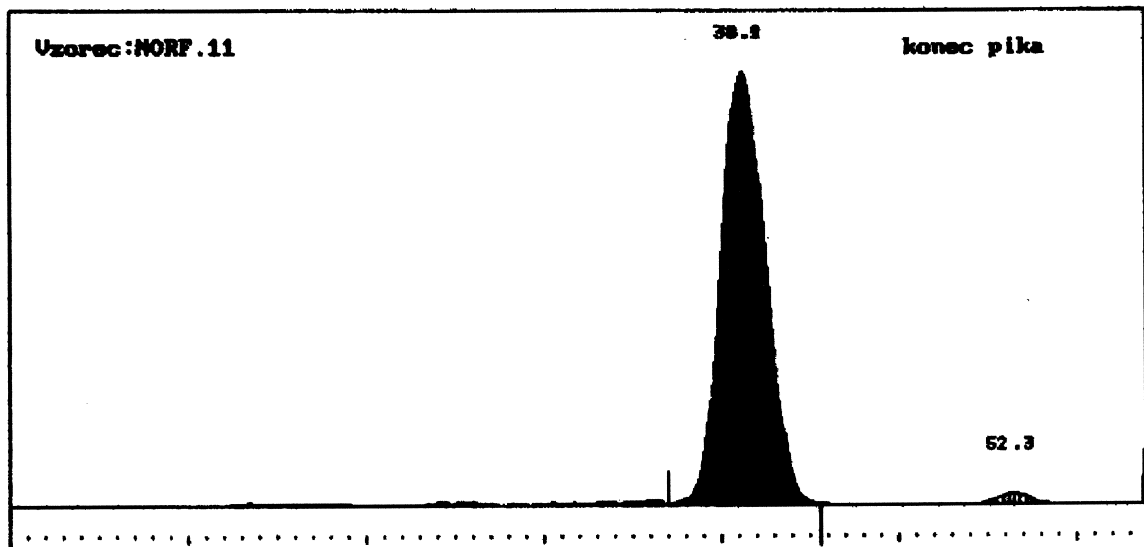

Fig. 3. Densitogram of 30 ng of norfloxacin on the HPTLC plate.

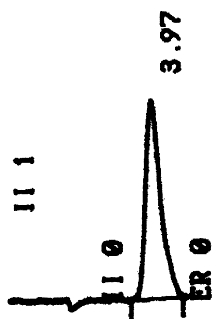

Fig. 4. Chromatogram of norfloxacin obtained by HPLC (c=100 ng/ml).

According to the present situation in the field of TLC, we can say that imaging techniques are becoming a more and more popular and necessary tool, not only for the documentation but also for the quantitative evaluation of TLC chromatograms [11–21]. The advantage of quantitative evaluation with a CCD camera is a high speed of data acquisition process, which is much faster compared to classical densitometry. Quantification with video systems will give results a few minutes after capturing the image. These advantages of quantitative evaluation with a video integration system are especially important and noticeable when there are a lot of samples (and spots or bands) on one TLC plate. As we are capturing the image of the whole plate and making the quantification thereafter, there are less possibilities for errors regarding the wrong positioning of the tracks compared to the quantification with a slit-scanning densitometer [22].

Furthermore, data acquisition with a CCD camera is much more suitable and simple in the case of two-dimensional TLC, circular and non-classical trapezoidal and triangular TLC [23]. On the other hand it is almost impossible to scan such plates with a slit-scanning densitometer, unless special software is used [24]. However, such scanning is time consuming and is not useful for the routine analysis.

There is also another fact called secondary chromatography which should be pointed out, when we are talking about quantitative TLC. It is obvious that the users are still not aware of the influence of secondary chromatography on the in-depth distribu-

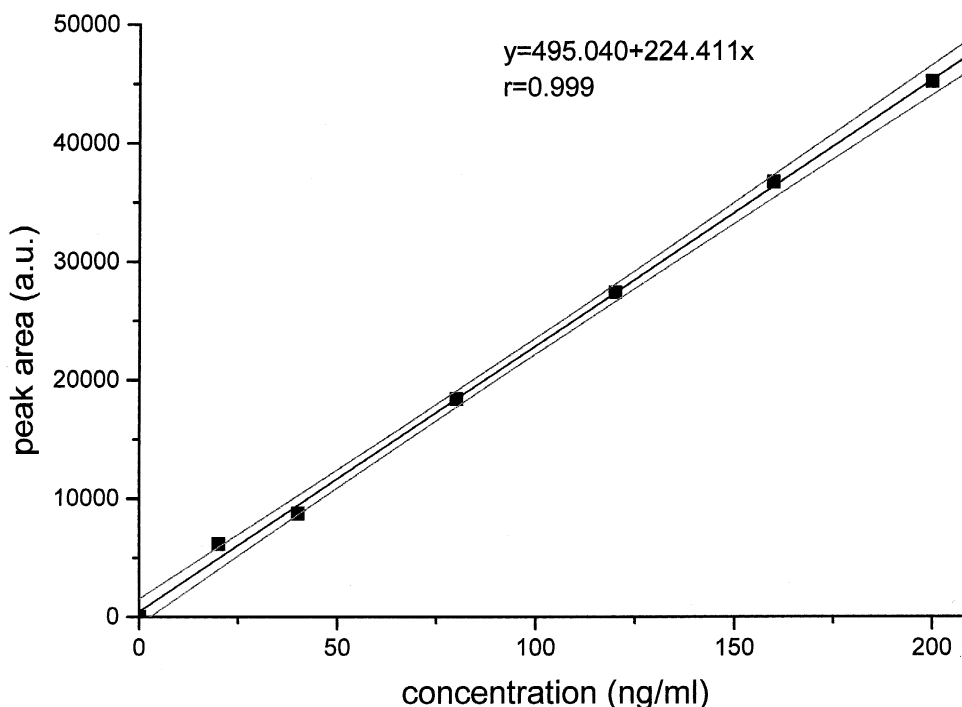

Fig. 5. Calibration curve for norfloxacin obtained by the HPLC method (α=0.05).

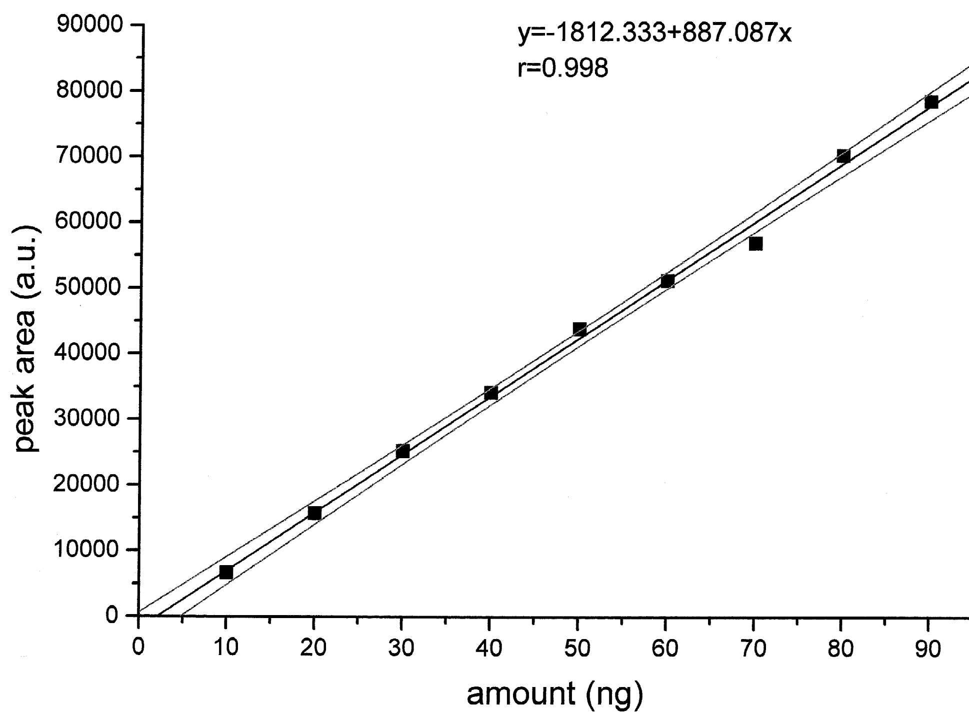

Fig. 6. Calibration curve for norfloxacin obtained by the HPTLC method (α=0.05); evaluation by densitometer.

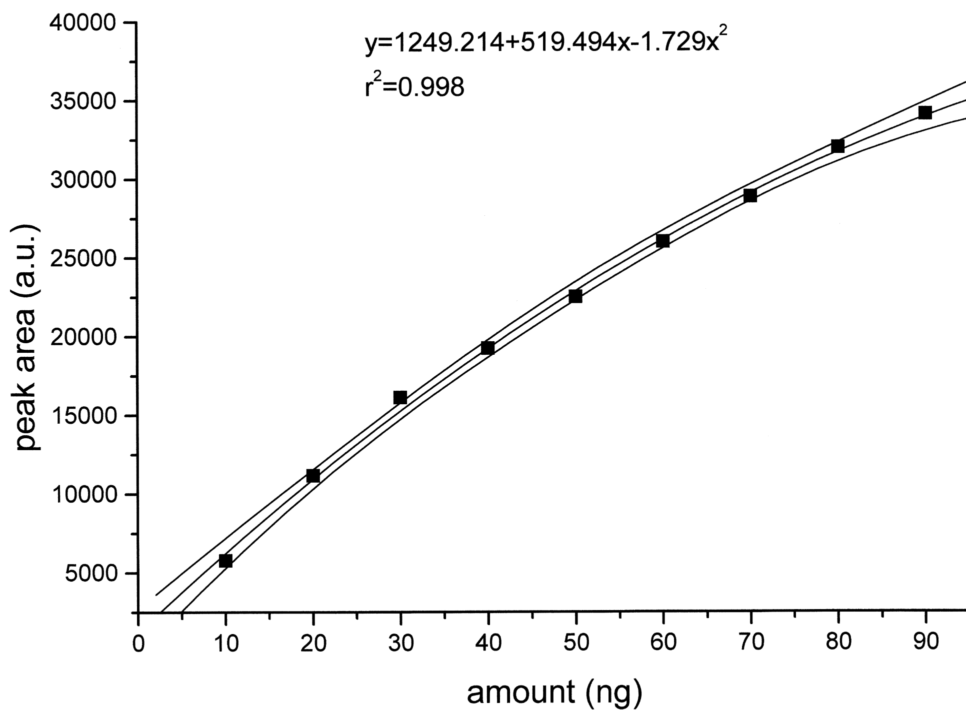

Fig. 7. Calibration curve for norfloxacin obtained by HPTLC method (α=0.05); evaluation by Camag video documentation system.

Table 1
Comparison of HPTLC and HPLC methods

	Mean recovery (%) (n=5)	Repeatability, RSD (%) (n=5)
HPLC	104.3	3.8
HPTLC (densitometer)	105.4	6.2
HPTLC (CCD camera)	104.9	3.5

tion of substances within the sorbent of the TLC plates. However, such differences in the in-depth distributions of samples and standards can cause erroneous interpretation of results [25–28]. The education of the users about this topic will certainly improve the reproducibility of results in TLC.

According to the state of the art in quantitative TLC, we can conclude that the analysis of residues of norfloxacin is only one among many analysis which could be done using TLC instead of HPLC.

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