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DENSITOMETRIC ANALYSIS OF SELECTED FLUOROQUINOLONES

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□ *Fluoroquinolones are the group of very important synthetic drugs, especially in the treatment of urinary tracks infections. The aim of this work was to find the best chromatographic conditions for analysis of ofloxacin and pefloxacin, as well as using the chromatograms for their densitometric analysis in drugs. The calibration curves were prepared for that purpose and the curves were described by mathematic functions. It permitted calculating the contents of fluoroquinolones in drugs investigated. The densitometric scanning was carried out for ofloxacin and pefloxacin. The quite similar values of quantity of spotted and determined ofloxacin and pefloxacin were stated.*

Keywords densitometry, fluoroquinolones, thin layer chromatography

INTRODUCTION

Fluoroquinolones are the group of very important synthetic drugs, especially in treatment of urinary tracks infections. They are also used in ophthalmology, dermatology, and gynecology.^[1–4]

The separation of fluoroquinolone antibiotics is very often carried out by using thin layer chromatography. Very often the TLC is connected with other instrumental methods, for example with densitometry,^[5–8] fluorescence,^[9,10] and mass spectrometry.^[9,11]

The aim of this work was to find the best chromatographic conditions for analysis of ofloxacin and pefloxacin, as well as, using the chromatograms for their densitometric analysis in pharmaceutical preparations of Tarivid and Abaktal.

EXPERIMENTAL

Substances Investigated

The subject of investigation was the standard solution of ofloxacin (Merck, Darmstadt) and pefloxacin (Chemos, Prague) as well as the pharmaceutical preparations of the contents of ofloxacin (Tarivid, Sanofi-Aventis, Frankfurt) and pefloxacin (Abaktal, Lek S.A., Ljubljana).

The solution of ofloxacin was prepared in 0.01 mol/L HCl and the solution of pefloxacin was prepared in 0.002 mol/L HCl. The concentration of initial solution was 25 mg/5 mL. The next solutions were obtained by dilution of the initial solution. The concentrations of these solutions were: 20, 12, 7.212, 4.3212, 2.529, 1.5552, 0.9331, 0.5599, 0.3359, 0.2015, 0.1209, 0.0726, 0.0435, 0.0261, 0.0157, and 0.0094 mg/5 mL.

Adsorption Thin-Layer Chromatography

Adsorption thin layer chromatography was performed on aluminium plates precoated with 0.2 mm layer of silica gel 60F₂₅₄ (E. Merck, #1.05548). The plates were activated at 120°C for 30 min. The mixture of acetonitrile (POCh, Poland), formic acid (80%, POCh, Poland), and water in volume ratio 40:3:7 (v/v/v) was used as mobile phase.

The chromatographic plates were developed to a height of 7.5 cm in the room temperature in a classical chamber (Camag, Switzerland), after their saturation with mobile phase (50 mL) during 30 min.

Compounds investigated were analyzed in pharmaceutical preparations as well. They were Tarivid, containing ofloxacin as active substance and Abaktal, containing pefloxacin. The tablets were ground in a mortar and solved in proper solution of HCl, as in the case of ofloxacin and pefloxacin. The solutions of the particular solution were spotted on chromatographic plates in the amount of 5 µL. The chromatographic conditions used were exactly the same in the case of ofloxacin and Tarivid as well as in the case of pefloxacin and Abaktal.

Spectrodensitometric Analysis

The spectrum was performed using Camag Scanner TLC 3. The radiation sources were deuterium and wolfram lamps. Start wavelength was 200 nm and end wavelength was 400 nm. The slit dimensions were 8.00 × 0.40 nm, the scanning speed was 100 nm s⁻¹. The measurement mode was absorption.

Spot Visualization Using Densitometer

Densitometric scanning was then performed with a Camag Scanner TLC 3 controlled by winCATS 1.4.1 software. The densitometric scanning was performed at various wavelengths depending on the compound investigated.

In the case of ofloxacin and Tarivid the scanning was performed at wavelength of 295 nm and in the case of pefloxacin and Abaktal –280 nm.

Regression Analysis

The regression equations describing relationships between contents of ofloxacin and pefloxacin and heights as well as between contents of ofloxacin and pefloxacin and areas of densitometric bands were achieved using computer program STATISTICA 7.1.

RESULTS AND DISCUSSION

On the basis of spectrodensitometric analysis the maximum wavelengths were settled for determination of particular compounds. The

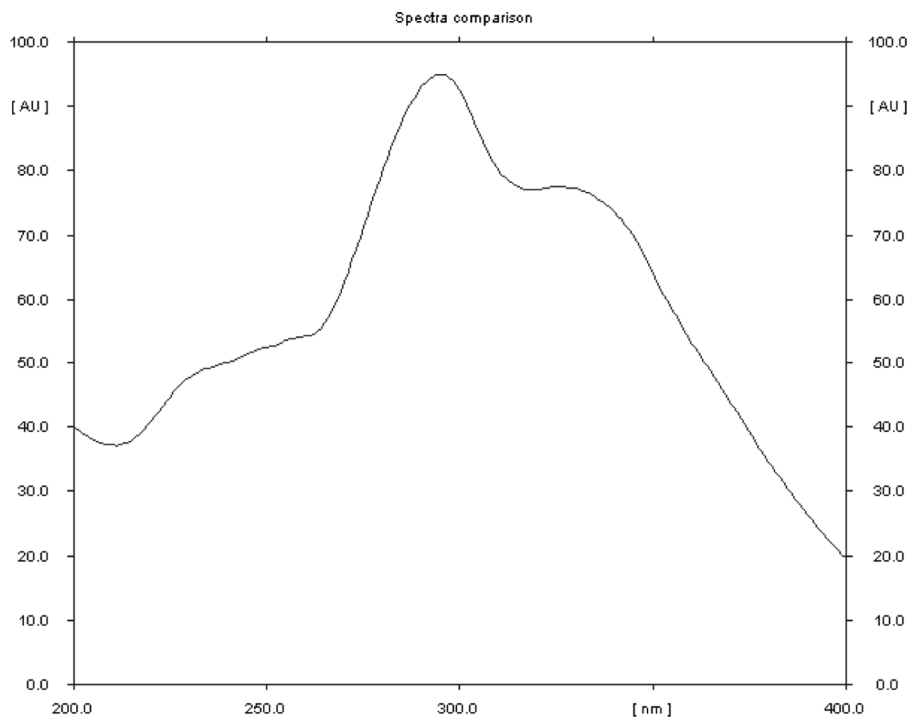


FIGURE 1 Spectrodensitogram of ofloxacin.

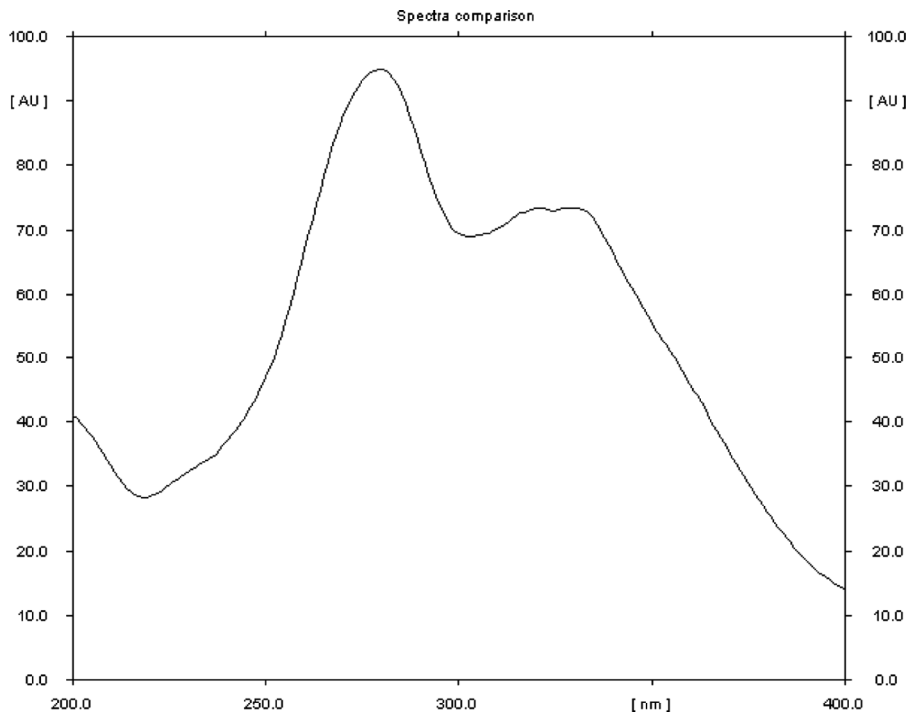


FIGURE 2 Spectrodensitogram of pefloxacin.

TABLE 1 The Values of Contents of Ofloxacin and Pefloxacin as well as the Adequate Values of Height and Area of Densitometric Peaks

Ofloxacin			Pefloxacin		
Contents [μg]	Peak height [AU]	Peak area [AU]	Contents [μg]	Peak height [AU]	Peak area [AU]
25,0000	557,6	58810,9	25,0000	459,6	53980,7
20,0000	507,7	64241,2	20,0000	454,2	52805,8
12,0000	428,7	47951,2	12,0000	397,4	42959,1
7,2120	420,2	46006,5	7,2120	283,1	27752,9
4,3212	349,0	37997,4	4,3212	270,9	24391,1
2,5920	284,8	27414,4	2,5920	242,6	19489,1
1,5552	239,3	18168,2	1,5552	181,4	13236,9
0,9331	192,0	12901,8	0,9331	145,6	8927,7
0,5599	184,2	8172,3	0,5599	110,9	5860,0
0,3359	89,7	4277,5	0,3359	87,7	4977,8
0,2015	90,5	3831,7	0,2015	61,7	2746,4
0,1209	62,0	2449,5	0,1209	40,3	1706,1
0,0726	36,9	1303,4	0,0726	27,7	1167,7
0,0435	20,2	618,6	0,0435	18,4	460,2
0,0261	26,3	933,0	0,0261	15,5	470,8
0,0157	15,4	452,8	0,0157	11,0	436,0
0,0094	17,0	490,1			

TABLE 2 The Equations Describing the Linear Relationship Between Height or Area of Densitometric Peak and Contents of Compound Investigated

Compound determined	Range of contents of compound investigated [µg]	Equation*	n	R	s	F	Nr Równania
Ofloxacin	0.0094 ÷ 0.2015	$h = 398.3090(\pm 28.0114)c + 10.4665(\pm 2.6658)$	7	97.59	4.8	202	1
	0.0094 ÷ 2.5290	$a = 10895.4000(\pm 344.3700)c + 937.7430(\pm 317.1020)$	11	99.01	895.3	1001	2
Pefloxacin	0.0157 ÷ 0.3359	$h = 241.6570(\pm 8.8464)c + 9.2957(\pm 1.4033)$	7	99.33	2.52	746	3
	0.0157 ÷ 0.3359	$a = 14509.2000(\pm 362.5770)c$	7	99.17	152.2	1601	4

*for all equations $p \leq 0.001$; h – peak height, a – peak area, c – contents of compound investigated.

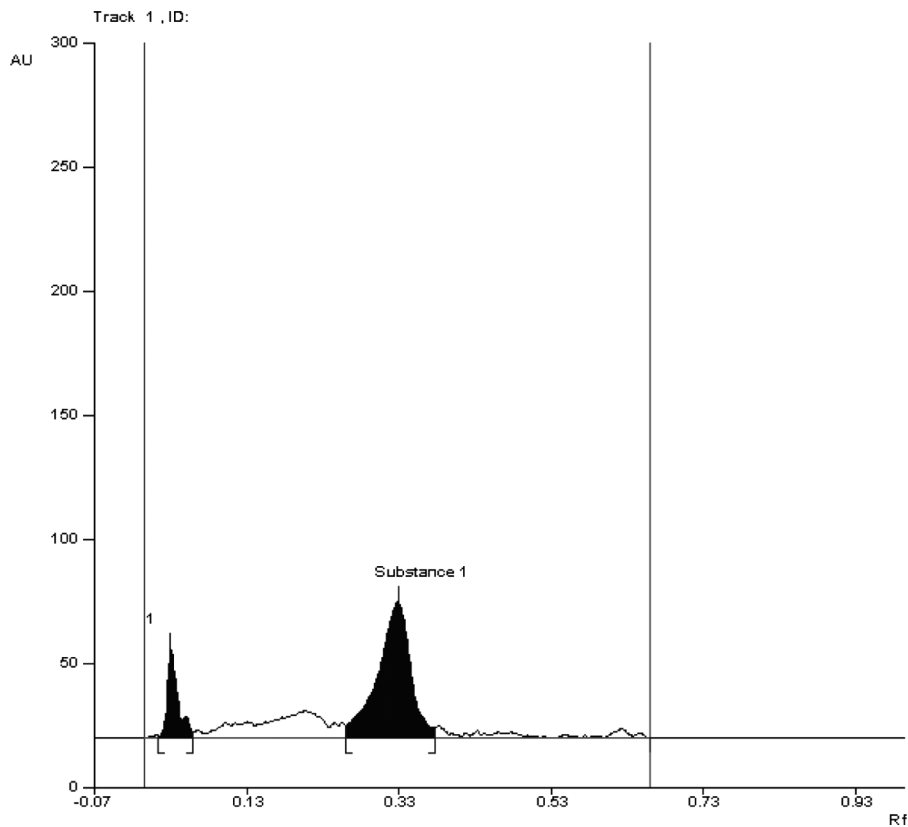


FIGURE 3 The example densitogram of ofloxacin determined in Tarivid.

spectrodensitograms of ofloxacin and pefloxacin are presented in Fig. 1 and 2, respectively.

Densitometric scanning was then performed at 295 nm (ofloxacin determination) and 280 nm (pefloxacin determination). The values of contents of ofloxacin and pefloxacin as well as the adequate values of height and area of densitometric peaks are presented in Table 1.

On the basis of results obtained the relationships between height or area of densitometric peaks and contents of ofloxacin or pefloxacin were settled. These relationships were described using proper equations. They are presented in Table 2. All presented equations are characterized by large determination coefficients. In the case of equations 2, 3, and 4 the determination coefficients were larger than 99%. The widest range of contents of compound investigated was obtained in the case of relationship between area of densitometric peak and contents of ofloxacin in solution ($0.0094 \div 2.5290 \mu\text{g}/5 \mu\text{L}$).

The linear relationships were used to determine the contents of compounds investigated in pharmaceutical preparations: Abaktal in the case of pefloxacin and Tarivid in the case of ofloxacin.

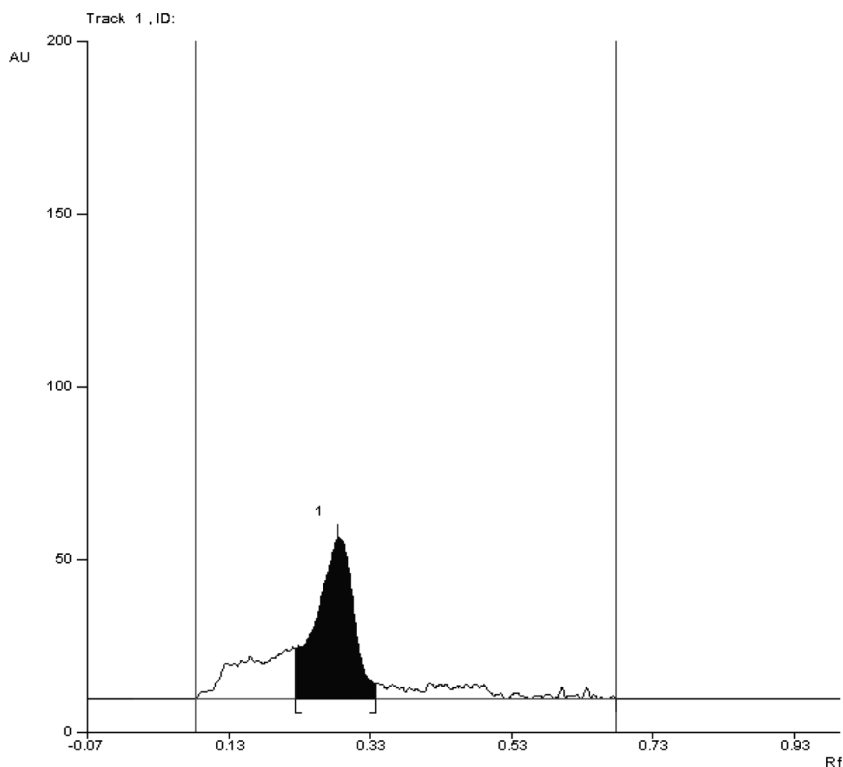


FIGURE 4 The example densitogram of pefloxacin determined in Abaktal.

TABLE 3 The Values of Height and Area of Densitometric Peaks as well as the Contents of Spotted and Determined Ofloxacin and Pefloxacin in Drugs Investigated

Compound investigated	Values of height and area of densitometric peaks obtained		Spotted contents of compound [μg]	Determined contents of compound [μg]
Ofloxacin	Peak height [AU]	53.7	0.1000	0.1085
	Peak area [AU]	2029.4	0.1000	0.1002
Pefloxacin	Peak height [AU]	47.7	0.1500	0.1589
	Peak area [AU]	2051.1	0.1500	0.1414

The Figures 3 and 4 present the example densitograms of ofloxacin and pefloxacin, respectively, in preparations investigated. The values of height and areas of densitometric peaks obtained served to calculate the contents of ofloxacin and pefloxacin in Tarivid and Abaktal. All these values, as well as the amount of spotted and calculated contents of compounds investigated, are presented in Table 3.

The values of contents of ofloxacin and pefloxacin, calculated on the basis of equations 1–4 only slightly differ from their contents in pharmaceutical preparations investigated. It certifies that the method can be used for quantitative determination of ofloxacin and pefloxacin in drugs. It can be used as well to determine the contents of active substances in the drugs quality control in production process.

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