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ARTICLES

Identification and Determination of Antibacterial Substances in Drugs by Capillary Electrophoresis

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Abstract—Methods of the quality control of finished dosage forms by capillary zone electrophoresis and micellar electrokinetic chromatography have been proposed. The optimum conditions for the separation and determination of different classes of antibacterial substances, penicillins, fluoroquinolones, nitrofurans, sulfanilamides, metronidazole, and chloramphenicol have been selected. The analytical range for the active ingredients of drugs was 1–1000 mg/g for solid and 0.001–0.50% for liquid drugs. The relative standard deviation of the results of analysis did not exceed 4%.

Keywords: antibiotics, penicillins, fluoroquinolones, nitrofurans, sulfanilamides, metronidazole, chloramphenicol, finished dosage forms, capillary electrophoresis

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INTRODUCTION

The modern level of the development of the chemical-pharmaceutical industry places serious requirements on the quality of manufactured products; this is due to both the therapeutic effect of the finished dosage forms (**FDF**) and their identity. Unlike the first problem, the problem of analytical control can be solved using modern chemical and physicochemical methods of analysis.

Antibiotics represent a large group of FDF. According to the WHO data, the most part (approximately 42%) of counterfeit FDF are antibiotics. Traditionally for the identification and determination of the active ingredients of antibacterial drugs analysts use spectrophotometry [1–5] and HPLC with different methods of detection [6–12]. Often these methods are labor-intensive or unavailable because of the high cost of the used equipment. In our opinion, a more available and rapid method of the analysis of FDF is capillary electrophoresis (**CE**), the efficiency of separation and the environmental compatibility of which are superior to many methods of analysis, including HPLC.

In the present work, we demonstrate a possibility of the rapid and effective evaluation of the quality of FDF based on antibiotics from different classes using CE.

EXPERIMENTAL

Equipment. A Capel-105M CE system (Lumex, Russia) equipped with a spectrophometric detector and an unmodified quartz capillary (d_{in} = 75 and 50 μm, l = 50 and 60 cm) was used. The registration and processing of the data was performed using MultiChrom and Elforan software (Ampersend, Russia). In the process of sample preparation, a Mini Spin ultracentrifuge (Eppendorf, Germany) was used.

Solvents and reagents. Standard samples of the following antibacterial substances were used (Fluka Analytical, Sigma Aldrich, and Dr. Ehrenstorfer): amoxicillin trihydrate, ampicillin trihydrate, danofloxacin mesylate, dicloxacillin sodium salt hydrate, cloxacillin sodium salt monohydrate, levofloxacin, lomefloxacin hydrochloride, metronidazole, nitrofurazone, oxacillin sodium salt hydrate, penicillin G sodium salt, pefloxacin methanesulfonate monohydrate, sulfadiazine, sulfadimethoxine, sulfamerazine, sulfachloropyrazidine, furazolidone, furaltadone, chloramphenicol, ciprofloxacin, enoxacin, and enrofloxacin.

Standard solutions of antibacterial substances (1 mg/mL) were prepared by dissolving a precisely weighed portion of a component in distilled water, 0.1 M HCl, or acetonitrile (depending on the substance nature) and stored at 4°C. Working solutions were prepared in the day of use by the dilution of stock solutions with distilled water.

For the preparation of the leading electrolyte, we used sodium dodecyl sulfate (**SDS**) (Merck), acetonitrile (Prolabo), sodium tetraborate decahydrate (Sigma-Aldrich), sodium phosphate dibasic dodecahydrate (Prolabo), sodium phosphate monobasic dihydrate (Prolabo), tetrabutylammonium phosphate (**TBA**) (Fluka Analytical), α-γ-cyclodex-

trines (**CD**) (Sigma-Aldrich), and twice-distilled water (GOST 7602-72)

Method of the determination of the active ingredient of antibacterial FDF. *Tablets*. A FDF tablet (0.1–0.8 g) was ground in a mortar, a precisely weighed portion (20–40 mg) was taken and dissolved in a centrifuge tube in 10 mL of a suitable solvent (0.1 M HCl was used for fluoroquinolones; acetonitrile for sulfanilamides, nitrofurans, chloramphenicol, and metronidazole, and distilled water was used for penicillins). An aliquot of 1 mL was transferred into a 100.0 mL flask and diluted with distilled water.

Solutions. Liquid dosage forms were sequentially diluted with distilled water to the concentration of analyzed substance in the range 0.5–20.0 mg/L. The solution obtained was centrifuged for 5 min at 5000 rpm and electrophoretic determination was performed.

RESULTS AND DISCUSSION

Fluoroquinolones (**FQ**) (lomefloxacin, danofloxacin, enoxacin, ciprofloxacin, levofloxacin, enrofloxacin, pefloxacin), sulfanilamides (**SA**) (sulfadimethoxine, sulfamerazine, sulfadiazine, sulfachloropyrazidine), nitrofurans (**NF**) (furazolidone, nitrofurazone, furaltadone), penicillins (**PC**) (amoxicillin, oxacillin, ampicillin, cloxacillin, penicillin G, dicloxacillin), metronidazole (imidazole derivative), and chloramphenicol were studied.

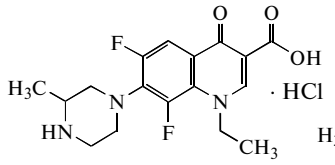
Selection of conditions of electrophoretic separation. As the analyzed antibacterial substances form organic anions (structural formulas and acidity constants are presented in Fig. 1) in neutral and alkaline solutions, it was preferable to use capillary zone electrophoresis (**CZE**) for their separation, identification, and determination. To select the optimum leading electrolyte for FQ and SA, we studied the capabilities of sodium tetraborate (pH 9.2) and a phosphate buffer solution (pH 7.0–8.5). The high selectivity of the separation of a mixture of 11 antibacterial substances is provided by a 25 mM phosphate buffer solution; however, because of its high conductivity, it is necessary to use a capillary with an inner diameter of 50 μm. For capillaries with a smaller diameter, higher efficiency of analyte separation because of the lower radial temperature gradient was characteristic, which resulted in a weaker peak broadening. In the CZE separation of ionogenic components, the pH of the leading electrolyte determines the selectivity of peaks and also affects the areas and heights of these peaks. The leading electrolyte with pH 8.5 was optimum for the separation of antibiotics of fluoroquinolone and sulfanilamide series. When the pH of the buffer solution was changed from 8.5 to 7.5, the selectivity of FQ and SA peaks decreased by 2–3 times (excluding ciprofloxacin and enoxacin), which was accompanied by the loss of the sensitivity of determination because of a decrease in the areas and heights of analyte peaks.

The separation of a PC mixture by CZE is low-efficient, because some of antibacterial substances have close pK_a values (Fig. 1). The application of micellar electrokinetic chromatography (**MEKC**) made possible the complete separation of a mixture of six PC. The presence of α-CD in the composition of the leading electrolyte did not substantially affect the electrophoretic behavior of the mixture components. On the contrary, γ-CD reduced the selectivity of the separation of the peaks of antibiotics. The presence of acetonitrile and TBA changed the distribution constants of ampicillin, amoxicillin, and oxacillin between the solution and the micellar phase and reduced the efficiency of their separation. The effect of the concentration of the micelle-forming agent on the electrophoretic behavior of analytes was evaluated: with an increase in the concentration of SDS in the background electrolyte from 20 to 50 mM, height of PC peaks decreased and, consequently, the sensitivity of the determination also decreased. The leading electrolyte containing 30 mM of SDS provided the high selectivity of separation with preserving the needed sensitivity.

It was found by a comparison of the capabilities of sodium tetraborate and a phosphate buffer solution that a 10 mM phosphate buffer solution (pH 6.0–8.5) provided better separation of the peaks of antibiotics of penicillin series at a slightly lower efficiency of separation. The pH of the background solution substantially affects the electrophoretic behavior of PC, by determining the speciation of the separated substances in the electrolyte phase and the degree of interaction with the micellar *pseudostationary phase*: in the pH range 6.0–8.5, the average separation efficiency reached a maximum at pH 7.0 (~364000 theoretical plates). The optimum was the leading electrolyte consisting of a 10 mM of phosphate buffer solution (pH 7.0) and 30 mM of SDS.

In the separation of HF, CZE cannot be efficient, because furazolidone and furaltadone have close pK_a values, while nitrofurazone is a very weak organic acid (Fig. 1). The use of a leading electrolyte consisting of 10 mM of sodium tetraborate and 30 mM of SDS ensures the complete separation of a mixture of three NF. The addition of CDs as members of competing reactions with analytes to the composition of the leading electrolyte does not affect the efficiency of separation. However, the presence of organic solvent acetonitrile (5–20 vol %) substantially increased the sensitivity of determination, which attained maximum values at the acetonitrile concentration 10 vol % which, on the other hand, detrimentally affected selectivity (Fig. 2). The further study of the electrophoretic behavior of HF at different compositions of the background phosphate buffer solution showed that the electrolyte consisting of 10 mM of sodium tetraborate, 40 mM SDS, and 10 vol % of acetonitrile was an appropriate electrolyte (Table 1).

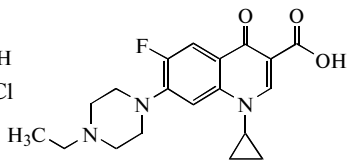
Lomefloxacin hydrochloride



$pK_a(COOH) = 2.4$

$pK_a(NH^+) = 8.8$ [12]

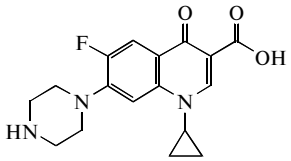
Enrofloxacin



$pK_a(COOH) = 6.2$

$pK_a(NH^+) = 7.2$ [13]

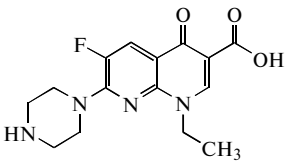
Ciprofloxacin



$pK_a(COOH) = 6.0$

$pK_a(NH^+) = 8.8$ [14]

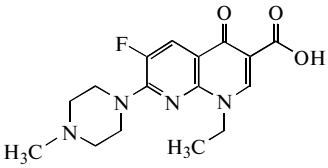
Enoxacin



$pK_a(COOH) = 6.2$

$pK_a(NH^+) = 8.8$ [14]

Pefloxacin



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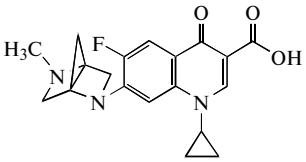
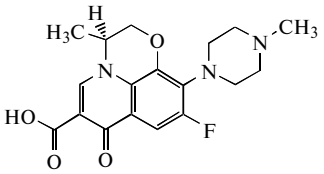
Danofloxacin



$pK_a(COOH) = 6.2$

$pK_a(NH^+) = 8.1$ [13]

Levofloxacin



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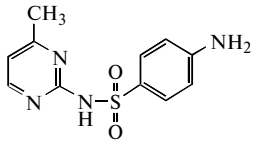
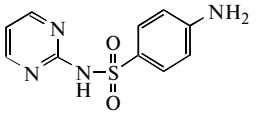
Sulfadiazine



$pK_a = 6.5$ [15]

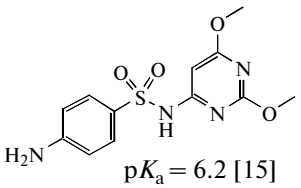
Sulfamerazine



$pK_a = 7.0$ [16]

Nitrofurazone (nitrofural)

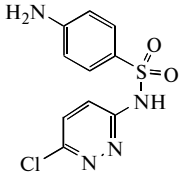
Sulfadimethoxine



$pK_a = 6.2$ [15]

Ampicillin

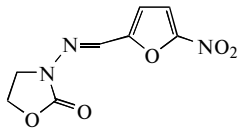
Sulfachloropyrazidine



$pK_a = 5.5$ [15]

Oxacillin monohydrate

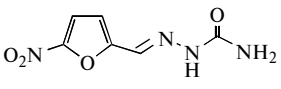
Furazolidone



$pK_a = 4.9$ [16]

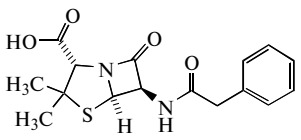
Cloxacillin sodium salt

Furaltadone



$pK_a = 10.0$ [16]

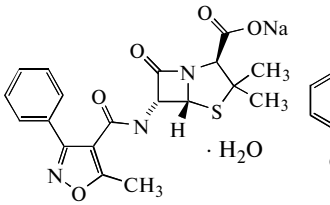
Amoxicillin



$pK_a(COOH) = 2.6$

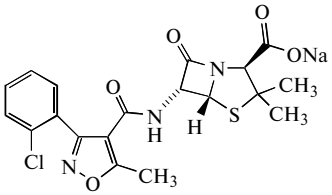
$pK_a(NH^+) = 7.3$ [17]

Penicillin G sodium salt

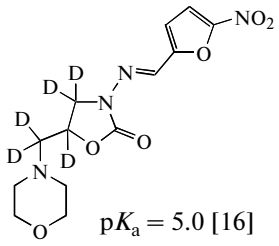


$pK_a = 2.7$ [17]

Dicloxacillin sodium salt hydrate

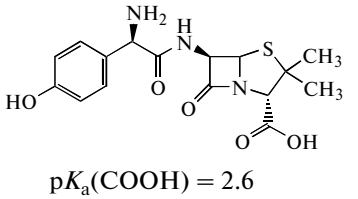


$pK_a = 2.7$ [17]



$pK_a = 5.0$ [16]

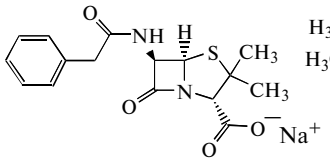
Chloramphenicol



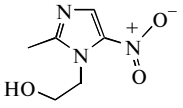
$pK_a(COOH) = 2.6$

$pK_a(NH^+) = 7.3$

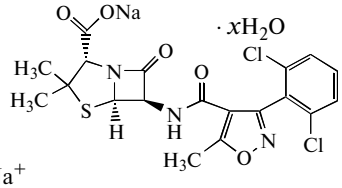
pK_a (ArOH) = 9.7 [17]



Metronidazole



$pK_a = 2.4$ [18]



$pK_a = 2.7$ [17]

Fig. 1. Structural formulas and acidity constants of the determined antibiotics; (–) means no data.

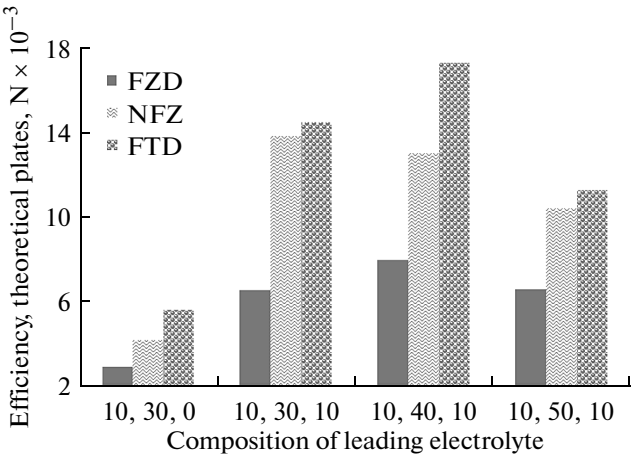


Fig. 2. Effect of the composition of leading electrolyte (mM of sodium tetraborate, mM of SDS, vol % of acetonitrile) on the efficiency of the separation of furazolidone (FZD), nitrofurazone (NFZ), and furaltadone (FTD).

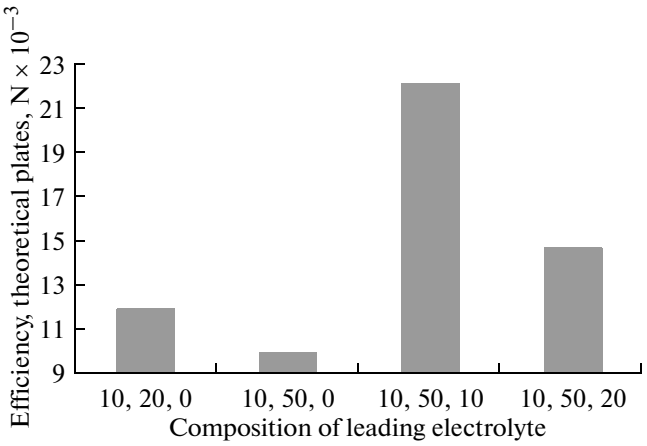


Fig. 3. Effect of the composition of leading electrolyte (mM of sodium tetraborate, mM of SDS, vol % of acetonitrile) on the efficiency of electromigration of metronidazole.

Metronidazole, which is a strong acid (Fig. 1), should be determined by CZE using a positive source of high-voltage power and a leading electrolyte with pH > 7. However, it was not observed in reality. It was found that the identification and determination of this substance are possible under MEKC conditions, when SDS and acetonitrile were used as a micelle-forming agent and an organic additive, respectively, because of which the hydrophobic interactions between the analyte and the micellar phase were reduced. Figure 3 demonstrates the effect of the composition of the leading electrolyte on the efficiency of metronidazole detection. An electrolyte consisting of 10 mM of sodium tetraborate, 50 mM of SDS, and 10 vol % of acetonitrile was the optimum.

It was found that chloramphenicol can be determined by CZE and MEKC methods. When MEKC was used, the results were more reproducible than in CZE determination. For the analysis of FDF containing chloramphenicol, an electrolyte consisting of 10 mM of sodium tetraborate, 40 mM of SDS, and 10 vol % of acetonitrile was used.

The optimum conditions for the identification and determination of antibiotics from different classes in FDF and electropherograms of standard solutions are presented in Table 1 and in Fig. 4, respectively. Table 2 presents the analytical characteristics of the developed methods of FDF analysis. The analytical range for antibiotics is 1–1000 mg/g for solid samples and 0.001–0.50% for liquid FDF.

Table 1. Optimum conditions of the electrophoretic separation of antibiotic mixtures

Mixture of antibiotics	Separation method	Conditions of separations	Composition of the leading electrolyte
Lomefloxacin, danofloxacin, enoxacin, ciprofloxacin, levofloxacin, enrofloxacin, pefloxacin, sulfadimethoxine, sulfamerazine, sulfadiazine, sulfachloropyrazidine	CZE	Capillary 75 cm × 50 μm, 280 nm, 35°C, +25 kV, sample injection 30 mbar × 10 s	25 mM of a phosphate buffer solution with pH 8.5
Furazolidone, nitrofurazone, furaltadone	MEKC	Capillary 60 cm × 75 μm, 362 nm, 20°C, +25 kV, sample injection 30 mbar × 10 s	10 mM of sodium tetraborate, 40 mM of SDS, and 10 vol % of acetonitrile
Metronidazole	MEKC	Capillary 60 cm × 75 μm, 312 nm, 20°C, +25 kV, sample injection 30 mbar × 10 s	10 mM of sodium tetraborate, 50 mM of SDS, and 10 vol % of acetonitrile
Chloramphenicol	MEKC	Capillary 60 cm × 75 μm, 220 nm, 20°C, +25 kV, sample injection 30 mbar × 10 s	10 mM of sodium tetraborate, 40 mM of SDS, and 10 vol % of acetonitrile
Amoxicillin, oxacillin, ampicillin, cloxacillin, penicillin G, dicloxacillin	MEKC	Capillary 60 cm × 75 μm, 210 nm, 20°C, +25 kV, sample injection 30 mbar × 10 s	10 mM of a phosphate buffer solution with pH 7.0, 30 mM of SDS

Table 2. Analytical characteristics of the method for the determination of PC, FQ, NF, SA, metronidazole, and chloramphenicol in FDF by CE

Antibacterial substance	Migration time, min	Equation of calibration characteristic	R^2	Analytical range, mg/g
Fluoroquinolones and sulfanilamides				
Lomefloxacin	7.2 ± 0.2	$y = 0.8714x$	0.9999	3–1000
Danofloxacin	7.6 ± 0.2	$y = 0.9560x$	0.9989	4–1000
Enoxacin	7.7 ± 0.2	$y = 1.3590x$	0.9999	5–1000
Ciprofloxacin	7.8 ± 0.2	$y = 0.8662x$	0.9999	3–1000
Levofloxacin	8.0 ± 0.2	$y = 0.8463x$	0.9996	3–1000
Enrofloxacin	8.3 ± 0.2	$y = 0.8245x$	0.9999	4–1000
Pefloxacin	8.6 ± 0.2	$y = 0.9123x$	0.9998	4–1000
Sulfadimethoxine	10.0 ± 0.3	$y = 0.7725x$	0.9997	4–1000
Sulfamerazine	10.2 ± 0.3	$y = 0.7642x$	0.9998	5–1000
Sulfadiazine	10.5 ± 0.3	$y = 1.0321x$	0.9997	6–1000
Sulfachloropyrazidine	10.7 ± 0.3	$y = 1.4685x$	0.9997	10–1000
Nitrofurans				
Furazolidone	5.2 ± 0.1	$y = 0.1017x$	0.9999	2–1000
Nitrofurazone	5.5 ± 0.1	$y = 0.1458x$	0.9999	2–1000
Furaltadone	5.7 ± 0.1	$y = 0.1971x$	0.9999	3–1000
Penicillins				
Amoxicillin	5.0 ± 0.1	$y = 0.2249x$	0.9997	2–1000
Oxacillin	6.1 ± 0.1	$y = 0.1243x$	0.9999	1–1000
Ampicillin	6.2 ± 0.1	$y = 0.1894x$	0.9999	2–1000
Cloxacillin	6.3 ± 0.1	$y = 0.1169x$	0.9998	1–1000
Penicillin G	7.1 ± 0.1	$y = 0.3171x$	0.9998	4–1000
Dicloxacillin	7.2 ± 0.1	$y = 0.1093x$	0.9998	1–1000
Metronidazole, chloramphenicol				
Metronidazole	4.7 ± 0.1	$y = 1.7626x$	0.9998	2–1000
Chloramphenicol	6.0 ± 0.1	$y = 0.3221x$	0.9999	4–1000

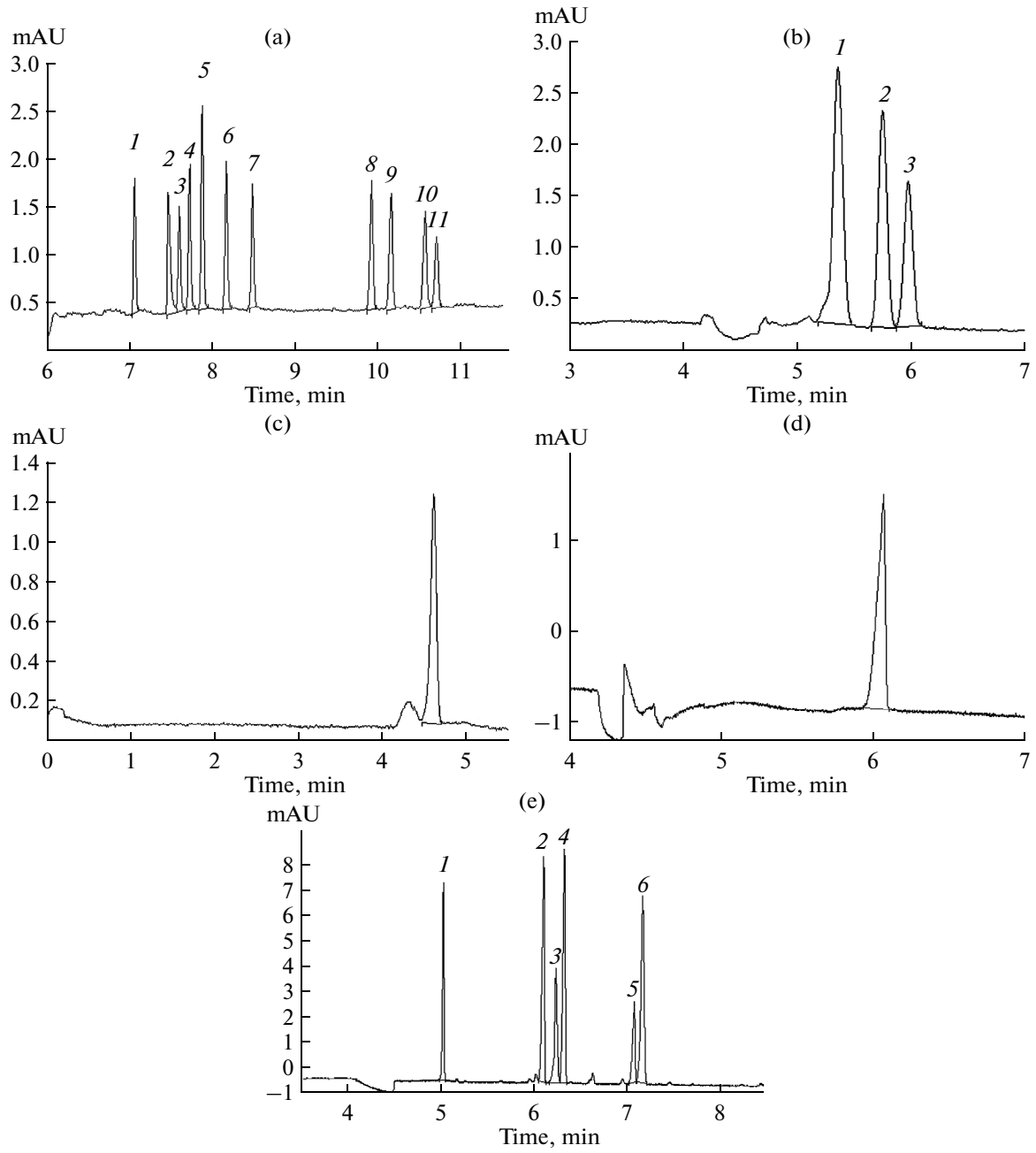


Fig. 4. Electropherograms of standard solutions of a mixture of (a) FC and SA 20 mg/L, (b) NF 10 mg/L, (c) metronidazole 10 mg/L, (d) chloramphenicol 20 mg/L, and (e) PC 20 mg/L obtained by (a) CZE and (b)–(d) MEKC respectively, (a) *1*, lomefloxacin; *2*, danofloxacin; *3*, enoxacin; *4*, ciprofloxacin; *5*, levofloxacin; *6*, enrofloxacin; *7*, pefloxacin; *8*, sulfadimethoxine; *9*, sulfamerazine; *10*, sulfadiazine; *11*, sulfachloropyrazidine; (b) *1*, furazolidone; *2*, nitrofurazone; *3*, furaltadone; (c) metronidazole; (d) chloramphenicol; (e) *1*, amoxicillin; *2*, oxacillin; *3*, ampicillin; *4*, cloxacillin; *5*, penicillin G; *6*, dicloxacillin.

The conditions of the electrophoretic separation of antibacterial substances selected in the work were used for the verification of the identity of drugs and analysis of the correspondence of the quantity of the active ingredient and the quantity indicated in the label. The migration times of the identified antibiotics correspond to the used standard samples, which confirms the identity of the studied drug forms. The obtained results of FDF analysis are presented Table 3. It can be seen that concentration of the active ingredient of some drugs did not correspond to the label claim.

The validity of the procedure of analysis was tested using the added–found method (Table 4). It was found that, on the addition of a known amount of antibacterial substances (from 20 to 125 mg), the recoveries of analytes were from 92 to 98%. The relative standard deviation of the results of analysis did not exceed 4%.

Table 3. Results of analysis of commercial FDF ($n = 4$; $P = 0.95$)

Brand name (active ingredient)	Label claim, mg in tablet (%)	Found concentration of active ingredient, mg in tablet (%)	
		$\bar{x} \pm \Delta x$	RSD, %
Tablets Ciprofloxacin (ciprofloxacin)	250	241 ± 14	2
Injection solution Baytril 5% (enrofoxacin)	(5)	5.7 ± 0.4	3
Tablets Ciprolet (ciprofloxacin)	500	292 ± 17	2
Tablets Sulfadimethoxine (sulfadimethoxine)	500	241 ± 9	1
Tablets Furazolidone (furazolidone)	50	34 ± 2	2
Tablets Furacilin (nitrofural)	20	19 ± 1	2
Tablets Furacillin (expired) (nitrofural)	20	22 ± 1	1
Tablets Metronidazole (metronidazole)	250	285 ± 2	1
Eye drops Levomycetin (chloramphenicol) (sample no. 1)	(0.25)	0.18 ± 0.01	3
Eye drops Levomycetin (chloramphenicol) (sample no. 2)	(0.25)	0.11 ± 0.01	1
Tablets Levomycetin (chloramphenicol) (sample no. 1)	250	211 ± 8	2
Tablets Levomycetin (chloramphenicol) (sample no. 2)	500	377 ± 20	3
Tablets Ampicillin (ampicillin)	250	246 ± 16	3
Tablets Amosin (amoxicillin)	500	532 ± 14	1

Table 4. Recoveries of antibiotics in the analysis of drugs by CE ($n = 4$, $P = 0.95$)

Antibacterial substance	Added, mg	Found, mg	Recovery, %	RSD, %
Fluoroquinolones and sulfanilamides				
Lomefloxacin	100	95 ± 4	95 ± 4	2
Danofloxacin	90	84 ± 4	93 ± 4	2
Enoxacin	95	88 ± 7	95 ± 8	4
Ciprofloxacin	105	97 ± 5	92 ± 4	2
Levofloxacin	125	117 ± 5	93 ± 4	2
Enrofloxacin	100	95 ± 4	95 ± 4	2
Pefloxacin	100	94 ± 2	94 ± 2	1
Sulfadimethoxine	85	81 ± 2	95 ± 2	1
Sulfamerazine	110	109 ± 4	95 ± 3	2
Sulfadiazine	85	81 ± 3	95 ± 3	2
Sulfachloropyrazidine	115	109 ± 4	95 ± 3	2
Nitrofurans				
Furazolidone	30	28 ± 1	93 ± 1	1
Nitrofurazone	20	19 ± 1	93 ± 4	2
Furaltadone	30	28 ± 1	95 ± 4	2
Penicillins				
Amoxicillin	110	107 ± 2	98 ± 2	1
Oxacillin	125	121 ± 2	97 ± 1	1
Ampicillin	95	92 ± 6	97 ± 6	2
Cloxacillin	95	92 ± 2	97 ± 2	1
Penicillin G	120	113 ± 7	94 ± 6	2
Dicloxacillin	110	105 ± 4	96 ± 4	2
Metronidazole, chloramphenicol				
Metronidazole	130	125 ± 5	96 ± 4	2
Chloramphenicol	120	110 ± 6	92 ± 5	2

Thus, the experimental data demonstrate the possibility of the application of the proposed methods to the determination of antibiotics in FDF with the aim of the quality control of pharmaceutical products.

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