Cefuroxime is a second-generation cephalosporin which exhibits a wide range of antibacterial activity. It is effective against most bacteria responsible for eye infections, in particular Staphylococcus spp. and Streptococcus spp. (1). It is also effective against Haemophilus influenzae, the majority of Enterobacteriaceae, Neisseria spp., Moraxella catarrhalis and against anaerobic bacteria such as Clostridium bacilli and Gram-negative bacteria of genus Fusobacterium. Apart from its special activity against Staphylococcus spp. and Streptococcus spp., the most important property of cefuroxime is its resistance to staphylococcal β-lactamases (penicillinases) and plasmid β-lactamases of broad substrate profile of TEM and SHV type. The antimicrobial activity of cefuroxime, similarly to other β-lactam antibiotics, depends mostly on the presence of the closed β-lactam ring in their molecules. Cefuroxime resistance to hydrolytic activity of bacterial enzymes of β-lactam group makes this antibiotic more efficient in comparison with e.g., penicillins in the treatment of the infections caused by bacteria producing plasmid and staphylococcal lactamases. β-Lactamases are responsible for rapid hydrolysis of β-lactam ring in the molecule of an antibiotic, loss of antimicrobial properties and, consequently, the resistance of the bacteria to the antibiotic. The application of cefuroxime in the eye drops for the treat-
ment of the infections, especially those caused by staphylococci and streptococci, is fully justified because these, as well as many other pathogens causing eye infections, show great ability to produce β-lactamases. Cefuroxime is applied in the form of drops, most often at the concentration of 1%, in topical treatment of such eye infections as blepharitis, conjunctivitis, and keratitis or in the form of intracameral injections in the treatment and prevention of ocular and periocular infections. In the case of suppurative keratitis, drops of increased activity are used, i.e., drops at the concentration of 5% (2–4). Cefuroxime permeates well from conjunctival sac into aqueous humor, and it is less toxic towards corneal epithelium in comparison with other topically applied antibiotics, e.g., aminoglycosides (5).

In aqueous solutions, cefuroxime undergoes hydrolysis whose mechanism is dependent on the pH of a solution (6, 7). Optimal pH for cefuroxime stability is in the range of 4.5–7.3 (8). Apart from pH, the rate of cefuroxime degradation in aqueous solutions is also influenced by temperature, composition of a solvent, exposure to light, type of packaging and the presence of bacteria. Cefuroxime in 1% and 5% aqueous solutions was not compatible with benzalkonium chloride at the concentration higher than 0.002% (9–11), thimerosal at the concentration over 0.002% (10, 11), and phenylmercuric acetate at the concentration of 0.002% in 5% drops (12). It was also not compatible with chlorhexidine diacetate, whose incompatibility with cefuroxime was observed at the concentrations of 0.0075 and 0.02% in 5% drops (9, 10) and at the concentration of 0.01% in 1% drops (11). Eye drops containing cefuroxime are not commercially available on account of the limited stability of the antibiotic in aqueous solutions. They are prepared in pharmacies exclusively for the needs of the patients of ophthalmological wards by dissolving sodium cefuroxime, a commercial dry form of the drug used for intravenous and intramuscular injections. Cefuroxime standard: sodium cefuroxime CRS (LGC, UK), acetonitrile (gradient grade), HPLC grade glacial acetic acid (JT Baker), citric acid monohydrate, sodium citrate dihydrate p.a., uracil p.a. (Sigma-Aldrich), HPLC standard: sodium cefuroxime CRS (LGC, UK), acetonitrile (gradient grade), HPLC grade glacial acetic acid (JT Baker), citric acid monohydrate, sodium citrate dihydrate p.a., uracil p.a. (Sigma-Aldrich), HPLC ultrapure water (System Synergy – Millipore).

The following solutions were used to prepare the drops: sterile solutions of citrate buffers: citrate buffer I (51.005 mM tri-sodium citrate dihydrate, 3.57 mM citric acid monohydrate, distilled water ad 1000.0 g), citrate buffer II (102.01 mM tri-sodium citrate dihydrate, 7.14 mM citric acid monohydrate, distilled water ad 1000.0 g) and citrate buffer III (204.02 mM tri-sodium citrate dihydrate, 14.28 mM citric acid monohydrate, distilled water ad 1000.0 g). The viscosity of the drops was enhanced with 6% sterile solution of polyvinyl alcohol. The drops were preserved with 0.04% sterile solution of phenylmercuric borate and β-phenylethyl alcohol. Kodym et al. examined antimicrobial activity of cefuroxime in 1% drops prepared in citrate buffer of pH 6.15–6.20 in the formulations whose viscosity was enhanced by polyvinyl alcohol and preserved with thiomersal at the concentration of 0.002% or phenylmercuric borate at the concentration of 0.001% mixed with 0.4% β-phenylethyl alcohol. After 30 days of storage of the drops at the temperature of 4°C, antimicrobial activity of cefuroxime, determined with cylinder-plate method according to PPh VI using the standard strain Staphylococcus aureus ATCC 6538P, did not decrease and remained stable at the initial level of 100% (11). The relatively long period of antimicrobial activity of cefuroxime in citrate buffer motivated the authors to examine the chemical stability of cefuroxime in 1% and 5% buffered drops using HPLC method.

**EXPERIMENTAL**

**Reagents and solutions**

The following reagents were used during the studies: Biofuroksym® (Cefuroximum natricum) IBA Bioton, 1.5 g ampoules, dry substance for intraocular and periocular infections. Cefuroxime standard: sodium cefuroxime CRS (LGC, UK), acetonitrile (gradient grade), HPLC grade glacial acetic acid (JT Baker), citric acid monohydrate, sodium citrate dihydrate p.a., polyvinyl alcohol molecular mass 72000 p.a. (POCh S.A.), β-phenylethyl alcohol p.a., phenylmercuric borate p.a., sodium acetate trihydrate p.a., uracil p.a. (Sigma-Aldrich), HPLC ultrapure water (System Synergy – Millipore).

The following solutions were used to prepare the drops: sterile solutions of citrate buffers: citrate buffer I (51.005 mM tri-sodium citrate dihydrate, 3.57 mM citric acid monohydrate, distilled water ad 1000.0 g), citrate buffer II (102.01 mM tri-sodium citrate dihydrate, 7.14 mM citric acid monohydrate, distilled water ad 1000.0 g) and citrate buffer III (204.02 mM tri-sodium citrate dihydrate, 14.28 mM citric acid monohydrate, distilled water ad 1000.0 g). The viscosity of the drops was enhanced with 6% sterile solution of polyvinyl alcohol. The drops were preserved with 0.04% sterile solution of phenylmercuric borate and β-phenylethyl alcohol.

**Apparatus**

Shimadzu high performance liquid chromatography system (Kyoto, Japan) equipped with two LC-20 AD piston pumps, DGU-20 A5 five-line degasser, SIL-20 AC autosampler, CTO-20 AC column oven and UV-VIS SPD-M20A detector were used to carry out the analysis. Ultrapure water was obtained using...
Synergy system (Millipore, France). Sonic 10 ultrasound washer (Polsonic, Poland) was used to degas the mobile phase. The drops containing cefuroxime were prepared in KL-21 laminar flow cabinet (Polon, Poland). Measurements of osmotic pressure were performed using Krioskop 800cl osmometer (Trident Med. S.C., Poland). To analyze the viscosity, Höppler KF-10 viscosimeter (Medingen, Germany) was used. pH measurements of the solutions and drops containing cefuroxime as well as the determinations of mobile phase for HPLC were done with CP-502 pH-meter (Elmetron, Poland). During the process of sterilization, SP-65W dry heat sterilizer (Wamed, Poland) and AS 446 WPA steam sterilizer (SMS, Poland) were used. Densito 30 PX densitometer (Mettler Toledo, Switzerland) was used to measure density of the solutions. The prepared drops were stored in MED-28 pharmaceutical coolers (Kirsch, Germany). The solutions and drops were dispensed for analysis with CP 100 and CP 1000 micropipettes (PZ HTL S.A, Poland), WPS720/C/2 precision balance (Radwag, Poland) and Sartorius Expert LE 225D balance (Sartorius, Germany) were used during the studies.

Preparation of sterile aqueous solutions of additives
Sterile aqueous solutions of citrate buffers, polyvinyl alcohol and 0.04% solution of phenylmercuric borate were prepared as described (13).

Preparation of eye drops containing cefuroxime
Drops (1% and 5% w/w) were prepared under aseptic conditions (laminar flow cabinet) according to the composition presented in Table 1. Biofuroksym® (Cefuroxime natricum) was dissolved in appropriate citrate buffer or in sterile water. Then, the prescribed quantity of auxiliary 0.04% solution of phenylmercuric borate and β-phenylethyl alcohol was added. After mixing, the drops were filtered through Sartorius microbiological membrane filter of 0.22 µm pore diameter. In case of formulations of enhanced viscosity, the prescribed quantity of sterile auxiliary solution of polyvinyl alcohol was added to the drops after their preservation and filtration through the Sartorius filter. The eye drops were poured into sterile infusion bottles, tightly closed with rubber corks and metal bottle caps. The drops were stored in pharmaceutical coolers at the temperature of 4°C and 20°C for 30 days, protected from light.

pH, osmotic pressure, viscosity of the eye drops and organoleptic analysis
Measurements of pH and osmotic pressure were performed in fresh solutions and in drops containing cefuroxime as well as during storage at the temperature of 4°C and 20°C. In addition, in formulations containing polyvinyl alcohol the viscosity was analyzed (Tab. 2). For organoleptic analysis the appearance of the eye drops was evaluated, i.e., clarity, color and odor.

Assessment of cefuroxime stability in drops using HPLC method
Chromatographic conditions
Chromatographic separation was carried out on Grace Smart C-18, 5 µm, 150 mm × 4.6 mm column
Table 2. pH, osmotic pressure and viscosity changes in eye drops containing sodium cefuroxime (n = 3).

<table>
<thead>
<tr>
<th>Versions</th>
<th>1% w/w</th>
<th>5% w/w</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>01%</td>
<td>I</td>
</tr>
<tr>
<td>pH</td>
<td>0.01</td>
<td>1</td>
</tr>
<tr>
<td>Initial pH</td>
<td>7.39 ± 0.01</td>
<td>6.23 ± 0.00</td>
</tr>
<tr>
<td>pH after 30 days of storage</td>
<td>7.86 ± 0.01</td>
<td>6.24 ± 0.00</td>
</tr>
<tr>
<td>temp. 4°C</td>
<td>8.15 ± 0.01</td>
<td>7.00 ± 0.00</td>
</tr>
<tr>
<td>Initial osmotic pressure</td>
<td>46 ± 1</td>
<td>368 ± 2</td>
</tr>
<tr>
<td>temp. 4°C</td>
<td>53 ± 1</td>
<td>359 ± 1</td>
</tr>
<tr>
<td>temp. 20°C</td>
<td>75 ± 2</td>
<td>363 ± 3</td>
</tr>
<tr>
<td>Initial viscosity</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>temp. 4°C</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>temp. 20°C</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Preparation of samples for HPLC analysis

A volume of 250 μL of 1% drops or 100 μL of 5% drops was added to 3 separate volumetric flasks of 25 mL volume using semiautomated pipettes. Then, 0.5 mL of 5% drops was added to each flask. Uracil solution at the concentration of 0.04 g/100 mL was added to each flask. Uracil was used as a marker to determine the column dead time $t_0$ and to calculate the retention factor $k$ for cefuroxime peaks. Then, the flasks were filled with HPLC water up to 25 mL total volume and mixed. The solutions were filtered through 0.45 μm membrane filter into vials. One injection was made from each of the three vials, which provided three results for each analyzed formulation version. Cefuroxime concentration in the analyzed samples was calculated from the registered peak areas using the equation of the calibration curve.

Validation

Specificity

The placebo solution of additives included in 1% and 5% drops containing cefuroxime was prepared, its concentration and composition were the same as in the formulation no. 4 of the drops. The placebo solution was analyzed in the same way as the drops containing cefuroxime. Additives, contained in the placebo, were monitored using HPLC. The wavelength of the mobile phase was 273 nm. The column temperature was 25°C. The injection volume was 10 μL for 1% drops and 5 μL for 5% drops.
polyvinyl alcohol, β-phenylethyl alcohol and phenylmercuric borate did not have any influence on the result of the determinations because at the retention time characteristic for cefuroxime and analytical wavelength of 273 nm they did not show any absorbance. Cefuroxime peak did not interfere with peaks of degradation products, their retention times were lower than cefuroxime retention time (Fig. 1). Average retention factor for cefuroxime was equal $k = 3.34$ during the whole cycle of examinations, whereas resolution $R_s$ of cefuroxime peaks was not lower than 3. The obtained number of theoretical plates $N$ was over 1500. The tailing coefficient $TF$ for cefuroxime did not exceed 1.5.

Accuracy and precision
Accuracy and precision of the method of qualitative determination of cefuroxime in drops were established by the analysis of model solutions of the drops of versions 4 and IV. The solutions contained, respectively, 5.0 mg/mL, 7.5 mg/mL, 10 mg/mL and 12.5 mg/mL of cefuroxime for version 4 and 25 mg/mL, 37.5 mg/mL, 50 mg/mL and 62.5 mg/mL for version IV. For each solution, 6 samples were prepared for injection as described in paragraph Preparation of samples for HPLC analysis and chromatographic analysis was performed following the point Chromatographic conditions. The percentage of recovery was adopted as the measure of the method accuracy and was calculated according to the following formula:

$$\text{Recovery (\%)} = \frac{\text{determined concentration}}{\text{calculated concentration}} \times 100 \%$$

Relative standard deviation (RSD) for six injections of the samples of model drops was from 0.14% to 0.82% for 1% drops and from 0.36% to 0.86% for 5% drops, respectively. The accuracy of the determinations of the changes in cefuroxime content in 1% solutions and in drops was from 98.71% to 101.99%, whereas for 5% solutions and drops it was from 99.8% to 102.01%.

Linearity
A 6-point calibration curve was prepared. It was based on the analysis of the content of standard cefuroxime solutions at the concentrations from 26 to 148.1 µg/mL. A volume of 10 µL of each standard solution was injected onto the column three times. The regression equation was prepared showing the relationship between the determined area of the peak and the concentration of cefuroxime standard in analyzed samples: $y = 22565x + 4970.4$, $R^2 = 0.9998$. The concentrations of cefuroxime standard solutions and determined peaks areas are presented in Table 3.

Limit of detection (LOD) and limit of quantitation (LOQ)
The limit of detection (LOD) of cefuroxime determined on the basis of the equation $\text{LOD} = 3.3 \times \text{Sy}/\text{a}$ was 2.03 µg/mL. The limit of quantitation of cefuroxime (LOQ) in accordance with the equation $\text{LOQ} = 10 \times \text{Sy}/\text{a}$ was 6.14 µg/mL, in which $\text{Sy}$ meant the scatter of y values calculated from the calibration curve and a meant the regression coefficient.

UV spectrophotometric analysis of cefuroxime and the main cefuroxime degradation product in 1% and 5% w/w drops stored at the temperature of 4°C and 20°C
The spectra of cefuroxime standard ($t_a = 8.5$ min) and those of the main degradation product ($t_a = 6.8$ min), determined with HPLC from 1% and 5% drops stored at the temperature of 4°C and 20°C, were analyzed. The spectrum of the solution of

<table>
<thead>
<tr>
<th>Concentration of cefuroxime standard solutions (µg/mL)</th>
<th>Peak area</th>
<th>Mean peak area</th>
</tr>
</thead>
<tbody>
<tr>
<td>26.0</td>
<td>585294</td>
<td>584920</td>
</tr>
<tr>
<td>53.0</td>
<td>1199417</td>
<td>1201112</td>
</tr>
<tr>
<td>74.7</td>
<td>1688730</td>
<td>1690143</td>
</tr>
<tr>
<td>98.2</td>
<td>2224666</td>
<td>2225473</td>
</tr>
<tr>
<td>123.3</td>
<td>2806735</td>
<td>2807756</td>
</tr>
<tr>
<td>148.1</td>
<td>3327462</td>
<td>3327936</td>
</tr>
</tbody>
</table>

Table 3. The concentrations of cefuroxime standard solutions and determined peaks areas for calibration curve.
Figure 1. Chromatogram of 5% drops containing cefuroxime, stored at the temperature of 20°C, version IV, day 30. A – uracil peak, B – peak of the main cefuroxime degradation product, C – cefuroxime

Figure 2 Chromatogram of freshly made 5% aqueous solution of cefuroxime heated for 10 min at the temperature of 60°C. A – uracil peak, B – peak of the main cefuroxime degradation product, C – cefuroxime

Figure 3. UV spectra in the range of 190-400 nm. I – UV spectrum of the main cefuroxime degradation product in aqueous solutions and in drops, appearing on chromatograms at the retention time $t_R = 6.8$, II – UV spectrum of cefuroxime
Table 4. Changes of cefuroxime concentration in aqueous solutions and in eye drops stored at the temperature of 4°C and 20°C for 30 days.

<table>
<thead>
<tr>
<th>Versions</th>
<th>1% w/w</th>
<th>5% w/w</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial conc. (mg/mL)</td>
<td>% initial concentration remaining</td>
</tr>
<tr>
<td></td>
<td>0(1%)</td>
<td>1</td>
</tr>
<tr>
<td>Storage time (temp. 4°C)</td>
<td>3 days</td>
<td>97.40 ± 0.35</td>
</tr>
<tr>
<td></td>
<td>6 days</td>
<td>96.61 ± 0.47</td>
</tr>
<tr>
<td></td>
<td>9 days</td>
<td>95.61 ± 0.15</td>
</tr>
<tr>
<td></td>
<td>12 days</td>
<td>94.28 ± 0.57</td>
</tr>
<tr>
<td></td>
<td>15 days</td>
<td>94.36 ± 0.88</td>
</tr>
<tr>
<td></td>
<td>18 days</td>
<td>93.50 ± 0.27</td>
</tr>
<tr>
<td></td>
<td>21 days</td>
<td>92.30 ± 0.18</td>
</tr>
<tr>
<td></td>
<td>24 days</td>
<td>92.38 ± 0.61</td>
</tr>
<tr>
<td></td>
<td>27 days</td>
<td><strong>90.02 ± 0.75</strong></td>
</tr>
<tr>
<td></td>
<td>30 days</td>
<td>88.52 ± 0.62</td>
</tr>
</tbody>
</table>

**Friedman’s test**

3 days: \( p = 0.00108 \)

6 days: \( p = 0.00108 \)

9 days: \( p = 0.00094 \)

12 days: \( p = 0.00120 \)

15 days: \( p = 0.00120 \)

18 days: \( p = 0.00086 \)

21 days: \( p = 0.00086 \)

24 days: \( p = 0.00086 \)

27 days: \( p = 0.00086 \)

30 days: \( p = 0.00086 \)

Friedman’s test

3 days: \( p = 0.00108 \)

6 days: \( p = 0.00108 \)

9 days: \( p = 0.00094 \)

12 days: \( p = 0.00120 \)

15 days: \( p = 0.00120 \)

18 days: \( p = 0.00086 \)

21 days: \( p = 0.00086 \)

24 days: \( p = 0.00086 \)

27 days: \( p = 0.00086 \)

30 days: \( p = 0.00086 \)

**% initial concentration remaining**

3 days: \( 97.40 ± 0.35 \)

6 days: \( 96.61 ± 0.47 \)

9 days: \( 95.61 ± 0.15 \)

12 days: \( 94.28 ± 0.57 \)

15 days: \( 94.36 ± 0.88 \)

18 days: \( 93.50 ± 0.27 \)

21 days: \( 92.30 ± 0.18 \)

24 days: \( 92.38 ± 0.61 \)

27 days: \( 90.02 ± 0.75 \)

30 days: \( 88.52 ± 0.62 \)

**In the table bold and underlined values of cefuroxime content in the drops refer to the last day of the analysis when the antibiotic content remained on the level higher or equal to the 90 % initial content.**
sodium cefuroxime in sterile water was also studied. The solution was heated earlier for 10 min at the temperature of 60°C according to the conditions specified in a previous publication (7). Sample chromatograms and UV spectra are presented in Figures 1, 2 and 3.

RESULTS AND DISCUSSION

In the studies of cefuroxime stability using HPLC method in 1% and 5% w/w eye drops, the results of the previous analyses (11) were taken into account. These results showed that the additives, i.e., citrate buffer of pH 6.15–6.20, phenylmercuric borate, β-phenylethyl alcohol and polyvinyl alcohol did not decrease antimicrobial activity of cefuroxime in 1% drops, which were stored for 30 days at the temperature of 4°C. Their stability was determined with cylinder-plate method in accordance with the PPh VI against standard strain Staphylococcus aureus ATCC 6538.

The application, in 1% and 5% drops, of phenylmercuric borate at the concentration of 0.001% and β-phenylethyl alcohol at the concentration of 0.4%, was also justified by previous results, which confirmed antimicrobial efficiency of these drops using the preservation test following the PPh VI, using test strains of bacteria and fungi: Staphylococcus aureus ATCC 6538, Pseudomonas aeruginosa ATCC 9027, Candida albicans ATCC 10231 and Aspergillus niger ATCC 16404 (9).

The validation of HPLC method applied in the studies of cefuroxime stability in 1% and 5% buffered drops was characterized by specificity, accuracy, precision and linearity. The time (days), during which cefuroxime concentration in drops remained at the level not lower than 90% of the initial concentration was chosen as the criterion of drops stability.

All the formulations of 1% and 5% buffered drops developed during the studies met the physical and chemical criteria set in the objective of the work and can be used in the treatment of eye infections. While choosing cefuroxime concentration in the drops and composition (formulation) of the drops one should take into account such factors as the condition and intensity of the infection, the infected eye area and the stage of the infection. The 1% and 5% drops prepared according to the composition of formulations no. 1 and no. I are particularly targeted at the application during the initial, acute stage of the infection, during which it is not recommended to use drops containing preservatives and of enhanced viscosity. The 1% and 5% drops of formulations no. 1 and no. I are also advisable in the treatment of the infections of the patients suffering from damaged epithelium of the cornea or the lack of tolerance towards preservatives. The 1% and 5% drops containing preservatives (formulations no. 2 and no. II) are recommended after the acute phase of the infection is managed, if there are no symptoms of damage to the epithelium of the cornea and in case of good tolerance of the patient’s eye towards preservatives. The application of 1% and 5% drops containing cefuroxime prepared in citrate buffer, including polyvinyl alcohol enhancing the viscosity of the drops and extending the contact time of the drops with the eye (formulations no. 3 and III), is advantageous if it is necessary to increase the penetration of cefuroxime through the cornea into the anterior eye chamber. The drops of formulations no. 3 and no. III can be applied after managing the acute phase of the infection and in case of the lack of tolerance of the patient’s eye towards preservatives. One percent and 5% drops containing preservatives and polyvinyl alcohol (formulations no. 4 and no. IV) can be used after managing the acute stage of the infection if there are no symptoms of damaging of the cornea and in case of good eye’s tolerance towards preservatives.

Citrate buffer of pH 6.05–6.28 turned out to be an appropriate solvent for cefuroxime in 1% and 5% drops. It guaranteed the stability of the drops required to perform the therapy, appropriate pH and osmotic pressure (Tabs. 2 and 4). The factor having a decisive influence on cefuroxime stability in buffered drops was, first of all, the storage temperature of the drops. The drops should be stored at the temperature of 4°C. The temperature of 20°C should not be used during the period of the storage of the drops containing cefuroxime because at this temperature the antibiotic undergoes very rapid degradation. A 10% decrease of cefuroxime content in 1% and 5% drops of enhanced viscosity (formulation no. 3 and 4 as well as no. III and no. IV) occurred between 3rd and 6th day of the storage at the temperature of 20°C. The remaining formulations (no. 1 and no. 2 as well as no. I and no. II) of 1% and 5% drops stored at the temperature of 20°C were characterized by the time of 10% antibiotic degradation shorter than 3 days. The stability of cefuroxime in 1% buffered drops (formulation no. 1) and in 5% drops (formulation no. 1) stored at the temperature of 4°C, determined with HPLC as the time of 10% cefuroxime degradation, was 15 days (Tab. 4). The preservatives added to the buffered drops did not lower their stability. The time of 10% cefuroxime degradation in 1% and 5% buffered and preserved
drops (formulations no. 2 and no. II) stored at the temperature of 4°C was similar to the time of 10% cefuroxime degradation in 1% and 5% buffered and not preserved drops (formulations no. 1 and no. I) and it equaled 15 days (Tab. 4). Polyvinyl alcohol increased the stability of cefuroxime in 1% and 5% buffered drops but the stabilizing influence of polyvinyl alcohol was particularly strong in case of 5% drops (formulations no. III and no. IV). The time of 10% cefuroxime degradation in 1% buffered drops of enhanced viscosity (formulations no. 3 and no. 4), stored at the temperature of 4°C was 18 days and it was 3 days longer in comparison with 1% drops without the addition of polyvinyl alcohol (formulations no. 1 and no. 2) (Tab. 4). The time of 10% cefuroxime degradation in 5% buffered drops of enhanced viscosity (formulations no. III and no. IV) stored at 4°C equalled 30 days and it was 15 days longer when comparing with 5% drops not containing polyvinyl alcohol (formulations no. I and no. II). The time of 10% cefuroxime degradation in not buffered drops prepared in sterile water, stored at 4°C, was 27 days in 1% drops (formulation no. 01%) and in 5% drops (formulation no. 05%).

The changes in cefuroxime content in specific formulations of the drops were evaluated with non-parametric assay for many correlation tests. During the statistical test, null hypothesis was made (H0) that all medians of the changes of the antibiotic content are equal. It was showed, using Friedman's test, that the null hypothesis was not true (p-value < 0.01). To perform Friedman’s test, Statistica software package version 8 was used.

On account of low osmotic pressure of not buffered drops, which differed considerably from the osmotic pressure of lacrimal fluid (280–300 mOsm/L) and amounted to 46 mOsm/L in 1% drops and 216 mOsm/L in 5% drops (formulations no. 01% and 05%), the eye drops containing 1% and 5% cefuroxime prepared in sterile water should not be used. Osmotic pressure of 1% and 5% buffered drops practically did not change during the period of 30-day storage at the temperature of 4°C. It was within the range of the osmotic pressure which is well tolerated by the eye and equalled 359–429 mOsm/L in 1% drops and 398–451 mOsm/L in 5% drops (Tab. 2).

The pH of 1% buffered drops stored at the temperature of 4°C after 30 days of storage was 6.13–6.43 while that of 5% drops equalled 7.20–7.84. pH stayed within the range acceptable for pH of the eye drops, i.e., pH 3.5–8.5.

The viscosity of 1% drops (formulation no.3 and no.4) after 30 days of storage at the temperature of 4°C did not change considerably and was 8.02–8.10 mPaxs, while that of 5% drops (formulation no. III and no. IV) was 8.12–8.33 mPaxs (Tab. 2).

One percent and 5% buffered drops of all formulations during the period of storage of 30 days at both temperatures were clear. Gradual yellowing was observed, which proceeded faster in the drops stored at the temperature of 20°C. The change of color after 30 days of storage at the temperature of 4°C in 1% and 5% drops stored was not significant. The change of odor of the drops stored at both temperatures was not observed.

Publications show that in aqueous solutions cefuroxime undergoes hydrolysis, as a result of which the side chain at the position 3 is removed from the molecule creating decarbamoylcefuroxime and preserving β-lactam group. The chromatographic analysis of the aqueous solution of cefuroxime, heated for 10 min at the temp. of 60°C, showed that the peak of the degradation product appears just before the peak of the analyzed antibiotic (7). The peak of the main degradation product of cefuroxime at retention time tR = 6.8 min (Figs. 1 and 2) appeared on chromatograms both during the analysis of cefuroxime stability in prepared aqueous solutions and eye drops for 30 days and during the studies of standard solution of cefuroxime heated for 10 min at the temperature of 60°C. The analysis of UV spectra showed that cefuroxime (tR = 8.6 min) and its main degradation product (tR = 6.8 min) detected with HPLC method in 1% and 5% w/w drops during the storage at the temperature of 4°C and 20°C were characterized by similar UV spectra with maximum absorbance at the wavelength of 273 nm (Fig. 3). It appears to confirm the preserved structure of β-lactam ring of the main degradation product. The presence of decarbamoylcefuroxime in the drops containing cefuroxime may explain the discrepancy between the cefuroxime stability in 1% drops, stored at the temperature of 4°C, determined with HPLC method and amounting to 15–18 days, after which cefuroxime concentration decreased by 10%, and the antimicrobial activity of cefuroxime, which after 30 days of drops’ storage remained at 100% initial level. The results of antimicrobial activity of cefuroxime in 1% drops may imply that decarbamoylcefuroxime in drops due to the preserved structure of β-lactam group may show antimicrobial activity.

CONCLUSIONS

The physical and chemical properties of all the formulations of 1% and 5% drops containing
cefuroxime, prepared in citrate buffer of pH 6.05–6.28, met the standards set in the objective of the studies. The stability of cefuroxime in buffered drops stored at the temperature of 4°C creates the opportunity for the preparation of the drops in pharmacies on the basis of prescriptions. Depending on the character and course of the infection, the drops can be prepared at the concentration of 1% and 5% according to the selected formulary composition, which would meet the individual needs of the patient’s therapy.

Acknowledgment

This study was supported by the research grant from the Nicolaus Copernicus University no. 21/2007.

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Received: 20. 04. 2010