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PHYSICAL AND CHEMICAL PROPERTIES AND STABILITY OF SODIUM CEFAZOLIN IN BUFFERED EYE DROPS DETERMINED WITH HPLC METHOD

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Abstract: The aim of the studies was to analyze the stability of 1% and 5% eye drops containing sodium cefazolin, prepared in citrate buffer of pH 6.11–6.27, which were stored at the temperature of 4°C and 20°C with light protection. The drops were prepared under aseptic conditions by dissolving sodium cefazolin (Biofazolin, IBA Bioton), dry injection form of the drug, in citrate buffer. The viscosity of the drops was increased using polyvinyl alcohol. The drops were preserved with phenylmercuric borate of 0.001% concentration mixed with β-phenylethyl alcohol of 0.4% concentration in the drops. The concentration of cefazolin was determined at every three days using HPLC method. Besides, the measurements of pH, osmotic pressure and viscosity were performed as well as the organoleptic analysis of the drops (clarity, color, odor). The concentration of cefazolin in 1% drops after the 30-day-storage at the temperature of 4°C, depending on their composition, decreased in the range of 2.17–6.02%. In 5% drops the decrease in cefazolin concentration was similar, i.e., after 30-day-storage at the temperature of 4°C it was 1.62–6.76%. In 1% and 5% drops stored at the temperature of 20°C the stability of the drops determined as the 10% degradation time of cefazolin was in the range of 9–15 days.

Keywords: cefazolin, eye drops, stability, HPLC

Cefazolin is a first-generation cephalosporin antibiotic which shows strong bactericidal activity towards Gram-positive cocci, among them staphylococci such as *Staphylococcus aureus* (except for MRSA), *Staphylococcus epidermidis* and streptococci: *Streptococcus pneumoniae* (except for penicilin-resistant strains), *Streptococcus pyogenes*, *Streptococcus viridans* etc. Cefazolin is also characterized by bactericidal activity towards some Gram-negative rods such as *Klebsiella pneumoniae* and *Escherichia coli* as well as towards anaerobic cocci and bacilli sensitive to penicilin.

Cefazolin is used in the form of intramuscular and intravenous injections in the treatment of infections of respiratory system, urogenital system, infections of skin and soft tissues, biliary tract, bones, joints and in case of endocarditis and septicaemia (1).

Cefazolin exhibits significant efficiency in the treatment of bacterial eye infections caused by staphylococci, pneumococci and Gram-negative bacilli (*Escherichia coli*, *Klebsiella spp*., *Proteus spp*.) (2).

Eye drops containing cefazolin, which constitute aqueous solutions, on account of their limited stability are not available as commercial products. They are formulary preparations at the concentration of 1% and 5%, which are made in pharmacies exclusively for the needs of the patients of ophthalmic departments.

Cefazolin in 1% aqueous solutions is not compatible with thiomersal at the concentration over 0.003%, benzalkonium chloride at the concentration over 0.005% or chlorhexidine diacetate at the concentration of 0.01% (3). The following substances were used as the solvents of sodium cefazolin in the eye drops: commercial preparations of artificial tears (4–6), acetate buffers of pH 4.5 and 5.7, phosphate buffer of pH 7.5, solutions of sodium chloride (6, 7) and glycerol (7). Cefazolin stability in aquous solutions is dependent mainly on pH and a storage temperature (6, 7). It is higher in the solutions of acidic pH, e.g., at pH 4.5 and 5.7 (6, 7).

The solutions of artificial tears preserved with thiomersal or benzalkonium chloride could not con-

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stitute the base for eye drops containing cefazolin because of the lack of drops' clarity (turbidity, sediment) (4–6). Kodym et al. examined antimicrobial activity of cefazolin in 1% drops prepared in citrate buffer of pH 6.11–6.27 (3). The viscosity of these formulations was increased with polyvinyl alcohol. They were preserved with thiomersal at the concentration of 0.002% or phenylmercuric borate at the concentration 0.001%, mixed with β-phenylethyl alcohol at the concentration of 0.4%. After 30 days of storage at the temperature of 4°C antimicrobial activity of cefazolin determined with the cylinder-plate method in accordance with P.Ph. VI, using reference strain *Staphylococcus aureus ATCC65389*, was not reduced and remained at the initial level of 100% (3).

EXPERIMENTAL

Materials

Biofazolin (Cefazolin sodium) IBA Bioton, Poland, vials a 1.0 g, dry substance for intramuscular and intravenous injections; eye drops containing cefazolin, prepared under aseptic conditions according to the formulary composition shown in Table 1; sterile solutions of cefazolin, citrate buffers, polyvinyl alcohol, phenylmercuric borate.

Reagents

Cefazolin standard: Cefazolin sodium CRS (LGC Promochem UK), cefuroxime standard: Cefuroxime sodium CRS (LGC Promochem UK), acetonitrile gradient grade, disodium phosphate anhydrous ultrapure, citric acid monohydrate ACS, sodium citrate dihydrate p.a., citric acid p.a., polyvinyl alcohol molecular mass 72000 p.a., β-phenylethyl alcohol p.a., phenylmercuric borate p.a., uracil p.a., ultrapure water (System Synergy – Millipore, Molsheim, France).

Apparatus

High performance liquid chromatography system (Shimadzu, Kyoto, Japan), equipped with two LC-20AD piston pumps, DGU-20A5 five-line degasser, SIL-20AC autosampler, CTO-20AC column oven, UV-VIS SPD-M20A detector.

C18 GraceSmart RP 18 column (Grace, USA) was used to carry out isocratic chromatographic separation (granularity of column packing: 5 μm, column dimensions 4.6 × 250 mm). Altima HP C18 pre-column (Altech, Belgium) of dimensions 4.6 × 7.5 mm (granularity: 5 μm) was used in order to protect the column. The drops containing cefazolin were prepared in KL-21 laminar flow cabinet (Polon, Poland). The measurements of osmotic pressure of the solutions and drops were carried out using Krioskop 800cl osmometer (Trident Med. S.C., Poland). The viscosity was determined in Höppler KF10 viscosimeter (Medingen, Germany). pH measurements of the solutions and drops containing cefazolin as well as of the mobile phase were taken using IQ150 pH-meter (IQ Scientific Instruments, USA & Canada). During the process of sterilization SP-65W dry heat sterilizer (Wamed, Poland) and AS 446 WPA steam sterilizer (SMS, Poland) were used. The density of the solutions and drops was measured using Densito 30 PX densitometer (Mettler Toledo, Switzerland). The drops were stored in MED-28 pharmaceutical coolers (Kirsch, Germany). WPS/720/C/2 precision balance (Radwag, Poland) and Sartorius Expert LE 225D balance (Sartorius, Germany) were used during the studies. The analyzed solutions and drops were dispensed for the studies with CP 100 and CP 1000 micropipettes (PZ HTL S.A, Poland).

Methods

Preparation of sterile solutions of additives

The solutions of additives, i.e., citrate buffers I, II and III, solution of polyvinyl alcohol, 0.04% solution of phenylmercuric borate, were prepared and examined using the methods described in previous publication (8).

Preparation of eye drops containing cefazolin

One percent and 5% w/w drops were prepared under aseptic conditions (laminar flow cabinet) in accordance with the formulary composition presented in Table 1. Biofazolin (Cefazolin sodium) was dissolved in the appropriate citrate buffer or in sterile water. The prescribed quantity of 0.04% auxiliary 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 the formulations of enhanced viscosity (the drops of versions no. 3, 4, III and IV) 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 MED-28 pharmaceutical coolers (Kirsch, Germany) at the temperature of 4°C and 20°C for 30 days, protected from light.

Table 1. Composition of eye drops containing sodium cefazolin.

Components (g) per 100 g of eye drops	**Formulary versions**									
	1 % w/w					**5% w/w**				
	$0_{(1\%)}$	**1**	**2**	**3**	**4**	**$0_{(5\%)}$**	**I**	**II**	**III**	**IV**
Biofazolin® (Sodium cefazolin)	1.0	1.0	1.0	1.0	1.0	5.0	5.0	5.0	5.0	5.0
Water for injection	99.00	–	–	–	–	95.00	–	–	–	–
Citrate buffer I (51.005 mM/kg trisodium citrate dihydrate 3.57 mM/kg citric acid monohydrate) pH 6.27, osmotic pressure: 161 mOsm/L	–	–	–	–	–	–	95.00	92.1	–	–
Citrate buffer II (102.01 mM/kg trisodium citrate dihydrate 7.14 mM/kg citric acid monohydrate) pH 6.22, osmotic pressure: 308 mOsm/L	–	99.00	96.1	–	–	–	–	–	47.5	46.05
Citrate buffer III (204.02 mM/kg trisodium citrate dihydrate 14.28 mM /kg citric acid monohydrate) pH 6.11, osmotic pressure: 581 mOsm/L	–	–	–	49.5	48.05	–	–	–	–	–
Solution of polyvinyl alcohol (PVA) viscosity η = 42.3 mPa·s, pH 4.48	–	–	–	49.5	48.05	–	–	–	47.5	46.05
Solution of phenylmercuric borate (0.04%)	–	–	2.5	–	2.5	–	–	2.5	–	2.5
2-Phenylethanol	–	–	0.4	–	0.4	–	–	0.4	–	0.4

Table 2. Changes of sodium cefazolin concentration in aqueous solutions and in eye drops stored at the temp. of 4°C and 20°C for 30 days.

Version		1% w/w					5% w/w				
		$\mathbf{0}_{(1\%)}$	**1**	**2**	**3**	**4**	$\mathbf{0}_{(5\%)}$	**I**	**II**	**III**	**IV**
Initial conc. (mg/mL)		10.15 ± 0.01	10.12 ± 0.01	10.30 ± 0.01	10.45 ± 0.02	10.49 ± 0.02	51.44 ± 0.03	50.15 ± 0.07	50.25 ± 0.01	53.04 ± 0.05	53.40 ± 0.05
temp. 4°C		**% initial concentration remaining**									
Storage time	3 days	99.20 ± 5.11	99,82 ± 0.94	100.10 ± 0.15	99.12 ± 0.24	98.59 ± 01.76	98.71 ± 3.11	99.57 ± 0.42	99.73 ± 1.20	100.29 ± 0.23	98.82 ± 0.62
	6 days	98.47 ± 1.49	99.42 ± 0.70	100.06 ± 0.81	97.76 ± 2.37	97.41 ± 0.71	96.74 ± 2.64	98.94 ± 3.15	100.09 ± 1.02	98.70 ± 0.73	97.74 ± 1.06
	9 days	97.35 ± 1.29	98.36 ± 3.25	99.10 ± 0.37	97.81 ± 1.09	97.61 ± 2.29	95.80 ± 1.77	98.27 ± 0.44	99.48 ± 1.97	98.56 ± 0.85	96.83 ± 1.83
	12 days	97.16 ± 0.30	99.26 ± 0.47	99.59 ± 0.42	97.19 ± 2.64	96.29 ± 1.29	94.62 ± 0.67	98.85 ± 2.03	99.09 ± 0.60	99.09 ± 0.21	98.27 ± 1.02
	15 days	97.64 ± 0.13	99.32 ± 1.28	98.18 ± 0.36	95.91 ± 0.50	95.85 ± 1.23	94.10 ± 1.30	96.56 ± 0.18	98.06 ± 1.76	97.87 ± 0.67	95.13 ± 2.25
	18 days	96.15 ± 0.55	99.31 ± 0.74	98.23 ± 1.29	95.75 ± 0.83	95.72 ± 1.14	93.94 ± 1.32	96.21 ± 1.81	97.65 ± 1.52	97.84 ± 1.81	95.07 ± 1.02
	21 days	96.19 ± 0.94	97.89 ± 2.02	98.75 ± 0.95	95.42 ± 1.61	94.99 ± 0.31	93.77 ± 1.92	97.34 ± 0.84	98.34 ± 2.49	96.22 ± 2.79	96.92 ± 1.14
	24 days	96.72 ± 0.60	97.12 ± 0.35	97.85 ± 0.28	95.24 ± 0.71	94.53 ± 1.24	93.45 ± 2.40	96.63 ± 0.80	97.83 ± 0.92	96.93 ± 1.45	96.83 ± 1.00
	27 days	96.57 ± 0.21	96.58 ± 0.49	97.55 ± 2.48	95.33 ± 2.02	94.18 ± 0.71	93.51 ± 1.00	95.47 ± 0.55	98.70 ± 3.63	96.95 ± 4.42	96.59 ± 1.05
	30 days	96.74 ± 1.18	96.05 ± 0.54	97.83 ± 0.37	93.99 ± 0.36	93.98 ± 1.47	93.24 ± 1.94	95.36 ± 3.16	98.38 ± 0.79	94.97 ± 1.97	95.40 ± 1.00
temp. 20°C		**% initial concentration remaining**									
Storage time	3 days	96.43 ± 2.28	99.64 ± 2.35	98.83 ± 1.07	95.98 ± 1.41	95.52 ± 2.69	98.34 ± 0.21	98.20 ± 0.65	97.52 ± 1.37	97.86 ± 0.83	96.36 ± 0.48
	6 days	95.23 ± 0.34	96.34 ± 0.83	95.36 ± 1.96	92.34 ± 0.93	92.84 ± 1.94	93.61 ± 0.66	95.74 ± 2.88	96.05 ± 0.16	95.23 ± 0.66	92.83 ± 0.38
	9 days	91.98 ± 0.84	94.44 ± 1.85	94.27 ± 0.41	90.54 ± 0.97	91.01 ± 2.00	92.48 ± 1.94	93.57 ± 2.20	94.63 ± 1.58	92.13 ± 1.25	92.22 ± 1.14
	12 days	91.38 ± 0.78	93.13 ± 1.14	92.20 ± 0.37	88.67 ± 0.52	88.25 ± 1.47	88.84 ± 0.72	92.44 ± 0.24	91.53 ± 1.16	91.86 ± 1.91	91.05 ± 0.49
	15 days	89.47 ± 2.95	93.50 ± 0.42	90.54 ± 2.37	87.50 ± 0.90	86.50 ± 1.80	88.45 ± 1.72	89.98 ± 0.63	89.62 ± 1.62	88.06 ± 0.81	86.66 ± 1.27
	18 days	87.36 ± 0.80	88.40 ± 0.49	88.40 ± 0.84	86.79 ± 0.85	86.00 ± 0.12	86.09 ± 0.50	85.76 ± 3.67	87.15 ± 0.36	85.07 ± 0.91	85.05 ± 0.21
	21 days	86.08 ± 0.89	87.04 ± 1.70	87.27 ± 1.01	84.78 ± 1.10	83.02 ± 1.23	83.65 ± 2.95	84.90 ± 0.62	85.63 ± 0.86	85.00 ± 1.07	85.16 ± 1.70
	24 days	84.85 ± 0.52	83.28 ± 0.55	86.34 ± 0.90	82.64 ± 0.52	81.55 ± 0.26	82.21 ± 0.85	80.53 ± 0.98	83.16 ± 0.17	83.33 ± 1.13	81.74 ± 0.92
	27 days	83.28 ± 0.45	82.35 ± 0.37	84.89 ± 2.09	81.58 ± 1.22	81.17 ± 0.14	81.00 ± 0.67	79.47 ± 3.35	82.49 ± 1.17	81.71 ± 0.43	79.86 ± 1.27
	30 days	81.69 ± 0.38	81.70 ± 0.74	82.01 ± 0.51	80.14 ± 0.67	78.02 ± 0.47	79.39 ± 0.35	79.15 ± 1.54	81.01 ± 1.30	78.95 ± 1.27	77.92 ± 1.45

Table 3. pH, osmotic pressure and viscosity changes in eye drops containing sodium cefazolin (n = 3).

Version	1% w/w					5% w/w				
	$0_{(1\%)}$	**1**	**2**	**3**	**4**	**$0_{(5\%)}$**	**I**	**II**	**III**	**IV**
Initial pH	5.60 ± 0.01	6.11 ± 0.01	6.07 ± 0.04	6.14 ± 0.03	6.13± 0.01	5.66 ± 0.00	6.12 ± 0.03	6.01 ± 0.02	6.15 ± 0.01	6.14 ± 0.03
pH after 30 days of storage										
temp. 4°C	6.06± 0.08	6.06 ± 0.08	6.23 ± 0.00	6.22± 0.05	6.23± 0.01	6.18 ± 0.01	6.18 ± 0.04	6.24 ± 0.04	6.26 ± 0.01	6.24 ± 0.04
temp. 20°C	6.64 ± 0.01	6.04 ± 0.01	6.25 ± 0.03	6.27± 0.05	6.26 ± 0.04	7.11 ± 0.04	6.37 ± 0.03	6.34 ± 0.01	6.49 ± 0.04	6.48 ± 0.01
Initial osmotic pressure (mOsm/L)	44 ± 3	334 ± 2	365 ± 4	382 ± 4	414 ± 6	181 ± 2	328 ± 2	365 ± 2	358 ± 3	377 ± 7
Osmotic pressure after 30 days of storage [mOsm/L]										
temp. 4°C	45 ± 3	334 ± 2	375 ± 5	391 ± 11	421 ± 4	188 ± 3	344 ± 9	365 ± 5	370 ± 5	399 ± 8
temp. 20°C	49 ± 2	342 ± 2	385 ± 0	399 ± 4	426 ± 4	219 ± 3	374 ± 2	391 ± 9	396 ± 5	414 ± 5
Initial viscosity (mPa×s)				7.95 ± 0.08	7.96 ± 0.05				8.53 ± 0.04	8.67 ± 0.08
Viscosity after 30 days of storage [mPa×s]										
temp. 4°C				7.99 ± 0.07	7.91 ± 0.17				8.52 ± 0.06	8.66 ± 0.12
temp. 20°C				8.01 ± 0.14	7.96 ± 0.15				8.53 ± 0.10	8.65 ± 0.16

Table 4. Clarity, color and odor of eye drops containing sodium cefazolin.

	Fresh eye drops					Storage temperature									
						4°C					20°C				
Formulary version	$0_{1\%}$	1	2	3	4	$0_{1\%}$	1	2	3	4	$0_{1\%}$	1	2	3	4
Clarity	Clear, colorless, delicate odor of antibiotic (in versions 2 and 4 the 2-phenylethanol odor dominated)					Clear, colorless, delicate odor of antibiotic (in versions 2 and 4 the 2-phenylethanol odor dominated)					Clear				
Color											yellowish since day 24	yellowish since day 24	yellowish since day 27	yellowish since day 24	yellowish since day 21
Odor											No changes				
Formulary version	$0_{1\%}$	1	2	3	4	$0_{1\%}$	1	2	3	4	$0_{1\%}$	1	2	3	4
Clarity	Clear, colorless, delicate odor of antibiotic (in versions 2 and 4 the 2-phenylethanol odor dominated)					Clear, colorless, delicate odor of antibiotic (in versions 2 and 4 the 2-phenylethanol odor dominated)					Clear				
Color											yellowish and more intense odor since day 12; yellow since day 30	yellowish and more intense odor since day 12; yellow since day 27	yellowish and more intense odor since day 15	yellowish and more intense odor since day 12; yellow since day 30	yellowish and more intense odor since day 12; yellow since day 27
Odor															

Physical and chemical evaluation of eye drops containing cefazolin after their preparation and after 30 days' storage at 4°C and 20°C

Measurements of pH, osmotic pressure and viscosity in the solutions and drops containing cefazolin were performed at determined time intervals. The organoleptic analysis regarding clarity, color and odor was also carried out. The results of the studies are presented in Tables 3 and 4.

Evaluation of cefazolin stability in the drops using HPLC method

Chromatographic conditions

Chromatographic separation was carried out on C18 Grace Smart column (Grace, USA). Mobile phase consisted of the solution of 78.0 g acetonitrile and 900.0 g phosphate buffer of pH 3.74 (7.73 mmol/L anhydrous disodium phosphate and 8.85 mmol/L citric acid monohydrate). It was filtered through 0.45 µm Millicup-LH membrane filter (Millipore Corporation, Bedford, USA) and degassed using ultrasounds. The flow rate of the mobile phase was 1 mL/min. The wavelength of the photodiode detector was 270 nm, the column temperature was 25°C and the injection volume of the sample was 10 µL. The injections were performed using autosampler. Chromatographic data were collected and analyzed using LCsolution software version 1.21 SP1 (Shimadzu Corporation, Japan).

Preparation of samples for HPLC analysis

Two hundred fifty microliters of 1% drops or 50 µL of 5% drops were placed into volumetric flasks of 25 mL volume using semiautomatic pipettes. Then, 1 mL of the uracil solution in HPLC water of 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 coefficient k for cefazolin peaks. Then, the flasks were filled with HPLC water up to the total volume and mixed. The solutions were filtered through 0.45 µm Chromafil membrane filter (Macherey-Nagel, Germany) into vials. The vials were placed into the autosampler. One injection was made from each of the three vials, which provided three results for each analyzed formulary version. Cefazolin concentration in the analyzed samples was calculated on the basis of the registered peak areas using the equation of the calibration curve.

Validation

The efficiency of the chromatographic system was examined using the method described in Ph. Eur. 6.0 in the monograph Cefazolin Sodium. The solution containing cefazolin and cefuroxime was prepared. The sample of the solution was injected onto the column. The obtained resolution between the peaks of cefuroxime and cefazolin was 4.674 (the required value amounts to at least 2.0). This confirmed the sufficient efficiency of the system (Fig. 1).

Specificity

The solutions of additives included in 1% and 5% drops containing cefazolin were prepared, at the concentrations the same as in the drops. Additives contained in the drops, i.e., citric acid, sodium citrate, polyvinyl alcohol, β-phenylethyl alcohol and phenylmercuric borate did not have any influence on the result of the determinations because at the reten-

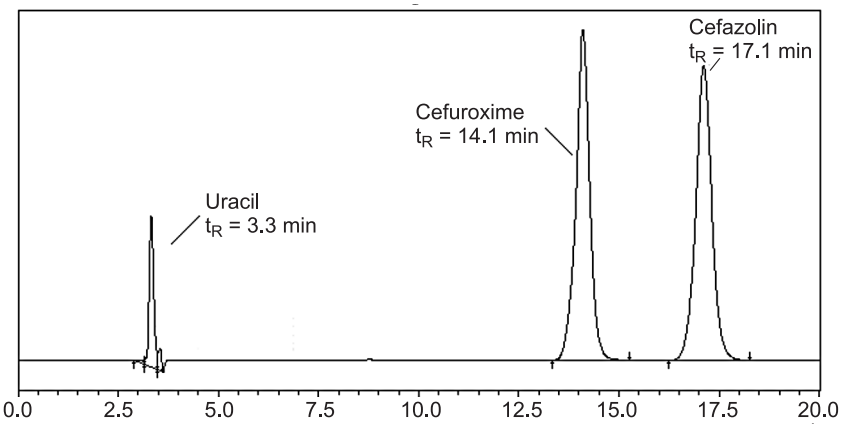

Figure 1. Resolution test R_S = 4.674

tion time characteristic for cefazolin and analytical wavelength of 270 nm they did not show any absorbance. The cefazolin peak did not interfere with the peak of degradation product of cefazolin t_R~8.5 min, which started to be more distinct after 30 days' storage of the drops at the temperature of 20°C (Fig. 2). Average retention coefficient for cefazolin equalled k = 3.8 during the whole cycle of the studies, whereas resolution R_s of cefazolin peaks was not lower than 4.67. The obtained number of theoretical

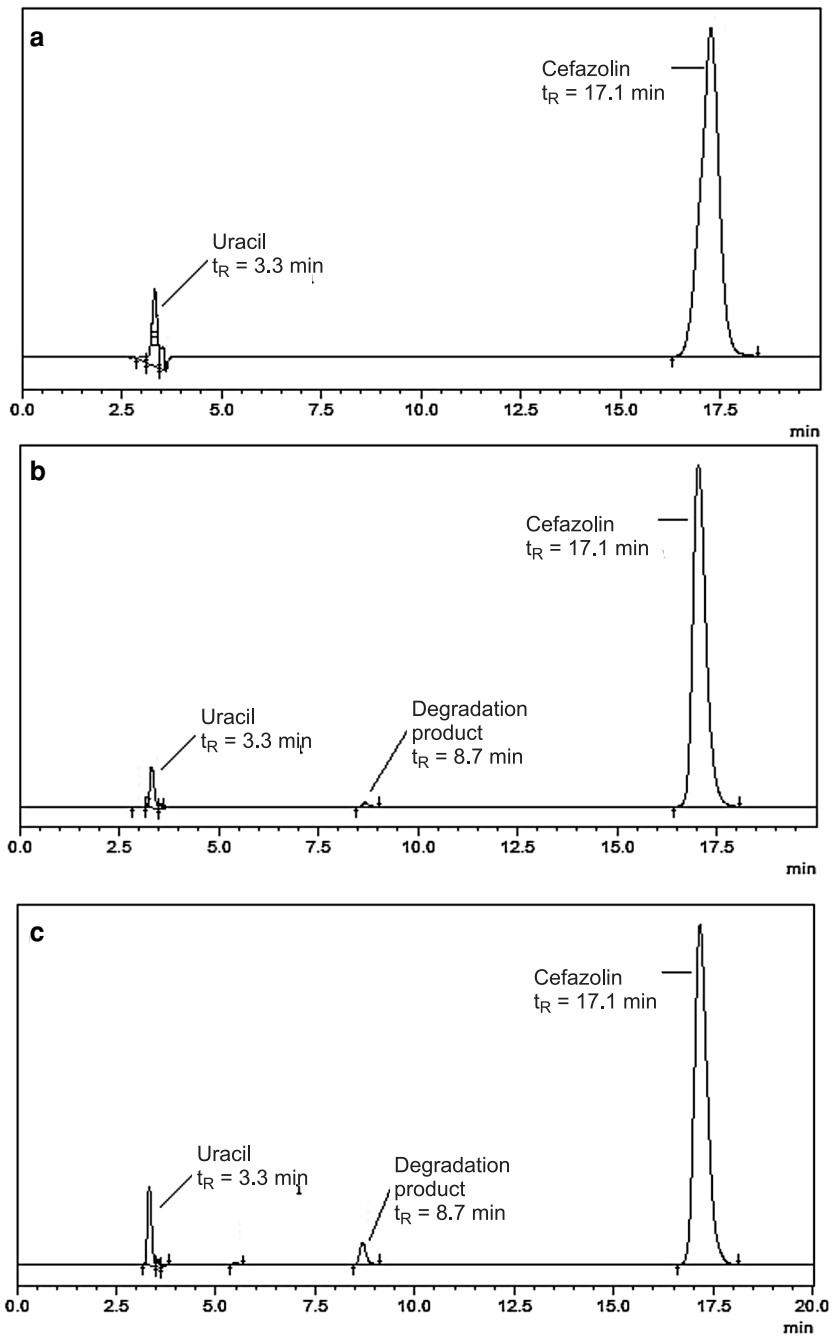

Figure 2. Eye drops of version IV freshly made (**a**) and after 30 days of storage at 4°C (**b**) and 20°C (**c**)

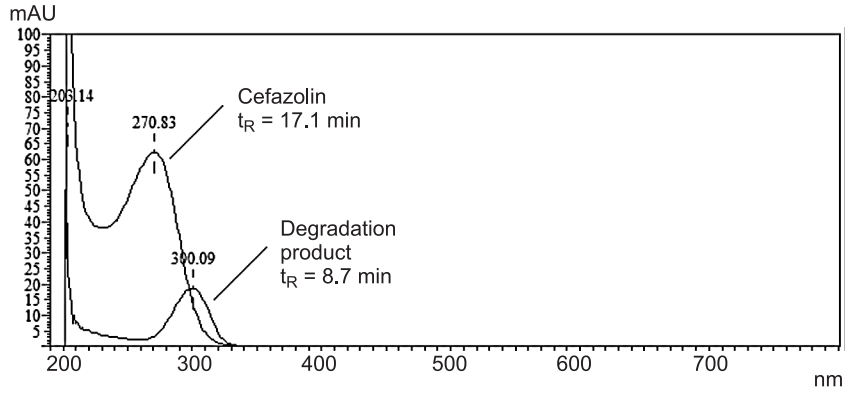

Figure 3. UV-VIS spectra of cefazolin and degradation product t_R = 8.7 min

plates N was over 25000/m. The tailing coefficient TF for cefazolin did not exceed 1.4.

Accuracy and precision

Accuracy and precision of the method of cefazolin quantitative determination in the drops were established by the analysis of reference solutions of the drops of formulations 4 and IV. The solutions contained respectively 0.8%, 1.0% and 1.2% cefazolin in drops of formulation no. 4 and 4.0%, 5.0% and 6.0% cefazolin in drops of formulation no. IV. Six samples were prepared for the injection out of each solution as it was described in the paragraph "Preparation of samples for HPLC analysis". The percentage of recovery was adopted as the measurement of the method accuracy, and it was calculated according to the following formula:

$$\% \text{ recovery} = \frac{\text{determined concentration}}{\text{calculated concentration}} \times 100$$

The precision of the method was presented as the value of relative standard deviation (RSD), which was in the range of 0.236% to 0.273% for 1% drops and from 0.513% to 0.617% for 5% drops. The accuracy of the determinations of cefazolin was from 102.11% to 102.54% in 1% drops, whereas for 5% drops it equaled from 101.82% to 102.59.

Linearity

A 6-point calibration curve was prepared. It was based on the analysis of the content of reference cefazolin solutions at the concentrations from 25 µg/mL to 150 µg/mL. The values of regression coefficients a and b were determined for the curve equation y = ax + b and then they were checked in terms of their significance. The value $t = a/S_a = 452.11 > t_\alpha$, f = 2.776 ($\alpha$ = 0.05 and f = 4) confirms the significance of the regression. The value $t = b/S_b = 0.694 < t_\alpha$, f = 2.776 ($\alpha$ = 0.05 and f = 4) confirms that the regression coefficient b is not significant. The regression equation showing the relationship between the determined area of the peak and the concentration of cefazolin standard is y = 16101.80 × x. The value of linear correlation coefficient R^2 = 0.9999 showed the linear relationship between the concentration of the analyte and the area of the peak.

The limit of detection (LOD) and limit of quantitation (LOQ)

The limit of detection (LOD) of cefazolin determined on the basis of the equation LOD = 3.3 × Sy/a was 0.75 µg/mL. The limit of quantitation of cefazolin in accordance with the equation LOQ = 10 × Sy/a was 2.27 µg/mL.

RESULTS AND DISCUSSION

In the studies of cefazolin stability using HPLC method in 1% and 5% w/w eye drops the results of the previous analyses (3) were taken into account. They 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 cefazolin in 1% drops, which were stored for 30 days at the temperature of 4°C and 20 °C. Their stability was determined with the cylinder-plate method in accordance with Polish Pharmacopoeia VI (PPh VI) against the test strain *Staphylococcus aureus ATCC 6538*. The application of the preservatives in 1% and 5% drops containing cefazolin was justified by the results of the preservation test of 1% drops carried out according to the methods following PPh VI. They proved that in buffered drops phenylmercuric borate at the concentration of 0.001% and β-phenylethyl alcohol

at the concentration of 0.4% were efficient towards pharmacopeal strains of test microorganisms: *Staphylococcus aureus ATCC6538*, *Pseudomonas aeruginosa ATCC9027*, *Candida albicans ATCC 10231*, *Aspergillus niger ATCC 16404* as well as MRSA and *Listeria monocytogenes* (3). The validation of HPLC method applied in the studies of cefazolin stability in the buffered 1% and 5% drops was characterized by specificity, accuracy, precision and linearity and the required limits of detection and quantitation. The concentration of cefazolin in the proposed formulations of 1% and 5% drops prepared in citrate buffer of pH 6.11–6.27, after 30 days' storage at the temperature of 4°C, remained within the range of 94–98% of the initial concentration (Tab. 2). Cefazolin stability in buffered eye drops stored at the temperature of 4°C for 30 days was not negatively influenced by its concentration or the presence of phenylmercuric borate, β-phenylethyl alcohol or polyvinyl alcohol in the eye drops' composition. The literature proves that similar stability characterized cefazolin in 5% drops stored at 4°C, prepared with 0.45% sodium chloride or 1% glycerol solution used as solvents. The stability of these drops, which amounted to 42 days, was ensured by their pH 4.92–6.25, similar to the optimal pH for the stability of cefazolin (7).

In 1% and 5% drops prepared in sterile water, i.e., formulations $0_{1\%}$ and $0_{5\%}$, the stability of cefazolin after 30 days of storage at the temperature of 4°C was very similar to the stability of cefazolin in 1% and 5% buffered drops (Tab. 2). However, on account of unacceptable osmotic pressure of those drops, very different from the osmotic pressure of the lacrimal fluid (280 mOsm/L), which equalled 44 mOsm/L in 1% drops and 181 mOsm/L in 5% drops (Tab. 3), not buffered 1% and 5% drops prepared in sterile water should not be applied. The influence of storage temperature on the stability of cefazolin in 1% and 5% buffered drops was much smaller in comparison with 1% and 5% buffered drops containing cefuroxime (9), ceftazidime (10) and cefepime (11). It was particularly noticeable in case of 1% and 5% buffered drops containing cefazolin stored at the temperature of 20°C, in which 10% decrease in cefazolin concentration ocurred only after 9–15 days (Tab. 2). In case of 1% and 5% drops containing cefuroxime, ceftazidime or cefepime, 10% decrease in the concentration of these antibiotics in the drops stored at the temperature of 20°C took place almost immediately after their preparation (9–11). The concentration of cefazolin in 1% buffered drops after 30 days' storage at the temperature of 4°C determined with HPLC method, which equalled 94–98% of the initial concentration (Tab. 2), corresponded with the antimicrobial activity of cefazolin in 1% buffered drops, evaluated after 30 days of storage at the temperature of 4°C, which remained at the initial level of 100% (3).

However, in case of 1% drops stored at the temperature of 20°C the results of the studies of cefazolin stability in the drops determined with HPLC method as the time of 10% degradation, which equalled 9–15 days (Tab. 2), did not correspond with the results of the previous studies related to the antimicrobial activity of cefazolin in 1% drops, which after 30 days of storage at the temperature of 20°C did not decrease (3). It may suggest that the product of cefazolin degradation ($t_R \sim 8.5$ min, Figs. 2 and 3) shows antimicrobial activity.

Citrate buffer of pH 6.11–6.27 turned out to be an appropriate solvent for cefazolin in 1% and 5% drops. It provided the drops with stable and acceptable pH, osmotic pressure and viscosity (Tab. 3) as well as the appropriate stability of cefazolin (Tab. 2). Buffered drops 1% and 5% of all formulary versions were clear during the 30-day storage at both temperatures, although in case of the drops stored at the temperature of 20°C, simultaneously with developing degradation of the antibiotic, the drops changed color into yellowish, which was accompanied by the appearance of the characteristic odor (Tab. 4).

Physical and chemical stability of 1% and 5% buffered drops containing cefazolin stored at the temperature of 4°C exceeding 30 days gives pharmacies the opportunity to prepare the drops on the basis of doctor's prescriptions. The choice of cefazolin concentration in drops and formulation composition should be made in relation to individual therapeutic needs of the patient.

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