Acta Poloniae Pharmaceutica – Drug Research, Vol. 68 No. 1 pp. 99–107, 2011 ISSN 0001-6837
Polish Pharmaceutical Society

PHARMACEUTICAL TECHNOLOGY

STABILITY OF CEFTAZIDIME IN 1% AND 5% BUFFERED EYE DROPS DETERMINED WITH HPLC METHOD

ANNA KODYM*, DOMINIKA HAPKA-ŻMICH, MARTA GOŁĄB and MAGDALENA GWIZDAŁA

Department of Pharmaceutical Technology, Nicolaus Copernicus University,
Collegium Medicum in Bydgoszcz, M. Skłodowskiej-Curie 9, Bydgoszcz, Poland

Abstract: The aim of the studies was to determine with HPLC method the stability of ceftazidime in buffered 1% and 5% eye drops of proposed formulary composition, which were stored for 30 days at the temperature of 4°C and 20°C and protected from light. The 1% and 5% eye drops were prepared under aseptic conditions by dissolving Biotum® (ceftazidimum), dry injection formulation, in citrate buffer of pH 6.10–6.24. The viscosity of the eye drops was increased with polyvinyl alcohol, phenylmercuric borate combined with 2-phenylethanol was used to preserve the eye drops. The eye drops were stored for 30 days in sterile glass bottles at the temperature of 4°C and 20°C and protected from light. The concentration of ceftazidime and pyridine was analyzed simultaneously with HPLC method every three days; pH, osmotic pressure and viscosity were examined as well as the organoleptic analysis of the eye drops (clarity, color, odor). Storage temperature had the biggest impact on ceftazidime stability in the eye drops. The stability of the drops depended also on ceftazidime concentration in the eye drops, the presence of preservatives and polyvinyl alcohol. The time of 10% ceftazidime degradation in buffered 1% and 5% eye drops, stored at the temperature of 4°C, was from 27 to 18 days in 1% eye drops and from 21 to 12 days in 5% eye drops, depending on their composition. In the eye drops which were stored at the temperature of 20°C 10% ceftazidime degradation occurred on the 3rd day of storage in all 1% and 5% formulary versions.

Key words: ceftazidime, eye drops, stability, HPLC

Ceftazidime is a third-generation semisynthetic cephalosporin which is active against a wide range of microorganisms. It works particularly strongly against Gram-negative bacteria, the pathogens which most often cause ophthalmological infections, i.e., *Pseudomonas aeruginosa*, *Haemophillus influenzae*, *Neisseria gonorrhoeae*, *Acinetobacter* as well as against all Gram-negative *Enterobacteriaceae* bacilli. The most important advantage of ceftazidime, apart from its particular activity against *Pseudomonas aeruginosa*, is its resistance to β-lactamases of TEM, SHV and PSE-1 type. Ceftazidime is applied in the form of intramuscular and intravenous injections in the treatment of serious lower airways' infections, urinary infections, septicemia, cerebrospinal meningitis, infections of skin, nose, and throat. The 1% or 5% eye drops containing ceftazidime, depending on the infected eye area and the intensity of the infection, are used in the treatment of ophthalmological infections caused in particular by *Pseudomonas aeruginosa* (1, 2). One percent eye drops are applied in the treatment of the infections of external eye structures, e.g., keratitis and conjunctivitis. Five percent eye drops, known as enhanced eye drops, are used under clinical conditions in case of severe infections of front or internal eye structures, including ceftazidime administered systemically. Eye drops containing ceftazidime, which are aqueous solutions, are not commercially manufactured because ceftazidime undergoes rapid degradation in aqueous solutions, as a result of which the β-lactam ring opens simultaneously with pyridine release (3–6). The rate of ceftazidime degradation in aqueous solutions depends on temperature, light, composition of a solvent, pH, ceftazidime concentration and the type of packaging. The maximal ceftazidime stability in aqueous solutions occurs at pH 4.5–6.5 (3, 4). The solution of 0.9% NaCl (6, 7), artificial tears – Sno tears (8) and balanced salt solution (9) were used as a vehiculum in the eye drops containing ceftazidime. The stability of the eye drops prepared in

* Corresponding author: e-mail: anna.kodym@cm.umk.pl

0.9% NaCl, stored at the temperature of 4°C, was 4 days for 5% eye drops (6) and 21 days in case of 2% eye drops (7). In 5% eye drops, based on artificial tears (8) and stored at the temperature of 7°C, 10% ceftazidime degradation occurred after 7 days. In 5% eye drops based on balanced salt solution (9), stored at the temperature of 4°C, the unchanged initial antimicrobial activity determined against *Pseudomonas aeruginosa* was maintained for 7 days. In 0.9% eye drops, prepared in citrate buffer of pH 6.18–6.30 (10), stored at the temperature of 4°C, ceftazidime antimicrobial activity remained at its initial unchanged level for the period of about 30 days. A relatively long period of ceftazidime antimicrobial activity in 0.9% eye drops prepared in citrate buffer encouraged to conduct research focused on the determination of chemical stability of ceftazidime with HPLC method in 1% and 5% eye drops prepared in citrate buffer, i.e., at the concentrations which are most frequently used in the topical treatment of eye infections.

The aim of the studies was to determine with HPLC method the stability of ceftazidime in buffered 1% and 5% eye drops of proposed formulary composition, which were stored for 30 days at the temperature of 4°C and 20°C and protected from light.

EXPERIMENTAL

Chemicals and reagents

Biotum® (ceftazidimum), IBA Bioton, of 1.0 g ampoules with dry active substance for intravenous or intramuscular injections, composed of ceftazidime pentahydrate (1.0 g) and sodium carbonate (0.118 g), citric acid monohydrate, sodium citrate p.a., polyvinyl alcohol 72000 (PVA) were from P.P.H. POCH Gliwice. Phenylmercuric borate, β-phenylethyl alcohol (2-phenylethanol) and uracil were from Sigma-Aldrich. Other reagents were: ceftazidime standard: ceftazidime CRS Strasburg Cedex 1, pyridine standard for GC (Z.D. Chemipan), water for HPLC (System Synergy – Millipore) and anhydrous disodium hydrogen-phosphate, potassium dihydrogen phosphate, acetonitrile (J.T. Baker, Holland).

Apparatus

High performance liquid chromatography: Shimadzu system (Kyoto, Japan) equipped with 20-A5 degasser, LC-20AD pumps, SIL-20AC autosampler, CTO-20AC column oven, DAD SPD-N20A detector, CMB-20A control system; CP-502 pH-meter (Elmetron, Poland), Krioskop 800cl osmometer (Trident Med. S.C. Warsaw, Poland), SP-65W

Table 1. Composition of eye drops containing ceftazidime.

Components (g) per 100 g of the eye drops	Formulary versions									
	1%					5%				
	$0_{(1\%)}$	1	2	3	4	$0_{(5\%)}$	I	II	III	IV
Biotum® (ceftazidimum)	1.259	1.259	1.259	1.259	1.259	6.295	6.295	6.295	6.295	6.295
calculated as ceftazidime pentahydrate	1.0	1.0	1.0	1.0	1.0	5.0	5.0	5.0	5.0	5.0
Solution of polyvinyl alcohol (PVA) viscosity η = 43.2 mPas, pH 5.51	-	-	-	49.5	48.05	-	-	-	47.5	46.05
0.04% solution of phenylmercuric borate	-	-	2.5		2.5	-	-	2.5	-	2.5
β-Phenylethyl alcohol	-	-	0.4		0.4	-	-	0.4	-	0.4
Water for injection	99.00	-	-	-	-	95.00	-	-	-	-
Citrate buffer I (102.01 mM/L tri-sodium citrate dihydrate 7.14 mM/L citric acid monohydrate) pH 6.24, osmotic pressure: 298 mOsm/L	-	99.00	96.1	-	-	-	95.00	92.1	-	-
Citrate buffer II (204.02 mM/L tri-sodium citrate dihydrate 14.28 mM/L citric acid monohydrate) pH 6.10, osmotic pressure: 586 mOsm/L	-	-	-	49.5	48.05	-	-	-	47.5	46.05

Table 2. pH, osmotic pressure and viscosity changes in eye drops containing ceftazidime (n = 3)

Versions	1%					5%				
	$0_{(1\%)}$	1	2	3	4	$0_{(5\%)}$	I	II	III	IV
Initial pH	7.53 ± 0.00	6.32 ± 0.04	6.25 ± 0.01	6.30 ± 0.00	6.21 ± 0.01	7.75 ± 0.02	6.48 ± 0.01	6.36 ± 0.00	6.43 ± 0.01	6.35 ± 0.01
pH after 30 day of storage										
temp. 4°C	7.31 ± 0.04	6.30 ± 0.00	6.25 ± 0.02	6.28 ± 0.00	6.20 ± 0.01	7.35 ± 0.01	6.41 ± 0.03	6.31 ± 0.03	6.36 ± 0.00	6.29 ± 0.02
temp. 20°C	7.23 ± 0.00	6.29 ± 0.00.	6.24 ± 0.02	6.26 ± 0.02	6.18 ± 0.01	7.10 ± 0.01	6.39 ± 0.01	6.29 ± 0.01	6.32 ± 0.00	6.27 ± 0.00
Initial osmotic pressure [mOsm/L]	40 ± 1	332 ± 0	358 ± 0	349 ± 1	386 ± 1	195 ± 2	475 ± 1	517 ± 1	488 ± 1	521 ± 2
Osmotic pressure after 30 day of storage [mOsm/L]										
temp. 4°C	44 ± 1	335 ± 2	364 ± 2	353 ± 1	394 ± 1	193 ± 1	484 ± 0	528 ± 2	499 ± 2	535 ± 2
temp. 20°C	52 ± 1	345 ± 0	37 2± 2	359 ± 1	402 ± 2	229 ± 3	520 ± 2	577 ± 1	538 ± 2	593 ± 3
Initial viscosity [mPa·s]	-	-	-	7.82 ± 0.00	7.48 ± 0.00	-	-	-	7.70 ± 0.00	7.57 ± 0.00
Viscosity after 30 day of storage [mPa·s]										
temp. 4°C	-	-	-	7.82 ± 0.00	7.48 ± 0.00	-	-	-	7.70 ± 0.00	7.57 ± 0.00
temp. 20°C	-	-	-	7.82 ± 0.01	7.50 ± 0.00	-	-	-	7.71 ± 0.00	7.59 ± 0.00

dry heat sterilizer (Wamed, Poland), AS 446 WPA steam sterilizer (SMS Poland), Sartorius Expert LE 225D balance (Sartorius, Germany), System Synergy – ultra pure water system (Millipore, France), pharmaceutical coolers MED-28 (Kirsch, Germany), Höppler viscosimeter KF 10 (Prüfgeräte Werk Medingen, Dresden).

Methods

Preparation of the additives

Preparation of sterile aqueous solutions of additives: citrate buffers I and II, solution of polyvinyl alcohol (PVA), 0.04% w/w solution of phenylmercuric borate were prepared following the description in (10). Physical properties of auxiliary solutions after sterilization are shown in Table 1.

Preparation of the eye drops containing ceftazidime

One percent and 5% w/w eye drops were prepared under aseptic conditions according to the formulary composition mentioned in Table 1. Biotum® (ceftazidimum) was dissolved in citrate buffer I or II. After preservation, the solution was filtered through membrane filter Sartorius with pore diameter of 0.22 µm. The viscosity of the filtered eye drops was increased with the solution of PVA. 0.04% solution of phenylmercuric borate and 100% β-phenylethyl alcohol were used for the preservation of the eye drops. The eye drops were poured into sterile glass infusion bottles and tightly closed with rubber corks and metal bottle caps. Storage conditions were as follows: the eye drops were stored in pharmaceutical coolers at 4°C and 20°C for 30 days, protected from light.

Physical and chemical evaluation of the eye drops containing ceftazidime after their preparation and after storage for 30 days at 4°C and 20°C

Organoleptic analysis

For organoleptic analysis, the appearance of the eye drops was evaluated, i.e., clarity, color and odor.

pH, osmotic pressure and viscosity of the eye drops

pH of the eye drops was determined with pH-meter, osmotic pressure was measured with osmometer, viscosity was established with Höppler viscosimeter (Table 2).

Chromatographic conditions

Chromatographic separation was carried out on GraceSmart RP 18, 5 µm, 250 × 4.6 mm column (Grace, USA). Mobile phase (w/w): phosphate buffer (0.029 M anhydrous disodium hydrogen phosphate, 0.019 M potassium dihydrogen phosphate) : acetonitrile (968.8 : 31.2), mobile phase pH 7.28, flow rate: 1.5 mL/min., temperature 25°C. Injection volume was 20 µL and detector wavelength was at 254 nm.

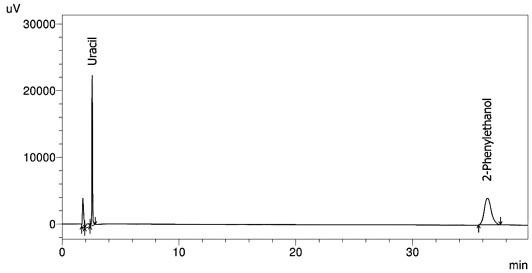

Figure 1. Specificity – a sample chromatogram of placebo version 2: detector signal generated only by 2-phenylethanol (r.t. 37 min.); uracil as a marker

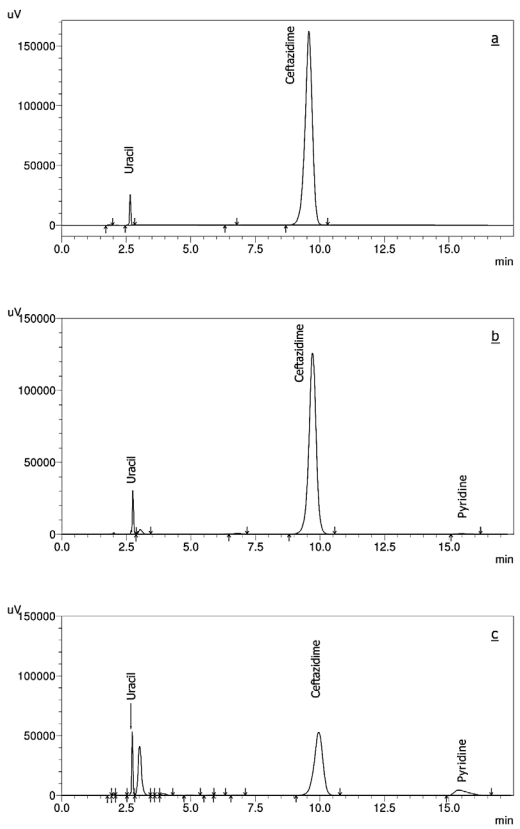

Figure 2. Chromatograms of the eye drops version 1 immediately after preparation (a) and after 30 days of storage at 4°C (b) and 20°C (c)

Table 3. Concentration of ceftazidime in eye drops during the storage (n = 3).

	Versions	1% w/w					5% w/w				
		$0_{(1\%)}$	1	2	3	4	$0_{(5\%)}$	I	II	III	IV
	Initial conc. (mg/mL)	10.08 ± 0.01	10.24 ± 0.01	10.01 ± 0,02	10.06 ± 0,01	10.03 ± 0,02	50.09 ± 0.74	50.70 ± 0.49	49.84 ± 0.86	50.11 ± 0.14	49.98 ± 0.28
	temp. 4°C	% initial concentration remaining									
Storage time	3 days	99.61 ± 0.77	99.92 ± 0.13	98.76 ± 0.56	97.48 ± 0.27	98.47 ± 0.45	98.62 ± 1.20	98.92 ± 0.21	99.10 ± 0.69	98.07 ± 0.83	98.38 ± 0.40
	6 days	97.95 ± 0.16	98.37 ± 0.40	98.27 ± 0.08	96.85 ± 0.34	97.32 ± 0.86	98.28 ± 0.12	97.57 ± 0.43	97.94 ± 0.80	96.28 ± 0.34	97.26 ± 0.54
	9 days	96.36 ± 0.20	96.80 ± 0.52	96.71 ± 0.30	95.67 ± 0.07	96.00 ± 0.39	96.32 ± 0.73	95.94 ± 0.02	96.27 ± 0.82	96.00 ± 0.16	95.56 ± 0.57
	12 days	95.96 ± 0.40	95.76 ± 0.63	95.45 ± 0.50	95.00 ± 0.27	95.71 ± 0.18	95.34 ± 0.99	95.12 ± 0.28	93.40 ± 0.26	93.21 ± 0.46	92.20 ± 1.35
	15 days	95.05 ± 0.32	95.46 ± 0.17	95.14 ± 0.16	93.37 ± 0.19	93.41 ± 1.06	93.02 ± 0.59	93.33 ± 0.13	90.42 ± 0.77	91.06 ± 0.33	89.38 ± 1.26
	18 days	93.79 ± 0.89	93.44 ± 0.34	93.95 ± 0.33	90.03 ± 0.10	92.24 ± 0.20	91.80 ± 1.05	91.16 ± 0.48	88.72 ± 1.07	87.55 ± 1.01	87.76 ± 1.05
	21 days	92.50 ± 1.25	92.53 ± 0.90	92.64 ± 0.05	88.96 ± 0.55	88.82 ± 1.56	90.09 ± 0.49	90.84 ± 0.34	87.17 ± 0.32	85.83 ± 0.26	83.98 ± 0.51
	24 days	92.41 ± 0.69	92.06 ± 0.60	90.97 ± 0.41	87.78 ± 0.40	87.29 ± 1.28	87.05 ± 0.72	87.14 ± 0.72	86.56 ± 0.96	84.68 ± 0.43	81.92 ± 0.22
	27 days	91.68 ± 0.60	91.05 ± 0.33	88.75 ± 1.33	84.28 ± 1.45	85.54 ± 0.42	85.12 ± 0.62	85.88 ± 0.23	84.65 ± 0.52	81.24 ± 1.12	78.91 ± 0.11
	30 days	89.45 ± 0.23	89.17 ± 0.15	88.66 ± 0.60	81.49 ± 0.37	83.94 ± 1.23	84.52 ± 1.12	85.00 ± 0.66	81.36 ± 0.29	79.60 ± 1.14	76.81 ± 0.39
	temp. 20°C	% initial concentration remaining									
Storage time	3 days	93.83 ± 0.89	93.89 ± 0.19	92.96 ± 0.59	92.06 ± 0.37	92.49 ± 1.12	88.74 ± 0.63	89.63 ± 0.40	91.81 ± 1.35	88.45 ± 0.65	89.79 ± 0.83
	6 days	87.60 ± 0.33	86.57 ± 0.26	86.78 ± 1.24	83.58 ± 0.90	88.27 ± 0.94	81.41 ± 0.76	79.65 ± 0.76	82.61 ± 0.72	85.65 ± 0.24	84.93 ± 1.61
	9 days	80.88 ± 0.66	79.94 ± 0.60	81.12 ± 0.16	77.49 ± 0.32	78.54 ± 1.20	73.80 ± 0.61	72.51 ± 0.96	74.53 ± 0.25	73.12 ± 0.72	76.52 ± 0.10
	12 days	76.71 ± 0.54	75.63 ± 0.64	75.28 ± 0.38	74.48 ± 0.07	74.71 ± 0.96	66.24 ± 0.31	64.45 ± 1.11	67.30 ± 0.61	66.86 ± 0.42	67.91 ± 1.88
	15 days	71.49 ± 1.03	69.68 ± 0.29	70.51 ± 1.22	64.20 ± 1.24	69.26 ± 1.23	59.25 ± 0.94	56.98 ± 0.02	60.44 ± 0.46	56.58 ± 0.27	60.06 ± 0.23
	18 days	66.35 ± 0.42	64.64 ± 0.64	63.67 ± 1.06	59.92 ± 1.52	64.26 ± 0.72	53.04 ± 0.88	52.08 ± 0.60	55.51 ± 2.66	51.69 ± 0.17	51.62 ± 0.54
	21 days	61.77 ± 0.22	59.74 ± 0.50	60.27 ± 0.74	56.43 ± 0.21	60.88 ± 0.56	47.72 ± 0.86	45.96 ± 0.26	48.94 ± 0.78	49.35 ± 0.71	48.54 ± 0.57
	24 days	57.84 ± 0.13	55.39 ± 0.58	54.50 ± 0.95	53.24 ± 0.76	55.08 ± 1.31	44.91 ± 0.84	42.79 ± 0.89	45.21 ± 0.62	44.45 ± 0.26	42.86 ± 0.34
	27 days	54.45 ± 0.23	53.39 ± 1.22	51.11 ± 0.17	47.16 ± 0.05	48.35 ± 0.66	40.01 ± 0.59	37.96 ± 0.48	39.21 ± 1.33	42.48 ± 0.44	41.81 ± 1.20
	30 days	50.82 ± 0.24	47.88 ± 0.68	47.16 ± 0.39	45.08 ± 1.61	44.53 ± 0.70	36.76 ± 0.51	35.43 ± 0.71	38.43 ± 1.10	39.84 ± 0.35	36.84 ± 0.65

Table 4. Pyridine concentration in eye drops containing ceftazidime after 30 day of storage.

Version	1% w/w					5% w/w				
	$0_{(1\%)}$	1	2	3	4	$0_{(5\%)}$	I	II	III	IV
Initial pyridine concentration in ceftazidime eye drops: below the detection limit										
Pyridine concentration [mg/mL] in ceftazidime eye drops after 30 day of storage:										
temp. 4°C	below the quantitation limit (below 0.2 mg/mL)					below the quantitation limit (below 1.0 mg/mL)				
temp. 20°C	0.590 ± 0.010	0.641 ± 0.020	0.703 ± 0.042	0.641 ± 0.051	0.645 ± 0.013	3.698 ± 0.118	3.797 ± 0.164	4.069 ± 0.090	3.755 ± 0.071	4.492 ± 0.094

Preparation of samples for HPLC analysis

Under aseptic conditions, the eye drops were transferred into glass test tubes using a sterile syringe and a needle. The samples for HPLC analysis were prepared as follows: the eye drops were diluted with water for HPLC (100× or 500× for 1% or 5% eye drops, respectively), the marker (uracil) was added and the solutions were then filtered through 0.45 µm membrane filter into vials. The vials were placed in an autosampler. Twenty microliters of the examined solutions was injected on the column. Content of ceftazidime and pyridine was calculated on the basis of standardization curve equations. The results of the analyses of ceftazidime and pyridine concentrations in 1% and 5% eye drops, stored at 4°C and 20°C for 30 days, are quoted in Tables 3 and 4.

Validation of HPLC method

Specificity

The components of the eye drops (citric acid, sodium citrate and phenylmercuric borate) did not show any absorption at the wavelength of 254 nm and no detector signal was generated during the analysis. β-Phenylethyl alcohol absorbed at the wavelength of 254 nm (A_{max} = 206 nm) and it was eluted from the column after ca. 37 min and did not interfere with the compounds determined. The analyses of versions 2, 4, II and IV were carried out till β-phenylethyl alcohol was eluted from the column, which significantly extended the time of determinations (a sample chromatogram of placebo version 2 – Fig. 1). It was possible to determine ceftazidime next to pyridine because the retention times of both compounds were 9.5 min and 15.5 min, respectively. Other degradation products, which appeared in the eye drops during the storage, did not interfere with the analysis and were eluted from the column in the time below 7.5 min, which was shown on sample chromatograms of version 1 on the 30th day of storage at the temperature of 4°C and 20°C (Fig. 2.). Resolution of ceftazidime peaks was not lower than 6.51, while the one of pyridine peaks did not fall below 8.86 during the 30-day-analysis for each version.

Linearity

Six point calibration curves were prepared for ceftazidime and pyridine. Ceftazidime calibration curve in the concentration range of 25–150 µg/mL was described by the equation: y = 314788465x + 8845, pyridine calibration curve in the concentration range of 2.0–20 µg/mL was described by the equation: y = 212502090x – 19894. The performed analysis of the regression of both curves confirmed

the relationship between the analyte concentration and the peak area: linear correlation coefficients were: r > 0.9999 for ceftazidime and r > 0.9996 for pyridine.

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

Limits of detection (LOD) and limits of quantitation (LOQ) were determined on the basis of the parameters of the analysis of calibration curves; regression of ceftazidime and pyridine worked out from the relationship:

$$LOD = 3.3 \cdot \frac{S_y}{a}, \qquad LOQ = 10 \cdot \frac{S_y}{a},$$

where: here S_y = value of standard deviation and a = directional coefficient of calibration curve.

Limits of detection (LOD) and limits of quantitation (LOQ) were: for ceftazidime LOD = 1.88 µg/mL, LOQ = 5.69 µg/mL and for pyridine they were LOD = 0.66 µg/mL and LOQ = 1.99 µg/mL.

Accuracy and precision

Ceftazidime

Accuracy and precision of the ceftazidime quantitation method in eye drops were determined by the analysis of standard solutions of the eye drops of formulary version No. 1 and No. I at 3 different ceftazidime concentrations. The solutions were prepared at the level of 80, 100 and 120% of the initial concentration of the eye drops and contained respectively 8.0, 10.0 and 12.0 mg/mL of ceftazidime for version No. 1 and 40.0, 50.0 and 60.0 mg/mL of ceftazidime for version No. I. For each standard solution, 3 samples for injections were prepared following the procedure mentioned in the paragraph "Preparation of samples for HPLC analysis", then chromatographic analysis was performed.

Percentage of the recovery calculated in accordance with the formula:

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

was adopted for the determination of the method accuracy:

Accuracy of the ceftazidime quantitation method, calculated as recovery, equaled: for version No. 1 and standard solutions of concentrations 8.0, 10.0, 12.0 mg/mL: 100.09%, 100.11%, 100.03%, respectively, whereas for version No. I and standard solutions of concentrations 40.0, 50.0, 60.0 mg/mL: 100.14%, 100.32%, 99.98%, respectively.

Precision, expressed as % of relative standard deviation (RSD) was not less than 0.51% for 1% eye drops and not less than 0.76% for 5% eye drops.

Pyridine

Accuracy and precision of pyridine quantitation method in eye drops were determined by the analysis of pyridine standard solutions containing 0.3, 0.5, 0.6 mg/mL of pyridine for version No. 1 and 2.0, 2.5, 3.0 mg/mL of pyridine for version No. I. Pyridine concentrations in standard solutions corresponded to the specified range.

Accuracy of the pyridine quantitation method, calculated as recovery, equaled: for version No. 1 and standard solutions of concentrations 0.3, 0.5, 0.6 mg/mL: 101.45%, 101.21%, 99.13%, respectively, whereas for version No. I and standard solutions of concentrations 2.0, 2.5, 3.0 mg/mL: 100.72%, 100.73%, 100.37%, respectively.

Precision, expressed as % of relative standard deviation (RSD) was not less than 1.75% for 1% eye drops and not less than 1.42% for 5% eye drops.

RESULTS

Ceftazidime stability in buffered eye drops stored at 4°C, determined by the time of 10% ceftazidime degradation, was higher in all formulary versions of 1% eye drops in comparison with its stability in 5% eye drops (Tab. 4.). The time of 10% ceftazidime degradation in buffered and not preserved 1% eye drops was 27 days, while in 5% eye drops it was 21 days. In buffered and preserved eye drops the time of 10% ceftazidime degradation was 24 days for 1% and 15 days for 5% eye drops. In the eye drops, which were buffered and of increased viscosity, the times of 10% ceftazidime degradation were 18 days for 1% and 15 days for 5% eye drops, while in case of buffered eye drops and those of increased viscosity containing preservatives they were 18 days for 1% and 12 days for 5% eye drops. Not buffered 1% and 5% eye drops, prepared in sterile water and stored at 4°C, were characterized by the same times of 10% ceftazidime degradation as buffered 1% and 5% eye drops, stored at 4°C. Due to the low osmotic pressure of these eye drops, i.e., 40 mOsm/L for 1% and 195 mOsm/L for 5% eye drops, which is not acceptable for eye drops, not buffered eye drops prepared in sterile water should not be used (Tab. 2.).

The concentration of pyridine in buffered 1% and 5% eye drops in all formulary versions, stored for 30 days at 4°C and 20°C was significantly below the concentration of 63 mg/mL, which was adopted as the concentration that causes no toxic effects following the topical application to the rabbit's eye.

The physical properties of buffered 1% and 5% eye drops (clarity, pH, osmotic pressure, viscosity)

after their preparation and during the time of their chemical stability (Tab. 2.) fulfilled the requirements of Ph. Eur. 6.0 quoted in the monograph Ophthalmica – Eye drops.

During the whole period of storage at both temperatures, the eye drops remained clear, gradual yellowing which was observed, occurred much faster in the eye drops stored at the temperature of 20°C. At the temperature of 4°C the odor change of the eye drops was almost imperceptible and it was only slightly more intense for 5% eye drops; pyridine odor appeared very quickly in the eye drops stored at the temperature of 20°C. Fast ceftazidime degradation in the eye drops stored at 20°C was accompanied by the increase of osmotic pressure, while no pH changes were observed in the eye drops in which ceftazidime degradation was well advanced.

The validation of HPLC method used for quantitative determinations of ceftazidime and pyridine concentration in the eye drops showed that the method was characterized by specificity, accuracy, precision and linearity.

DISCUSSION

Previously published results of the studies (10), which indicated that the application of citrate buffer of pH 6.18–6.30 in 0.9% eye drops containing ceftazidime, stored at the temperature of 4°C, guaranteed no change of 100% initial antimicrobial activity of ceftazidime in the eye drops during their storage for 30 days, were taken into account in the process of developing the composition of 1% and 5% buffered eye drops.

Due to the different nature, condition and course of infection, the composition of the eye drops was developed at two ceftazidime concentrations (1% and 5%) and in five formulary versions for each concentration.

The HPLC analysis of ceftazidime concentration in buffered 1% and 5% eye drops, prepared under aseptic conditions according to the composition of the formulary versions No. 1–4 (1% eye drops) and No. I–IV (5% eye drops), stored at 4°C and 20°C, provided data concerning the influence of ceftazidime concentration, particular additives and storage temperature on the stability of ceftazidime, measured by the time of 10% ceftazidime degradation in the eye drops.

The selection of preservatives for 1% and 5% w/w eye drops containing ceftazidime was related to the results of the preservation efficiency test of 0.9% eye drops, carried out in accordance with the methods of Polish Pharmacopoeia VI, which showed that in 0.9% buffered eye drops phenylmercuric borate at the concentration of 0.001% in the drops combined with 2-phenylethanol at the concentration of 0.4% was effective against the test strains of bacteria *Staphylococcus aureus ATCC 6538*, *Pseudomonas aeruginosa ATCC 9027*, *Candida albicans ATCC 10231* and *Aspergillus niger ATCC 16404*. Apart from that, it showed a high antimicrobial activity against *Listeria monocytogenes*, the test strain which was not mentioned in the preservation test (10).

Citrate buffer turned out to be an appropriate solvent for ceftazidime because in comparison with other solvents, which were used so far in the eye drops containing ceftazidime such as 0.9% NaCl (6, 7), artificial tears (8) and balanced salt solution (9), citrate buffer guaranteed much longer stability of the drops. Besides, citrate buffer was essential in the proposed formulary versions of 1% and 5% eye drops on account of the fact that it put the eye drops to the acceptable osmotic pressure and pH similar to pH of lacrimal fluid (Tab. 2.). Citrate buffer of pH 6.10–6.24 did not decrease the stability of ceftazidime in 1% and 5% eye drops. It results from the comparison of ceftazidime stability in not buffered eye drops (versions $0_{1\%}$ and $0_{5\%}$) and buffered ones (versions 1 and I) (Tab. 3).

Auxilliary substances: phenylmercuric borate, β-phenylethyl alcohol and polyvinyl alcohol decreased the stability of ceftazidime in the eye drops, but in a degree which did not exclude their application in the composition of the eye drops. The lowest stability of ceftazidime was observed in the eye drops containing preservatives including polyvinyl alcohol, formulary versions No. 4 (1% eye drops) and No. IV (5% eye drops). Stability of ceftazidime in 1% eye drops was 18 days and in 5% eye drops it was 12 days (Tab. 3).

CONCLUSION

Storage temperature had the biggest impact on ceftazidime stability in the eye drops. The eye drops, protected from light, should be stored at the temperature of 4°C. The stability of the eye drops depended also on ceftazidime concentration in the eye drops as well as on the presence of auxiliary substances in their composition: phenylmercuric borate, 2-phenylethanol and polyvinyl alcohol.

Several days' stability of ceftazidime in 1% and 5% buffered eye drops, stored at the temperature of 4°C, established with HPLC method and determined by the time limit of 10% ceftazidime degradation in the eye drops, gives pharmacies the oppor-

tunity to prepare eye drops containing ceftazidime on the basis of prescriptions. Depending on the nature and course of infection, the eye drops may be prepared at the concentration of 1% or 5% and according to chosen formulary version in order to meet the needs of the individual patient.

Acknowledgments

The studies were supported by the UMK research grant no. 20/2008.

REFERENCES

1. Kremer I., Robinson A., Braffman M., Drucker M., Goldenfeld M., Samra Z., Myszne-Daye N., Gaton D.: Cornea 13, 360 (1994).
2. Robinson A., Kremer I., Avisar R., Gaton D., Savie H., Yassur Y.: Graefes Arch. Clin. Exp. Ophthalmol. 237, 177 (1999).
3. Fubara J.O., Notari R.E.: J. Pharm. Sci. 87, 53 (1998).
4. Zhou M., Notari R.E.: J. Pharm. Sci. 84, 534 (1995).
5. Zając M., Siwek J., Muszalska I.: Acta Pol. Pharm. Drug Res. 55, 275 (1998).
6. Achach K., Peroux E.: J. Pharm. Clin. 18, 65 (1999).
7. Peyron F., Ibrahim E., Elias R., Bues-Charbit M., Balansard G.: J. Clin. Pharmacol. 18, 48 (1999).
8. Barnes A.R, Nash S.: J. Clin. Pharm. Ther. 24, 299 (1999).
9. Karampatakis V., Papanikolaou T., Giannousis M., Goulas A., Mandraveli K., Kilmpasani M., Alexiou-Daniel S., Mirtsou-Fidani V.: Acta Ophthalmol. 87, 555 (2009).
10. Kodym A., Zawisza T., Napierała B., Kukuła H.: Acta Pol. Pharm. Drug Res. 63, 507 (2006).
11. Hughes W.F. Jr: Appendix I. Bull. Johns Hopkins Hosp. 82, 338 (1948).

Received: 8. 12. 2009