Department of Drug Form Technology[1], Collegium Medicum in Bydgoszcz, Nicolaus Copernicus University, Bydgoszcz; Department of Organic Chemistry[2], Nicolaus Copernicus University, Toruń, Poland

Stability of cefepime in aqueous eye drops

A. Kodym[1], M. Pawłowska[1], J.K. Rumiński[2], A. Bartosińska[1], A. Kieliba[1]

Received July 23, 2010, accepted August 2, 2010

Monika Pawłowska, Department of Drug Form Technology, Collegium Medicum in Bydgoszcz, Nicolaus Copernicus University, M. Skłodowskiej-Curie 9, 85-940 Bydgoszcz, Poland
mjak19@wp.pl

Pharmazie 66: 17–23 (2011) *doi: 10.1691/ph.2011.0214*

The aim of the studies was to determine HPLC the stability of cefepime in 1% and 5% buffered eye drops of developed formulary composition, which were stored for 30 days at the temperature of 4 °C and 20 °C, protected from light. Separation was performed on RP18 Gemini octadecylsilane column (250 mm × 4.6 mm, 5.0 μm) at a temperature of 25 °C. The mobile phase consisted of 0.015 M solution of sodium salt of pentane sulphonic acid brought to pH 4.0 with glacial acetic acid and 45% KOH solution and acetonitrile 94:6 w/w, with detection of 254 nm. The method was linear in the range of 12.6–125 μg/ml ($R^2 = 0.9996$). The limit of detection (LOD) was 3 μg/ml and limit of quantification (LOQ) was 10 μg/ml. 10% degradation of cefepime in 1% and 5% buffered eye drops stored at the temperature of 4 °C, depending on the composition of the eye drops, occurred after 21–27 days in 1% eye drops and 18–21 days in 5% eye drops. In the eye drops, which were stored at the temperature of 20 °C, 10% degradation of cefepime took place on the third day of storage regardless of formulary composition of 1% and 5% drops. Cefepime stability lasting a couple of weeks in 1% and 5% solution allows extemporaneous preparation of buffered eye drops containing cefepime.

1. Introduction

Cefepime – a fourth generation cephalosporin is characterised by a wide range of antimicrobial activity towards Gram-negative and Gram-positive micro organisms including most strains resistant to aminoglycosides and third-generation cephalosporins.
The use of cefepime in the eye drops developed for the topical treatment of infections induced by pathogens which are particularly dangerous for the eye (for example bacteria such as *Pseudomonas*) seems to be fully justified. In scientific literature there is no data concerning the application of the antibiotic in ophthalmology.
Cefepime undergoes rapid degradation in aqueous solutions, which results in hydrolysis (opening) of the β-lactam ring and simultaneous release of the side chain in R-2 position from its particle. Hydrolysis of the β-lactam group and cleavage of the N-methylpyrrolidine particle cause the appearance of two degradation products, neither of which demonstrates any antimicrobial activity. One of them was identified as 2-[((2-amino-4 tiazolyl)(methoxyimino)acetyl)amino]acetaldehyde (Sprauten et al. 2003).
The rate of cefepime degradation in aqueous solutions, similarly to other β-lactam antibiotics, is influenced by temperature, light, composition of a solvent, pH, antibiotic concentration and the type of packaging (Steward et al. 1997; Fubara et al. 1998). Maximum stability of cefepime in aqueous solutions occurs at pH 4.0–6.0 (Evangelou et al. 2003). In aqueous solutions of room temperature cefepime remains stable for about 24 h, while at the temperature of 4 °C its stability lasts 7 days. Stability of cefepime in 0.8% solutions of 0.9% NaCL and in 5% solutions of dextrose stored at the temperature of 4 °C, determined by HPLC, is 15 days (Raubon-Gauyon et al. 1997). The antibiotic degradation at high temperatures (24 °C ± 2 °C) is accompanied by the increase of pH and the change of colour from yellow to amber or red (Baririan et al. 2003).
Special antimicrobial properties of cefepime and its exceptional resistance to plasmid and chromosomal β-lactamases motivated the authors to conduct research on the application of cefepime in eye drops designed for topical treatment of eye infections. Eye drops containing cefepime 1% and 5% (Table 1) were developed and stability of cefepime in eye drops was determined using a HPLC method. The influence of storage temperature, concentration and applied excipients on the stability of cefepime in eye drops was examined as well.

2. Investigations and results

It was found that cefepime in 1% aqueous solutions was pharmaceutically compatible with citric acid, sodium citrate, polyvinyl alcohol, β-phenylethyl alcohol, phenylmercuric borate, sodium chloride. In solutions containing 5% of cefepime an interaction with phenylmercuric borate of 0.001% concentration was observed. Moreover, cefepime in 1% and 5% solutions interacted with thiomersal in concentrations above 0.002% (precipitation appeared after 6 days of storage at the temperature of 4 °C), benzalkonium chloride of 0.005% concentration and chlorhexidine diacetate of 0.01% concentration (strong turbidity of solutions occurred immediately after adding benzalkonium chloride or chlorhexidine diacetate to the cefepime solutions).
Freshly prepared eye drops were clear and colourless in case of 1% drops and slightly yellow in case of 5% eye drops. The characteristic odour of cefepime was observed regardless of the

Table 1: Composition of formulary versions of cefepime in 1% and 5% eye drops

Components (g) per 100 g of the eye drops	1% w/w					5% w/w				
	$0_{(1\%)}$	1	2	3	4	$0_{(5\%)}$	I	II*	III	IV
Maxipime® (Cefepime)	1.725	1.725	1.725	1.725	1.725	8.625	8.625	8.625	8.625	8.625
calculated as cefepime dihydrochloride	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 $\eta = 42.0$ mPas, pH 5.75	–	–	–	49.13	47.70	–	–	–	–	–
Content in % of polyvinyl alcohol for formulation 3 and 4				2.94	2.86					
Solution of polyvinyl alcohol (PVA) viscosity $\eta = 17.0$ mPas, pH 5.75	–	–	–	–	–	–	–	–	45.70	44.23
Content in % of polyvinyl alcohol for formulation III and IV									1.83	1.77
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	98.30	–	–	–	–	91.40	–	–	–	–
Citrate buffer I (pH 6.24, osmotic pressure: 298 mOsm/l)	–	98.30	95.40	–	–	–	91.40	88.47	45.70	44.23
Citrate buffer II (pH 6.05, osmotic pressure: 586 mOsm/l)	–	–	–	49.13	47.70	–	–	–	–	–

* Pharmaceutical interaction in formulation ll of 5% eye drops containing cefepime.

concentration. After 30 days of storage at the temperature of 4 °C eye drops remained clear. No significant changes in colour and odour were noticed in 1% eye drops, while the colour of 5% eye drops changed gradually from light yellow to amber and their odour became more intense and distinctively unpleasant. The colour of 1% and 5% eye drops stored at the temperature of 20 °C changed quickly, after 18 days of storage the eye drops turned red. The odour of the eye drops was also affected and became very intense.

Values pH of fresh 1% and 5% eye drops were in the range of 5.83–6.21, after 30 days of storage at the temperature of 4 °C pH of the eye drops did not change (Table 2). At the temperature of 20 °C pH of 5% drops gradually increased and after 30 days of storage it changed from the initial pH of 5.83–6.00 to pH 6.79 (formulation no. I) and to 7.11 (formulation no. III) (Table 2).

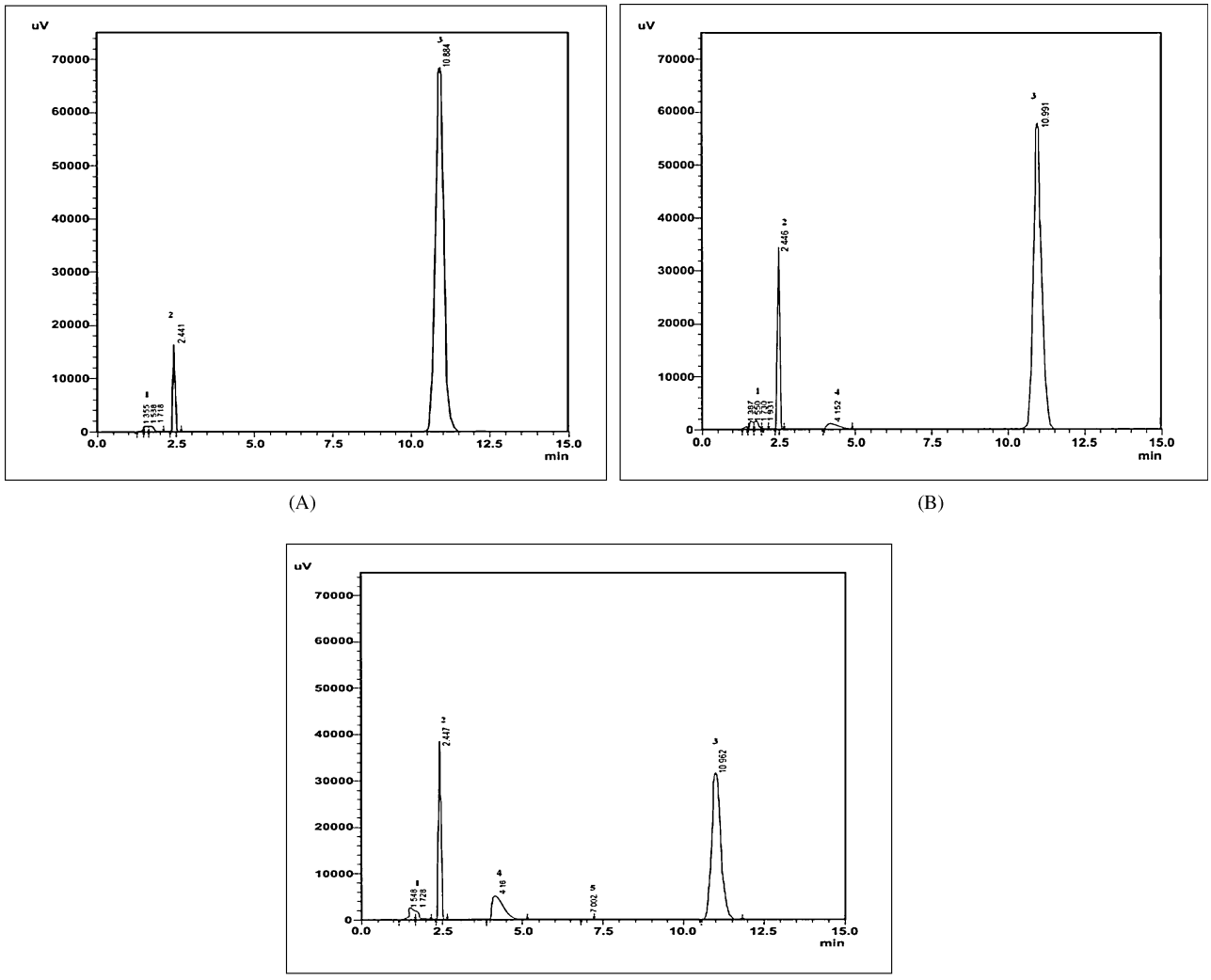
The osmotic pressure of freshly prepared 1% eye drops was in the range of 375–467 mOsm/l, whereas for 5% eye drops it was 586–720 mOsm/l. Osmotic pressure of 1% and 5% eye drops changed slightly after 30 days of storage at the temperature of 4 °C (Table 2).

The viscosity of 1% fresh drops was in the range of 8.54–9.16, for 5% drops 4.95–5.37 mPa·s. After 30 days of storage at the temperature of 4 °C viscosity of eye drops did not change significantly (Table 2).

The stability of cefepime was determined with a validated reversed-phase high performance liquid chromatography method (Table 3). Cefepime stability in 1% buffered eye drops at the temperature of 4 °C, determined by the time of 10% degradation of cefepime, was the highest in eye drops of formulations no. 1 and no. 2 and equalled 27 days. In the eye drops of formulations no. 3 and no. 4 the stability was 21 days. The stability of 5% eye drops was the highest in formulations no. III and no. IV and was the same as the stability of 1% eye drops of formulations no. 3 and no. 4, and equalled 21 days. Shorter stability characterised formulation no I. The stability of 1% and 5% eye drops stored at the temperature of 20 °C was low, 10% degradation of cefepime occurred in all formulations of eye drops on the third day of storage. The results of the stability evaluation are shown in Tables 4 and 5.

A placebo solution containing excipients included in 1% and 5% eye drops was prepared. Its concentration and composition was the same as in the formulation no. 4 of 1% eye drops (Table 1). L-Arginine at the concentration of 0.725% was also added to the placebo solution, which corresponds to its content in 1% drops containing cefepime. The analysis of the placebo solution followed the same procedure as the analysis of the eye drops (see section 4.4.4.). The presence of excipients in 1% and 5% eye drops, i.e., citric acid, sodium citrate, polyvinyl alcohol, β-phenylethyl alcohol, phenylmercuric borate and L-arginine did not in fluence the results of the determination because at the retention time characteristic for cefepime (t_R about 10.990 min) and at the analytical wavelength of 254 nm they showed no retention or absorbance. No interference was observed between the cefepime peak and the peak of the degradation product, whose retention time was shorter (t_R = about 4.180) than the retention time of cefepime (t_R = about 10.990 Fig. 1B and C). During the whole cycle of the analysis the average retention factor for cefepime was k = 3.45, whereas the resolution Rs of the cefepime peak in relation to other peaks was over 1.5. The number of theoretical plates N over 1500 was achieved and the tailing factor (TF) for cefepime peak did not exceed 1.7 (USP, 2006).

A six-point standardization curve was prepared on the basis of the analysis of cefepime content in its standard solutions of concentrations ranging from 12.6 μg/ml to 125 μg/ml. In order to do so, the analytical samples of cefepime standard were weighed up to the accuracy of 0.0001 g, transferred into volumetric flasks of 50 ml and filled up with HPLC water to the required volume. Uracil was added as a marker to every flask. Each standard solution 10 μl was injected onto the column three times. Chromatograms were registered at the wavelength of 254 nm. Limit of detection (LOD) was determined according to the equation $LOD = 3.3 \cdot S_y/a$. Limit of quantitation (LOQ) was determined on the basis of the equation $LOQ = 10 \cdot S_y/a$, where Sy is the standard deviation of y values calculated from the standardization curve and a is the regression factor. The regression equation was $y = 239414.23 \times -2982.99$. Correlation coefficient R^2 was 0.9996. The limit of detection (LOD) was 3 μg/ml whereas the limit of quantitation (LOQ) equalled 10 μg/ml (Table 3).



(A) (B)

(C)

Fig. 1: A sample chromatogram of 1% cefepime in buffered eye drops of version 1 after the preparation (A), after the storage at 4 °C for 30 days (B) and after the storage at 20 °C for 30 days (C). Labels on the chromatograms denote: (A) 1-components of citric buffer l, 2 - uracil - marker, 3 - cefepime, (B) 1 - components of citric buffer l, 2 -uracil - marker, 3 - cefepime, 4 - undefined product of cefepime degradation; (C) 1 - components of citric buffer l, 2 - uracil - marker, 3 - cefepime, 4–5 undefined products of cefepime degradation

Accuracy and precision of the method were determined by analysing model solutions of 1% eye drops (formulation no. 1) and 5% eye drops (formulation no. I) (Table 3). The solutions of eye drops contained 8.0, 10.0 and 12.0 mg/ml of cefepime in 1% eye drops and 40.0, 50.0 and 60.0 mg/ml of cefepime in 5% eye drops. Six samples for injections were prepared for each model mixture according to section 4.4.4.. The percentage of recovery was adopted as the measure of the method accuracy and was calculated according to the following formula: Recovery = determined concentration • 100%/calculated concentration (%). Accuracy and precision expressed as the percentage of relative standard deviation (RSD) is presented in Table 3.

To assess robustness of the method constant values of parameters of chromatographic analysis were determined, i.e. retention time, tailing factor, number of theoretical plates and accuracy and precision (Table 6). The analysis of the robustness of the method at the applied small changes in the composition of the eluent and column temperature did not show any significant influence of parameters of chromatographic analysis (Table 6).

Spectrophotometric analysis of the degradation product of cefepime was carried out using LC Solution software which controlled the liquid chromatography system. UV spectrum of the degradation product (t_R = 4.152 min), which appeared on chromatograms of 1% eye drops after 6 days of storage at the temperature of 4 °C, was prepared (Fig. 2 B). The UV absorption spectra of the degradation product of cefepime in 1% and 5% eye drops of retention time t_R = 4.152 min (for the eye drops stored at the temperature of 4 °C) and t_R = 4.166 min (for the eye drops stored at the temperature of 20 °C) were identical but they differed considerably from the UV absorption spectra of cefepime of fresh eye drops (Fig. 2A and B). The disappearance of the maximum at the wavelength of 257 nm, characteristic for cefepime, in the absorption spectra of the degradation product, and the appearance of the maximum at the wavelength of 287 nm shows that the β-lactam ring opened in the molecule of the analysed degradation product causing the loss of antimicrobial properties.

3. Discussion

The aim of the study was to develop eye drops containing cefepime (1% and 5%) and to determine their stability over a couple of weeks. Topical administration of medicaments in case of eye diseases is justified because the amount of a drug reaching directly the anterior chamber of the eye is much bigger than it is after general administration. The smaller amount of

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

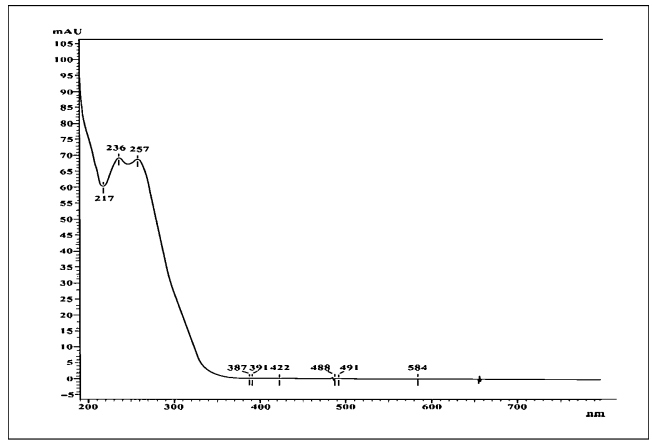
Versions	1% w/w					5% w/w				
	$0_{(1\%)}$	1	2	3	4	$0_{(5\%)}$	I	II	III	IV
Initial pH	4.93 ± 0.01	6.15 ± 0.01	6.21 ± 0.00	5.99 ± 0.01	5.92 ± 0.01	4.82 ± 0.00	6.00 ± 0.01	Pharmaceutical interaction Formulation II was excluded from further research	5.92 ± 0.01	5.83 ± 0.01
pH after 30 days of storage										
Temp. 4 °C		6.02 ± 0.01	6.15 ± 0.01	6.14 ± 0.01	6.16 ± 0.01	6.20 ± 0.01	5.67 ± 0.01	6.25 ± 0.02	5.62 ± 0.02	5.42 ± 0.01
Temp. 20 °C		7.21 ± 0.03	6.29 ± 0.01	6.33 ± 0.01	6.24 ± 0.01	6.26 ± 0.01	7.23 ± 0.01	6.79 ± 0.01	7.11 ± 0.02	7.05 ± 0.01
Initial osmotic pressure (mOsm/l)	87 ± 1	375 ± 1	399 ± 1	426 ± 6	467 ± 3	407 ± 1	720 ± 1		586 ± 2	611 ± 3
Osmotic pressure after 30 days of storage [mOsm/l]										
Temp. 4 °C	90 ± 1	402 ± 1	402 ± 1	451 ± 1	480 ± 1	390 ± 1	699 ± 1		650 ± 2	640 ± 1
Temp. 20 °C	102 ± 1	412 ± 1	412 ± 1	446 ± 1	489 ± 1	431 ± 1	742 ± 1		652 ± 4	673 ± 2
Initial viscosity (mPa.·s)	–	–	–	9.16 ± 0.15	8.54 ± 0.05	–	–		5.37 ± 0.1	4.95 ± 0.03
Viscosity after 30 days of storage [mPa.·s]										
Temp. 4 °C	–	–	–	9.16 ± 0.15	8.57 ± 0.05	–	–		5.44 ± 0.1	5.06 ± 0.03
Temp. 20 °C	–	–	–	9.18 ± 0.15	8.62 ± 0.05	–	–		5.62 ± 0.1	5.25 ± 0.03

Table 3: Validation parameters of HPLC method

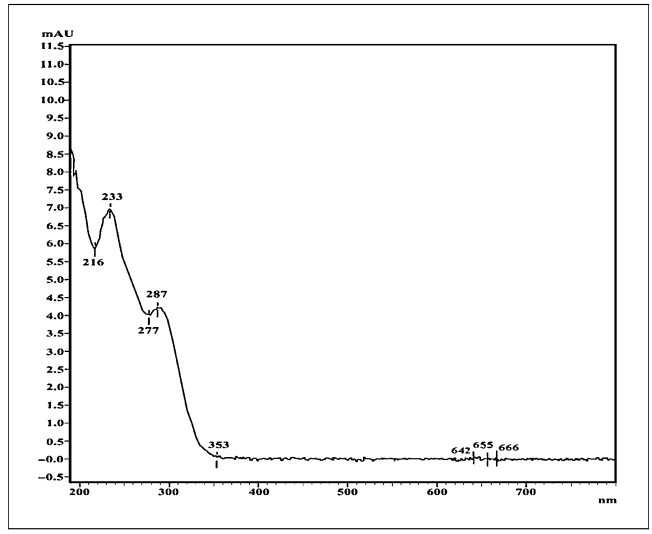
Analyte		Cefepime
Range of applicability μg/ml	12.6–125	
Correlation coefficient (R^2)	0.9996	
LOD μg/ml	3.00	
LOQ μg/ml	10.00	
Accuracy % $n = 6$	1% eye drops	100.25
	5% eye drops	99.57
Precision (RSD) % $n = 6$	1% eye drops	0.30
	5% eye drops	0.51

drug required, consequently reduces the danger of potential side effects. It also applies to cefepime as its general administration may involve many serious harmful side effects (Capparelli et al. 2005; Yahav et al. 2007; Garces et al. 2008; Martin Herrera et al. 2009).

To evaluate the stability of cefepime in 1% and 5% buffered eye drops the influence of the following agents was examined using HPLC method: storage temperature, concentration of cefepime in eye drops, excipients – citric buffers, phenylmercuric borate, β-phenylethyl alcohol and polyvinyl alcohol. Time (number of days) after which 10% degradation of the antibiotic occurred in



(A)



(B)

Fig. 2: Absorbance spectra of cefepime peak showed on the chromatogram (Fig. 1) after the preparation (A) Absorbance spectra of the peaks of cefepime degradation products. The spectrum of the peak at the retention time tR = 4.166 min (B)

Table 4: Cefepime stability - concentration in eye drops containing cefepime (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)	10.06 ± 0.01	10.06 ± 0.01	10.07 ± 0.01	10.08 ± 0.01	10.06 ± 0.01	51.89 ± 0.30	50.50 ± 0.05	Pharmaceutical interaction. Formulation II was excluded from further research.	50.20 ± 0.12	50.80 ± 0.21
Temp. 4 °C	% initial conc. remaining										
Storage time	3 days	99.61 ± 0.77	98.62 ± 0.52	99.18 ± 0.98	96.53 ± 0.10	99.00 ± 1.71	99.12 ± 0.44	98.05 ± 0.33		98.73 ± 0.25	97.86 ± 0.72
	6 days	95.96 ± 0.16	97.92 ± 0.33	98.38 ± 1.12	96.34 ± 0.55	95.74 ± 0.41	97.77 ± 0.87	97.38 ± 0.55		98.50 ± 0.24	97.86 ± 0.12
	9 days	95.45 ± 0.32	97.10 ± 0.44	97.05 ± 0.57	95.56 ± 1.13	91.49 ± 0.01	95.68 ± 0.55	94.89 ± 0.88		98.04 ± 0.12	94.76 ± 0.18
	12 days	93.07 ± 0.22	96.33 ± 0.62	96.67 ± 0.82	94.60 ± 0.43	95.35 ± 0.14	96.39 ± 0.25	94.30 ± 0.58		97.06 ± 0.58	94.66 ± 0.97
	15 days	92.97 ± 0.56	96.06 ± 0.71	94.26 ± 0.66	93.85 ± 0.78	94.46 ± 1.22	93.45 ± 0.18	92.70 ± 0.55		95.46 ± 0.66	93.30 ± 0.36
	18 days	91.52 ± 0.48	92.40 ± 0.50	91.48 ± 0.98	92.38 ± 0.62	93.34 ± 0.09	90.43 ± 0.89	90.91 ± 0.97		93.97 ± 0.21	92.75 ± 0.27
	21 days	90.59 ± 0.22	91.53 ± 0.41	91.68 ± 0.89	92.09 ± 0.32	91.19 ± 0.06	89.70 ± 0.96	89.26 ± 0.57		90.37 ± 0.81	90.78 ± 0.54
	24 days	90.35 ± 0.27	90.49 ± 0.77	91.50 ± 0.55	89.80 ± 0.11	89.44 ± 0.06	89.08 ± 0.35	87.68 ± 0.99		87.37 ± 0.34	89.62 ± 0.21
	27 days	88.82 ± 0.62	90.36 ± 0.32	91.28 ± 0.76	89.09 ± 0.04	86.58 ± 0.14	89.07 ± 0.44	86.58 ± 0.36		88.39 ± 0.54	86.95 ± 0.21
	30 days	88.81 ± 0.43	89.28 ± 0.51	89.19 ± 0.88	88.95 ± 0.76	84.56 ± 0.10	86.96 ± 0.84	85.35 ± 0.74		87.23 ± 0.56	85.70 ± 0.42
Temp. 20 °C	% initial conc. remaining										
Storage time	3 days	95.05 ± 0.28	98.95 ± 0.19	98.83 ± 0.57	94.19 ± 0.01	96.52 ± 2.09	93.42 ± 0.55	89.47 ± 0.33	Pharmaceutical interaction. Formulation II was excluded from further research.	91.22 ± 0.62	89.53 ± 0.33
	6 days	86.63 ± 0.33	87.68 ± 0.25	86.87 ± 0.08	87.66 ± 0.62	86.55 ± 0.05	85.07 ± 0.74	78.93 ± 0.22		87.15 ± 0.33	82.41 ± 0.63
	9 days	82.37 ± 0.22	84.25 ± 0.46	83.74 ± 0.22	85.04 ± 0.20	84.06 ± 0.02	76.51 ± 0.25	72.19 ± 0.56		77.47 ± 0.08	76.75 ± 1.05
	12 days	76.72 ± 0.55	78.00 ± 0.18	79.07 ± 0.55	79.07 ± 0.01	78.56 ± 0.03	68.25 ± 0.38	63.20 ± 0.99		69.01 ± 0.34	69.13 ± 0.81
	15 days	71.19 ± 0.64	73.15 ± 0.26	73.50 ± 0.46	74.24 ± 0.04	74.05 ± 0.05	59.95 ± 0.24	56.74 ± 0.44		61.57 ± 0.94	61.33 ± 0.66
	18 days	63.98 ± 0.41	68.29 ± 0.58	69.25 ± 0.44	72.40 ± 0.03	70.92 ± 0.06	47.46 ± 0.99	55.47 ± 0.68		56.20 ± 0.56	54.60 ± 0.58
	21 days	58.56 ± 0.89	63.41 ± 0.97	62.83 ± 0.74	66.04 ± 0.04	65.80 ± 0.04	42.55 ± 0.84	48.61 ± 0.99		46.64 ± 0.87	43.71 ± 0.15
	24 days	52.83 ± 0.65	61.04 ± 0.67	59.72 ± 0.69	57.27 ± 1.18	56.96 ± 0.36	36.73 ± 0.69	47.56 ± 0.90		47.46 ± 0.18	41.06 ± 0.42
	27 days	43.40 ± 0.85	60.25 ± 1.22	56.22 ± 0.25	57.50 ± 0.43	56.72 ± 0.36	29.23 ± 0.94	41.20 ± 0.38		27.05 ± 0.28	33.07 ± 0.31
	30 days	42.53 ± 0.97	50.14 ± 0.50	50.04 ± 0.98	53.59 ± 0.04	52.98 ± 0.36	21.69 ± 0.21	30.77 ± 0.35		19.65 ± 0.25	24.14 ± 0.55

Table 5: Stability of cefepime in 1% and 5% eye drops stored at the temperature of 4 °C

Versions	1% w/w				
	$0_{(1\%)}$	1	2	3	4
Stability (4 °C)	24 days	27 days	27 days	21 days	21 days
Content of cefepime after 30 days of storage (%)	88.81%	89.28%	89.19%	88.95%	84.56%
Versions	5% w/w				
	$0_{(5\%)}$	I	Pharmaceutical interaction in formulation II of 5% eye drops containing cefepime	III	IV
Stability (4 °C)	18 days	18 days		21 days	21 days
Content of cefepime after 30 days of storage (%)	86.96%	85.35%		87.23%	85.70

the eye drops stored at the temperature of 4 °C and 20 °C was adopted as the determining factor of the stability of cefepime. Selecting the right concentration of cefepime and the composition (formulation) of eye drops (Table 1) one should consider the condition and intensity of an infection, the infected area of the eye and the stage of the infection. For example, 1% and 5% eye drops prepared according to the composition of the formulation no. 1 and formulation no. I (Table 1.) can be applied for the initial acute stage of infection, when the use of eye drops containing preservatives and excipients increasing viscosity is not recommended, and also in the treatment of infections of patients with damaged epithelium of the cornea or with no tolerance towards preservatives. The use of polyvinyl alcohol, which increases viscosity and extends contact time of drops with the eye (formulation no. 3, 4, III, IV, Table 1), in 1% and 5% eye drops containing cefepime, may be beneficial if there is a need for an increased penetration through the cornea to the anterior chamber of the eye.

Citric buffer was essential in 1% eye drops to bring the drops to the acceptable osmotic pressure. 1% not buffered eye drops (formulation $0_{1\%}$) demonstrated strong hypoosmoticity towards the lacrimal fluid. Osmotic pressure of those eye drops was only 87 mOsm/L (Table 2). Citric buffers (pH 6.05 and 6.24) did not decrease the stability of cefepime in 1% and 5% eye drops stored at the temperature of 4 °C. It results from the comparison of the stability of cefepime in 1% buffered eye drops (formulation no. 1) with the stability of cefepime in 1% not buffered eye drops (formulation no. $0_{1\%}$) as well as from the comparison of the stability of cefepime in 5% buffered eye drops (formulation no. I) with the stability of cefepime in not buffered eye drops (formulation no. $0_{5\%}$, Tables 4 and 5).

Concentration of cefepime in buffered eye drops stored at the temperature of 4 °C influenced the stability of the antibiotic: cefepime was more stable in 1% eye drops (10% degradation time of cefepime in those drops was 27 days (formulation no. 1), whereas in 5% eye drops (formulation no. I) 10% degradation time of cefepime was 18 days (Table 4)).

The preservatives in 1% buffered eye drops (phenylmercuric borate of 0.001% concentration in the drops and β-phenylethanol of 0.4% concentration in the drops) did not reduce the stability of cefepime. 1% preserved eye drops of the formulation no. 2 were characterised by the same stability as the one of the eye drops of formulation no 1, which were not preserved. In both cases, i.e. for preserved and not preserved eye drops, 10% degradation of cefepime occurred after 27 days of storage (Tables 4 and 5).

In 5% eye drops (formulation no. II) phenylmercuric borate of 0.001% concentration interacted with cefepime causing turbidity and sediment. As a result 5% eye drops of the formulation no. II were not studied further.

The influence of polyvinyl alcohol on the stability of cefepime depended on its concentration in the eye drops. The negative influence of polyvinyl alcohol in 1% eye drops was observed at its concentration of 2.86% (formulation no. 4) and of 2.94% (formulation no. 3). 10% degradation time of cefepime was shortened by about 6 days in comparison with the eye drops not containing polyvinyl alcohol (formulation no. 1 and 2) and was 21 days (Tables 4 and 5).

In 5% eye drops the concentration of polyvinyl alcohol was lower than in 1% eye drops, i.e. 1.77% (formulation no. IV) and 1.83% (formulation no. III). Polyvinyl alcohol in 5% drops prevented the pharmaceutical interaction between cefepime and phenylmercuric borate used at 0.001% concentration. Furthermore, polyvinyl alcohol extended the time of 10% degradation of cefepime from 18 days (formulation no. I) to 21 days (formulation no. III and IV, Tables 4 and 5).

Specificity and accuracy of the HPLC method made it possible to separate cefepime from the excipients and from the degradation products, which occurred during storage. It also enabled the quantitative determination of cefepime concentration in fresh drops and in those stored at the temperature of 4 °C or 20 °C. The storage temperature was the most important factor for the stability of cefepime. 1% and 5% buffered eye drops containing cefepime stored at the temperature of 4 °C and protected from light have been stable for a couple of weeks. The eye drops stored at the temperature of 20 °C lost their stability just after three days regardless of their formulary composition.

4. Experimental

4.1. Material

Aqueous and buffered solutions of eye drops containing cefepime 1% and 5% were prepared under aseptic conditions according to the composition shown in Table 1; sterile solutions of excipients were used for buffering, preserving and increasing viscosity.

4.2. Reagents

Maxipime (cefepime dihydrochloride), Bristol-Myers Squibb, vials à 1.725 g, in the form of dry powder for intramuscular and intravenous injections, containing cefepime dihydrochloride (1.0 g) and L-arginine (0.725 g), standard of cefepime hydrochloride in accordance with USP 29, citric acid monohydrate p.a., sodium citrate PPhVIII p.a., polyvinyl alcohol mol. mass 72000 p.a. - POCH S.A Gliwice, thiomersal p.a., chlorhexidine diacetate p.a.; β-phenylethyl alcohol p.a., phenylmercuric borate p.a. - Sigma - Aldrich, Germany; 1- pentane sulphonic acid, sodium salt for ion pair chromatography - LiChropur® Merck KGaA, Germany; potassium hydroxide and glacial acetic acid (purity for HPLC) - J. T. Baker, Holland; acetonitrile (gradient grade) - J. T. Baker, Holland; uracil (purity for HPLC) Sigma-Aldrich, Germany, ultrapure water for HPLC obtained using Synergy system - Millipore, France.

Table 6: Robustness of HPLC method

Variations	Temperature	Retention time	Tailing factor	Theoretical plates	Accuracy (%)	RSD (%)
Mobile phase (94:5.7)	25 °C	12.21	1.086	48576.03	103.8	0.07
Mobile phase (94:6)	23 °C	11.41	1.160	46979.84	103.4	0.14
Mobile phase (94:6)	27 °C	10.32	1.102	56003.27	103.7	0.13
Mobile phase (94:6)	25 °C	10.81	1.099	52596.56	103.8	0.14
Mobile phase (94:6.3)	25 °C	9.39	1.139	52073.38	103.5	0.185

Mobile phase: Acetonitrile: 0.015 M sodium salt of pentane sulphonic acid brought to pH 4.0 with 45% KOH, oven temprature

4.3. Apparatus

Shimadzu system for liquid chromatography (Shimadzu Corporation, Japan) equipped with two two-piston pumps (LC 20 AD), degasser, autosampler (SIL 20A/20AC), diode detector (SPD-M20A), column oven (CTO-20A/20AC), C18 Gemini column with column guard (250 mm + 5 mm) × 4.6 mm (Phenomenex), 800 cl osmometer (Trident Med., Poland), Höppler KF-10 viscometer (Medington, Germany), Densito 30PX densimeter (Mettler - Toledo), SP-65 W air steriliser (Wamed, Poland), AS 446 WPA vapour steriliser (Spółdzielnia Mechaników SMS, Poland), CP-502 pH-meter (Elmetron, Poland), 720 g WPS720/C/2 precision balance (Radwag, Poland), Sartorius Expert LE 225D scales (Sartorius, Germany), LM-100/100 µl and LM1000/1000 µl micropipettes (LabMate, Poland), Med-28 pharmaceutical refrigerator (Kirsch, Germany), Synergy system for ultrapure water (Millipore, France), Sonic 10 supersonic washer (Polsonic, Poland).

4.4. Methods

4.4.1. Preparation of solutions for the evaluation of pharmaceutical compatibility of cefepime with excipients

Under aseptic conditions excipients were added separately to 1% and 5% aqueous solutions of cefepime. The volume of excipients corresponded to their actual concentration in the composition of eye drops prepared for industrial and non-industrial purposes, i.e. sodium chloride (0.9%), sodium citrate (6%), citric acid (0.3%), polyvinyl alcohol (1.77% to 2.94.%), benzalkonium chloride (0.005%), thiomersal (0.002% and 0.02%), chlorhexidine diacetate (0.01%), phenylmercuric borate (0.001%), β-phenylethyl alcohol (0.4%). Sample aqueous solutions of cefepime containing dissolved excipients were stored at the temperature of 4 °C and 20 °C for 10 days, protected from light and observed for changes in colour, clarity and odour.

4.4.2. Preparation of sterile aqueous solutions of excipients

The following solutions of excipients were used to prepare eye drops: sterile solutions of citric buffers, i.e. citric buffer I (102.01 mM tri-sodium citrate dihydrate, 7.14 mM citric acid monohydrate, distilled water ad 1000.0 g), citric buffer II (204.02 mM tri-sodium citrate dihydrate, 14.28 mM citric acid monohydrate, distilled water ad 1000.0 g), 4% and 6% solutions of polyvinyl alcohol (PVAL), auxiliary aqueous solutions of preservatives: 2% thiomersal, 0.5% benzalkonium chloride, 1% chlorhexidine diacetate, 0.04% phenylmercuric borate and β-phenylethyl alcohol. The method of preparation of the above mentioned solutions was described in the previous publication (Kodym et al. 2006).

4.4.3. Preparation of eye drops containing cefepime

Eye drops were prepared under aseptic conditions (laminar air flow chamber) in accordance with formulary compositions shown in Table 1. Maxipime® (cefepimum) was dissolved in the appropriate citric buffer or in sterile water. Defined volume of the auxiliary solution of 0.04% phenylmercuric borate and β-phenylethyl alcohol was added. After mixing, the eye drops were filtered through a Sartorius microbiological membrane filter of 0.22 µm pore diameter. In case of the formulations of increased viscosity, the defined volume of sterile auxiliary solution of polyvinyl alcohol was added after the eye drops had been preserved and filtered through Sartorius filters. The eye drops were poured into 100 ml sterile infusion bottles, sealed with rubber plugs and metal caps. Eye drops were stored for 30 days in pharmaceutical refrigerators (Med.-28 Kirsch, Germany) at 4 °C and 20 °C and protected from light.

4.4.4. HPLC evaluation of cefepime stability in 1% and 5% eye drops

Chromatographic separation was performed on a RP 18 Gemini column with column guard (250 mm + 5 mm) × 4.6 mm (Phenomenex, USA), particle size 5 µm, porosity 110 Å. Mobile phase: acetic buffer (0.015 M sodium salt of 1-pentane sulphonic acid brought first to pH 3.4 with glacial acetic acid and then to pH 4.0 with 45% potassium hydroxide): acetonitrile (940:60, w/w) (mobile phase pH 4.0), flow rate: 1.5 ml/min, column temperature: 25 °C. The wavelength of the detector was fixed at 254 nm. The injection volume for 1% samples was 10 µl, whereas for 5% samples the injection volume was 5 µl. Each sample was injected three times.

500 µl of 1% eye drops and 200 µl of 5% eye drops were transferred with automatic pipettes to three separate volumetric flasks of 50 ml. 100 µl of the aqueous solution of uracil at the concentration of 0.4 g/L was added to each sample. Uracil was applied as a marker to determine dead-time (t_o) of the analysis and to calculate the retention factor (k) for cefepime. The volume of flasks was supplemented with water for HPLC. After mixing, the solutions were filtered through a 0.45 µm membrane filter into 1.5 ml vials. The vials were put into the autosampler. One shot was made from each of the three vials (10 µl of 1% eye drops and 5 µl of 5% eye drops). Concentration of cefepime in the analysed samples was calculated from the equation of the standardization curve, using the recorded areas of peaks as the base for the calculation. The concentration of cefepime in the eye drops was determined every three days. The results of the determination of cefepime stability in the aqueous solutions and in the eye drops are presented in Tables 4 and 5.

References

Baririan N, Chanteux, H, Viaene E, Servais H, Tulkens P (2003) Stability and compatibility study of cefepime in comparison with ceftazidime for potential administration by continuous infusion under conditions pertinent to ambulatory treatment of cystic fibrosis patients and to administration in intensive care units. J. Antimicrob Chemother 51: 651–658.

Capparelli F, Diaz M, Hlavnika A, Wainsztein N, Leiguarda R, Del Castillo ME (2005) Cefepime and cefixime induced encephalopathy in a patient with a normal renal function. Neurology 65: 1840.

Evangelou V, Tsantili-Kakoulidou A, Koupparis M (2003) Determination of the Dissociation Constants of the Cephalosporins Cefepime and Cefpirome Using UV Spectrometry and pH Potentiometry. J Pharm Biomed Anal 31: 1119–1128.

Fubara JO, Notari RE (1998) Influence of pH, temperature and buffers on cefepime degradation kinetics and stability predictions in aqueous aolutions. J Pharm Sci 87: 1572–1576.

Garces E, Andrade de Azambuja M, Silva D, Bragatti J, Jacoby T, Saldanina T (2008) Renal failure is a risk factor for cefepim induced encephalopathy. J Nephrol 21: 526–534.

Kodym A, Zawisza T, Napierała B, Kukuła H (2006) Influence of additives and storage temperature on physicochemical and microbiological properties of eye drops containing ceftazidime. Acta Pol Pharm 6: 507–513.

Martin Herrera C, Navarro M (2009) Cefepim induced encephalopathy in patients with renal failure. Nefrologia 29: 181.

Polish Pharmacopeia Edition VIII (2008) Warsaw PZWL.

Sprauten P, Beringer PM, Louie SG, Synold TW, Gill MA (2003) Stability and antibacterial activity of cefepime during continous infusion. Antimicrob Agents Chemother 6: 1991–1994.

Stewart JT, Warren WFW, Madoxx FC (1999) Stability of cefepime hydrochloride injection in polypropylene syringes at –20 °C, 4 °C, and 22–24 °C. Am J Health Syst Pharm 56: 457–459.

US Pharmacopeia XXIX (2006) The United States Pharmacopeial Convention, Rockville, MD, p. 409–411.

Yahav D, Paul M, Fraser A, Sarid N, Leibovici L (2007) Efficacy and safety of cefepime; systematic review and meta-analysis. Lancet Infect Dis 7: 338–348.