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Stability study of cefepime in different infusion solutions

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Abstract

The stability of cefepime in the formulation Axepim® diluted with 0.9% sodium chloride or with 5% glucose in a three layer laminate bag Clear-Flex® (polyethylene container) was investigated at 24 ± 2°C in daylight and at 4 ± 2°C in the dark (initial concentration was 8 mg ml^{-1}). Stability was defined as at least 90% of the initial concentration. Analyses were performed by reversed phase high performance liquid chromatography, with a method previously validated. This was specific, linear ($r \geq 0.9995$), sensitive (CV < 0.44%) and reproducible (CV < 1.26%). The limit of detection was 0.1 $\mu g\ ml^{-1}$ and limit of quantification was 1 $\mu g\ ml^{-1}$. This study showed the changes in the stability of cefepime over 48 h at 24 ± 2°C in daylight, or 15 days at 4 ± 2°C in the dark. The yellowish coloration which appeared in some solutions, indicated visually a degradation of cefepime. IR and NMR spectra showed that this coloration was linked to the destruction of the Δ-cephem ring of the molecule. © 1997 Elsevier Science B.V.

Keywords: Stability; Cefepime; Polyethylene; Infusion

1. Introduction

Continuous intravenous infusions of drugs may have advantages over conventional repeated injection. For intravenous infusion, the containers used may be glass or plastic. Glass has the appeal of wide compatibility with all types of drugs but drawbacks are the large size of the container (relative to the fluid volume), its fragility and necessity of air entrance for fluid delivery. Conversely plastic bags are collapsible (without requiring the entrance of air), unbreakable and easily stockable but are not always compatible with drugs. The majority of the plastic bags available on the market are polyvinyl chloride (PVC),

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which contains the plasticizer diethylhexylphthalate (DEHP) for flexibility. Therefore leaching of DEHP can occur in some solutions and its toxicity has been reported (Jouko et al., 1982; Downie et al., 1985; Jaeger and Rubin, 1972; Barthes et al., 1994). Another type of plastic bag available on the market is composed of a three layer laminate of polypropylene, polyamide and polyethylene, the latter being the inert and biocompatible material in contact with the injectable solution. Of course, as no DEHP is used for manufacturing of polyethylene, no leaching can be generated. This study was carried out in polyethylene-lined bags.

It is interesting to know if these infusions can be prepared and dispensed for use over several days, in order to plan the reconstitution of antibiotic infusions in the pharmaceutical service. The widespread use of intravenous infusion bags makes stability considerations important.

The purpose of this study was to determine the stability of cefepime, which is a new antibiotic, in the formulation Axepim® diluted with 0.9% sodium chloride or with 5% glucose in a three layer (Clear-Flex®) laminate bag (initial concentration: 8 mg ml^{-1}). The mechanism of degradation of cefepime was also studied.

Cefepime is [2-aminothiazol-4-yl]-2(*Z*)-[methoxyimino - acetamido] - 3 - [methyl - 1 - pyrrolidino] methyl-ceph3-em4-carboxylic acid as shown in Fig. 1. It is a fourth generation cephalosphorin antibiotic, (Knapp and Washington, 1989; Kessler, 1985; Jones and Fuchs, 1989; Voutsinas et al., 1989; Fung-Tomc et al., 1991). It is a broad spectrum antibiotic.

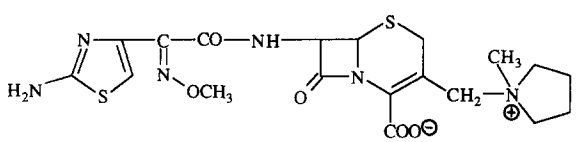

Fig. 1. Molecular structure of cefepime.

2. Materials and methods

2.1. Reagents

Axepim® lot A3280 was purchased by Bristol-Myers-Squibb, Paris, France. Each vial contained 1 g of cefepime. L-Arginine is added in Axepim® for its basic properties, and it allows the achievement of a final pH of about 5 after reconstitution. Indeed cefepime aqueous solution gives $pK_1 = 1.5$ and $pK_2 = 3.1$, and it would be too acidic for parenterals.

Axepim® was further diluted with 0.9% sodium chloride or 5% glucose, already packaged in the infusion container, to give a concentration of 8 mg ml^{-1}, usual in therapeutic.

All substances and solvents were analytical reagent grade or HPLC grade.

2.2. Standards

Cefepime (lot CDD3U2005) was provided by Bristol-Myers-Squibb, Paris, France.

2.3. Containers

Polyethylene bags (Clear-Flex®) with 250 ml of 0.9% sodium chloride or with 5% glucose were obtained from Bieffe Medital, St. Germain en Laye, France.

2.4. HPLC analysis

All assays were performed by high performance liquid chromatography (HPLC) at room temperature (24 ± 2°C). An automatic sample injector Wisp 717 (Millipore Corporation, St. Quentin, France) and L-6000 Merck pump (Nogent sur Marne, France) were used. Isocratic reversed-phase chromatography was performed with a Merck Lichrocart® 100 RP_{18} column (4×125 mm, 5 mm particle size) and an acetonitrile: pH 4.8 buffer (10:90) used as mobile phase (flow rate was 1.0 ml min^{-1}). The pH 4.8 buffer was prepared by mixing CH_3COOH 0.2 M, CH_3COONa 0.2 M, H_2O in the ratio 10:15:75 (v:v:v). Samples were previously diluted 1 to 100 in water, and 100 μl were injected. The column effluent was moni-

tored with a diode array detector (Waters 990 Millipore Corporation, St. Quentin, France), at 257 nm. Peak areas were registered with a software HPLC Merck Manager via a computer and interface Merck-Hitachi (Merck, Nogent sur Marne, France). Under these conditions, the cefepime eluted at 2.7 min. Method validations were determined using the included Merck software validation method manager (Caporal-Gautier et al., 1992) (Nogent sur Marne, France).

2.5. Spectrum analysis:

UV spectra were monitored with a Hewlett Packard 8450A UV-Visible spectrophotometer (Les Ulis, France), on a 15-day-old solution of Axepim® stored at 24 ± 2 and 4 ± 2°C. Prior to recording IR and NMR spectra, the aqueous solutions were evaporated in a vacuum desiccator. IR spectra were obtained with Ati-Mattson Genesis series FTIR (Madison, USA) from a 5% dispersion in KBr, after evaporation of a 500 ml 60-day-old solution, stored at 24 ± 2 and 4 ± 2°C. NMR spectra were obtained with Varian EM 360A (Les Ulis, France) on the powder obtained after evaporation and also on Axepim® solution reconstituted directly in D_2O and stored for 60 days at 24 ± 2 and 4 ± 2°C.

For each type of spectrum, the standards with Axepim® recently reconstituted were recorded and showed that the evaporation did not change the compound.

2.6. Cefepime

The peak for cefepime showed the same UV spectrum as a standard. Neither L-arginine absorbed at 257 nm, nor was there any interference with the cefepime peak. Fig. 2 shows a chromatogram of the Axepim® sample at the initial time.

Calibration curves were drawn from a linear plot of peak area versus concentration (20–120 μg ml^{-1}). The correlation coefficient of the standard curves was greater than 0.9995. No statistical difference (Student's t-test) appeared between cefepime standard curve and curve.

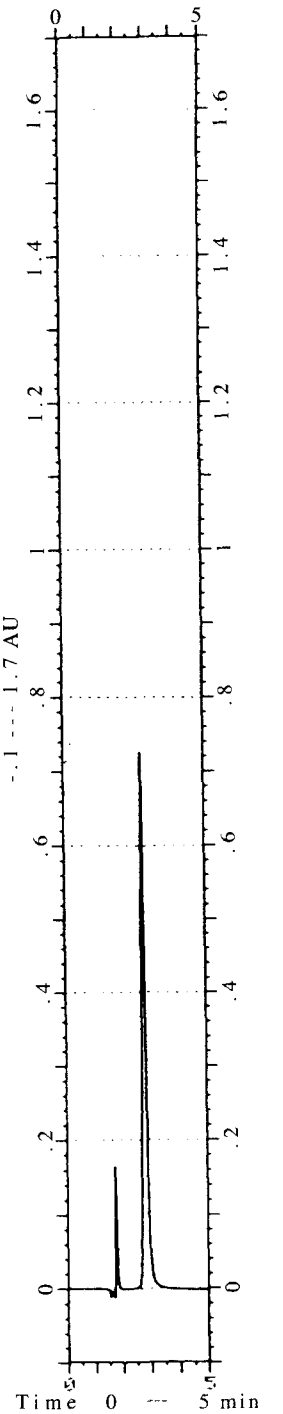

Fig. 2. Chromatogram of AXEPIM®, at initial time (column Lichrocart® 100 RP_{18}; mobile phase acetonitrile/pH 4.8 buffer 10/90; flow-rate 1.0 ml min^{-1}; detection wavelength 257 nm).

The fidelity of the method was determined on three series of $n = 7$ measures at theoretical concentration of 80 μg ml^{-1}. The repeatability (within-day) and reproducibility (between-day) coefficients were respectively 0.44 and 1.26%. The limit of detection was 0.1 μg ml^{-1} and the limit of quantification was 1 μg ml^{-1}.

2.7. Stability study

The study was realized on two different solutions for infusion in 250 ml polyethylene containers, 0.9% sodium chloride and 5% glucose. For each, 2 g of cefepime were introduced into the container, with previous dissolution in a small aliquot to give a final concentration of 8 mg ml^{-1}. The bags were agitated by bending, flexing and shaking for about 1 min and then the containers were stored either at 24 ± 2°C (room temperature, daylight), or 4 ± 2°C (refrigerated temperature, dark). Triplicate measures were realised for each temperature. Samples of 2 ml were withdrawn at 0, 2, 4, 6, 8, 24 h, then at 2, 3, 4, 7 and 15 days. After agitation, solutions were visually inspected for color and clarity (European Pharmacopeia, 1983). Samples were immediately subjected to pH measure and chromatographic analysis. Drug concentrations were determined by HPLC ($n = 3$ for each infusion solute/temperature). The initial concentration of drug was designated as 100%; all subsequent concentrations were expressed as a percentage of the initial one. Stability was defined as 90–105% of the initial concentration.

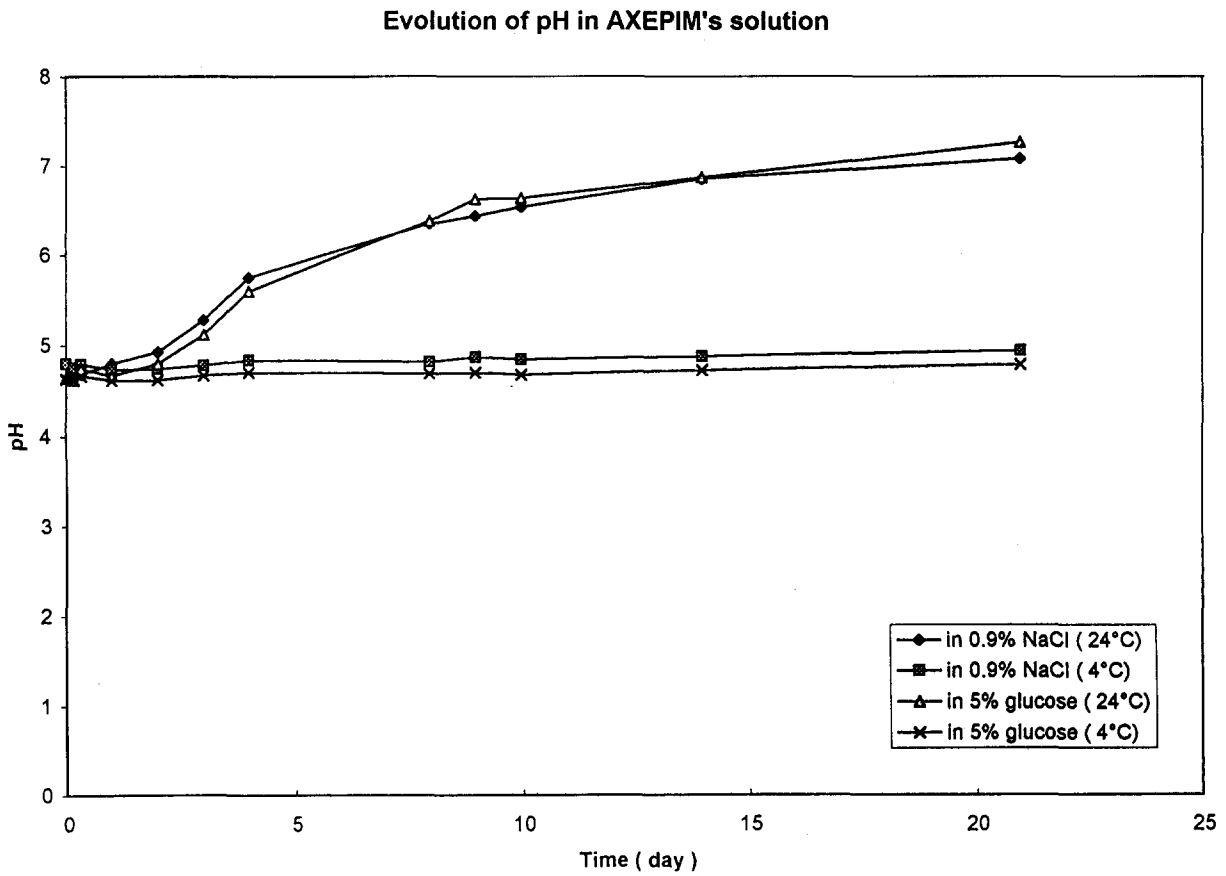

Fig. 3. pH values in AXEPIM® reconstituted with 0.9% sodium chloride or with 5% glucose, in polyethylene infusion containers, stored at 4 ± 2 and 24 ± 2°C.

3. Results and discussion

Both in 0.9% sodium chloride, or in 5% glucose, visual observations showed the appearance of an amber colour during the protocol. When stored at 24 ± 2°C in daylight, the coloration started after 2 days, and after 15 days when stored at 4 ± 2°C, in the dark of the refrigerator. Squibb (Vidal, 1995) recommends storage of the powder protected from light. pH measures indicated no variation at 4 ± 2°C and an increase of 2 pH-units at 24 ± 2°C, as shown in Fig. 3.

Cefepime assays were found in the range 100–68% of the initial concentration, and are reported in Table 1, for the stability study in 0.9% sodium chloride. Cefepime was stable in these solutions at 8 mg ml^{-1} for 72 h at 24 ± 2°C in daylight, and 15 days at 4 ± 2°C in the dark. On the other hand in 5% glucose solution, assays in the range 100–30% of the initial concentration were found, and are also reported in Table 1. It indicates that cefepime was effectively stable in these solutions for 48 h at 24 ± 2°C in daylight, and 15 days at 4 ± 2°C in the dark.

Table 1
Cefepime (Axepim®) stability study in 0.9% sodium chloride or 5% glucose, in polyethylene infusion containers, stored at 4 ± 2 and 24 ± 2°C

	Percentage of initial cefepime concentration (S.D.)			
	In 0.9% NaCl		In 5% glucose	
	+4°C	+24°C	+4°C	+24°C
To	100.0	100.0	100.0	100.0
2 h	96.7 (0.8)	98.7 (0.8)	98.7 (1.2)	99.0 (0.2)
4 h	96.4 (1.9)	97.9 (1.2)	98.2 (1.9)	99.1 (0.7)
6 h	96.7 (0.6)	98.9 (1.1)	96.4 (1.9)	97.1 (1.0)
8 h	96.9 (1.8)	97.8 (0.5)	97.4 (1.9)	98.1 (1.1)
24 h	94.4 (1.8)	95.0 (0.4)	94.2 (1.6)	94.3 (1.1)
2 days	95.4 (0.6)	92.9 (1.3)	94.4 (1.0)	91.7 (1.0)
3 days	96.3 (1.1)	91.8 (0.5)	94.4 (0.8)	89.7 (0.8)
4 days	95.9 (1.8)	88.6 (0.6)	93.3 (0.6)	86.5 (1.0)
7 days	95.8 (1.2)	83.8 (0.3)	94.1 (1.2)	80.1 (1.0)
15 days	92.6 (0.7)	68.8 (1.1)	91.6 (1.8)	30.0 (0.6)

Values expressed as a percentage of initial concentrations ($n = 3$).

Up to 24 h, a slight decrease of cefepime was similar in 0.9% NaCl at 24 ± 2°C compared to 4 ± 2°C, but after this period, the decrease was faster in daylight than in the refrigerator.

A coloration appeared in all 60-day solutions, but the 24°C stored samples were strongly ambered. However in our chromatographic conditions and with a diode array detector, no new peaks appeared although the cefepime peak decreased. A comparison of the UV-visible spectrum of Axepim® freshly reconstituted ($t = 0$) and after 15 days ($t = 15$) showed a slight decrease in absorbance at 257 nm; this was more accentuated at 24 ± 2 than at 4 ± 2°C. The IR at $t = 0$ and $t = 60$ days showed just one difference; the lack of the C = O vibration of the lactam ring, when the solution was stored at 24 ± 2°C. If stored at 4 ± 2°C, this vibration peak only decreased. The NMR spectrum shows the two coupled doublets corresponding to the lactam ring missing. The degradation of the lactam ring was confirmed by UV, IR and NMR spectra, simultaneously with the decrease of the cefepime's peak in HPLC. In addition, in the NMR spectra, the disappearance of the peak at 3.8 ppm, corresponding to the CH_2N^+ of cefepime, could result from the breaking of the pyrrolidine cycle.

The classic hydrolysis of the Δ3-cephem ring occurs in cephalosporin in aqueous solution, but is obviously not sufficient to explain all these observations. On the other hand, our data corroborate the well-known yellowish coloration of cephalosporin during ageing as described in the literature. Lerner et al. (1988) reported competitive photolysis of cefotaxime consisting of at least two processes (one on the Δ3-cephem ring and the second on the methoxyimino group) which led to an intense yellowing of the solution corresponding to the destruction of Δ3-cephem ring. Fig. 4 shows that during photolysis, the ring opening path was found to be different from the one observed during hydrolysis.

The absorbance decrease observed at 257 nm can be linked to the destruction of the Δ3-cephem ring. On the other hand the amount of photolysis cannot be quantified because the formed photoproducts can have a low UV absorption. The direct effects of irradiation on the antibiotic activity make this photochemical degradation path an important one, but it cannot be separated from degradation by hydrolysis.

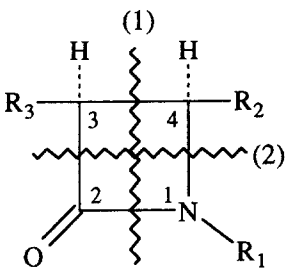

Fig. 4. Degradation paths of the β-lactam ring: (1) hydrolysis (2) photolysis.

4. Conclusion

The study of cefepime diluted with 0.9% sodium chloride or 5% glucose to 8 mg ml^{-1}, in polyethylene containers showed stability of cefepime for 48 h at 24 ± 2°C in daylight, or 15 days at 4 ± 2°C in the dark, for both. The Δ3-cephem ring photolysis which is in competition with the isomerization and the hydrolysis gives several compounds and at least one of them has a yellow colour that could be used as a visual index of degradation. For reconstitution of the antibiotic, polyethylene bags can be recommended, with storage in the dark at 4 ± 2°C for 15 days, for both 0.9% sodium chloride, or 5% glucose solutions.

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