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Stability of reconstituted solutions of ceftazidime for injections: an HPLC and CE approach

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Abstract

The stability of aqueous reconstituted ceftazidime injection vials containing ceftazidime pentahydrate blended with anhydrous sodium carbonate was investigated in different storage conditions (4°C and 10°C for 7 days in a refrigerator, 20 and 30°C for 24 h) with validated HPLC and (micellar) CE methods. Stability indicating data were obtained for ceftazidime and two degradation products: pyridine and the Δ^2-ceftazidime isomer. Other degradation products were also identified (the complementarity of the two used experimental procedures was useful in such exercise) and characterized by their UV spectra and retention times. Stability data (7 days at 4°C in a refrigerator and 18 h at room temperature) resulted in agreements with the manufacturers prescription and point out the need of a strict temperature control of the refrigerator's compartment used to store the reconstituted solution. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Ceftazidime; HPLC; CE; Stability; Degradation products

1. Introduction

Ceftazidime (I) is a well known third-generation cephalosporin antibiotic [1,2] having, in its Z (syn) configuration [3,4], an extended spectrum of activity against gram-negative bacteria especially *pseudomonas* spp; with its oxymino moiety it is also more resistant than second-generation cephalosporins to most β-lactamases produced by various species of bacteria. Like all cephalosporins, aqueous solutions of ceftazidime are however unstable and subject to hydrolytic degradation; indeed several studies have examined its stability under different conditions [5–9], its degradation kinetics [8] influenced by various catalysts (buffers, carbohydrates, etc.), the pathway of the hydrolytic process itself [7]. The hydrolysis, facilitated in alkaline solution [9], involves, inter alia, the formation of pyridine, the cleavage of the β-lactame ring, epimerization and isomerization processes (see Fig. 1). Quite recently *N*-{(2-aminothiazol-4-yl)-[(2-carboxy-2-methyl)-ethoxyimino]acetyl}-aminoacetaldehyde has been described as a degradation product whose polymeric form is a specific inhibitor of lentiviral RNases H [10].

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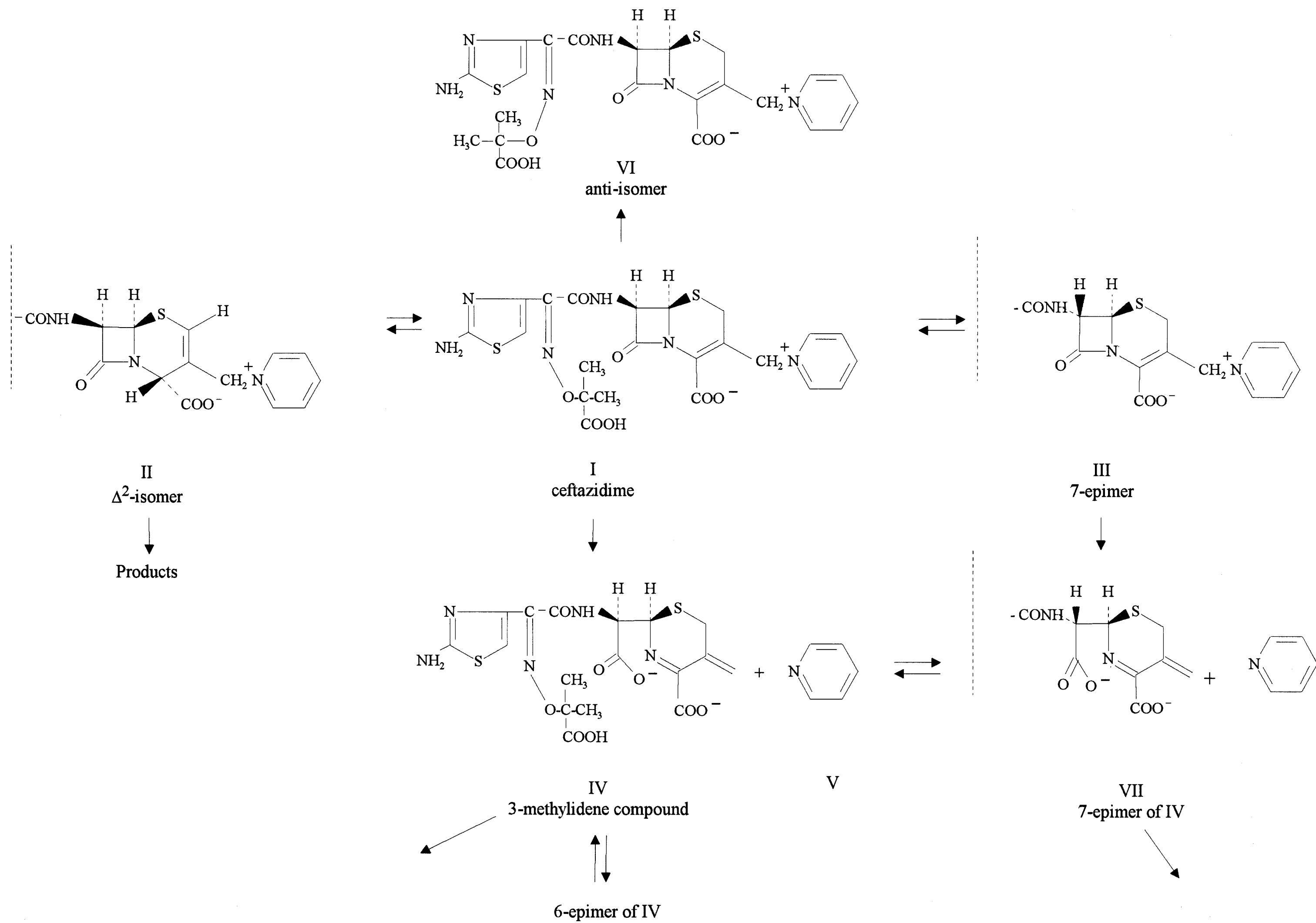

Fig. 1. Structure of ceftazidime (I) and its main impurities and degradation products.

Ceftazidime (I) is an acid which crystallizes as the pentahydrate; it is available for clinical use as a sterile blend with anhydrous sodium carbonate or L-arginine since in this solid forms is stable and can be stored at room temperature protected from light, for a long period of time, without apparent loss of activity. The pyridine content in the dry mixture ceftazidime pentahydrate-sodium carbonate allowed by the Food and Drug Administration [11] and USP 23 [12], is 'not more than 0.4%', furthermore the Food and Drug Administration specify that 'at the issuance of a certificate for each batch of the sodium carbonate formulation, the pyridine content is not more than 0.12%'. For intramuscular or intravenous administration the solid blend is brought in solution by the addition of water for injections; such reconstituted solutions are not stable; indeed the reported instructions for ceftazidime–sodium carbonate blend indicate that reconstituted solutions are to be used within 18 h if stored at room temperature or within 7 days if stored at 4°C. Several studies have examined ceftazidime stability under simulated usage patterns for reconstituted commercial products. However, for ceftazidime injections there is still a need for a simple procedure that can be used for the simultaneous analysis of ceftazidime, ceftazidime impurities and degradation products including pyridine. The aim of our study has been:

1. the development and validation of an HPLC method to characterize the evolution of the decomposition processes of ceftazidime;
2. the application of the validated procedure to the investigation of the reconstituted solutions stability, with specific reference to ceftazidime, Δ^2-isomer and pyridine, taking into account the influence of temperature and light;
3. to compare the HPLC stability data with simultaneously obtained CE stability data using an elsewhere described method [13].

2. Experimental

2.1. Chemicals and reagents

Standards of ceftazidime pentahydrate (84.7% anhydrous ceftazidime), Δ^2-and anti-isomers were kindly supplied by Glaxo Wellcome (Verona, Italy) and were used as received.

Pharmaceutical dosage forms (ceftazidime injection vials containing ceftazidime pentahydrate blended with anhydrous sodium carbonate corresponding to 1 g of anhydrous ceftazidime and 3 ml water vial for injection) were commercial samples.

Analytical grade sodium tetraborate was from Fluka (Buchs, Switzerland); sodium dodecylsulphate was 99% pure from Aldrich (Buchs, Switzerland) and was used without further purification; all other chemicals and solvents were obtained from Merck (Darmstad, Germany) and were of analytical or HPLC grade.

2.2. Instruments

2.2.1. High performance liquid chromatography

A Hewlett-Packard Model 1050 instrument (Avondale, PA, USA) consisting of a HP 1050 quaternary pump, diode array detector (190–600 nm), autosampler, and a Violet T-55 S column heater compartment (Violet, Rome, Italy) was used. Chromatographic data were monitored and processed with a HP. ChemStation using an HP computer Model Vectra 90.

2.2.2. Capillary electrophoresis

An HP3D System (Hewlett-Packard, Waldbrom, Germany) equipped with a diode array detector (190–600 nm) was used. Data were collected on a HP Chemstation.

2.2.3. UV spectroscopy

UV absorption spectra of ceftazidime, its impurities and degradation products were obtained with the diode array detector; a Unicam UV 4–100 double-beam spectrometer was used to obtain numerical values of molar extinction coefficients.

2.3. Analytical conditions

2.3.1. Chromatographic conditions

The HPLC separation of ceftazidime and its

related impurities was achieved by means of Alltima C18, 5 μm (length 250 mm, I.D. 4.6 mm) column. In the development stage different eluents were tested; what follow refers to the final method. Eluent: 93% of a 43.5 mM $NH_4H_2PO_4$ solution in water (pH 3.9 with H_3PO_4) plus 7% of acetonitrile, at a flow rate of 1 ml min^{-1} at 35°C. The injection volume was 10 μl for all samples. UV detection was carried out at 254 nm.

The CE separation was achieved using a fused silica capillary tube (Hewlett-Packard) with extended path light. The dimensions of the capillary were 64.5 cm long (56 cm effective length) × 0.05 mm I.D.

The final background electrolyte (BGE) consisted of 25 mM sodium tetraborate, pH 9.2 (unadjusted), added with sodium dodecylsulfate (SDS) up to a 150 mM concentration. The appropriate amount of SDS was added to the BGE daily.

Before CE separation the capillary was cleaned using 0.1 M NaOH (5 min) followed by an H_2O rinse (5 min). Injection conditions: 50 mbar for 8 s; the applied voltage was 20 kV (constant). The capillary was thermostated at 25°C. The electropherograms were detected at a fixed UV wavelength (254 nm).

2.4. Procedure

2.4.1. Standard solutions

Aqueous standard solutions of ceftazidime (1 mg ml^{-1} anhydrous ceftazidime), Δ^2-isomer (100 μg ml^{-1} and pyridine (100 μg ml^{-1}) were prepared and used immediately after appropriate dilution for standardization purposes.

2.4.2. Sample solutions

One vial corresponding to 1.23 g of ceftazidime pentahydrate blended with sodium carbonate was diluted with 3 ml of water for injection. The concentration of the obtained solution correspond to about 270 mg ml^{-1} of anhydrous ceftazidime.

2.4.3. Calibration curves

Calibration curves were obtained by injection of standard solutions and plotting the analytical data.

2.4.4. Qualitative, quantitative analysis and storage conditions

Qualitative and quantitative analysis were made using ceftazidime vials for injections (from different manufacturers), reconstituted with water for injection according to the manufacturer's instructions. Each obtained solution was divided in four equals parts and stored in the following conditions:

1. 4°C for 7 days in a refrigerator;
2. 10°C for 7 days in a refrigerator;
3. 20°C for 24h;
4. 30°C for 24 h.

Samples of 100 μl were taken from the stored solutions daily for 7 days, during storage at 4 and 10°C; after 3, 6, 18, 24 h during storage at 20 and 30°C. Such samples were immediately analysed after a suitable dilution with water.

Ceftazidime, Δ^2-isomer and pyridine were quantified by peak area measurements using calibration curves.

3. Results and discussion

3.1. Performance of the HPLC method

The performance of the HPLC method was evaluated with respect to specificity, system and method precision, linearity and limits of quantitation and detection.

3.1.1. Specificity

Well resolved peaks corresponding to ceftazidime, its known impurities and degradation products is shown in Fig. 2 together with their relative retention times. Ceftazidime samples separately spiked with pyridine, Δ^2- and anti- isomers were used for the identification of the peaks corresponding respectively to each compound.

The UV absorption spectra (see below) and the correlation with the increasing concentration of pyridine support the peak attribution to the 3-methylidene compound. The peak appearing at RRT 2.2 was tentatively attributed to the 7 epimer of I [9].

For each observed peak the UV absorption spectra of the specific corresponding compound

has been recorded; indeed the shape of the UV absorption is an useful parameter in supporting the identification of the compound responsible of each specific peak area. Fig. 3 show the various spectra recorded with the diode array during the most appropriate chromatographic experimental conditions. A comparison of their shape (absorption intensities are not comparable between different spectra) allow the following considerations.

The syn → anti conformational transition of ceftazidime induces small but significant variations in the observed spectra of the syn-conformer:

- the shoulder in the on-set of the first absorption band is blue-shifted;
- blue-shifts and small intensity changes are also clearly evident in the 210–270 nm range.

As expected, the Δ^2-isomer, deriving from an isomerization process involving a double-bond, shows, with respect to syn-ceftazidime, evident differences in some electronic transition energies:

- the shoulder in the 300 nm region is better defined;
- the observed red-shift of the minimum in the 210–270 nm range is a clear consequence of some transition energies shifts.

The 3-methylidene derivative shows a broad absorption in the 260 nm region; the quoted low intensity [9] of such band is in agreement with the absence of the pyridine moiety in the chromophore itself and with the opening of the β-lactam ring.

The recorded spectra of the compound with RRT 2.2 is in agreement with the attribution of the corresponding peak area to the 7-epimer of syn-ceftazidime. Indeed the 7-epimer of I is said to have the same UV spectrum of I itself [9]; such statement is acceptable in the assumption that small but significant differences characterize the spectrum of each epimer or isomer of syn–ceftazidime.

Ceftazidime pentahydrate has been also characterized measuring the molar extinction coefficients in 0.1 M HCl solutions ($\varepsilon_{260} = 25\,740 \pm 700$).

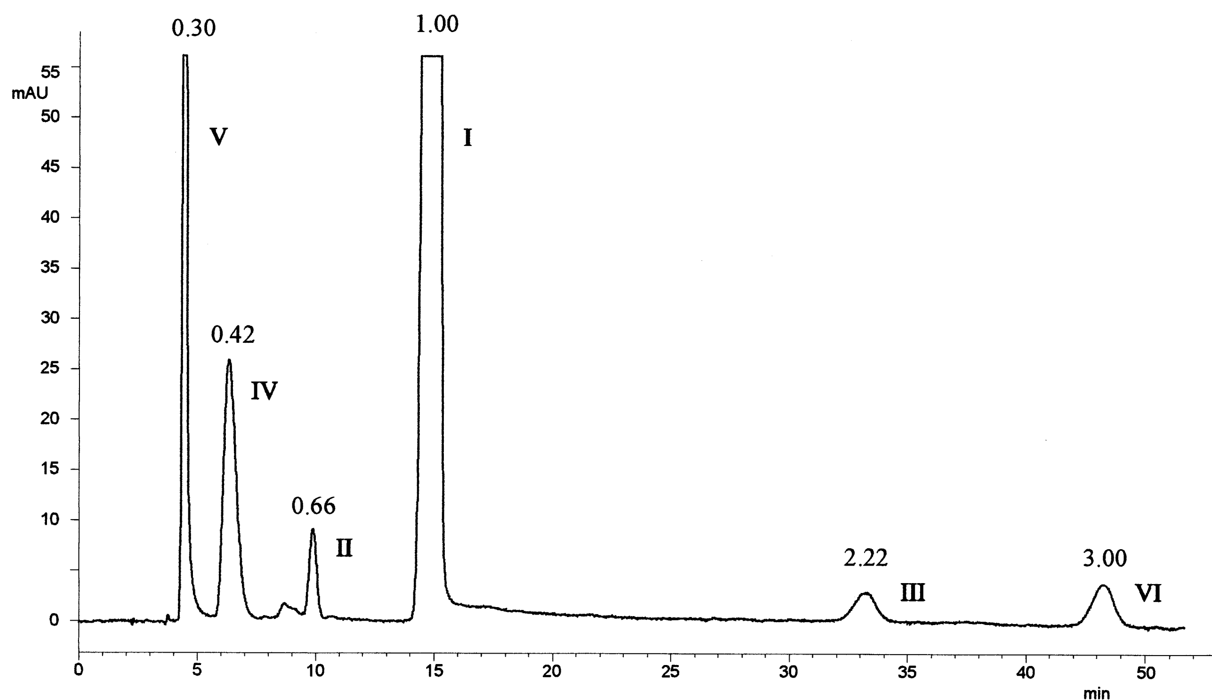

Fig. 2. HPLC chromatogram of degraded ceftazidime (I) solution, spiked with pyridine (V), Δ^2- (II) and anti-isomers (VI). Peaks IV (RRT 0.42) and III (RRT 2.22) are, respectively, attributed to 3-methylidene compound and 7-epimer of I.

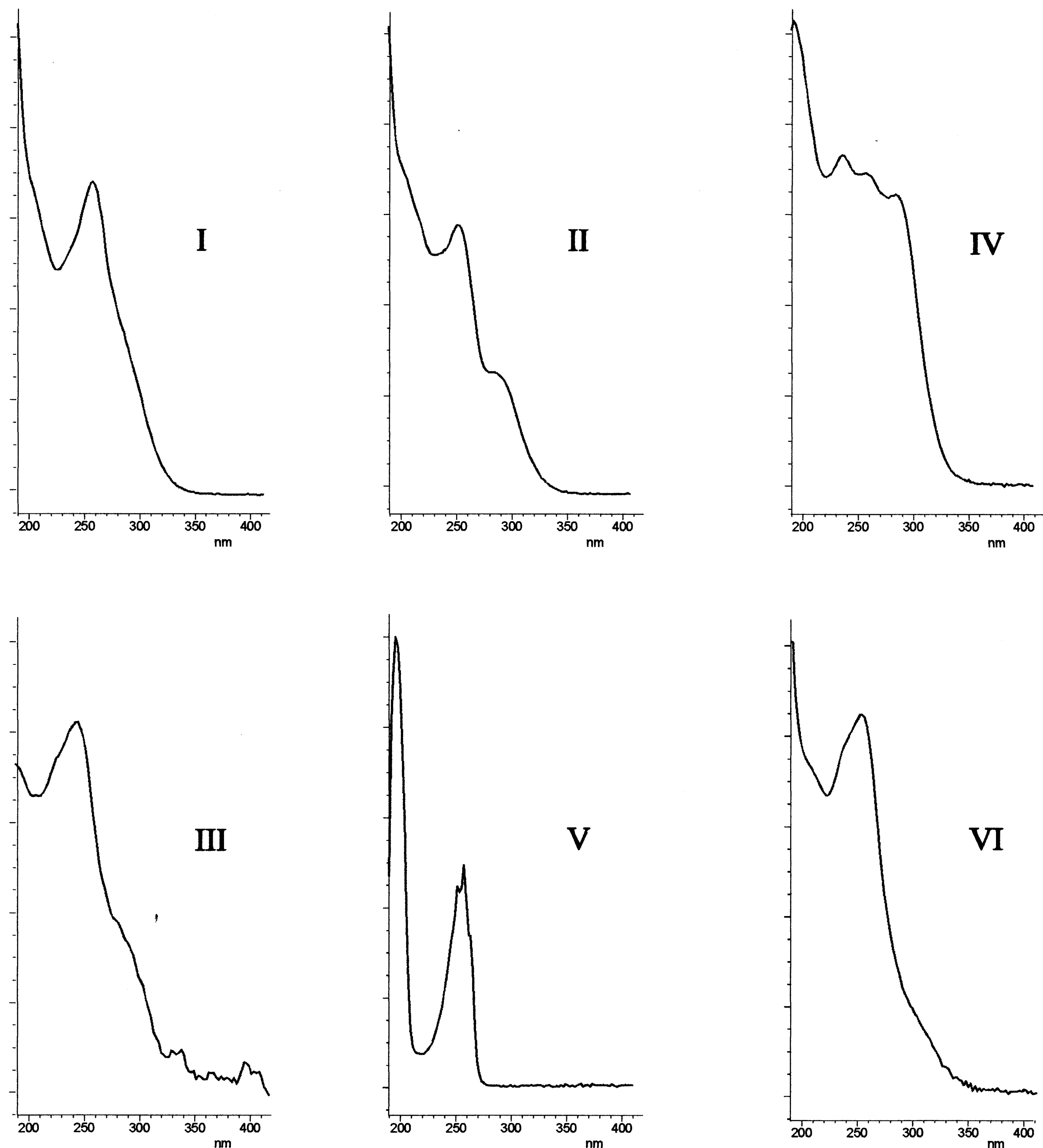

Fig. 3. UV spectra of ceftazidime (I), Δ^2-isomer (II), 3-methylidene compound (IV), 7-epimer (III), pyridine (V), anti-isomer (VI).

3.1.2. System precision

System precision was measured by the reproducibility of the retention time and peak areas of ceftazidime for six replicate injections (10 μl) of a single standard solution preparation containing 100 μg ml^{-1}. The following values are obtained:

time (min) $\bar{x} = 14.40 \pm 0.07$, %RSD 0.49; peak area $\bar{x} = 2967 \pm 4.2$, %RSD 0.14.

3.1.3. Method precision

Method precision was obtained on different days by making injections of six standard preparations of ceftazidime (100 μg ml^{-1}). The retention times and values for the peak area/sample weight for these injections are: $\bar{x} = 14.47 \pm 0.08$, %RSD 0.56; $\bar{x} = 2.42 \pm 0.02$, %RSD 0.62, respectively.

3.1.4. Linearity

Linear calibration plots were obtained over the concentration ranges tested, i.e. 0.1–2 μg for I, 10–250 ng for pyridine and 10–100 ng for Δ^2-isomer; the corresponding linear regression equations, with correlation coefficients ≥ 0.999, were, respectively, $y = 2375.6x + 10.655$, $y = 3.549x + 44.12$ and $y = 2.737x + 5.333$.

3.1.5. Limits of detection and quantitation

The limits of detection (signal-to-noise ratio of 2) for pyridine and Δ^2-isomer were determined to be approximately 0.05 and 0.2 μg ml^{-1}, respectively.

Results for the limit of quantitation (calculated as three times the LOD value) for the two degradation products were, respectively, 0.40 ± 0.02 and 0.70 ± 0.04 μg ml^{-1}.

These limits of detection and quantitation are sufficient for this work in which the stability of ceftazidime is monitored also as a function of pyridine content and not only as a function of ceftazidime concentration.

3.1.6. Recovery of pyridine

The recovery of pyridine from a ceftazidime blend sample (containing 77.4% of anhydrous ceftazidime) spiked with three different concentrations of pyridine was determined by comparing the peak areas with those of standard solutions of pyridine.

Four samples of a solution of ceftazidime blend (corresponding to 10 mg ml^{-1} of anhydrous ceftazidime) each spiked with known quantities of pyridine (0, 0.4, 1.0 and 1.5% w/w, respectively) were analyzed. For each spiked level 4 replicated analyses were performed.

The ratio of the found to the expected pyridine content with spiked samples (recovery) was $99.7 \pm 1.34\%$ ($n = 9$).

3.2. Performance of CE method

The performance of the CE method was previously evaluated [13] with respect to specificity, linearity, precision, limit of detection and quantitation and recovery of pyridine.

An electropherogram showing well resolved peaks is presented in Fig. 4. Peaks corresponding to ceftazidime (I), its Δ^2 and anti-isomers (II and VI, respectively) and pyridine (V) were identified spiking with the relative compounds specific analyzed samples. Compounds IV and III were tentatively considered to give rise, in the order, to the two small peaks whose elution time is around 9.2–9.6 min.

Calibration graphs for ceftazidime were linear within the studied range (0.02–0.9 mg ml^{-1}); the same holds true for pyridine (0.5–100 μg ml^{-1}) and Δ^2-isomer (0.5–35 μg ml^{-1}). Correlation coefficients > 0.999 were found. The limit of quantitation for pyridine and Δ^2-isomer has been determined as 0.50 ± 0.02 μg ml^{-1}.

3.3. Stability of ceftazidime injection in water after reconstitution

In agreement with previously reported data, a typical first order kinetic for the loss of ceftazidime was observed using HPLC and CE methods for the stability study of reconstituted ceftazidime solutions. In addition the obtained results confirm that, in our experimental conditions, the primary route of degradation might be the formation of pyridine and the concomitant rearrangement involving the opening of the β-lactam ring of the remaining moiety.

Table 1 shows the calculated percentages of ceftazidime remaining, after the specified degradation time, assuming 100% the initial concentration of ceftazidime itself in the reservoirs stored in different conditions. Absolute concentration values for pyridine and Δ^2-isomer are also reported.

In the assumption that a ceftazidime solution is considered acceptable when retains 90% or more

of its ceftazidime initial concentration such data are in agreement with the manufacturers indication on stability of ceftazidime injection in water after reconstitution: 7 days at 4°C (condition A) and 18 h at room temperature (condition C).

Data relative to storage conditions D and B point out a crucial factor: the temperature's controls of the different sites of the 'medium' used to store the reconstituted vials. Indeed:

1. the various compartments of a refrigerator normally used in hospitals or houses might not be controlled with respect to their real temperature; differences of few °C can change significantly the stability of the reconstituted solutions;
2. room temperature near to 30°C significantly decreases the stability time range.

Data relative to the two quantified degradation products point out that, irrespective of the storage conditions, with a 10% degradation of ceftazidime in the reconstituted solutions:

- the pyridine content in the same solutions increases from about 0.05% (w/v) to approximately about 0.35% (w/v);
- the Δ^2-isomer concentration do not changes appreciably.

Such data seem to indicate the opportunity to consider also the pyridine content in defining 'indications' on stability of ceftazidime injections in water after reconstitution. Indeed, considering that the pyridine content allowed by USP for a dry mixture of ceftazidime pentahydrate-sodium carbonate is not more that 0.4% w/w (corresponding to a pyridine content of 0.11 w/v in the reconstituted solutions), the acceptance, as unique stability parameter, of a 10% reduction in the initial ceftazidime concentration imply to consent pyridine levels, in the reconstituted solutions, of the order of 0.35% w/v.

The described analytical procedures, taking into account that running cost and run times of CE are lower than for HPLC and that reproducibility of CE is often not as good as that of HPLC, do not show significant advantages or disadvantages of the use of one method compared to the other. Vice versa it emerges that the separation of ceftazidime and its degradation products is improved by the HPLC method; in this respect the two methods seem to be complementary for a wide stability study referring to all ceftazidime degradation products. For instance the HPLC approach clearly point out a peculiar behaviour of

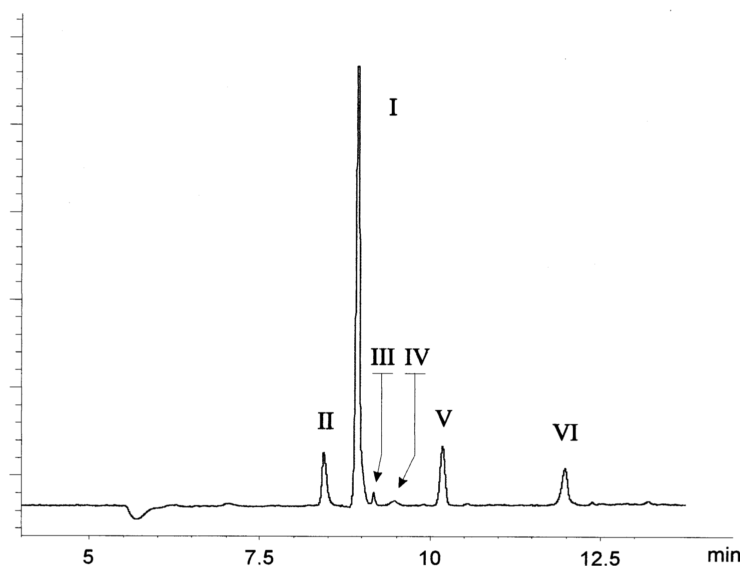

Fig. 4. Electropherogram of degradated ceftazidime (I) solution, spiked with pyridine (V), Δ^2- (II) and anti- (VI) isomers. Peaks IV (RMT 1.06) and III (RMT 1.03) are tentatively attributed to 3-methylidene compound and 7-epimer of I.

Table 1
Stability of reconstituted solutions of ceftazidime for injection. (1.23 g of ceftazidime pentahydrate blended with sodium carbonate reconstituted with 3 ml of water corresponding to about 270 mg ml^{-1} of anhydrous ceftazidime) [a]

Storage time	Ceftazidime % initial concentration remaining		Pyridine[b] (g% w/v)		Δ^2-Isomer (g% w/v)	
	HPLC	CE	HPLC	MEKC	HPLC	CE
Condition A[c]						
Initial concentration	–	–	0.05 ± 0.01	0.04 ± 0.01	0.04 ± 0.01	0.04 ± 0.01
3 Days	98.9 ± 0.8	97.3 ± 1.2	0.11 ± 0.02	0.12 ± 0.02	0.05	0.05
5 Days	94.6 ± 0.5	94.6 ± 0.3	0.12 ± 0.02	0.13 ± 0.02	0.04	0.06
7 Days	91.8 ± 0.6	91.1 ± 0.5	0.30 ± 0.03	0.28 ± 0.02	0.04	0.06
Condition B[d]						
1 Day	98.8 ± 0.3	99.3 ± 0.6	0.11 ± 0.01	–	0.04	0.03
3 Days	94.9 ± 0.9	95.0 ± 1.5	0.25 ± 0.02	0.20 ± 0.03	0.05	0.06
4 Days	91.5 ± 1.2	90.6 ± 0.5	0.31 ± 0.02	0.32 ± 0.02	0.06	0.07
5 Days	87.9 ± 1.2	87.7 ± 0.8	0.37 ± 0.02	–	0.06	–
7 Days	83.6 ± 1.8	84.4 ± 1.0	0.49 ± 0.03	0.43 ± 0.04	0.07	0.11
Conditions C[e]						
3 h	99.8 ± 0.7	100.1 ± 0.8	0.07 ± 0.01	0.06 ± 0.01	0.05	0.05
6 h	98.7 ± 0.8	97.6 ± 0.5	0.09 ± 0.01	0.11 ± 0.02	0.06	0.05
18 h	95.1 ± 1.0	96.6 ± 1.3	0.12 ± 0.02	0.13 ± 0.02	0.05	0.06
24 h	92.7 ± 0.9	92.0 ± 1.5	0.26 ± 0.02	0.25 ± 0.03	0.05	0.06
Conditions D[f]						
3 h	97.9 ± 0.9	97.7 ± 1.3	0.11 ± 0.01	0.12 ± 0.02	–	0.05
6 h	95.5 ± 0.5	–	0.17 ± 0.01	–	0.05	0.05
18 h	88.4 ± 0.1	89.6 ± 1.6	0.39 ± 0.07	–	0.07	0.08
24 h	83.7 ± 1.7	84.0 ± 2.1	0.46 ± 0.05	0.45 ± 0.07	0.08	0.10

[a] Each reported data represents the mean value ± SD of duplicate determinations for each of at least three samples of pharmaceutical formulations.
[b] Pyridine content in the bulk samples claimed by the manufacturer was about 0.14% w/w, corresponding to <0.05% w/v in the reconstituted solution.
[c] Storage in a refrigerator at 4°C.
[d] Storage in a refrigerator at 10°C.
[e] Storage at 20°C.
[f] Storage at 30°C, no significative differences were observed in the experimental values obtained in dark or daylight conditions.

the peak area at RT ~ 30 min (RRT 2.2) which, after 24 h at 30°C, seems to decrease while a new peak start at RT ~ 7.7.

4. Conclusion

The HPLC and MEKC methods used for stability studies of ceftazidime in different storage conditions both separate this compound from its potential impurities and degradation products. In addition the use of two complementary methods increases the likelihood of detecting any unknown impurities which may be present in the examined material.

Both methods meet acceptable criteria for system precision, method precision and linearity. The limits of detection and quantitation for the two degradation products are also adequate.

The quantitative results obtained with the two methods are consistent.

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