

Fortum[®] stability in different disposable infusion devices by pyridine assay[☆]

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Abstract

The stability of ceftazidime in 5% dextrose injection and 0.9% sodium chloride injection when stored in a different disposable infusion device was determined. Solutions of ceftazidime 40 mg/ml were used to fill the drug administration devices. Stability was determined for both 5% dextrose injection and 0.9% sodium chloride injection solutions at 37 °C in four disposable infusion devices. Ceftazidime and its mean degradation product, pyridine, were simultaneously assayed in triplicate by a stability-indicating high-performance liquid chromatographic (HPLC) method. This method was simple, sensitive (limit of quantitation (LOQ), 2 ng injected for both compounds), rapid (run time was 7 min) and precise (mean recovery was 100.5 ± 2.9 and $103.6 \pm 1.9\%$ for pyridine and ceftazidime, respectively). The ceftazidime stability in the 5% dextrose solution was lower than in the 0.9% sodium chloride solution. When stored at 37 °C in a disposable infusion device, the stability of the ceftazidime is included in large hourly range, depending strongly on the manufacturer. The stability of ceftazidime exceed 19 h in none studied cases. The pyridine formed in 24 h was in the range of 100–400 mg depending on devices and infusions. © 2002 Elsevier Science B.V. All rights reserved.

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1. Introduction

Ceftazidime is a third-generation cephalosporin that is widely used for the treatment of serious infections caused by gram-negative bacteria including *Pseudomonas aeruginosa*. It is more resistant than second-generation cephalosporin to most β -lactamases produced by various species of

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bacteria [1]. β -Lactam antibiotics most effectively kill bacteria when their concentration remains above the minimum inhibitory concentration (MIC) of the infecting organism throughout the dosing interval; thus, intermittent dosing may not always provide optimal therapy [2]. The rising incidence of gram-negative infections, especially for patients with cystic-fibrosis, and the availability of improved intravenous drug delivery systems have led to the investigation of continuous-infusion β -lactam therapy [3,4]. Administration of ceftazidime at home by continuous I.V. infusion was safe, effective and cost-effective [3]. Therefore, to obtain maximum benefit from this method of drug delivery, it is necessary to know the behaviour of drugs under therapeutic drug concentration, in different available infusion devices [5]. But, like all cephalosporins, aqueous solutions of ceftazidime are subject to hydrolysis reaction [6]. Indeed, influence of pH, temperature, buffers and concentration on the kinetics of ceftazidime degradation in aqueous solutions was studied [7,8]. The kinetic analysis of ceftazidime degradation showed first-order hydrolysis [9].

Other studies have examined its stability under several storage conditions: in plastic syringes and/or glass vials at different temperatures [10,11], in normal saline and dextrose in minibags [12], in drug reservoirs in an insulated pouch enclosed between two frozen gel packs [13], in an elastomeric infusion device [14]—Singleday Infusor Baxter—and in PVC infusion bags [15].

The products of degradation are multiple [16], but one more than the others draw attention, i.e. pyridine. Indeed, the underestimated toxicity of pyridine by intravenous way and FDA or USP texts hint at a maximum tolerated concentration of pyridine in the medicine [17,18]. Since, several studies followed the appearance of pyridine during the degradation of Fortum in aqueous solutions, in infusion device with two different mobile phases [19], simultaneously in an eye drop formulation [20,21] by HPLC, in aqueous solution by capillary electrophoresis [16] and in OutBound[®] ambulatory infusion device [22]; this last one estimates the stability of the ceftazidime by measuring the pyridine according to time.

In spite of the important number of articles telling the stability of the ceftazidime, any of them do not report such a study on these types of infusion devices, especially by referring on the dosage of the pyridine. So, the purpose of this study was to evaluate the stability of ceftazidime in 5% dextrose injection or 0.9% sodium chloride injection, based on the measure of pyridine concentration as described in previous study [22], when stored at 37 °C in four disposable infusion devices (Infusor[®], EasyPump[®], UltraFlow[®], OutBound[®]).

2. Materials and methods

2.1. Materials

Ceftazidime in FortumSet, containing ceftazidime pentahydrate (1 or 2 g) with sodium carbonate, was obtained from Glaxo-Wellcome (Glaxo-Wellcome, batches V9D04 and V9M03). The drug was assayed in different disposable infusion devices to simulate ambulatory treatment, the types of containers used were Infusor[®] LV 10 (Baxter Laboratories, batches 99D012, 99EO43 and 99DO73), a 240 ml poly-isoprene latex free device, Easypump[®] LT 125 (Braun, lot 992091), a 120 ml synthetic elastomer latex free bag, UltraFlow[®] (Fresenius-Kabi, lots 7284, 7359 and 7498), a 110 ml poly-vinyl chloride (PVC) bag, and Outbound[®] (Zambon, lots D990609B, D990121B and D990316B), 100 ml polyethylene device. The drug stability was tested in each of these four systems with 0.9% sodium chloride injection (Baxter, batches 00A10G50, 00A28G50, 00A31G53, 00C09C2G51 and Aguettant, batch 29201A02) and 5% dextrose injection (Baxter, batches 00A27S51 and 00B23G50).

Ceftazidime pentahydrate (lot AWS 27E) 84.6% pure powder was donated by Glaxo-Wellcome (Evreux, France) and pyridine, 99 + % (lot 24263-039) was obtained from Sigma-Aldrich (St Quentin-Fallavier, France). All other chemicals and reagents were of analytical grade or high-performance liquid chromatography (HPLC) grade.

2.2. Principles of different ambulatory infusion devices

These infusion ambulatory systems work according to three different principles. Infusor and Easypump have an inflatable ‘balloon’ reservoir surrounded by a protective shell; the pressure created by the inflated balloon forces the medication through the tubing. In UltraFlow system, the pressure is exerted on the infusion bag by a reusable, spring-loaded, two-part cylindrical housing with spring. Outbound[®] system works by exerting pressure on an internal syringe plunger equipped with vacuum chamber.

2.3. Preparation of admixtures

Solutions of ceftazidime injection were prepared under sterile conditions at a concentration of 40 mg/ml. That concentration reflects the recommended dosage of ceftazidime in commercial sources for stability and compatibility studies. More, working in concentration, and not in infused quantity like in cystic fibrosis therapy [3], simplified analytical handling and data analysis.

The stability of ceftazidime was determined at 37 °C in four brands of disposable infusion devices. A pooled solution of ceftazidime was prepared by dissolving Fortum[®] in the infusion fluids. The manufacturers’ directions were used to fill drug administration devices with the following volumes, Infusor LV 10, 240 ml; EasyPump, 120 ml; UltraFlow, 82.5 ml with 0.9% sodium chloride solution and 70 ml with 5% dextrose solution; OutBound, 100 ml.

Three of each device, each being a different lot, excepted for Braun system, were used to test concentration–time evolutions of pyridine and ceftazidime. Devices were stored in the dark in a steamer at 37 °C. Every 2 days, one type of device filled with one type of injection solution was studied. Every hour, samples were taken in a collecting glass tube placed at the end of device capillary outlet. This volume was diluted 1:160 with mobile phase to about 250 µg/ml of ceftazidime. The simultaneous measure of ceftazidime and pyridine was made alternately in each of the three shares. Triplicate injections were done onto HPLC.

2.4. Analysis by high-performance liquid chromatography

The HPLC assay used was the same method as Favetta et al. [22]. The instrumentation included a Kromasil C₁₈ reversed phase column (Interchim, Montluçon, France), an isocratic pump model LC-10AD (Shimadzu, Touzart et Matignon, France), an ultraviolet detector model SPD-10Avp (Shimadzu) set at 257 nm, a Rheodyne manual valve model 7125 (Cotati, USA) with 20 µl loop and a recording integrator model C-R6A Chromatopac (Shimadzu). The mobile phase consisted of acetonitrile–ammonium acetate (25 mM) (10:90, v/v) adjusted to pH* 5 with concentrated acetic acid. The flow rate was 0.8 ml/min and retention times were about 3.5 and 4.4 min for ceftazidime and pyridine, respectively.

Ceftazidime and pyridine concentrations were determined by comparing peak areas with standard curves. Standard curves were determined daily by using ten standard solutions. Five with ceftazidime concentrations ranging from 25 to 250 µg/ml and five with pyridine concentrations ranging from 0.1 to 50 µg/ml. The correlation coefficients for the standard curves were all greater than 0.999.

Equations for the calibration curves were; for Ceftazidime, $Y(\text{area}) = 36\,119 \pm 1998X(\mu\text{g/ml}) + 187\,170 \pm 15\,875$; $R^2 = 0.9992$ (95% confidence limit) and for pyridine, $Y(\text{area}) = 2249 \pm 489X(\mu\text{g/ml}) + 2999 \pm 142$; $R^2 = 0.9999$ (95% confidence limit).

The intraday coefficients of variation were less than 1.12% ($n = 9$), and the interday coefficients of variation were less than 1.8% ($n = 9$), for ceftazidime. The intraday coefficients of variation were less than 3.16% ($n = 9$), and the interday coefficients of variation were less than 4.37% ($n = 9$), for pyridine.

The limits of quantitation were 2 ng for both compounds and the limits of detection were 50 and 20 pg for ceftazidime and pyridine, respectively. The first term was defined as the lowest level assayed with an R.S.D. of 10% and the second was based on signal-to-noise ratio of 3:1.

Mean recovery was estimated at $103.6 \pm 1.9\%$ for ceftazidime in the range of 2–250 µg/ml and

100.5 ± 2.9% for pyridine in the range 0.1 and 50 µg/ml. Homogeneity of S.D. was proved with the Cochran statistical test.

To establish the stability-indicating nature of the assay method, ceftazidime was undergone forced neutral, acidic and basic hydrolysis. Three water solutions of ceftazidime 100 µg/ml were made, one was adjusted at pH 1 with concentrated hydrochloric acid, the second at pH 12 with concentrated sodium hydroxide, the last one was used as a standard. Solutions were incubated at 80 °C for 1 h.

2.5. Statistical analysis

Linear regression of pyridine formation versus time was used. Comparison of different ambulatory pumps was based on median value of pyridine concentration. General Linear Model-analysis of variance (ANOVA), Student's *t*-paired test and One way-ANOM analysis led statistical intra- and inter-device comparison study. A *P*-value < 0.05 was considered statistically significant.

3. Results and discussion

3.1. Chromatography

In the accelerated degradation studies, the ceftazidime and pyridine peaks were well resolved from any observed degradation products. The other degradation product peak did not interfere with drug quantification. The purity of chromatographic peaks, ceftazidime and pyridine, was confirmed by liquid chromatograph HP 1100 Series (Agilent Technologies, Palo Alto, USA) equipped with diode array detector. Both peaks showed a purity index superior at the limit chosen by the software HPchem[®]. No other compounds were co-eluted with two peaks of interest.

3.2. Stability study

Estimation of ceftazidime stability by monitoring pyridine was a choice based on several studies previously reported. Indeed, ceftazidime showed a

concentration-dependent first-order rate constants for its hydrolysis in advanced works on chemical stability of the molecule [7–9]. Besides, the pyridine concentration according to time was additional in that of ceftazidime like shown by other studies [16,20–22]. According to these different results, it would seem so that monitoring of pyridine can be a sure label of ceftazidime stability in portable infusion device or all containers. The only critical parameter was the behaviour of pyridine with wall of filled containers. The sorption phenomenon was weak and will be developed in other work [23].

Drug stability was considered clinically acceptable if 90% or more of the original concentration remained. Here, the stability was also estimated by pyridine concentration. At the initial time, concentration of pyridine should not be higher than 0.016% (w/v) in infusion solutions, corresponding to 0.4% w/w allowed by USP [17] in dry mixture. The stability was calculated when percentage of pyridine was equal to 0.052% weight (g) by volume unity, or 520 µg/ml in infusion, corresponding to a 10% reduction in the initial ceftazidime concentration, in the same solutions. This method was based on two previous studies [15,16].

The stability results are summarised in Table 1 and appearance of pyridine versus time was noted in Table 2.

Behaviour of different lots of device was statistically identical for the same type of solution. Nevertheless, formation of pyridine was statistically different in 0.9% sodium chloride solution and 5% dextrose solution. These calculations were shown by a multivariate ANOVA called Linear General Model at 12 h. This value was defined by the pyridine concentration median. So, the stability of Fortum[®], in each device, was in accordance with the infusion solution kind, as described previously [12]. The ceftazidime was more stable in sodium chloride solution, excepted for OutBound where it was statistically identical after 12 h at 37 °C.

An inter-batch variability was exhibited in Baxter Infusor and Fresenius Ultraflow with 0.9% NaCl solution. Braun Easypump and Zambon Outbound did not show variability. For Braun,

availability of a single lot explained this fact. The Zambon's supplies exhibited, in 0.9% NaCl as well as 5% Dextrose, weak S.D. values. The ceftazidime stability was lot-independent for this device, in NaCl and dextrose injections.

In 5% dextrose, a inter-lot variability was found in Fresenius OutBound. Therefore, the statistical study, by one-way ANOM analysis, showed that the stability in D5% was significantly greater than other ones.

In 0.9% sodium chloride injection, there was not significant differences of the stability between manufacturers. Easypump showed the better behaviour, but the conclusion was careful. Indeed, only one lot was studied.

Attention could be given on infused pyridine quantity in virtual patient after 24 h therapy, when the 40 mg/ml concentration was used. A previous study reported that quantity of pyridine was equal at 50 mg in 100-ml pump after 14 days of storage at 4 °C [19], with an initial ceftazidime concentration of 60 mg/ml. All premature conclusion should be avoided for the numbers shown in

Tables 1 and 2. The reason was the following, study was led in ceftazidime concentration. So, the device possessing the greater volume will infuse the greater mass of pyridine. In accordance with that all device had an identical degradation kinetic. Nevertheless, these pyridine levels, by its toxicity, were certainly clinical sound.

4. Conclusion

The method described in this study allows to quantify ceftazidime and pyridine simultaneously in single run. It was fast, simple and sensitive. Although estimation of a compound stability by monitoring this degradation compound was seldom, this method appeared, in the case of Fortum, like a good alternative, as reported by several authors.

Fresenius device showed an injection independent ceftazidime stability and exhibited weaker pyridine concentration in D5% after 12 h of infusion. The four systems did not have statistically

Table 1
Appearance of pyridine in µg/ml in four portable infusion devices filled with 40 mg/ml ceftazidime injection

Device	Injection	Initial concentration of pyridine (µg/ml)	Concentration of pyridine in µg/ml					
			4 h	8 h	12 h	16 h	20 h	24 h
Baxter	0.9% NaCl	1.8 ± 0.7	119.4 ± 6.0	268.8 ± 14.3	418.2 ± 9.9	567.6 ± 34.9	717.0 ± 4.8	883.5 ± 17.8*
Infusor®	Dextrose 5%	18.6 ± 2.9	199.3 ± 13.9	412.7 ± 58.6	626.1 ± 6.5	839.6 ± 80.9	1052.9 ± 50.9	1266.4 ± 69.7
Braun	0.9% NaCl	16.1 ± 1.7	104.1 ± 4.8	256.7 ± 23.9	409.3 ± 12.4	561.8 ± 46.1	714.4 ± 235.2	866.9 ± 2.8
Easypump®	Dextrose 5%	86.7 ± 1.0	225.5 ± 2.5	378.0 ± 1.7	530.6 ± 10.4	683.2 ± 10.5	835.7 ± 12.1	988.3 ± 5.2
Fresenius	0.9% NaCl	9.2 ± 1.4	166.0 ± 12.9	352.9 ± 26.4	539.8 ± 89.1	726.6 ± 23.7	913.5 ± 17.2	1100.4 ± 162.9
Ultraflow®	Dextrose 5%	90.7 ± 0.7	255.0 ± 2.1	390.5 ± 5.7	525.9 ± 14.6	661.4 ± 21.6	796.8 ± 7.3	932.3 ± 6.2
Zambon	0.9% NaCl	8.6 ± 3.3	133.1 ± 5.8	289.5 ± 2.4	445.8 ± 5.3	602.2 ± 1.9	758.5 ± 0.85	914.9 ± 4.7
Outbound®	Dextrose 5%	19.4 ± 5.7	279.3 ± 3.5	512.4 ± 13.0	745.6 ± 14.9	978.7 ± 11.4	1211.9 ± 146.4	1445.1 ± 71.4

Values are mean ± S.D. of triplicate determination for three samples, excepted two samples for *, because one Baxter Infusor has infused solution in time less than 24 h.

Table 2
Calculation of inter-batch variability, stability of Fortum and quantity of pyridine infused in different infusion devices

Brand	Injection	Batch	Pyridine, $t = 0$ h	Pyridine, $t = 12$ h	Inter-batch variability	Pyridine, $t = 24$ h	Stability (h)	Initial mass of ceftazidime (g)	Mass of pyridine infused in 12 h (mg)
Baxter	NaCl	Batch 1	0.0235	6.137	Yes	13.012	14.4	9.6	251 ± 16
		Batch 2		5.454		11.105	16.3	9.6	
		Batch 3		4.611		9.420	18.9	9.6	
	D5	Batch 1	0.235	8.154	No	16.628	10.7	9.6	392 ± 16
		Batch 2		5.775		12.299	10.2	9.6	
		Batch 3		7.015		13.540	12.7	9.6	
Braun	NaCl	Batch 1	0.202	5.063	No	10.818	18.2	4.8	114 ± 15
		Batch 2		4.800		9.657	20.0	4.8	
		Batch 3		5.552		12.332	16.9	4.8	
	D5	Batch 1	1.097	6.163	No	11.707	16.0	4.8	168 ± 13
		Batch 2		7.015		13.49	14.0	4.8	
		Batch 3		7.035		12.532	14.5	4.8	
Fresemsius	NaCl	Batch 1	0.116	5.615	Yes	11.560	18.4	3.3	86 ± 14
		Batch 2		6.921		14.115	14.3	3.3	
		Batch 3		8.010		16.535	12.4	3.3	
	D5	Batch 1	1.146	7.521	Yes	13.254	13.1	2.8	97 ± 15
		Batch 2		6.760		12.182	15.6	2.8	
		Batch 3		5.639		9.708	19.8	2.8	
Zambon	NaCl	Batch 1	0.109	5.663	No	11.668	15.4	4	112 ± 8
		Batch 2		6.122		12.3263	14.4	4	
		Batch 3		5.122		10.8505	16.7	4	
	D5	Batch 1	0.245	9.475	No	18.240	8.8	4	197 ± 9
		Batch 2		9.632		18.319	8.6	4	
		Batch 3		9.161		18.279	9.4	4	

Values of pyridine concentration are expressed in mM.

significant differences in NaCl. Zambon did not show intra-batch variability, but apparition of pyridine was more marked than other ones in D5%, on 12 h.

The infusion solution should be chosen as NaCl for ceftazidime. Also, the selection of infusion device for use in ambulatory care is function of flow-rate accuracy, the cost of disposable supplies, the type of therapy administered and the comfort of patients.

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