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Stability studies of antipyocyanic beta-lactam antibiotics used in continuous infusion

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In intensive care, beta-lactams can be reconstituted in 50 mL polypropylene syringes with NaCl 0.9 % and administered for 8 to 12 h at various concentrations with motor-operated syringe pumps. The feasibility and/or the stability of these antibiotic therapies are often poorly known by clinicians. The purpose of this study was to determine the stability of seven antipyocyanic beta-lactam antibiotics and cilastatin under real-life conditions. Stability indicating HPLC methods allowing quantification in pharmaceutical preparations and subsequent stability studies were performed. The stability studies showed that continuous infusion of piperacillin/tazobactam 80/10 mg/mL, of cefepime 20 and 40 mg/mL and of aztreonam 40 and 120 mg/mL can be used over 12 h. Moreover, continuous infusion of cefepime 120 mg/mL can be used over 10 h, whereas meropenem 10 and 20 mg/mL and ceftazidime 40 mg/mL remained stable only over 8 h, and meropenem 40 mg/mL was significantly degraded after 6 h. Finally, imipenem/cilastatin 5/5 mg/mL and piperacillin/tazobactam 320/40 mg/mL should not be used as continuous infusion. These data allow the establishment of protocols of administration of antipyocyanic beta-lactams by continuous infusion. Some of them are not appropriate to this mode of administration (imipenem/cilastatin, piperacillin/tazobactam 320/40 mg/mL) or must be avoided if possible (ceftazidime 40 mg/mL).

1. Introduction

While survival benefits with continuous versus intermittent infusion has been largely discussed for β-lactams (Roberts et al. 2016; Lee et al. 2018), recent publications evidenced the efficiency of their continuous infusion in the treatment of *Pseudomonas aeruginosa* infections (Moriyama et al. 2010; Verdakas et al. 2018). Antipyocyanic β-lactams like piperacillin/tazobactam, cefepime, ceftazidime, imipenem/cilastatin, meropenem and aztreonam are often administered as a continuous infusion. In intensive care, these antipyocyanic β-lactams are usually reconstituted in 50 mL polypropylene syringes with NaCl 0.9 % and administered for 8 to 12 h at various concentrations with a motor-operated syringe pump. Therefore, stability of these molecules must be determined under conditions that are in accordance with those of their clinical use. Numerous studies investigated the stability of piperacillin/tazobactam (Moon et al. 1995; Donnelly 2009), cefepime (Gupta et al. 1997; Stewart et al. 1999; Gupta and Ling 2001; Trissel and Xu 2003), ceftazidime (Arsène et al. 2002), imipenem/cilastatin (Bigley et al. 1986; Allen et al. 1996), meropenem (Krämer 1997; Patel and Cook 1997; Berthoin et al. 2010; Tomasello et al. 2015) and aztreonam (Marble et al. 1986; Belliveau et al. 1994). However, most of these studies were performed on only one of these β-lactams, in other reconstituting solvents than NaCl 0.9 %, or in other containers than polypropylene syringes. Moreover, data are lacking for concentrations administrated in intensive care.

Therefore, we determined the stability of all these antipyocyanic β-lactams under the conditions of use in clinical practice. Thus, we monitored the stability of piperacillin/tazobactam, cefepime, ceftazidime, imipenem/cilastatin, meropenem and aztreonam solutions reconstituted with NaCl 0.9 % in ready-to-use 50 mL polypropylene syringes at various concentrations administered in intensive care. Stability of these solutions was monitored at ambient temperature during eight to twelve hours.

Significant alteration in visual aspect, pH, and concentration of active molecules, as well as formation of degradation products in these solutions were determined. All the dosing methods were statistically validated and performed to be stability-indicating. This complete study allows the protocolization of β-lactams administration in intensive care for the treatment of *Pseudomonas aeruginosa* infections.

2. Investigations and results

2.1. HPLC analytical conditions and methods validation

Beta-lactams were quantified by HPLC under various experimental conditions. The analytical method used was first developed for ticarcillin quantification (Curti et al. 2017), but slight variations (mobile phase pH, gradient, detector wavelength) allowed the quantification of five other β-lactams and cilastatin. For aztreonam, tazobactam and piperacillin, new conditions were implemented. Analytical conditions are summarized in Table 1.

The following parameters were evaluated in the methods validation: selectivity, specificity, repeatability (within-day variation), intermediate precision (between-day variation), accuracy and uncertainty. Selectivity and specificity were evaluated with forced degradation studies. Within-day and between-day measurements were carried out at two concentrations for each beta-lactam and cilastatin. For within-day measurement, at least 15 samples by concentration levels were analyzed on the same day. Between-day measurements were carried out at the same concentrations, on at least 20 samples on 3 consecutive days. The results of repeatability, intermediate precision and accuracy are summarized in Table 2.

Table 1: Analytical conditions for beta-lactams quantification by HPLC

Samples	Mobile phases	pH PBS	Flow (mL/min)	λ
Piperacillin	Isocratic PBS/methanol/acetonitrile (70/15/15)	5	1	220 nm
Cefepime	Gradient T0 min PBS, T20 min PBS/methanol (80/20)	7	0.8	230 nm
Ceftazidime	Gradient T0 min PBS, T20 min PBS/methanol (80/20)	5	0.8	254 nm
Imipenem	Gradient T0 min PBS, T20 min PBS/methanol (90/10)	7	0.8	295 nm
Meropenem	Gradient T0 min PBS, T20 min PBS/methanol (80/20)	7	0.8	230 nm
Aztreonam	Isocratic PBS/acetonitrile (70/30)	3	1	220 nm
Tazobactam	Isocratic PBS/methanol/acetonitrile (70/15/15)	5	1	220 nm
Cilastatin	Gradient T0 min PBS, T20 min PBS/methanol (90/10)	7	0.8	210 nm

Table 2: Repeatability, intermediate precision and accuracy

Samples (Concentration levels, µg/mL)	Repeatability (% RSD within-day)		Intermediate precision (% RSD between-day)		Accuracy (Bias in %)	
Piperacillin (200/400)	2.07 %	2.13 %	0.83 %	2.80 %	0.75 %	0.10 %
Cefepime (100/200)	0.80 %	1.25 %	2.47 %	2.96 %	3.71 %	0.33 %
Ceftazidime (200/500)	0.18 %	0.52 %	0.46 %	1.18 %	2.60 %	0.50 %
Imipenem (75/100)	0.38 %	0.36 %	0.98 %	0.91 %	1.18 %	0.02 %
Meropenem (100/250)	0.65 %	2.15 %	0.79 %	2.16 %	2.17 %	2.02 %
Aztreonam (100/200)	3.11 %	3.36 %	2.30 %	2.14 %	4.20 %	3.05 %
Tazobactam (25/50)	2.47 %	2.42 %	3.08 %	3.34 %	4.57 %	0.72 %
Cilastatin (200/250)	1.16 %	0.69 %	1.17 %	2.34 %	0.13 %	0.19 %

Retention times and calibration ranges for analyzed beta-lactams are summarized in Table 3, followed by representative chromatograms shown in Fig. 1.

2.2. Stability study design

Stability was tested over 12 h for piperacillin/tazobactam, cefepime, meropenem and aztreonam and over 8 h for ceftazidime and imipenem/cilastatin. Each β-lactam was reconstituted according to its respective summary of product characteristic, if possible with normal saline. When possible, dilutions were also done with normal saline in 50 mL polypropylene syringes. Concentrations and pH of reconstituted solutions were measured in triplicate every 2 h. Visual examination of reconstituted syringes were also done at each measurement point.

Table 3. Retention time and linearity

Samples	Retention time	Calibration range (µg/mL)
Piperacillin	13.6 min	100 - 800
Cefepime	16.6 min	100 - 400
Ceftazidime	16.6 min	25 -1000
Imipenem	12.0 min	50-100
Meropenem	19.9 min	100 - 400
Aztreonam	2.7 min	12.5 - 200
Tazobactam	2.8 min	12.5 - 100
Cilastatin	27.0 min	125 - 500

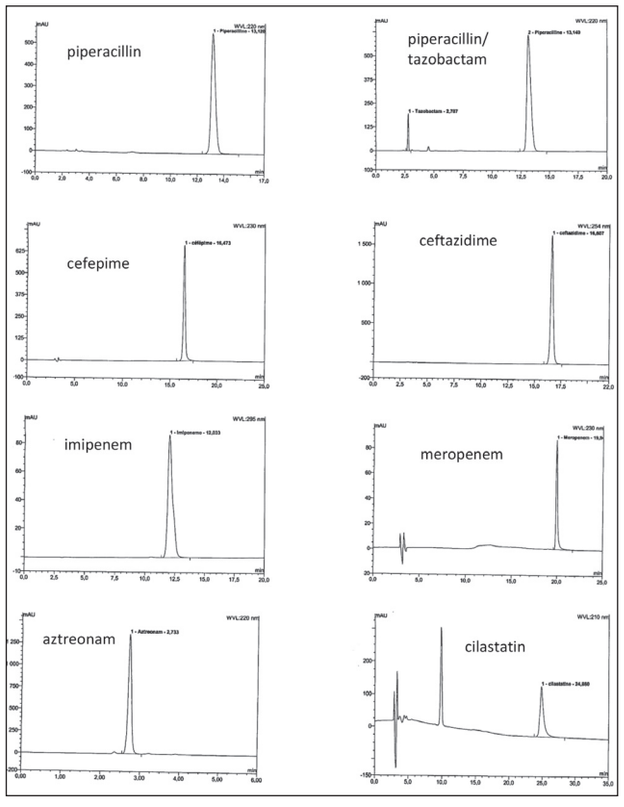

Fig. 1: Representative chromatograms

During stability studies, significant changes are defined by the International Conference on Harmonization as "a 5 % change in assay from its initial value". Applied in pharmaceutical industry, these 5 % limits were often increased to "10 % change in assay from its initial value" for hospital compounded preparations done in a smaller scale (Chennell et al. 2017; Curti et al. 2017; Ezquer-Garin et al. 2017; Friciu et al. 2017). In the present study, both the 5 % and the 10 % changes in assay are reported, even if the 10 % limits were used to define stability. Forced degradation studies were performed for each beta-lactam and cilastatin under several experimental conditions: thermal, oxidation, alkaline and acidic conditions.

First, beta-lactams were reconstituted according to their respective Summary of Product Characteristics (SPC).

For thermal stress, beta-lactams were placed in an oven at 80 °C. For oxidation conditions, hydrogen peroxide (1.5 or 3 %) was

Table 4: pH of reconstituted solutions of β-lactams

		T0	2 h	4 h	6 h	8 h	10 h	12 h
Piperacillin/tazobactam	80/10 mg/mL	5.74	5.68	5.60	5.50	5.42	5.40	5.31
	320/40 mg/mL	6.15	6.12	6.01	5.93	5.92	5.86	5.80
Cefepime	20 mg/mL	4.53	4.44	4.57	4.57	4.56	4.60	4.62
	40 mg/mL	4.62	4.63	4.65	4.64	4.66	4.69	4.71
	120 mg/mL	4.54	4.57	4.59	4.54	4.60	4.58	4.61
Ceftazidime	40 mg/mL	6.62	6.62	6.62	6.68	6.60	-	-
Imipenem/cilastatin	5/5 mg/mL	7.34	7.21	7.05	7.12	7.03	-	-
Meropenem	10 mg/mL	7.87	7.92	7.96	7.95	7.91	7.87	7.85
	20 mg/mL	7.82	7.92	7.94	7.93	7.84	7.88	7.79
	40 mg/mL	7.88	7.89	7.88	7.86	7.78	7.81	7.75
Aztreonam	40 mg/mL	5.01	4.72	4.78	4.95	4.94	4.93	4.92
	120 mg/mL	5.02	5.01	5.01	5.01	5.03	4.99	5.03

added to reconstituted beta-lactams. For alkaline conditions sodium hydroxide (pH 10 or pH 12) and for acidic conditions hydrochloric acid (pH 0.7, pH 1 or pH 2), were added to the beta-lactam solutions.

Forced degradation studies showed no interferences with dosing methods, even if, for aztreonam forced degradation, two degradation products were observed close to API peak. The results are summarized in supplementary material.

3. Discussion

3.1. Piperacillin/tazobactam

Quantification of piperacillin/tazobactam showed no significant variation up to 12 h. Nevertheless, the 320/40 mg/mL samples were all initially under dosed.

For the 80/10 mg/mL samples, one vial of commercial piperacillin/tazobactam (4 g/500 mg) was reconstituted with 8 mL of water

Table 5: Quantification of reconstituted solutions of β-lactams (% of theoretical values)

		T0	2 h	4 h	6 h	8 h	10 h	12 h
Piperacillin	320 mg/mL	91.6±2.2	91.6±1.9	91.9±1.7	90.2±2.9	90.8±3.0	89.8±2.2	91.4±2.0
	80 mg/mL	94.6±2.7	95.7±1.5	95.2±3.1	94.4±3.0	94.4±1.3	93.5±1.5	94.6±1.8
Tazobactam	40 mg/mL	93.7±1.9	93.6±2.5	94.9±2.0	93.0±2.3	93.6±2.7	93.0±2.8	94.1±2.0
	10 mg/mL	97.5±1.6	97.4±1.7	96.9±3.1	96.1±2.8	96.3±1.3	97.2±1.6	97.7±0.6
Cefepime	20 mg/mL	102.9±2.6	104.6±2.9	98.7±2.8	97.8±1.0	95.6±2.1	97.5±4.2	99.3±0.5
	40 mg/mL	98.6±0.7	99.4±2.4	97.1±7.7	102.9±1.6	99.0±8.7	97.3±3.3	95.5±6.0
	120 mg/mL	100.3±0.1	98.7±2.0	98.7±0.3	95.4±0.2	92.1±0.7	90.5±0.5	90.4±1.3
Ceftazidime	40 mg/mL	96.9±1.5	97.7±0.6	97.6±0.4	97.6±0.3	97.1±0.6	-	-
Imipenem	5 mg/mL	90.0±3.7	83.4±2.4	83.9±3.6	78.4±2.5	74.8±6.3	-	-
Cilastatin	5 mg/mL	83.2±0.7	82.7±2.3	82.5±2.5	81.0±2.2	80.2±2.8	-	-
Meropenem	10 mg/mL	99.5±0.6	97.7±0.6	95.3±1.5	93.0±2.3	92.2±1.1	89.1±1.4	86.8±1.2
	20 mg/mL	99.6±1.3	98.8±2.0	96.4±1.5	93.6±5.4	91.8±1.6	87.0±0.4	85.0±0.2
	40 mg/mL	100.1±0.1	98.8±1.6	94.3±3.5	93.1±3.3	87.9±1.0	84.6±0.1	80.0±2.2
Aztreonam	40 mg/mL	98.4±0.4	96.8±0.7	94.6±2.1	94.9±2.6	95.1±1.6	98.2±2.6	94.3±2.6
	120 mg/mL	94.6±2.5	95.9±3.5	98.1±2.6	97.7±2.8	95.5±1.2	96.5±3.5	96.0±1.2

2.3. Results

No significant variations exceeding 5 % of the initial value of the pH were observed over the duration of the study for each of the β-lactams studied (Table 4). Visual examination showed no difference over time, except for ceftazidime for which the appearance of a slight brownish coloration was noticed.

Quantification of reconstituted solutions of β-lactams showed substantial variability between the tested APIs. Results are summarized in Table 5 and then discussed molecule by molecule. For each API, statistical significances were determined by simple linear regression (slope of the regression line is different from 0).

for injection as recommended in the SPC. Subsequent volume was aliquoted in the syringe; the vial was rinsed with normal saline (afterward added in the syringe). Finally, normal saline was added qs 50 mL in the syringe. At this concentration, piperacillin/tazobactam was stable during 12 h, as reported in the literature in polypropylene syringes/polyvinyl chloride bags with normal saline or dextrose 5 % as solvent (Moon et al. 1995) and in polyvinyl chloride bags with dextrose 5 % as solvent (Donnelly 2009).

For the 320/40 mg/mL samples, four vials of commercial piperacillin/tazobactam (4 g/500 mg) were reconstituted each with 8 mL of water for injection. We observed a volume expansion (8 mL added in

vial giving more than 10 mL after solubilization of the active pharmaceutical ingredients and excipients) which not allowed us to rinse vials with normal saline, as we had to obtain 50 mL of solution. We hypothesized that the initial under-dosage was due to this absence of vial washout. Moreover, even if the clinical interest of piperacillin/tazobactam continuous infusion was shown in *Pseudomonas aeruginosa* infections (Lodise et al. 2007; Cotrina-Luque et al. 2016), high doses of piperacillin by continuous infusion were recently suggested to be neurotoxic in critically ill patients (Quinton et al. 2017).
Therefore, even if piperacillin/tazobactam appeared to be stable over 12 h at the two concentrations studied, only 80/10 mg/mL concentrations should be prepared for clinical use in a 50 mL syringe.

3.2. Cefepime

Quantification of cefepime showed slight variations, indicating that the cefepime degradation rate increased with concentration.
For the 20 mg/ml samples, one vial of commercial cefepime (2 g) was reconstituted with 40 mL of normal saline. 20 mL were aliquoted in a 50 mL syringe with normal saline qs 50 mL. At this concentration, cefepime remained stable up to 12 h.
For the 40 mg/ml solutions, one vial of commercial cefepime (2 g) was reconstituted with 40 mL of normal saline, aliquoted in 50 mL syringe with normal saline qs 50 mL. Average cefepime concentration remained within the bounds of ±5 % up to 10 h and ±10 % up to 12 h, but these variations were not statistically significant.
For the 120 mg/ml solutions, three vials of commercial cefepime were reconstituted with 15 mL of normal saline, aliquoted in 50 mL syringe with normal saline qs 50 mL. Average cefepime concentration varied by around ±5 % up to 6 h and then ±10 % up to 10 h. A significant decrease ($p < 0.001$) was noticed over 12 h.
During this study, increases of two degradation products were noticed. The first one was identified during forced degradation studies under basic conditions, with retention time of 10.6 min. For the 120 mg/mL solutions, its peak area was multiplied by 2.6 from T0 to T12 h. The second one, at 22.5 min, developed under heat stress conditions, and its peak area was multiplied by 2.7 during the twelve hours of the study.
In the literature, the same positive correlation between degradation rate and concentration was found (Trissel and Xu 2003), with stabilities (concentration within the bounds of ±10 %) as good as 48 h for 20 mg/mL (Gupta et al. 1997; Gupta and Ling 2001), and 24 h for 40 mg/mL (Trissel and Xu 2003) and 120 mg/mL with normal saline as solvent and at ambient temperature (Stewart et al. 1999).

3.3. Ceftazidime

For the 40 mg/ml samples, two vials of commercial ceftazidime (1 g) were reconstituted with 10 mL of normal saline. These 20 mL were aliquoted in a 50 mL syringe with normal saline qs 50 mL. At this concentration, ceftazidime concentration does not significantly change up to 8 h. At this concentration, ceftazidime is described to be stable in normal saline and in plastic bags up to 20 h at 20 °C (Arsène et al. 2002).

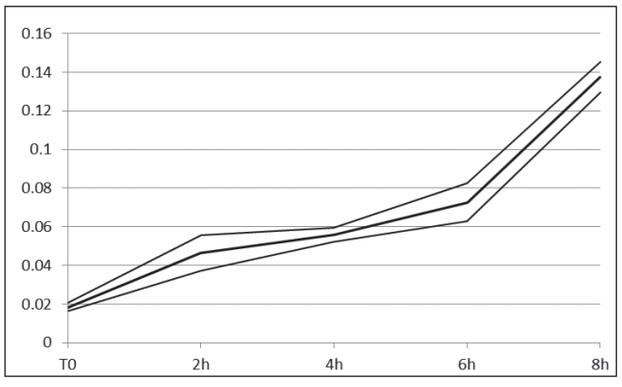

Fig. 2: Pyridine concentrations (mg/mL) in 40 mg/mL ceftazidime syringes

Even if ceftazidime degradation was not sufficient over 8 h to be detected under our experimental conditions, a small, undetectable degradation could occur. It is well known that ceftazidime can release pyridine as a toxic degradation product. In the US Pharmacopeia, the upper limit of pyridine concentration is fixed 1.1 mg/mL (U.S. Pharmacopeia 1995), but the EMA guideline for residual solvents established a permitted daily exposure for pyridine at 2 mg per day (European Medicines Agency 2010).
In addition to ceftazidime quantification, we therefore evaluated by HPLC pyridine releasing under our experimental conditions.
Briefly, pyridine concentration was evaluated with a Kromasil® C18 column with 5 µm particle size (250 mm x 4.6 mm), at wavelength 254 nm, flow 1 mL/min, using a mobile phase ammonium acetate buffer pH5/acetonitrile (9/1). An 8 points calibration line was done between 0.01 and 50 mg/mL, and samples were analyzed by direct injection of 20 µL of the ceftazidime solution without dilution (tested in triplicate). Results are summarized in Fig. 2.
Just after reconstitution, 50 mL syringes of 40 mg/mL ceftazidime contained 0.0184±0.0020 mg/mL of pyridine (0.920 mg of pyridine in a 50 mL syringe). After 8 h, pyridine concentrations increased to 0.1373±0.0079 mg/mL (6.865 mg of pyridine in a 50 mL syringe). Schematically, the extemporaneous preparation of 40 mg/mL ceftazidime in 50 mL syringe administered over 8 h under continuous flow corresponds to the intake of 3.8925 mg of pyridine to the patient. Our results show that the USP upper limit of pyridine concentration was not reached when ceftazidime 40 mg/mL in normal saline was infused during an 8 h infusion at ambient temperature. Moreover, the concentration of pyridine we found after 8 h is in the same scale that previously reported findings (Favetta et al. 2002; Stendal et al. 1998) but yields to a patient daily exposure above the EMA guideline limit. This implies that ceftazidime continuous infusion should be avoided or at least strictly limited to well-documented infections.

3.4. Imipenem/cilastatin

Initially, we decided to test three different concentrations of imipenem/cilastatin: 5 mg/mL, 10 mg/mL and 20 mg/mL. Taking into consideration possible solubility problems, preliminary tests were done in normal saline and in dextrose 5 %. We observed similar results with both solvents. 20 mg/mL concentration conducted to an insoluble suspension. With 10 mg/mL concentration, we noticed solubilization in more than 15 min, with small residual particles. Finally, 5 mg/mL concentration allowed a good solubilization in 5 min, as reported in the SPC.
Therefore, as the commercial imipenem/cilastatin product has a nominal content of 500 mg/500 mg, 10 mL of normal saline (coming from a 100 mL bag) were used for reconstitution. The suspension such obtained was added in the 100 mL normal saline bag. 10 mL were taken again from the bag to wash out the vial, and to be returned into the bag.
With this reconstitution procedure, imipenem/cilastatin solutions were underdosed (from approximatively 10 %), because of the well-known dead volume of normal saline bags. Moreover, even if cilastatin concentrations only suffered from small variations over 8 hours ($p < 0.001$), imipenem concentrations quickly and significantly ($p < 0.001$) decreased. Average imipenem concentrations only remained within the bounds of ±5 % up to 2 h and ±10 % up to 4 h.
At 5 mg/mL, 10 mg/mL and 20 mg/mL, imipenem/cilastatin cannot be reconstituted in 50 mL syringes due to their lack of solubility. Moreover, in a 100 mL bag of normal saline, imipenem stability does not appear sufficient for its clinical use in continuous infusion.

3.5. Meropenem

Quantification of meropenem showed variations indicating that the meropenem degradation rate increased with concentration. For each concentration, a significant decrease of meropenem concentration was noticed over 12 h ($p < 0.001$ for 10 and 20 mg/mL and $p < 0.01$ for 40 mg/mL).

Table 6: Quantification of reconstituted solutions of beta-lactams

		Stability	Continuous infusion (50 mL syringe)	Comment
Piperacillin/ tazobactam	80 mg/mL – 10 mg/mL	12 h	yes	
	320 mg/mL – 40 mg/mL	12 h	no	Under dosed
Cefepime	20 mg/mL	12 h	yes	
	40 mg/mL	12 h	yes	
	120 mg/mL	10 h	yes	
Ceftazidime	40 mg/mL	8 h	yes but avoided if possible	Administration for well-documented infections asap after reconstitution (pyridine releasing)
Imipenem/ cilastatin	5 mg/mL	4 h	no	Under dosed and low stability
	10 mg/mL	-	no	poor solubility
	20 mg/mL	-	no	insoluble
Meropenem	10 mg/mL	8 h	yes	
	20 mg/mL	8 h	yes	
	40 mg/mL	6 h	yes	
Aztreonam	40 mg/mL	12 h	yes	
	120 mg/mL	12 h	yes	

For the 10 mg/ml samples, one vial of commercial meropenem (1 g) was reconstituted with 20 mL of normal saline. 10 mL were aliquoted in 50 mL syringe with normal saline qs 50 mL. At this concentration, meropenem remained within the bounds of ±5 % up to 4 h and ±10 % up to 8 h.
For the 20 mg/ml solutions, one vial of commercial meropenem (1 g) was reconstituted with 20 mL of normal saline, aliquoted in 50 mL syringe with normal saline qs 50 mL. Average meropenem concentration remained within the bounds of ±5 % up to 4 h and ±10 % up to 8 h.
For the 40 mg/ml solutions, two vials of commercial meropenem (1 g) were each reconstituted with 20 mL of normal saline, aliquoted in 50 mL syringe with normal saline qs 50 mL. Average meropenem concentration varied by around ±5 % up to 2 h and then ±10 % up to 6 h.
During this study we noticed relative increases of two degradation products. The first one was detected during forced degradation studies under acido-basic conditions with a retention time of 12.2 min. For the 20 mg/mL solutions, its peak area was multiplied by 17.2 during the 12 h of the study. The second one, at 19.0 min, was present under oxidative stress conditions, and its peak area was multiplied by 7.5 during the 12 h of the study.

3.6. Aztreonam

Quantification of aztreonam showed no significant variation up to 12 h. To obtain 40 mg/mL samples, one vial of commercial aztreonam (2 g) was reconstituted with 3 mL of water for injection as recommended in the SPC. Subsequent volume was aliquoted in a 50 mL syringe; the vial was rinsed with 4 mL of normal saline (afterward added in the syringe). Finally, normal saline was added qs 50 mL in the syringe. To obtain 120 mg/mL samples, the same procedure was applied with 3 vials of commercial aztreonam (2 g). At 40 mg/mL and 120 mg/mL, with normal saline and in 50 mL polypropylene syringe, average aztreonam concentrations remained within ±5 % up to 12 h. This high stability was previously reported for other concentrations (Marble et al. 1986; Belliveau et al. 1994), but from our knowledge, 40 and 120 mg/mL were never evaluated before.
In conclusion, simple and efficient β-lactams and cilastatin dosing methods by high performance liquid chromatography were developed and successfully validated. These methods were proved to be stability indicating after forced degradation studies and were applied to evaluate the stability of β-lactams during continuous infusion with 50 mL polypropylene syringes.
Our stability studies allow the establishment of a protocol of administration of antipyocyanic β-lactams by continuous infusion. Results are summarized in Table 6.
Some of the antibiotics are not appropriate to this mode of administration. Stability and solubility of imipenem/cilastatin impose higher dilution and a shorter mode of administration. Ceftazidime release of pyridine among time makes it to be avoided if possible with regards to EMA permitted daily exposure limits.
As cefepime 120 mg/mL remained stable for 10 h only, its administration by continuous infusion must be completed within 8 h, such as meropenem 10 and 20 mg/mL.
Without prejudging *Pseudomonas aeruginosa* susceptibility, piperacillin/tazobactam 80-10 mg/mL, cefepime 20 and 40 mg/mL and aztreonam 40 and 120 mg/mL appeared to be the best β-lactams to be administered by continuous infusion in 50 mL syringes.

4. Experimental

Beta-lactams were obtained from the medicinal products and diluted in NaCl 0.9 % (sodium chloride 0.9 % Viaflo 500 mL, Baxter). The syringes used for stability testing were BD PlastipakTM Luer-LokTM polypropylene.
The HPLC mobile phases were prepared using ultrapure water (HiPerSolv Chromanorm, VWR International), methanol (HiPerSolv Chromanorm, VWR International) and acetonitrile (HiPerSolv Chromanorm, VWR International) of HPLC grade. Phosphate buffered saline (PBS) pH7 was prepared from ammonium phosphate dibasic (Emsure ACS , Reag. Ph Eur), acetic acid 100 % (AnalaR Normapur VWR International) and sodium hydroxide (Fisher Scientific). PBS pH5 was prepared from ammonium phosphate dibasic (Emsure ACS , Reag. Ph Eur), ammonium acetate (Emsure ACS , Reag. Ph Eur) and phosphoric acid 85 % (RPE ACS Carlo Erba). PBS pH3 was prepared from potassium phosphate monobasic (Prolabo) and phosphoric acid 85 % (RPE ACS Carlo Erba). The mobile phases were filtered using 0.45 µm Millipore cellulose filters. Volumes were aliquoted with a precision pipette (Thermo Scientific Finnpipette® F2 500 µL).
PH values were determined with a Thermo Scientific Orion 4 Star pH-meter, calibrated with Radiometer Analytical standard etalons (pH 4.005, pH 7.000 and pH 10.012).
The chromatographic method was carried out on an automatic HPLC apparatus Dionex Ultimate 3000 with a UV diode array detector. The apparatus was connected to an HP 1702 computer equipped with chromatographic data processing software (Chromeleon Chromatography Management System, Version 6.80 SRH Biold 3161, 1994-2011 Dionex Corporation). Beta-lactams separations were achieved by using a Lichrospher® 100 RP18 column with 5 µm particle size (250 mm x 4.6 mm).

Conflicts of interest: None reported.

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