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Short communication

Simultaneous determination of three carbapenem antibiotics in plasma by HPLC with ultraviolet detection

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ABSTRACT

A simple, precise and accurate high-performance liquid chromatography (HPLC) method using ultraviolet (UV) detection has been developed for simultaneous determination of carbapenem antibiotics: imipenem, meropenem and ertapenem in human plasma. Samples were spiked with ceftazidime as internal standard and proteins were precipitated by acetonitrile. Separation was achieved on a C8 column with a mobile phase composed of phosphate buffer 0.1 M (pH 6.8) and methanol in gradient elution mode. Detection was performed at 298 nm. Calibration curves were linear from 0.5 to 80 mg/L for each compound, with correlation coefficients over 0.997. Intra- and inter-day validation studies showed accuracy between −4.5 and 8.1% and precision below 10.4%. Mean recoveries were 82.2, 90.8 and 87.7% for imipenem, meropenem and ertapenem, respectively. This method provides a useful tool for the therapeutic drug monitoring of carbapenems.

1. Introduction

Carbapenems are β-lactam antibiotics, which inhibit the bacterial cell wall synthesis by binding to penicillin binding proteins (PBP). The three carbapenems currently available in France are imipenem, meropenem and ertapenem (Fig. 1). These parenteral antibiotics have similar broad spectrum activity against most of gram positive and gram negative bacteria. However, ertapenem is less active against *Pseudomonas aeruginosa* and *Acinetobacter* sp. than imipenem and ertapenem. These antibiotics are used in a wide range of serious infections like intra-abdominal, urinary tract, lower respiratory tract or skin infections [1,2].

Imipenem is hydrolyzed in the kidney by human dehydropeptidase-1 (DHP-1) and require to be co-administered with cilastatin, a dehydropeptidase inhibitor, contrary to meropenem and ertapenem, which are naturally resistant to DHP-1, due to their 1-β-methyl group [3]. Ertapenem has a longer plasma half-life (about 4 h versus 1 h for imipenem and meropenem), because of its high plasma protein binding (approximately 94%) allowing once-a-day administration [1,4].

Like other β-lactam antibiotics, the parameter best correlated with treatment efficiency of carbapenems is the time that plasma concentration remains above the minimal inhibitory concentration (MIC) and, according to many authors, the optimal bactericidal activity is obtained when this time is greater or equal to 40% of the time interval between two doses [2,5–7]. Several studies evidenced that the currently recommended doses of imipenem, meropenem and ertapenem do not guarantee the achievement of this pharmacodynamic endpoint. For instance, Moczygemba et al. found that for infections with bacteria producing extended spectrum β-lactamases, the 1 g once-a-day ertapenem dose does not provide the optimal exposure in 22% of the patients [6]. Burgess and Frei also evidenced that around 30% of the patients suffering from pulmonary infections with *Pseudomonas aeruginosa* or *Acinetobacter baumannii* cannot achieve a satisfying imipenem exposure with a 0.5 g every 6-h dose [8]. This result was consistent with the study of DeRyke et al. who found that, for the same bacteria and equivalent doses, around 15–30% of the patients do not achieve the optimal efficacy with imipenem or meropenem [9]. Similarly, Kuti et al. showed that for MICs of 2 and 4 mg/L, only 60 and 15% of the patients, respectively attained the pharmacodynamic endpoint during skin infections with the recommended 0.5 g three times a

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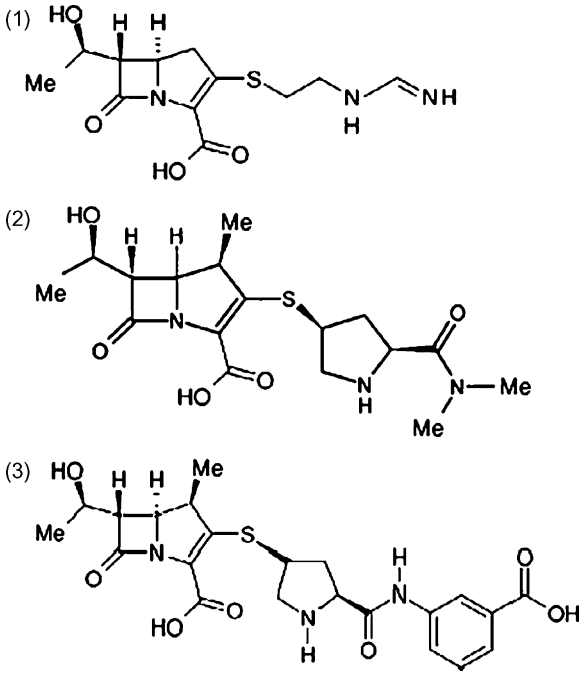

Fig. 1. Chemical structures of imipenem (1), meropenem (2), and ertapenem (3).

day meropenem dose [10]. All these results strongly suggest the need for an individualized dose adjustment depending on the site of infection, the causative bacteria, and the corresponding MIC. Moreover, high concentrations of carbapenems may induce adverse reactions, like excitement and seizures [11–14]. For these reasons, therapeutic drug monitoring of carbapenems represents a useful tool in order to optimize treatment efficiency, and to prevent any overdose.

Several analytical methods based on high-performance liquid chromatography (HPLC) using ultraviolet (UV) detection have been reported for the analysis of a carbapenem in plasma or other biological fluids [4,15–31]. Few of them required a simple sample preparation such as protein precipitation [16,17,29], but most of them required material, which is not available in every laboratory like column switching [4,21–23,25,27] or ultra-filtration [4,15,19,26,28]. Only one method described a simultaneous determination of several carbapenems in plasma but this method used capillary electrophoresis to separate the different compounds [32]. No simultaneous method using HPLC/UV has been reported to date.

Although these antibiotics are not supposed to be co-administered, a single analytical method for the simultaneous determination of three carbapenems could be very useful in a routine laboratory to save time and solvents. The objective of this study was therefore to develop a rapid, simple and low-cost method for simultaneous quantification of imipenem, meropenem and ertapenem in human plasma.

2. Experimental

2.1. Chemicals and reagent

Imipenem and ertapenem, were kindly supplied by Merck® (Rahway, USA), meropenem trihydrate by AstraZeneca® (Rueil-Malmaison, France). Ceftazidime hydrate used as internal standard (IS) was obtained from Sigma® (Saint-Quentin Fallavier, France).

Potassium dihydrogenophosphate, di-sodium hydrogenophosphate, sodium hydroxide and methanol (HPLC grade) were purchased from VWR® (Fontenay-sous-bois, France), acetonitrile (HPLC grade) and 3-[*N*-morpholino]propanesulfonic acid (MOPS) were from Sigma® (Saint-Quentin Fallavier, France).

Ultra-pure water was obtained using a Milli-Q water purification system (Millipore®, Saint-Quentin-en-Yvelines, France). Drug free human plasma was received from the French Blood Center EFS (Etablissement Français du Sang).

2.2. Apparatus and chromatographic conditions

Chromatographic system consisted of a P680 HPLC quaternary pump, a TCC-100 column oven, an ASI-100 thermostated autosampler and an UVD340U detector from Dionex® (Voisins-le-Bretonneux, France). Data analysis was performed using Chromeleon® software.

Separation was achieved at 20 °C using a Satisfaction C8 Plus guard column (15 mm × 3 mm, 5 μm) and a Satisfaction C8 Plus analytical column (250 mm × 3 mm, 5 μm) purchased from Cluzeau® (Sainte-Foy la Grande, France). Mobile phase consisted of a mixture of phosphate buffer 0.1 M (pH 6.8) and methanol delivered at 0.5 mL/min in a gradient elution mode. Initial mobile phase composition was 100% of phosphate buffer. Between 0 and 25 min, the percentage of methanol was linearly increased to 30%. Then composition of mobile phase was back to the initial conditions for 10 min for re-equilibration. The autosampler temperature was kept at +8 °C and the injection volume was 50 μL. The detection wavelength was set at 298 nm.

2.3. Preparation of solutions

Stabilizer solution was prepared by dissolving 2.64 g of MOPS in 100 mL of purified water and pH was adjusted to 6.8 with 2.5N sodium hydroxide.

Three stock standard solutions of imipenem, meropenem and ertapenem at 1 mg/mL were prepared in stabilizer solution, aliquoted and stored at −80 °C. The day of analysis, aliquots were thawed and a working solution was prepared in stabilizer solution at a concentration of 100 mg/L for each compound. Calibration standards (0.5, 1, 2.5, 5, 10, 25, 50 and 80 mg/L) were obtained by spiking 100 μL of drug free plasma with 100 μL of appropriate dilution of the working solution.

Three other stock solutions of imipenem, meropenem and ertapenem at 1 mg/mL were prepared in stabilizer solution to spike quality controls (QCs). QC samples were prepared by adding an appropriate volume of the stock solutions to drug free plasma to obtain a final concentration of 0.5, 2, 40 and 60 mg/L (respectively LOQ, low, medium and high QC) for each compound and then an equal volume of stabilizer solution was added. QC samples were divided into 500 μL aliquots and stored at −80 °C.

Stock solution of ceftazidime (IS) was prepared in stabilizer solution at a concentration of 1 mg/mL and stored at −80 °C. Working solution was prepared at a 100 mg/L concentration.

2.4. Sample preparation

Heparinized patients' blood samples were transported to the laboratory in ice and centrifuged just after reception. Then plasma was diluted with an equal volume of stabilizer solution and stored at −80 °C until analysis. The day of analysis, after thawing, 200 μL of stabilized samples (plasma/stabilizer solution 50/50 (v/v)), were spiked with 100 μL of IS 100 mg/L. Then, the proteins were precipitated by adding 500 μL of acetonitrile. The mixture was vortexed, then left at room temperature for approximately 5 min and centrifuged at 15,800 × *g* for 5 min. The supernatant was evaporated at 30 °C under a gentle nitrogen stream. The residue was dissolved in

200 μL of stabilizer solution and 50 μL was injected into the HPLC system.

2.5. Validation

2.5.1. Linearity

Linearity study was performed by analysis of standards in triplicate on 3 days between 0.5 and 80 mg/L and QC samples were assayed twice with each standard curve. Calibration curves of imipenem, meropenem and ertapenem were established using peak height ratio analyte/IS (y) versus plasma concentration (x). For each analyte, the best weighting factor, determined according to the relation between variance and concentration was $1/y^2$. Slopes, intercepts and correlation coefficients (r^2) were obtained by linear regression analysis. Calculated QC values and back-calculated standard concentrations could not differ from ±15% of the theoretical value (except ±20% for the lowest standard and LOQ QC) [33].

2.5.2. Accuracy and precision

Intra-day study was performed by assaying the four QC six times during the same day, while inter-day study was performed by assaying the four QC concentrations once-a-day on 6 separate days. Mean measured concentrations and their standard deviations (S.D.) were calculated. Precision was reported by calculating the coefficient of variation (CV) expressed as (S.D./mean) × 100 and accuracy by the bias expressed as ((measured concentration − theoretical concentration)/(theoretical concentration)) × 100.

Acceptance criteria for accuracy and precision were: bias within ±15% (except ±20% for the LOQ QC) and CV lower than 15% (except 20% for the LOQ QC) [33].

2.5.3. Limit of quantitation (LOQ)

LOQ was defined as the lowest drug plasma concentration, which can be determined with acceptable accuracy and precision. In this method, LOQ was set at the lowest standard calibration 0.5 mg/L.

2.5.4. Recovery

Imipenem, meropenem and ertapenem recoveries were quantified at concentration levels corresponding to the lowest, medium and highest standards values (0.5, 5 and 80 mg/L) analysed in triplicate. They were calculated by comparing peak height ratio of analytes spiked before protein precipitation to analytes spiked after protein precipitation (on the dry residue).

2.5.5. Selectivity and specificity

Selectivity of the method was evaluated by analysing five different drug free plasma samples and then by comparing retention times of endogenous compounds with those of carbapenems.

Specificity of the method was evaluated by injecting solutions containing other anti-infective drugs largely used in broad spectrum antibiotic treatment: cilastatin, amoxicillin, clavulanic acid, cloxacillin, ticarcillin, ceftriaxone, cefotaxime, cefazolin, cefoxitin, cefamandole, vancomycin, teicoplanin, amikacin, gentamycin, tobramycin, ofloxacin, pefloxacin, ciprofloxacin, clarithromycin, azithromycin, josamycin, spiramycin, clindamycin, doxycyclin, rifampicin, trimethoprim, sulfamethoxazole, metronidazole, voriconazole, posaconazole, fluconazole, itraconazole, flucytosine, amphotericin B, caspofungin.

2.6. Stability

2.6.1. Long-term and freeze-thaw stability

Stability of stock solutions was determined by comparing peak heights in triplicate between solutions stored at −80 °C for 1 month and freshly prepared solutions. Difference between mean peak heights has to be less than 5%. Stability of QC samples stored at −80 °C was assessed in triplicate on the four QC levels for 50 days by measuring the bias compared to the theoretical concentration. Freeze-thaw stability was determined by assaying QC samples on the four QC levels in triplicate over three freeze-thawing cycles by measuring the bias compared to the theoretical concentration (maximum bias accepted: ±15%).

2.6.2. Stability in autosampler

Stability in autosampler at +8 °C of QC extracts was assessed in triplicate on the four QC levels by injecting extracts immediately after preparation and re-injected 24 h later and by measuring the bias compared to the theoretical concentration (maximum bias accepted: ±15%).

2.6.3. Short-term stability

Short-term stability was assessed on the medium QC sample (40 mg/L) with or without stabilizer solution and at different temperatures (−80, +4 °C and room temperature). QC samples (stabilized or not) were analysed in duplicate after different times of storage (0, 2, 4, 6, 8, 24, 30, 48 and 72 h). Stability was measured by comparing mean peak height ratios. Samples with peak height ratios representing at least 85% of the ratio of the QC sample analysed at time 0 were considered stable.

2.6.4. Patients' sample stability

Concerning stability of patients' blood samples, plasma concentrations of imipenem, meropenem and ertapenem were compared between samples centrifuged and stabilized immediately after collection and samples stored in ice and centrifuged and stabilized 8 h after collection.

3. Results and discussion

3.1. Chromatographic conditions

Phosphate buffer 0.1 M (pH 6.8) was used as aqueous part of mobile phase, because of carbapenems low stability at basic or acidic pH [22,27,28,34]. Initial tests were performed in isocratic mode using 20% methanol. In these conditions, meropenem and ertapenem were not efficiently separated, and imipenem was not sufficiently retained by the column, so it was detected near the solvent front. Optimal chromatographic conditions were obtained in gradient elution mode (0–30% methanol from 0 to 25 min), allowing better separation of imipenem from solvent front and improving resolution between meropenem and ertapenem. In these conditions, retention times were about 10.7, 17.8, 22.5, and 23.8 min for imipenem, IS, meropenem and ertapenem, respectively. Representative chromatograms of blank plasma, plasma samples spiked with 0.5 and 10 mg/L of each antibiotic and plasma from patients treated with imipenem, meropenem and ertapenem are shown in Fig. 2.

3.2. Validation

3.2.1. Linearity

The method was found to be linear over a range of 0.5–80 mg/L for the three analytes. Mean ± S.D. slopes were 0.060 ± 0.004, 0.070 ± 0.003 and 0.031 ± 0.001; mean intercepts ± S.D. were 0.004 ± 0.002, 0.010 ± 0.003 and 0.004 ± 0.002, respectively for imipenem, meropenem and ertapenem. All back-calculated concentrations did not differ from ±15% of the theoretical value.

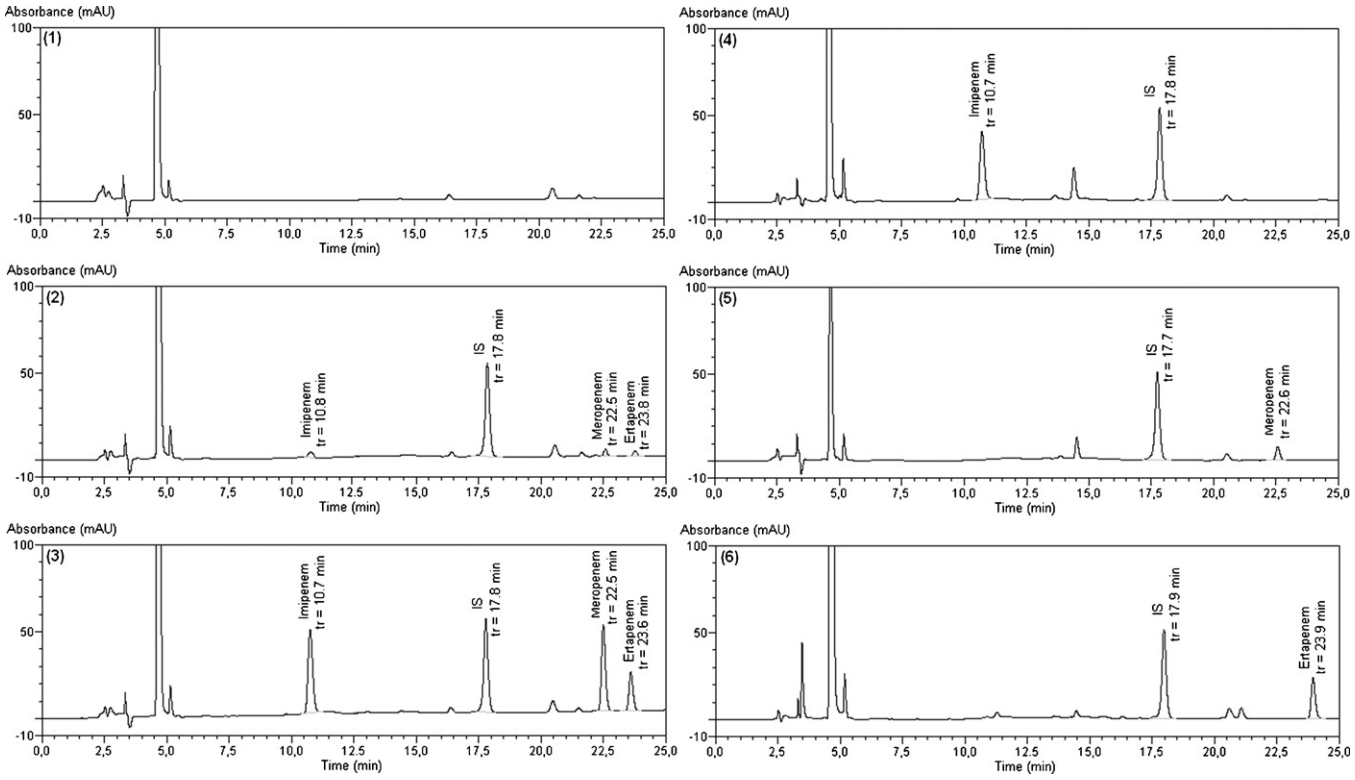

Fig. 2. Chromatogram of blank plasma (1), plasma spiked with imipenem, meropenem, ertapenem at 0.5 mg/L (2) and 10 mg/L (3) and plasma from patient treated with imipenem (concentration: 8.38 mg/L) (4), meropenem (concentration: 1.65 mg/L) (5), and ertapenem (concentration: 11.5 mg/L) (6).

3.2.2. Accuracy and precision

Acceptance criteria for accuracy and precision were met in all case. Intra-day accuracy ranged from −4.5 to 8.1% and inter-day accuracy ranged from −4.3 to 5.9% for the three compounds. Intra-day precision was less than 5.8%, while inter-day precision was less than 10.4% for each compound (Table 1).

3.2.3. Recovery

Mean ± S.D. recoveries of imipenem, meropenem and ertapenem calculated by three determinations of three standards concentrations were 82.2 ± 6.6, 90.8 ± 3.5 and 87.7 ± 4.7%, respectively (Table 2). The mean recovery of IS was 89.8 ± 1.9%.

3.2.4. Specificity and selectivity

The method was found to be selective as no interference was found with biological compounds on five different blank plasmas. Otherwise, the method was specific as no interference was found with solutions containing other anti-infective drugs.

3.2.5. LOQ

This method showed a LOQ of 0.5 mg/L for each compound and required only a 100-μL plasma sample, which is a major advantage in paediatric care. Lower LOQs (from 0.05 to 0.25 mg/L) were previously reported with methods using ultra-filtration or column switching [4,19,22,26,28]. Other published methods using protein precipitation showed LOQs similar to our method

Table 1
Intra- and inter-day precision (CV) and accuracy (bias) of imipenem, meropenem and ertapenem

Theoretical concentration (mg/L)	Intra-day (n = 6)			Inter-day (n = 6)		
	Mean measured concentration ± S.D. (mg/L)	CV (%)	Bias (%)	Mean measured concentration ± S.D. (mg/L)	CV (%)	Bias (%)
Imipenem						
0.5[a]	0.52 ± 0.03	5.8	3.1	0.50 ± 0.02	4.4	0.9
2	1.98 ± 0.07	3.3	−1.1	1.97 ± 0.04	1.8	−1.6
40	38.70 ± 1.08	2.8	−3.3	38.30 ± 1.10	2.9	−4.3
60	57.31 ± 1.78	3.1	−4.5	57.62 ± 2.53	4.4	−4.0
Meropenem						
0.5[a]	0.49 ± 0.02	3.3	−1.0	0.50 ± 0.05	10.4	0.2
2	2.02 ± 0.04	2.1	1.0	1.98 ± 0.06	2.9	−1.1
40	39.50 ± 0.45	1.1	−1.3	38.79 ± 1.22	3.1	−3.0
60	57.67 ± 0.87	1.5	−3.9	57.81 ± 3.05	5.3	−3.6
Ertapenem						
0.5[a]	0.49 ± 0.03	5.3	−1.2	0.48 ± 0.04	9.0	−3.3
2	2.16 ± 0.03	1.4	8.1	2.12 ± 0.09	4.1	5.9
40	40.29 ± 0.44	1.1	0.7	40.01 ± 1.50	3.8	0.0
60	57.37 ± 0.88	1.5	−4.4	58.19 ± 3.11	5.3	−3.0

[a] Corresponding to the LOQ.

Table 2
Mean ± S.D. recoveries (%) of imipenem, meropenem and ertapenem

Concentration (mg/L)	*n*	Imipenem	Meropenem	Ertapenem
0.5	3	75.0 ± 0.0	88.9 ± 9.6	84.0 ± 9.6
5	3	83.8 ± 0.0	88.7 ± 1.3	86.1 ± 0.7
80	3	87.9 ± 1.6	94.9 ± 1.8	92.9 ± 2.0
Total	9	82.2 ± 6.6	90.8 ± 3.5	87.7 ± 4.7

(0.25–1 mg/L), but required much higher volume of plasma (up to 500 μL) [15,17,29]. LOQ was sufficient for clinical applications, as reported minimal concentrations were higher than 0.5 mg/L at recommended dosages [2,4,5,35,36]. Moreover, according to the European Committee on Antimicrobial Susceptibility Testing (http://www.srga.org/eucastwt/MICTAB/index.html), clinical breakpoints of carbapenems (ranging from 0.5 to 2 mg/L) are greater than our LOQ, allowing clinical applications of this method.

3.3. Stability

Stock solutions were stable for at least 1 month at −80 °C because no significant degradation was found between solutions stored at −80 °C for 1 month and solutions freshly prepared (difference between mean peak heights inferior to 1.1%). QC samples stored at −80 °C were stable for at least 50 days (all biases were comprised between −10.7 and 14.8%). Three consecutive freeze-thawing cycles showed no significant degradation for the three compounds (all biases were comprised between −13.3 and 0.4%).

Otherwise, QC extracts were stable at least 24 h in the autosampler at +8 °C (all biases were comprised between −13.1 and 13.0%).

Short-term stability showed that at −80 °C, concentration of the three carbapenems remained stable for at least 72 h in stabilized and non-stabilized plasma. At +4 °C, only meropenem and ertapenem remained stable for at least 72 h in stabilized and non-stabilized plasma, while imipenem were stable for 48 h in stabilized plasma and 30 h in non-stabilized plasma. At room temperature and in stabilized plasma, meropenem and ertapenem were stable for 30 h and imipenem for 8 h. In non-stabilized plasma, meropenem and ertapenem were stable for 24 h and imipenem for 2 h. These results, displayed in Fig. 3, showed that carbapenems were stable at −80 °C for at least 72 h and that adding the stabilizer increased their stability at higher temperatures.

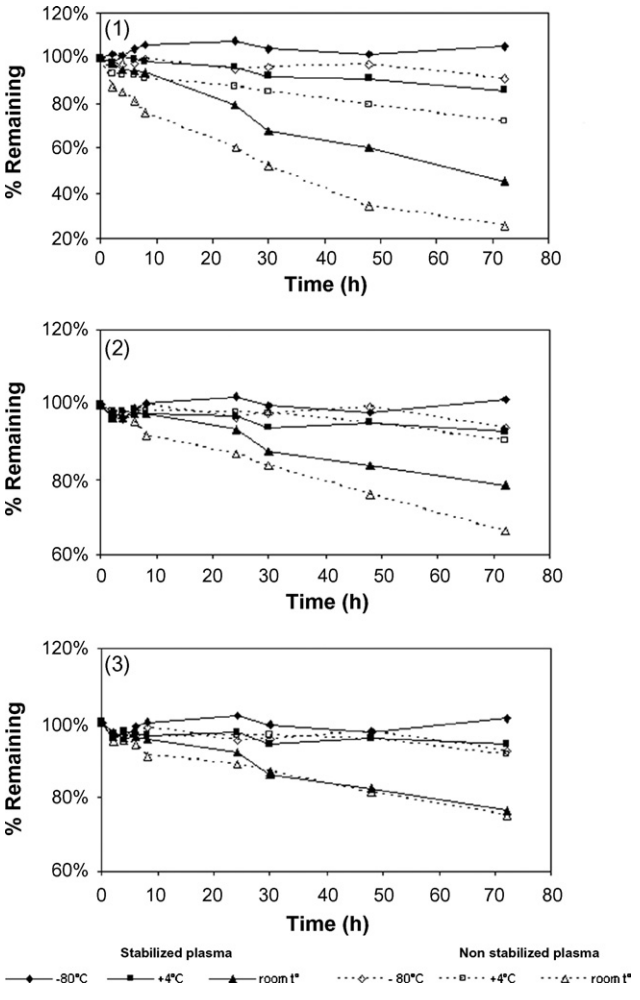

Fig. 3. Short-term stability of imipenem (1), meropenem (2) and ertapenem (3) at 40 mg/L in plasma with or without stabilizer solution at −80 °C, +4 °C and room temperature.

Carbapenem plasma concentrations of patients' samples were not different between samples immediately centrifuged and stablilized and samples centrifuged and stabilized after being kept at +4 °C for 8 h after collection (data not shown). Consequently, blood samples have to be transported in ice to the laboratory and have to be centrifuged, stabilized and stored at −80 °C within 8 h after collection. The results of the short-term stability at +4 °C suggested that patients' samples can be stored in ice for 30 h. However we did not investigate the stability of patients' samples over a delay greater than 8 h since samples are not supposed to necessitate more than 8 h to be transported to the laboratory.

4. Conclusion

A simple analytical method for the simultaneous determination of three carbapenems in plasma was developed. This method has been found to be precise and accurate. Sample treatment was simple and required a small volume of plasma (100 μL), which is well adapted to paediatric patients. Simultaneous determination of these three antibiotics is useful for routine application in a clinical laboratory. Furthermore, this method allowed therapeutic drug monitoring of carbapenems, an interesting tool in the treatment of patients with serious infections.

References

[1] P.M. Shah, R.D. Isaacs, J. Antimicrob. Chemother. 52 (2003) 538.
[2] D.M. Livermore, A.M. Sefton, G.M. Scott, J. Antimicrob. Chemother. 52 (2003) 331.
[3] M.L. Hammond, J. Antimicrob. Chemother. 53 (Suppl. 2) (2004) ii7.
[4] A.K. Majumdar, D.G. Musson, K.L. Birk, C.J. Kitchen, S. Holland, J. McCrea, G. Mistry, M. Hesney, L. Xi, S.X. Li, R. Haesen, R.A. Blum, R.L. Lins, H. Greenberg, S. Waldman, P. Deutsch, J.D. Rogers, Antimicrob. Agents Chemother. 46 (2002) 3506.
[5] J.L. Kuti, P.K. Dandekar, C.H. Nightingale, D.P. Nicolau, J. Clin. Pharmacol. 43 (2003) 1116.
[6] L.R. Moczygemba, C.R. Frei, D.S. Burgess, Clin. Ther. 26 (2004) 1800.
[7] W.A. Krueger, J. Bulitta, M. Kinzig-Schippers, C. Landersdorfer, U. Holzgrabe, K.G. Naber, G.L. Drusano, F. Sorgel, Antimicrob. Agents Chemother. 49 (2005) 1881.
[8] D.S. Burgess, C.R. Frei, J. Antimicrob. Chemother. 56 (2005) 893.
[9] C.A. DeRyke, J.L. Kuti, D.P. Nicolau, Pharmacotherapy 27 (2007) 333.
[10] J.L. Kuti, C. Ong, M. Lo, D. Melnick, N. Soto, D.P. Nicolau, Int. J. Antimicrob. Agents 28 (2006) 62.
[11] S.R. Norrby, Drug Saf. 22 (2000) 191.
[12] C. Ruffmann, G. Bogliun, E. Beghi, Expert Rev. Neurother. 6 (2006) 575.
[13] A.H. Seto, J.C. Song, S.S. Guest, Ann. Pharmacother. 39 (2005) 352.
[14] B.S. Koppel, W.A. Hauser, C. Politis, D. van Duin, M. Daras, Epilepsia 42 (2001) 1590.
[15] C.M. Myers, J.L. Blumer, Antimicrob. Agents Chemother. 26 (1984) 78.
[16] G. Carlucci, L. Biordi, C. Vicentini, M. Bologna, J. Pharm. Biomed. Anal. 8 (1990) 283.
[17] H. Elkhaili, S. Niedergang, D. Pompei, L. Linger, D. Leveque, F. Jehl, J. Chromatogr. Biomed. Appl. 686 (1996) 19.

[18] M. Matsuoka, T. Maki, K. Banno, T. Sato, J. Chromatogr. Biomed. Appl. 685 (1996) 273.
[19] L. Garcia-Capdevila, C. Lopez-Calull, C. Arroyo, M.A. Moral, M.A. Mangues, J. Bonal, J. Chromatogr. Biomed. Sci. Appl. 692 (1997) 127.
[20] S.H. Kim, J.W. Kwon, J. Yang, M.G. Lee, J. Chromatogr. Biomed. Sci. Appl. 688 (1997) 95.
[21] S. Bompadre, L. Ferrante, M. De Martinis, L. Leone, J. Chromatogr. 812 (1998) 249.
[22] D.G. Musson, K.L. Birk, A.M. Cairns, A.K. Majumdar, J.D. Rogers, J. Chromatogr. Biomed. Sci. Appl. 720 (1998) 99.
[23] M. Tanaka, K. Kato, J. Chromatogr. Biomed. Sci. Appl. 724 (1999) 73.
[24] Y. Ozkan, I. Kucukguzel, S.A. Ozkan, H.Y. Aboul-Enein, Biomed. Chromatogr. 15 (2001) 263.
[25] D.G. Musson, C.J. Kitchen, J.Y. Hsieh, K.L. Birk, J. Chromatogr. Analyt Technol. Biomed. Life Sci. 779 (2002) 341.
[26] D.G. Musson, K.L. Birk, C.J. Kitchen, J. Zhang, J.Y. Hsieh, W. Fang, A.K. Majumdar, J.D. Rogers, J. Chromatogr. Analyt Technol. Biomed. Life Sci. 783 (2003) 1.
[27] C.J. Kitchen, D.G. Musson, A.L. Fisher, J. Chromatogr. Analyt Technol. Biomed. Life Sci. 799 (2004) 9.
[28] J.B. Gordien, E. Boselli, C. Fleureau, B. Allaouchiche, G. Janvier, O. Lalaude, M.C. Saux, D. Breilh, J. Chromatogr. Analyt Technol. Biomed. Life Sci. 830 (2006) 218.
[29] M. Soltani, A.P. MacGowan, A.M. Lovering, Int. J. Antimicrob. Agents 27 (2006) 165.
[30] R.G. Mundkowski, J. Majcher-Peszynska, O. Burkhardt, T. Welte, B. Drewelow, J. Chromatogr. Analyt Technol. Biomed. Life Sci. 832 (2006) 231.
[31] C. Sutherland, D.P. Nicolau, J. Chromatogr. Analyt Technol. Biomed. Life Sci. 853 (2007) 123.
[32] S. Taniguchi, K. Hamase, A. Kinoshita, K. Zaitsu, J. Chromatogr. Biomed. Sci. Appl. 727 (1999) 219.
[33] V.P. Shah, K.K. Midha, J.W. Findlay, H.M. Hill, J.D. Hulse, I.J. McGilveray, G. McKay, K.J. Miller, R.N. Patnaik, M.L. Powell, A. Tonelli, C.T. Viswanathan, A. Yacobi, Pharmcol. Res. 17 (2000) 1551.
[34] L. Basaez, P. Vanysek, J. Pharmcol. Biomed. Anal. 19 (1999) 183.
[35] R. Kitzes-Cohen, D. Farin, G. Piva, S.A. De Myttenaere-Bursztein, Int. J. Antimicrob. Agents 19 (2002) 105.
[36] S. Jaruratanasirikul, N. Raungsri, J. Punyo, S. Sriwiriyajan, J. Antimicrob. Chemother. 56 (2005) 1163.