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A liquid chromatography assay for a quantification of doripenem, ertapenem, imipenem, meropenem concentrations in human plasma: Application to a clinical pharmacokinetic study

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ABSTRACT

A simple chromatographic assay based on ultra high performance liquid chromatography with ultraviolet detection at 295 nm is proposed to determinate simultaneously human plasma concentrations of imipenem, doripenem, meropenem and ertapenem. After deproteinization by acetonitrile, carbapenems are separated on a PentaFluoroPhenyl column with a binary gradient elution. This method is specific, accurate, precise (the intra-day and inter-day imprecision and inaccuracy are lower than 15%), sensitive (the limit of quantitation is equal to 0.50 mg/L for imipenem, doripenem, ertapenem, meropenem) and not time consuming (run time = 7 min). An application of this method to measure ertapenem plasma concentrations in burn patients is presented.

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1. Introduction

Carbapenem is a class of beta-lactam antibiotic which is widely used to treat especially severe infections [1–4]. Various chromatographic methods with UV detection were developed to measure total plasma concentrations of carbapemens in plasma [5–25]. These assays concern mainly imipenem [5–9,21], meropenem [10,12,14–19,21] and ertapenem [11,21–25]. Two assays based on ultrafiltration [20] and solid phase extraction [13] were only reported for doripenem. A single assay proposed a simultaneous determination of these drugs except doripenem in human plasma [21]. The objective of this work is to present a simultaneous determination of imipenem, doripenem, ertapenem, meropenem total concentrations in human plasma by an assay based on a simple extraction by protein precipitation and a rapid chromatrographic run using the separative performance of columns packed with the new shell Kinetex® particles. The core–shell technology allows to improve resolution, throughput, and sensitivity as well as reduce solvent consumption relative to conventional monolith column technology by performing ultra high efficiency chromatography. Moreover, this technology uses common 400 bar liquid chromatography instruments which are less expensive than ultra performance liquid chromatography instruments [26]. Since this technology is recent, just a few assays with UV detection were developed [27]. This work contributes to propose a new application of this technology in therapeutic drug monitoring of antibiotics.

2. Materials and methods

2.1. Chemical and reagents

Imipenem and ertapenem were supplied by Merck (Rahway, USA) doripenem, meropenem, by respectively, Johnson & Johnson (Raritan, USA) and Astra Zeneca (Rueil-Malmaison, France). Ceftazidime (internal standard) was obtained from Sigma (Saint-Quentin Fallavier, France). Acetonitrile, methanol (HPLC grade), sodium hydroxide (NaOH), orthophosphoric acid 85% (H_3PO_4) were purchased from VWR International (Fontenay sous Bois, France), disodiumhydrogenophosphate dihydrate ($Na_2HPO_4{\cdot}2H_2O$) was from Merck (Darmstadt, Germany) and 2-[N-morpholino]ethanesulfonic acid, monohydrate (MES) was from Research Organics (Cleveland, USA).

2.2. Instruments

The chromatographic system consisted of Agilent (Palo Alto, USA) 1100 Series components including a quaternary pump, degasser, autosampler, and a photodiode array detector. Chromatographic separations were achieved using a Kinetex® (Phenomenex,

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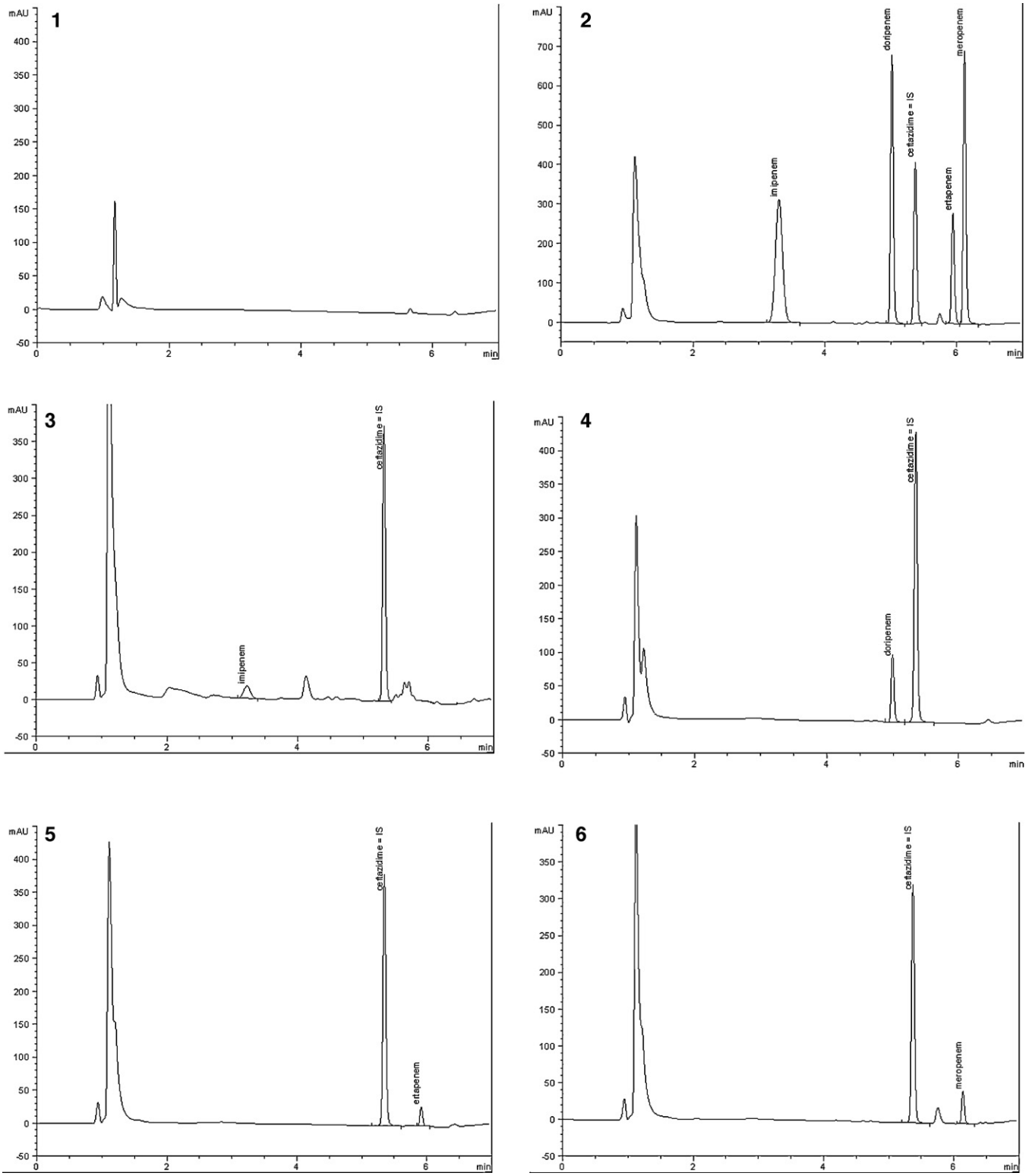

Fig. 1. Chromatogram of a blank plasma (1), plasma spiked with imipenem, meropenem, ertapenem, doripenem at 100 mg/L (IS = internal standard) (2), plasma from patient receiving imipenem 500 mg twice a day (imipenem trough plasma concentration = 7.1 mg/L) (3), doripenem 500 mg three times daily (doripenem trough plasma concentration = 9.0 mg/L) (4), ertapenem 1000 mg once a day (ertapenem trough plasma concentration = 8.5 mg/L) (5) and meropenem 500 mg three times daily (meropenem trough plasma concentration = 6.6 mg/L) (6).

USA). 2.6 μm PentaFluoroPhenyl column (100 mm × 4.6 mm ID). This stationary phase is associated with various interaction mechanisms such as hydrogen bonding, dipole–dipole, aromatic pi–pi and hydrophobic interactions which allows to improve resolution between compounds.

2.3. Chromatographic conditions

The mobile phase which a mixture of methanol (solvant A) and extemporaneously prepared sodium phosphate buffer [$Na_2HPO_4 \cdot 2H_2O$ 0.1 M adjusted to pH = 7 using H_3PO_4 85%] (solvent

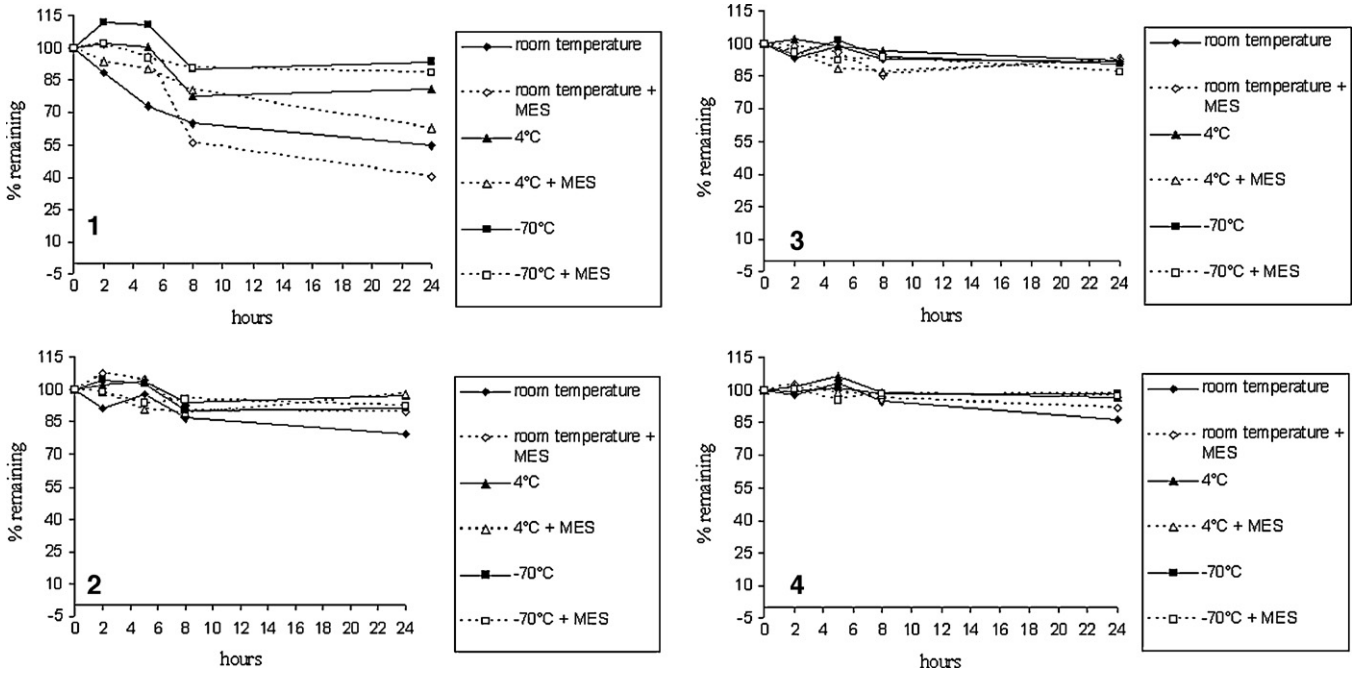

Fig. 2. Stability of plasma imipenem (1), doripenem (2), ertapenem (3), meropenem (4) samples (30 mg/L).

B) was delivered at 1 mL/min with a gradient program: from time 0 (solvent A 0% to solvent B 100%) to 5 min (solvent A 30% to solvent B 70%), the percentage of methanol is progressively increased allowing better separation of imipenem from solvent front and relatively short retention times for other carbapemens associated with a satisfactory resolution between compounds; from time 5 to 7 min the column is stabilized with the initial gradient (solvent A 0% to solvent B 100%) before the next injection. Chromatographic separation was performed at ambient temperature and the UV detection was performed at 295 nm which corresponds to an absorbance maxima for carbapenems.

2.4. Preparation of standards

Stock solutions of imipenem, ertapenem, meropenem, doripenem and ceftazidime are prepared at concentrations of 1000 mg/L for carbapenems and 100 mg/L for ceftazidime in water, aliquoted and stored at −70 °C. Two working solutions were prepared by diluting the stock solutions in MES buffer (MES 1 M adjusted to pH = 6 using NaOH 5 M and stored at room temperature one week) to final concentrations of 100 mg/L and 10 mg/L for imipenem, ertapenem, meropenem and doripenem. Seven-points calibration curves (0.5, 2, 5, 10, 25, 50, 100 mg/L) were prepared for the calibration of each carbapenem by diluting known volumes of these working solutions in MES buffer (pH = 6) to obtain a 100 μL final volume. These diluted solutions (100 μL) are mixed with 100 μL of drug free human plasma. Quality controls (low-level 6 mg/L, medium-level 30 mg/L and high-level 60 mg/L for each carbapenem) were obtained by diluting the working solutions in MES buffer (pH = 6) and mixing these diluted solution in drug free human plasma (1:1, v/v).

These mixtures (calibration standards and quality controls) of plasma and MES buffer (pH = 6) are further treated as described in section sample preparation.

Table 1
Inter- and intra-day precision and inaccuracy (bias) for carbapenems: inaccuracy is defined as the percent of deviation from the nominal level and precision as the coefficient of variation. The units of plasma concentration is mg/L.

Theoretical concentration	Intra-day (n = 3)			Inter-day (n = 5)		
	Mean measured concentration ± S.D.	CV (%)	Bias (%)	Mean measured concentration ± S.D.	CV (%)	Bias (%)
Imipenem						
6	5.67 ± 0.21	3.7	5.4	5.72 ± 0.29	5.08	4.6
30	28.45 ± 0.91	3.2	5.1	28.53 ± 1.26	4.4	4.9
60	53.8 ± 0.94	1.75	10.3	55.05 ± 2.56	4.65	8.25
Doripenem						
6	5.98 ± 0.22	3.6	0.3	6.04 ± 0.40	6.6	0.7
30	29.21 ± 0.79	2.7	2.6	28.94 ± 0.84	2.9	3.5
60	55.67 ± 0.92	1.65	7.2	56.11 ± 1.35	2.4	6.5
Ertapenem						
6	6.7 ± 0.13	1.9	12.1	6.2 ± 0.76	12.2	7.8
30	28.28 ± 0.48	1.7	5.7	27.66 ± 0.91	3.3	7.8
60	56.58 ± 0.68	1.2	5.7	55.98 ± 1.68	3.0	6.7
Meropenem						
6	5.89 ± 0.05	0.8	1.8	5.91 ± 0.05	0.9	1.5
30	29.33 ± 0.85	2.9	2.2	29.35 ± 0.59	2.0	2.2
60	57.07 ± 1.31	2.3	4.9	57.77 ± 3.98	2.3	3.7

2.5. Sample preparation

After blood collection (lithium heparinate as anticoagulant), samples were quickly centrifuged and the plasma was stabilised by combining an aliquot of plasma (1 mL) with MES buffer (pH = 6) (1:1, v/v) in conventional plastic vial. A 200 μL aliquot of this mixture (patient samples, calibration standards, quality controls) is vortexed with 100 μL of the internal standard solution (100 mg/L) and 500 μL of acetonitrile. After centrifugation (15,800 × *g* for 5 min), the supernatant is transferred in glass vial and evaporated to dryness under a gentle stream of nitrogen at +30 °C. The residue is reconstituted in 200 μL of MES buffer (pH = 6), transferred in autosampler glass vial and 50 μL are injected in the chromatographic system.

2.6. Method validation

Calibration curves (peak area ratio using the internal standard versus nominal concentration) were fitted by a linear regression (equal weight and 1/*x* weight). The concentrations were back-calculated and the model with the lowest deviation between the calculated and nominal concentrations was retained. Inaccuracy and imprecision were determined from the analysis of 5 replicates on 5 separate assays (inter-day inaccuracy and imprecision) and 3 replicates on the same assay (intra-day inaccuracy and imprecision) of the quality controls (low-level, medium-level and high-level). Inaccuracy is defined as the percentage of deviation from the nominal level and imprecision as the coefficient of variation within a single run (intra-assay) and between different assays (inter-assay). The imprecision and the inaccuracy should not exceed 15% except for the limit of quantitation which is defined as the lowest concentration in a plasma sample such that the imprecision and inaccuracy are less than 20% [28]. The selectivity was investigated by analyzing 5 different blank plasma samples, patients samples and plasma spiked with drugs having chemical structures similar to carbapenems such as other beta lactamin antibiotics (cefazoline, cefepime, cefotaxime, cloxacilline, oxacilline, piperacilline, ticarcilline) and hypnotic drugs (midazolam, thiopental and pentobarbital) which can be associated to carbapenems in intensive care unit such as burn care department. The recovery ratio was determined by comparing the peak areas of the quality controls samples after extraction with the peak areas of the standard solutions at the same concentration and not extracted for the three levels of quality controls. The stability of plasma samples spiked with ertapenem, imipenem, ertapenem, doripenem (30 mg/L) stored at room temperature,+4 °C, −70 °C stabilized or not by MES was investigated. Stability was assured when 85–115% of the nominal concentration was found in the stored stability samples compared with a freshly prepared calibration curve. The stability of plasma extracts on the rack of the autosampler at room temperature and the stability of stocks solutions stored at −70 °C were also evaluated.

3. Results and discussion

The calibration curves were better fitted by an un-weighted linear regression. The linearity was observed until 100 mg/L for all carbapenem since the correlation coefficients for all calibration curves were above 0.995. The intra-assay ($n = 3$) and inter-assay ($n = 5$) imprecision and inaccuracy were less than 15% for quality control samples of each carbapenem. These results are presented in Table 1. The limit of quantitation was 0.5 mg/L for imipenem, doripenem, ertapenem and meropenem which is consistent with previous results [13,21]. No interference was found in blank sample, spiked sample, patient samples

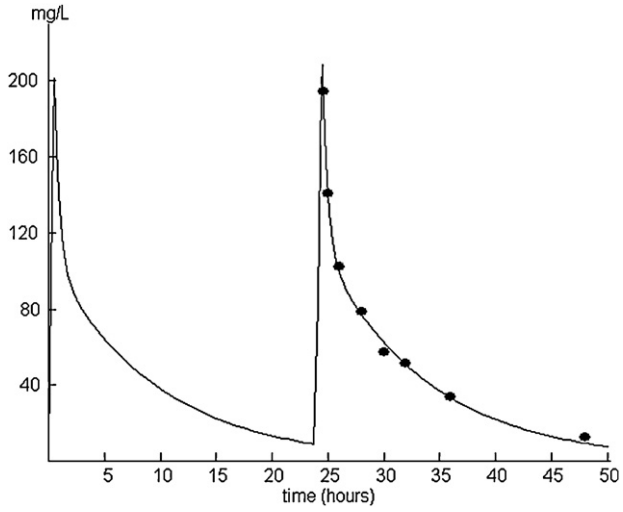

Fig. 3. Time course of ertapenem plasma concentration in a patient after 1000 mg administered once a day. Concentrations are fitted according to a two compartment model.

(Fig. 1) between endogenous compounds or xenobiotics and carbapenems or internal standard. This selectivity was confirmed by frequently analyzing patients samples without finding interferences. No interference was detected with drugs listed in sections 2–6. The retention times of co-administered drugs established from spiked human plasma samples are respectively 6.5 min and 6.7 min for cefazoline and cefepime. The others compounds are not detected. The average values for recovery calculated with the quality control samples were from 81% to 92%.

At room temperature, the stability of not stabilized carbapenems plasma sample is checked up to 2 h for imipenem, 8 h for doripenem and at least 24 h for ertapenem and meropenem which is consistent with previous results [13,21] (Fig. 2). At +4 °C, the stability of stabilized or not stabilized carbapenems plasma samples was checked up to 5 h for imipenem and at least 24 h for ertapenem, doripenem, meropenem. At −70 °C, the stability of stabilized carbapenems plasma samples and stock solutions was observed for at least 6 months for imipenem, doripenem, ertapenem, meropenem and ceftazidime. In the autosampler, the stability of extracts is checked up to 8 h.

The chromatographs of patients samples are presented in Fig. 1. The run time was decreased in comparison with the single method previously published allowing a simultaneous determination of carbapenem plasma concentration except doripenem (7 min versus 25 min [21]). A pharmacokinetic study based on our assay was performed to investigate ertapenem pharmacokinetics in burn patients. Burn patients received a 0.5 h infusion of ertapenem (1000 mg) every 24 h. Plasma samples were collected before the second infusion, 5 min after the end of the second infusion and 1 h, 2 h, 4 h, 6 h, 12 h, 24 h after the beginning of second infusion. Ertapenem plasma total concentrations were fitted according to a two compartment model (Fig. 3). Since ertapenem is highly plasma protein bound contrary to other carbapenems and unbound ertapenem was responsible for the antimicrobial activity [29], the chromatographic conditions defined in this assay were also applied to plasma filtrates obtained according to a previously validated method [30] to measure ertapenem free plasma concentration (Fig. 4). Briefly, a 1 mL plasma aliquot was transferred to a Centrifree® Centrifugal Filter Device (Millipore, USA), the device was centrifuged at 1500 × *g* for 15 min at room temperature and 50 μL of the filtrate were injected in the chromatographic system.

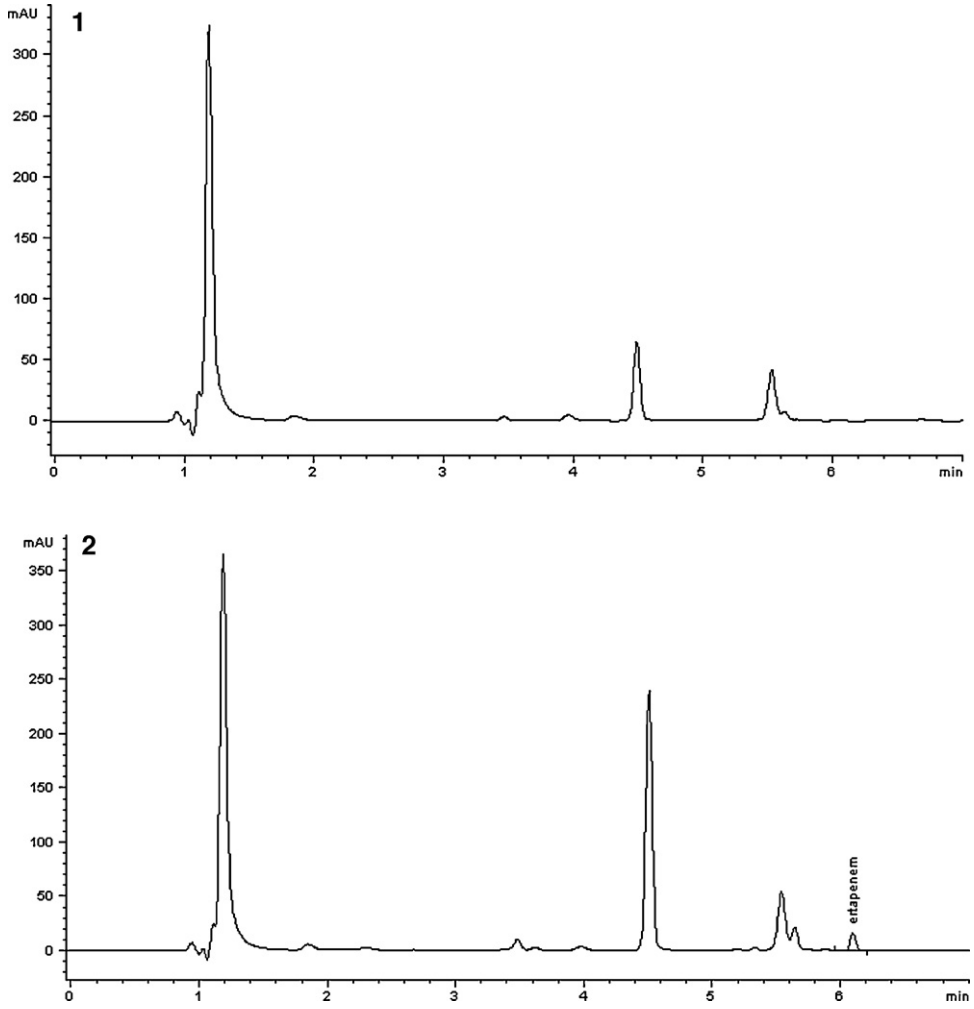

Fig. 4. Chromatograms of blank plasma filtrate (1) and plasma filtrate obtained from a patient receiving ertapenem 1000 mg once a day (ertapenem plasma concentration 12 h after the beginning of second infusion = 1.78 mg/L) (2).

4. Conclusion

This method allows an accurate, precise, specific determination of total plasma concentration of four carbapenems (imipemem, doripenem, ertapenem and meropenem) in a single run which is shorter than run previously described in other assays thanks to the core shell technology. The simple liquid extraction less expensive than extraction based on centrifugal filter device [20] or extraction cartridge [13], the simultaneous determination of 4 carbapenems plasma concentrations and the short chromatographic run can contribute to improve the efficiency of clinical laboratory in routine therapeutic drug monitoring of carbapenems. Moreover, this assay can be performed in most of the laboratory since this assay is not requiring a sophisticated chromatographic system such as ultra performance liquid chromatography or liquid chromatography coupled with mass spectrometry.

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