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Short communication

A simple and sensitive liquid chromatography–tandem mass spectrometry assay for the quantification of ertapenem in microdialysate

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Abstract

A new liquid chromatography assay with isocratic elution and tandem mass spectrometry detection (LC–MS/MS) using an electrospray ionization interface in the multiple reaction monitoring mode was developed and validated for ertapenem determination in microdialysate samples. Linearity was demonstrated between 10 ng mL^{-1} (lower limit of quantification, LLoQ) and 160 ng mL^{-1}. The precision (CV%) and accuracy (bias%) in microdialysates at the LLoQ were respectively 2.2% and 17.3% within-day and 10.6% and 2.7% between-days. Ertapenem was stable for 1 month at −20 °C and −80 °C but unstable at +4 °C. This new LC–MS/MS assay is simple, rapid and more sensitive than previously described assays.
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Keywords: LC–MS/MS; Ertapenem; Microdialysis; Pharmacokinetics

1. Introduction

Ertapenem is a new broad-spectrum antibiotic that has been shown to provide effective treatment against a wide range of community-acquired infections [1]. Because most infections occur in the tissue extracellular fluid (ECF), free ECF antibiotic concentrations at the target site are more useful than total plasma concentrations to predict the time course of antimicrobial activity [2]. Microdialysis appears to be a technique of choice for investigating unbound drug concentrations in tissue ECF [2–5]. However because of the small dialysates volume combined with low concentrations, drug analysis may become challenging. Ertapenem is extensively (84–96%) bound to plasma proteins [1], and high-performance liquid chromatography (HPLC) assays with UV detection may lack sensitivity to determine free tissue concentrations. Several chromatographic methods have been developed in plasma. An HPLC technique with ultraviolet detection at 300 nm was initially published to measure total drug concentrations in plasma and urine [6] with a reported lower limit of quantification (LLoQ) at 0.125 μg mL^{-1}. It was then slightly modified for the determination of unbound ertapenem concentrations in plasma with a limit of quantification of 0.25 μg mL^{-1} for an injected volume of 50 μL [7]. These techniques were used for the first published pharmacokinetic studies of ertapenem in young healthy volunteers [8] and then in healthy elderly subjects [9]. Other HPLC assays have been described, in particular for ertapenem determination in cerebrospinal fluid, with a limit of quantification at 0.025 μg mL^{-1}, but requiring a 150-μL injection volume [10]. Several simplified HPLC assays with UV detection have recently been reported for ertapenem determination in plasma [11] and/or tissues or fluids such as broncho-alveolar fluid [12,13], with reported limits of quantification ranging from 0.25 μg mL^{-1} for an injected volume of 40 μL [12] to 0.02 μg mL^{-1} for an injected volume of 10 μL [13]. An assay using LC–MS/MS has been used for a pharmacokinetic study of ertapenem in humans [14], but with a poor description and a reported limit of quantification in plasma of 1 μg mL^{-1}. A liquid chromatography coupled with mass spectrometry assay (LC–MS) was published and appropriately described but its limit of quantification was still equal to 1 μg mL^{-1} in plasma with an injected volume of

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20 μL [15]. Finally a solid-phase extraction LC–MS/MS method was recently used to study ertapenem distribution in skeletal and subcutaneous adipose tissue using microdialysis [16]. Injected volume was not indicated but LLoQ was reported to 0.04 μg mL^{-1} with a run time equal to 14 min [16]. The objective of the present study was therefore to develop and validate a simple, rapid and sensitive LC–MS/MS assay for the quantification of ertapenem concentrations in microdialysate samples.

2. Experimental

2.1. Reagents and materials

Ertapenem (Invanz®) was supplied by Merck Sharp and Dohme pharmaceutical company (Paris, France). Ethylene glycol and 2-(4-morpholino) ethylsulfonic acid (MES buffer) were obtained from Sigma–Aldrich (Saint Quentin Fallavier, France). Ringer solution was supplied by CMA (Phymep, Paris, France). Acetonitrile was HPLC graded from Carlo Erba Reagenti (Milan, Italy). Formic acid was of reagent grade from Merck (Darmstadt, Germany). HPLC quality water was prepared using a Millipore Milli-Q purification system. Blank drug-free plasma was obtained from the Poitiers University Hospital blood bank.

2.2. Preparation of standard and quality control samples

Two aqueous ertapenem stock solutions (1 μg mL^{-1}) were prepared, one for standard curves construction and one for quality control (QC) samples preparation. They were stored at −20 °C until being used. Calibration curves and QC were prepared extemporaneously for each chromatographic session. Calibration samples were prepared by dilution of the stock solution with a mixture (1:1, v/v) of Ringer and stabilizer (0.1 M MES buffer pH 6.5, ethylene glycol (1:1, v/v)) to obtain final concentrations ranging from 10 ng mL^{-1} to 160 ng mL^{-1}. QCs were prepared by diluting the stock solution with the Ringer–stabilizer solution, at low (10 ng mL^{-1}), medium (50 ng mL^{-1}) and high (150 ng mL^{-1}) concentrations.

2.3. Dialysate sample preparation

Immediately after collection microdialysate samples were diluted (1:1, v/v) with the MES buffer stabilizer and directly transferred to the autosampler maintained at 4 °C for analysis. Because microdialysate samples were directly injected, no internal standard was added.

2.4. LC–mass spectrometry apparatus and conditions

Samples (5 μL) were injected directly onto the Xterra® MS C18 (150 mm × 3.9 mm; 5 μm) column (Waters, Saint Quentin en Yvelines, France). The mobile phase was composed of a mixture of 82% solvent A (water, formic acid (99.9/0.1, v/v)) and 18% solvent B (acetonitrile, formic acid (99.9/0.1, v/v)), delivered at a flow rate of 0.8 mL min^{-1} in an isocratic elution mode. The flow was split (1/4) before entry in the mass spectrometer. Nitrogen was used as desolvation gas (300 L h^{-1}) and as cone gas (100 L h^{-1}). The pressure of argon collision cell was 4.5×10^{-3} mbar. The source temperature was maintained at 120 °C, the desolvation temperature was 300 °C, the cone voltage was 17 V and the capillary voltage was 2.75 kV. A Quattro Micro Mass Spectrometer (Waters, Saint Quentin en Yvelines, France) equipped with an electrospray ionization interface, a nitrogen generator and a 2695 Separation module (Waters, Milford, MA, USA) was used. The analytes were detected in the positive ion mode by multiple reaction monitoring (MRM). The product ion mass spectra of the protonated molecules of ertapenem at *m/z* 476.2 indicated the presence of an intense fragment ion at *m/z* 432.1 (Fig. 1). Selected ion monitoring was accomplished at *m/z* 476.2 → *m/z* 432.1 with *m/z* resolution of 0.1 amu. Data acquisition was in centroid mode with 0.5 s for total scan time. Processing was performed by Masslynx software (Version 3.1).

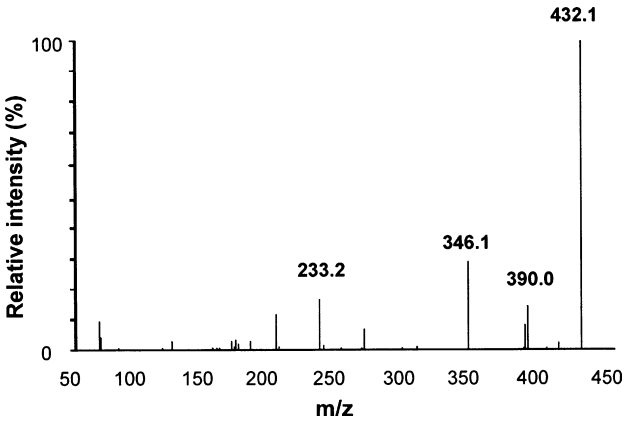

Fig. 1. MS/MS product ion spectra from *m/z* 476.2 by positive ion electrospray ionization (nitrogen as desolvation gas (300 L h^{-1}) and cone gas (100 L h^{-1}); pressure of argon collision cell: 4.5×10^{-3} mbar; source temperature: 120 °C; desolvation temperature: 300 °C; cone voltage: 17 V; capillary voltage: 2.75 kV and ion energy: 11 eV).

2.5. Method validation

2.5.1. Ion suppression

Ertapenem (10 ng mL^{-1}) solution in mobile phase was continuously infused post-column (10 μL min^{-1}) and mixed with the column effluent before entering the mass spectrometer. After a steady baseline was obtained, blank microdialysate samples were injected into LC–MS/MS, eluted as previously described, and chromatograms were recorded.

2.5.2. Calibration and sample quantification

Calibration curves in Ringer were calculated by linear least-square regression of the peak area of ertapenem versus the corresponding concentrations. Ertapenem concentrations in unknown samples were determined by interpolation from the calibration curves.

2.5.3. Accuracy, precision, limit of quantification, and linearity

Accuracy, within-day and between-day precisions of the method were determined for dialysate analysis. Six replicates

spiked Ringer samples were assayed between-day and within-day at three different ertapenem concentrations. Concentrations were calculated using a calibration curve prepared within the same medium and analyzed in the same run. Accuracy was calculated as percent deviation from nominal concentration (bias). Within-day and between-day precisions were expressed as the coefficient of variation (CV% = 100 × standard deviation (S.D.)/mean). At the LLoQ, the percent deviation from the nominal concentration (mean accuracy) and precision (CV%) have to be within the limit of 20% [17,18]. Linearity was checked within the 10–160 ng mL^{-1} range in dialysates. The linear least-square regression was carried out to determine the mean intercepts, mean slopes and correlation coefficients of calibration curves (n = 6).

2.5.4. Stability

Ertapenem stability in Ringer stabilized with MES buffer was evaluated at a concentration of 100 ng mL^{-1} over 31 days at various temperatures: +4 °C, −20 °C and −80 °C. Freeze-thawed stability of ertapenem solution in Ringer stabilized with MES buffer at 100 ng mL^{-1} was assessed over three cycles: samples were frozen, thawed and analyzed three times.

2.6. Application

The method was used to determine unbound ertapenem concentrations in microdialysates collected from blood and muscle extracellular fluid of anesthetized rats treated by ertapenem (30 mg kg^{-1} administered as a 30-min intravenous infusion) as previously described [5]. This work was done in accordance with the Principles of Laboratory Animals Care (NIH Publication #85-23, revised 1985).

3. Results and discussion

3.1. Mass spectra, MS/MS detection, chromatographic conditions and ion suppression

Under the chromatographic conditions used, the short retention time of ertapenem (1.97 min) allowed injections every 3 min, which is much shorter than the 14-min period of time required with the other LC–MS/MS assay recently reported [16]. An ion suppression peak eluting at about 1.4 min was observed but had no consequence on ertapenem detection (Fig. 2). No additional peaks due to endogenous substances that could have interfered with the detection of the compounds of interest were observed. Typical ion chromatogram of a blank and microdialysate samples are shown in Fig. 3.

3.2. Linearity, accuracy, precision and limit of quantification

Standard curves were linear in dialysates, with a mean ± S.D. slope and mean ± S.D. y intercept equal to 8.11 ± 1.25 and 3.91 ± 8.43 in Ringer, respectively. Calibration curves correlation coefficients (r^2) were at least equal to 0.997. The bias and precision for an injected volume of 5 μL were less than 20% at 10 ng mL^{-1} which was considered as the LLoQ. The most sensitive assay previously described in the literature [13] reported an LLoQ in broncho-alveolar lavage fluids equal to 20 ng mL^{-1} for an injected volume of 10 μL, that is four times less sensitive than this new LC–MS/MS assay. Results from the validation of the method are listed in Table 1. The method proved to be accurate within-day and between-day with bias at the three investigated concentrations between 2.7% and 17.3% in Ringer (Table 1). The within-day and between-day precision (CV%) ranged from

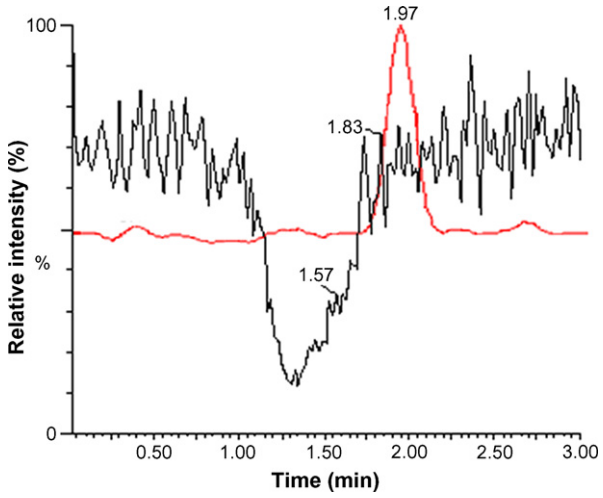

Fig. 2. Ion suppression profile of microdialysate samples analyzed during a post-column continuous ertapenem solution (10 ng mL^{-1}) infusion. Ertapenem standard (10 ng mL^{-1}) ion chromatograms were overlaid to indicate retention times.

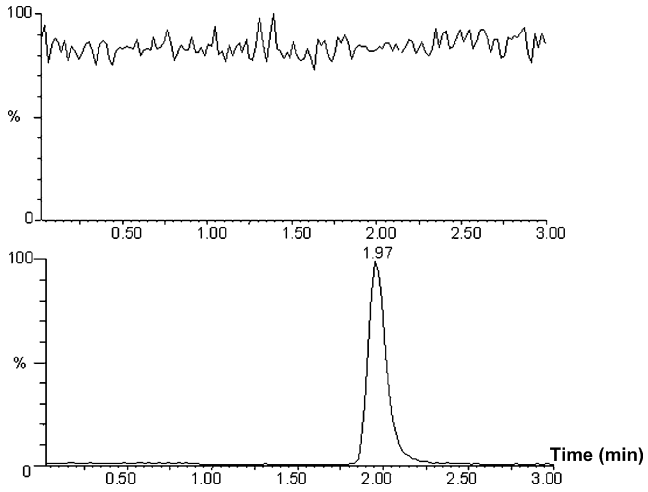

Fig. 3. Ion chromatograms of a blank microdialysate and a microdialysate sample (5 μL) with ertapenem present at a concentration close to 110 ng mL^{-1}.

Table 1
Within-day and between-day precision and accuracy of ertapenem in Ringer samples stabilized with a MES buffer (n = 6)

	Within-day			Between-day		
	10	50	150	10	50	150
Mean	11.7	52.6	142.3	9.8	47.2	139.0
S.D.	0.2	2.6	4.2	1.0	3.4	6.5
CV (%)	2.2	5.0	3.0	10.6	7.3	4.6
Bias (%)	17.3	5.2	4.9	2.7	4.4	8.4

10, 50 and 150 denote the values of concentration in ng mL^{-1}.

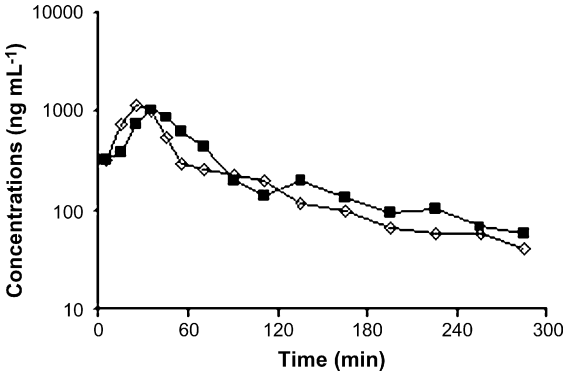

Fig. 4. Semi-logarithmic plot of unbound ertapenem concentrations vs. time measured in dialysates collected from blood (◊) and muscle (■), following an intravenous administration of ertapenem (30 mg kg^{-1}, infused over 30 min) in a rat.

2.2% to 10.6% (Table 1). These results are within the acceptable criteria for precision and accuracy.

3.3. Stability

Because carbapenem antibiotics including ertapenem are potentially unstable in aqueous solutions [19], it is of common practice to dilute biological samples with a MES buffer stabilizer immediately after collection [9,10,13,14]. In these conditions ertapenem was stable over 31 days of storage at −20 °C and −80 °C, but unstable at +4 °C (data not shown). After three freeze-thawed cycles, average stability results ranged from 98.9% to 101.7%.

3.4. Application

An example of ertapenem pharmacokinetics in rats is presented in Fig. 4. Concentrations measured in dialysates are presented, but note that actual concentrations in tissues should be higher and estimated after correction for microdialysis probes recovery [5].

4. Conclusion

The LC–MS/MS assay described in this paper was developed for microdialysate sample analysis but could also be used for the quantification of ertapenem in biological liquids such as broncho-alveolar lavage fluid or cerebrospinal fluid. It presents the advantages of being easy to implement in particular because it does not use a gradient mode elution, more rapid and more sensitive than any other previously reported method.

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