Development and validation of a simple High-Performance Liquid Chromatography method for the quantification of meropenem in human plasma.

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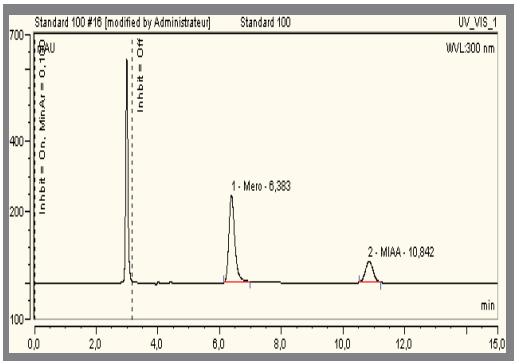
Introduction

Meropenem is an antibiotic of the β-lactam family belonging to the Carbapenem class. Marketed under the name of Meronem®, it is often used in hospitals particularly in intensive care units, for treatments of nosocomial infections.
The purpose of our study was tp develop a rapid High-Performance Liquid Chromatography Method, for the quantification of meropenem in human plasma.



Representative chromatogram :
1 = Meropenem (6,3 min)
2 = MIAA, internal standard (10,8 min)

Methods

For calibration standards and controls, drug free, commercially available, human plasma was spiked with meropenem and fixed concentration of MIAA (5-methoxiyndol-3-acetic acid) used as an internal standard. MES (2-N-morpholinoethanesulfonic acid) was added to avoid meropenem hydrolysis in plasma. The assay procedure involved a liquid-liquid extraction. The separation was performed with a reversed-phase column (µBondapack C18 with a pre-column RP C18). The mobile phase consisted of acetonitrile, 25mM phosphate buffer (pH 6.5) and 0.1% acetic acid (10:90:0,1; v/v/v;) and was used at a constant flow rate of 1ml/min. The detector wavelength was set at 300nm. The linearity was observed by plotting the peak response (area counts) against the standards over the range of concentrations (100 to 0.5 µg/mL). Replicate analyses of 3 levels of quality controls samples were done: on the same day and on 6 different days, so as to assess intra-assay and inter-assay variability, respectively. Precision was represented as coefficients of variation, accuracy was determined by comparison between target and determined concentrations. Specificity was studied by testing potential interference with other frequently used drugs.

Results

The retention times were 6.3 minutes for the meropenem and 10.8 minutes for the MIAA (Figure 1). The recovery was 66%. The method was sensitive to 0.5µg/mL and linear to 100µg/mL with a 40µL injection. The low intra-day and inter-day variability at all measured control quality levels, when compared to the target concentrations, indicated sufficient accuracy. The coefficients of variation were less than 15%, defining the precision of the assay at low and high concentrations.

No interference was observed with Caffeine, Aspirin, Paracetamol, Ibuprofen, Gentamicin, Netilmicin nor Teicoplanin (drugs frequently associated with meropenem).

Conclusion

In conclusion, the method meets the guidelines required for diagnostic laboratories. It will be soon proposed for patient therapeutic drug monitoring specially in those suffering from nosocomial meningitis.

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