Original Article

DEVELOPMENT AND VALIDATION OF A RP-HPLC METHOD FOR THE DETECTION OF MEROPENEM AS A PURE COMPOUND, IN A PHARMACEUTICAL DOSAGE FORM AND POST THERMAL INDUCED DEGRADATION

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ABSTRACT

Objective: As Meropenemis a potential drug for drug-delivery systems, a reliable reverse-phase high performance liquid chromatographic (RP-HPLC) method was developed and validated, for the detection of various Meropenem samples.

Methods: Chromatographic conditions involved a reverse-phase C-18 column with the mobile phase consisting of 0.03 M di-hydrogen phosphate buffer adjusted to a pH of 3 and acetonitrile at a ratio of 80:20, at an isocratic flow rate of 1 mL/min and UV detection wavelength of 298 nm with 20 µL sample volumes. The method was validated for its limit of detection (LOD), limit of quantification (LOQ), linearity, precision and accuracy with analytical samples. Specificity of the method was determined by analysing the drug content in a marketed preparation of Meropenemand heated Meropenem solution.

Results: Upon validation, the developed method effectively detected the drug as a pure compound, in a pharmaceutical dosage form and upon degradation with thermal exposure. The linearity was found to be greater than 0.999 and precision and accuracy values were less than 3.5% and from 98 to 102% respectively.

Conclusion: The developed method was found to be linear, accurate, precise and specific in detecting Meropenem.

Keywords: Meropenem, RP-HPLC, method validation, marketed preparation, degradation product.

INTRODUCTION

Meropenem is a broad spectrum antibiotic of the Carbapenemclass of antibacterials, effective against many Gram-positive and Gram-negative pathogens including anaerobic microorganisms [1]. It is chemically termed as (4R,5S,6S)-3-[[(3S,5S)-5-dimethylcarba-moylpyrrolidin-3-yl]-thio]-6-[(1R)-1-hydroxyethyl]-4-methyl-7-oxo-1-azabicyclo[3,2,0]hept-2-ene-2-carboxylic acid [2] and its structure is shown in Figure 1. The antibacterial activity of Meropenem is associated with its high affinity for a variety of penicillin-binding proteins (PBPs), which are its main targets. Meropenem exerts its action by readily penetrating bacterial cells and interfering with the synthesis of vital cell wall components, which ultimately leads to cell death [3].
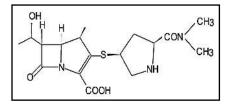

Fig. 1: It shows the chemical structure of Meropenem

Meropenemis commonly used as an intravenous solution (I.V.S). It has good clinical efficacy, low side effects and is well tolerated in patients [1, 4]. Meropenem has also been used for combinatorial drug therapy alongside the Aminoglycosides, Tobramycin [5] and the antifungal Fluconazole [6], in various cases of clinical infections. It is a good candidate for drug delivery systems (DDS) and previously has been successfully incorporated into liposomes [7] and nanoparticles [8]. Methods used to formulate DDSs such as solvent evaporation or solvent displacement techniques, require the drug to be exposed to high temperatures. This occurs in steps associated with high pressure homogenisation (HPH) and solvent evaporation under pressure [9]. These high temperature exposures may lead to degradation of the drug entity. Thus, the objectives of the present study were to develop and validate a reliable RP-HPLC method for the detection of (i) pure Meropenem, (ii) the drug content of Meropenemin a commonly used marketed preparation and (iii) the degradation products of pure Meropenem, post exposure to high temperatures.

MATERIALS AND METHODS

Materials

Meropenemtrihydrate (CAS#119478-56-7) was purchased from PI Chemicals, (Shanghai, China) with reported 98% purity. The marketed preparation of Meropenem for injection (IP) was purchased from a pharmacy as Meromac, 0.5g (Macleods Pharmaceuticals Ltd, India), containing 500 mg Meropenem and 45.10 mg sodium carbonate. Chromatographic grade acetonitrile (Friendemann& Schmidt, Germany), analytical grade di-potassium hydrogen phosphate (KH_2PO_4) buffer salts (Friendemann& Schmidt, Germany), 85% orthophosphoric acid (Merck, Darmstadt, Germany), triethylamine (Friendemann& Schmidt, Germany) and ultra-pure water (UPW, Milipore®, 0.22 µm filtered) were purchased from local suppliers. Acetonitrile and potassium (KH_2PO_4) buffer were filtered through 0.45 µm nylon membrane filters and degassed prior to usage for HPLC.

HPLC analyses were conducted with a Hypersil GOLD C-18 analytical column with the dimensions of 250 mm x 4.6 mm ID x 5 µm (Fisher

Scientific, USA), 50 µL glass syringe for chromatography (SGE, Australia) and the High Performance Liquid Chromatography instrument (Agilent Technologies, 1200 Series USA), equipped with a ultra-violet (UV) detector, rheodyne manual injector and 20 µL sample loop.

Methods

HPLC analytical conditions

The mobile phase for detection of Meropenem consisted of acetonitrile and di-hydrogen potassium phosphate (KH_2PO_4) buffer prepared to 0.03 M, adjusted to pH 3 with 85% orthophosphoric acid. The mobile phase was fixed at a ratio of 20% acetonitrile and 80% buffer for isocratic elution. All samples were analysed at a flow rate of 1 mL/min and a UV wavelength of 298 nm as determined in prior with UV spectrophotometry and supported by literature [10].

Preparation of stock and working standard solutions

A stock solution of Meropenemtrihydrate at 1 mg/mL was prepared by diluting accurately weighed drug powder with ultra-pure water. From the stock solution, 100 µg/mL, 10 µg/mL and 1 µg/mL dilutions were prepared.

A standard calibration curve was obtained from analysing aqueous solutions of Meropenem prepared at concentrations of 2, 6, 10, 14, 18 and 20 µg/mL. Quality Control (QC) concentrations were then prepared at 2, 12 and 19 µg/mL, as the respective low, medium and high concentration control samples. These concentrations were selected based on recommended guidelines by the International Conference on Harmonisation (ICH) and Food and Drug Administration (FDA) for analytical methods validation [11].

Preparation of working standard solutions from a marketed preparation of Meropenem

The injectable powder of Meropenem (for IV) equivalent to 10 mg of Meropenem was transferred to a 100 mL volumetric flask. To this, 100 mL of ultra-pure water was added and the drug solution was ultra-sonicated for 20 minutes. This was followed by filtration through a nylon syringe filter (200 µm cut-off), creating a stock solution with a concentration of 1 mg/mL.A lower concentration of the marketed Meropenem product at 10 µg/mL was further prepared with appropriate dilutions.

Method Validation

Determinations of limit of detection (LOD) and limit of quantification (LOQ)

The limit of detection (LOD), as the lowest detectable concentration of the drug compound, was determined by successively injecting sequentially lower concentrations of Meropenem until a signal (peak) to noise ratio of 3:1 was obtained. The limit of quantification (LOQ), as the lowest quantifiable concentration, was also determined from the range of concentrations analysed for the LOD determination.

Evaluation of linearity

The linearity of the method was evaluated by standard solutions (QC sample preparations) within the concentration range of 2 to 20 µg/mL. The peak area was plotted against drug concentration and the linearity was thus calculated by the linear regression equation,*y = mx + c*. A correlation coefficient of approximately 0.999 or more was considered as desirable for all calibration curves.

Determination of accuracy and precision

Inter-day validation was conducted with six sets of three QC samples of different concentrations. These samples were evaluated for six days by generating a calibration curve each day. As for the intra-day validation, six sets of three different drug concentration samples were assayed and evaluated with reference to one calibration curve on the same run. The accuracy and precision values were calculated using a standard formula. Under the stated experimental conditions, acceptable precision (RSD)values were a maximum of 5% and accuracy values were within a range of 95 to 105%.

Specificity

The specificity of the developed HPLC method was evaluated by analysis of aqueousMeropenem solution heated to 70°C for 120 minutes. Observations were made for interfering peaks generated by degradation products during the analyses.

RESULTS

The most suitable isocratic condition to resolve Meropenem with a C-18 column after the chromatographic conditions were optimised for specificity, resolution and retention timewas a mobile phase consisting of 0.03 M phosphate buffer adjusted to a pH of 3 and acetonitrile, used at a ratio of 80:20. When the pH of the mobile phase was increased or a higher percentage of acetonitrile was used, the resultant chromatogramseither had an increase in background noise or peaks indicated the tailing effect. Pure Meropenem was found to be eluted at a retention time of 5.8 to 6 minutes, as shown in the chromatogram below (Figure 2).
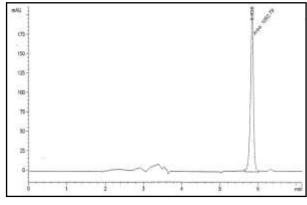

Fig. 2: It shows a chromatogram of pure Meropenem (10 µg/mL)

Method Validation

Limit of detection (LOD) and limit of quantification (LOQ)

The LOD for Meropenem was determined to be 0.5 µg/mL and the LOQ was 2 µg/mL. The calculated co-efficient of variance (CV) for the sample was 3.41% and the accuracy was 98.99%.

Linearity

A linear relationship was obtained between the peak area of Meropenem and the corresponding concentration. The mean standard calibration curve is presented in Figure 3. The calibration curve exhibited linearity over the concentration range of 2 to 20 µg/mL with a regression quotient value greater than 0.999.
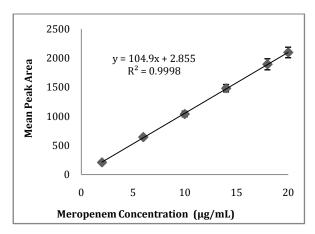

Fig. 3: It shows the mean calibration curve for Meropenem

Accuracy and precision

The intra-day and inter-day precision and accuracy values for the quality control samples are represented in Table 1. The intra-day precision and accuracy results of the method indicated CV percentages of between 2.21% and 3.41%, and accuracy percentages between 98.99% and 102.00%. On the other hand, the inter-day precision and accuracy data generated from the samples with the method, indicated CV percentages of between 2.06% and 3.76%, and accuracy percentages between 98.67% and 100.55%.

Table 1: It shows the accuracy and precision *values* of quality control (QC) samples of Meropenem

QC sample (µg/mL)	Accuracy (%)		Precision (%)	
	Intra-day	Inter-day	Intra-day	Inter-day
2 µg/mL	98.99 ± 3.38	100.55 ± 3.78	3.41	3.76
12 µg/mL	99.47 ± 2.56	99.70 ± 2.65	2.58	2.66
19 µg/mL	102.00 ± 2.25	98.67 ± 2.03	2.21	2.06

Specificity

Upon subjection to a higher temperature, pure Meropenem solution produced a consistent secondary peak. These peaks generated by the degradation product of Meropenemeluted at a retention time of 3.6 to 3.8 minutes and was non-interfering with that of the main drug compound, as shown in Figure 4.
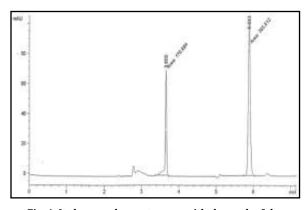

Fig. 4: It shows a chromatogram with the peak of the degradation product of Meropenemappearing at 3.665 minutes, followed by the peak of the main drug compound at 5.883 minutes

HPLC analysis of the marketed preparation of Meropenem

Analysis of the marketed preparation of Meropenem, with the validated assay method found the drug content eluting at the same retention time as the pure drug with no interfering peaks generated by excipients in the marketed product. The analysis produced an accuracy percentage of 101.06% ± 3.76%. A representative chromatogram of the HPLC analysis of the marketed preparation is shown in Figure 5.
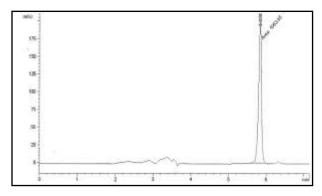

Fig. 5: It shows a HPLC chromatogram generated by a marketed preparation of Meropenemat 10 µg/mL

DISCUSSION

Meropenem is a common broad-spectrum antibiotic administered intravenously to hospitalised patients. Being a hydrophilic drug with innate insolubility in organic solvents, Meropenem was successfully detected with reverse phase HPLC utilising a higher percentage of buffered water in its mobile phase. The reliability and sensitivity of the validated method was ensured with good linearity, accuracy and precision, within the ICH and FDA limits for method validation of analytical samples. In addition, the method was specific in enabling repeated detection of Meropenem in a common marketed preparation of the drug with undetectable interferences from excipients during the HPLC analyses.

Furthermore, being a Carbapenem analogue of the β-lactam class of antibiotics, Meropenem is known to undergo degradation both in its solid state and in aqueous solutions. Meropenem also degrades under thermal pressure, leading to the formation of degradation products such as 4-methyl-3-(1Hpyrrol-3-ylsulfanyl)-5H-pyrrole-2-carboxylic acid and experiences changes of its side chain as a result of decarboxylation, aromatisation and hydrolysis of its rings [12]. Hence, aqueous solutions of Meropenem subjected to high temperatures may form various degradation products. However, with the method developed and validated in this study, chromatograms generated by assaying heated drug solutions produced good peak separation between the degraded drug product and the main drug entity.

CONCLUSION

The UV reverse-phase HPLC method developed for detection of Meropenem was successfully validated. The method allowed reliable, sensitive and specific detection of Meropenem in a common marketed preparation. Good separation of the main drug and thermal degradation products in degraded samples was furthermore achieved. This method will be useful and reliable in the detection of both pure and degraded compounds of Meropenem incorporated into drug delivery systems utilising excipients and exposed to high temperatures.

ACKNOWLEDGEMENT

CONFLICT OF INTEREST

We report no conflict of interest in this original scientific study.

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