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Qualitative analysis of 5th Generation of Carbapenem Antibiotics by UV Spectrophotometric Method

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Abstract

In the present study the Quantitative analysis of 5th generation Carbapenems viz. Meropenem and Imipenem was done. They are used in case of severe infections like: infection in urinary tract, respiratory tract, etc.as they have broad spectrum of activities can be able to work on gram positive, gram negative as well as aerobics and anaerobic bacteria's. For analysis of both antibiotics we took two samples from each Meropenem and Imipenem named as 1st Control i.e. Pharmaceutical formulation 2nd was extracted which was extracted from Synthetic urine. Here, UV spectrophotometer was used for the estimation of meropenem and imipenem in pharmaceutical formulations. The λ max for both the samples were estimated with meropenem having 305nm while imipenem having 295nm. At this wavelength extracted sample absorbance was observed. Similarly, %amount of extracted antibiotics from urine sample was also calculated. As it is usually not used in criminal activities till now, overdosing symptoms can be possible. As most of the elimination is by kidney so urine sample are used for analysis. Here quantitative analysis was done as at what amount these antibiotics can be detected in both standard as well as in synthetic urine by UV spectrophotometry.

Keywords: Carbapenems, UV spectrophotometry, detection.

Introduction

Carbapenems belongs to the group of β-Lactam antibiotics which is having a broad spectrum of antibacterial activities that means they are able to act on gram positive, gram negative as well as anaerobic and aerobic bacteria's[1]. They are having a strong resistant structure that delivers them mostly resistant to β-lactamases. Carbapenem antibiotics were originally generated from carbapenem Thienamycin, which was considered as a model and originated from *Streptomyces cattleya.* Some common FDA approved carbapenems are meropenem, imipenem, doripenem and ertapenem. These were administrated via (IV)as they taken parenterally by intravenous infusion because it show maximum absorption than oral administration till now. Carbapenems get metabolised by an enzyme i.e. Renal Dihydropeptidase-I except imipenem all other can metabolised directly by an enzyme but Imipenem require co-administration with an inhibitor i.e. Cilastain which helps in breakdown[2]. After all procedure they eliminated by kidney. There are some adverse effects[3] after taken into body parenterally which are very common in all carbapenems as reaction at injection site, nausea, diarrhea, rashes on skin, abdominal pain etc not lasted more than day/(s) depends upon physical conditions of patients.In previous studies[4],[5] various methods were opted for carbapenems to get information about its stability, compatibility, quantification in its bulk as well as in pharmaceutical forms[6] or determination from different biological matrices either by animal or human samples like urine as well as human plasma[7]. HPLC method was also used for quantitative analysis of certain carbapenems in human bile and peritoneal fluid[8]in the study it was found that Meropenem to be effective against Mycobacterium tuberculosis by using HPLC-DAD method[9].

Material and Methods

Meropenem (MEPM) was purchased from MT Biopharm pvt.ltd. It was in powdered form quantity 500mg/10ml. Similarly, Imipenem (IMPM) was purchased from Knoll pvt. Ltd. its quantity was 1000mg/10ml.These drugs were stored under 4^0C to avoid degradation. A double beam PERKIN ELMER software WIN LAB UV spectrophometric was used. Its range was 190 to 1100nm and bandwidth 1nm (fixed). Room temperature was in between 20-25^{0}C.

Selection of solvent: Selection of solvent was based on solubility and stability of antibiotics in solvent system as well as extraction of antibiotics from its formulation. Meropenem is soluble in water as well as chloroform[3] while Imipenem is soluble in methanol and ethanol. Hence, these two solvents were selected for UV-Spectrometric determination.

Preparation of samples: Synthetic urine was prepared as 100ml H_2O: 4drops of ammonia: pinch of salt and used for further analysis.

20mg from both antibiotics was taken and added into two urine chamber prepared for analysis. This chamber was kept undisturbed for 3hrs. For the Liquid-liquid extraction for both the samples was done as chloroform: acetone: urine in ratio of 20:20:10 for Meropenem while, in case of Imipenem

Chloroform: Di-ethyl ether: ethanol: urine in the ratio of 20:20:20:10. After this process the extracted sample was kept aside. Standard sample having only pharmaceutical formulation of antibiotics dissolved with solvent in which they are easily soluble like for meropenem 10mg/ 10ml in chloroform while 10mg/10ml imipenem in ethanol.

Preparation of Stock solution: A standard stock solution of MEPM was prepared by adding 0.020g of antibiotic into flask in which 100ml of chloroform was added to prepare 100ml of stock solution for MPEM. This stock solution was further used for dilution. Similarly, a standard stock solution of IMPEM was prepared by adding 0.010g of antibiotic into standard flask in which 100 ml of methanol was added to prepare 100ml of stock solution for IMPEM. Then this solution was used for further dilution.

Determination of λ max[4]: The absorbance of standard stock solution of MPEM and IMPEM was scanned in the UV spectrometer ranging 200-800nm. The plot shows max. Absorbance at 305nm and for MPEM while the plot shows max. Absorbance at 295nm for IMPEM.

Method Validation: From the standard stock solution of meropenem dilution were made by chloroformas 5, 10, 15, 20,25,30,35, 40 µg/ml concentration. Absorbance values of then stock solution were measured at *λmax* 305nm. The calibration curve was plotted between con.[n] of MPEM and respective measured absorbance. Similarly, for the standard stock solution of IMPEM various dilutions were made by ethanol to obtained 1, 5,10,15,25 µg/ml con.[n] Absorbance values of these solution were measured *at* λmax 295nm. The calibration curve was then plotted between concentration of IMPEM and respective measured absorbance as shown in table-1.

Table-1
Linearity and range of imipenem (λ max in Ethanol)

S.No	Concentration µg/ml)	Absorbance
1	1	0.0215
2	5	0.0573
3	10	0.1189
4	15	0.1749
5	20	0.2607
6	25	0.3467

Assay of Extracted sample: The extracted sample of meropenem was scanned in the UV spectrophotometer to measure absorbance at 305nm. Similarly, from the extracted sample of imipenem 0.1ml was taken and diluted to 10ml by adding methanol. The resulting solution was scanned in the UV spectrophotometer to measure the absorbance at 295nm.

$$\%\ extracted\ in\ solvent = \frac{calculacted\ amount}{total\ added\ amount\ initially} * 100$$

Table-2
Linearity and range of Meropenem (λ max in chloroform)

S.no	Concentration µg/ml)	Absorbance
1	5	0.0028
2	10	0.0066
3	15	0.0076
4	20	0.0108
5	25	0.0139
6	30	0.0188
7	35	0.0204
8	40	0.0217

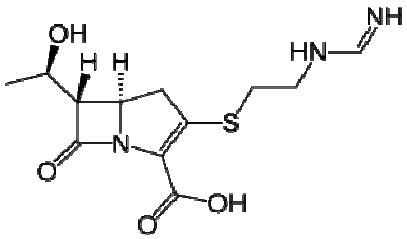
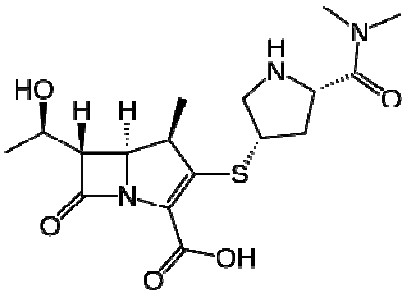
Antibiotics	Structure	Properties
1. Imipenem		Molecular formula $C_{12}H_{17}N_3O_4S$ Molecular weight 299.34g/mol Melting point 193-198°c pH 6.5-8.5 solubility- easily in water and methanol
2. Meropenem		Molecular formula $C_{17}H_{25}N_3O_5S$ Molecular weight 383.46g/mol Melting point 150-153°c pH 7.3-8.3 Solubility- soluble in chloroform,H_2O

Figure-1
Chemistry of Imipenem and Meropenem

Results and Discussion

From above analysis the λ max for both the samples were estimated as shown in figure-2, 3 for both antibiotics respectively (as we know λ max value is achieved by standard stock solutions) meropenem having 305nm while imipenem having 295nm. It is important to estimate the λ max at considered λ max different concentration was observed linearity graph is shown in figure-4, 5. At this wavelength extracted sample absorbance was observed. Quantity of extracted antibiotic was calculated i.e. total amount of extracted antibiotic was measured by using calibration curve equation Y= mx + c, similarly %amount of extracted antibiotics from urine sample was also calculated result are as follows: for meropenem (73.85%) while for Imipenem it is (93.81 %). In case of imipenem as shown in figure-4. Dilution factor (100) was multiplied into it for accuracy. But in case of meropenem it was not used because the result itself was in accurate form i.e. in visible form.

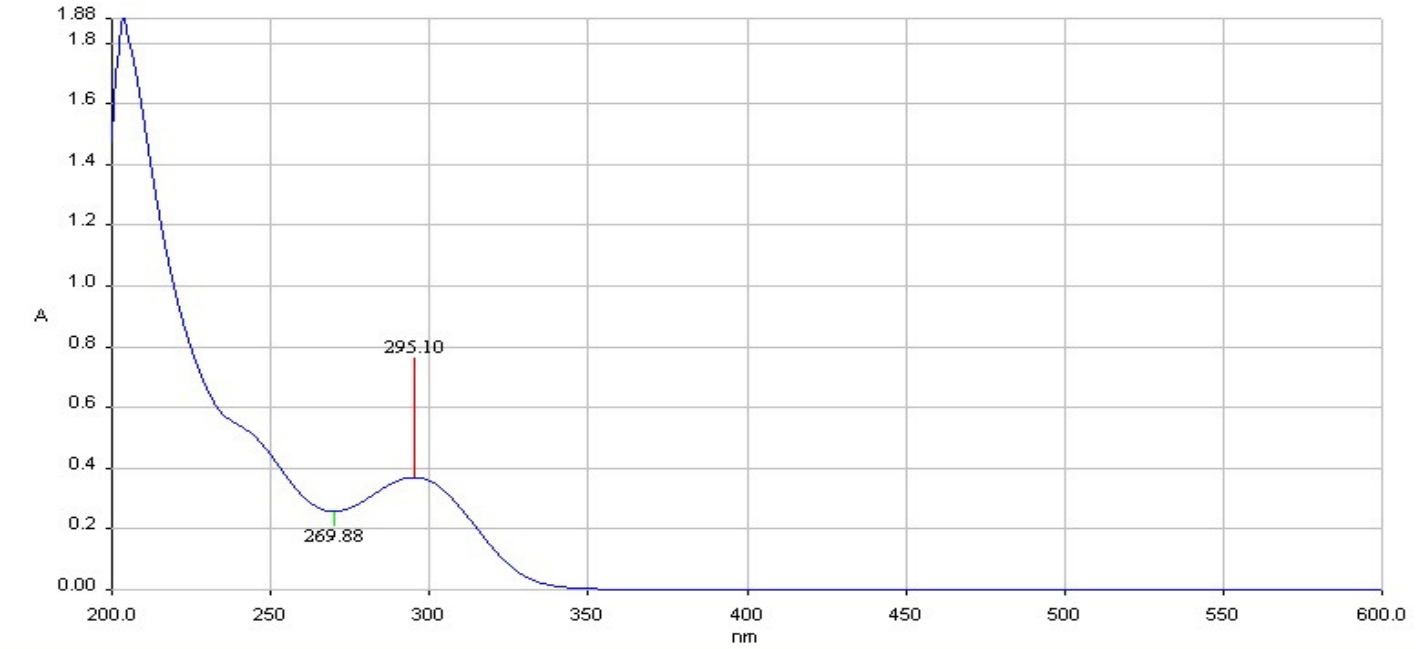


Figure-2
UV-Visible Absorption Spectra of Imipenem: λ max in ethanol

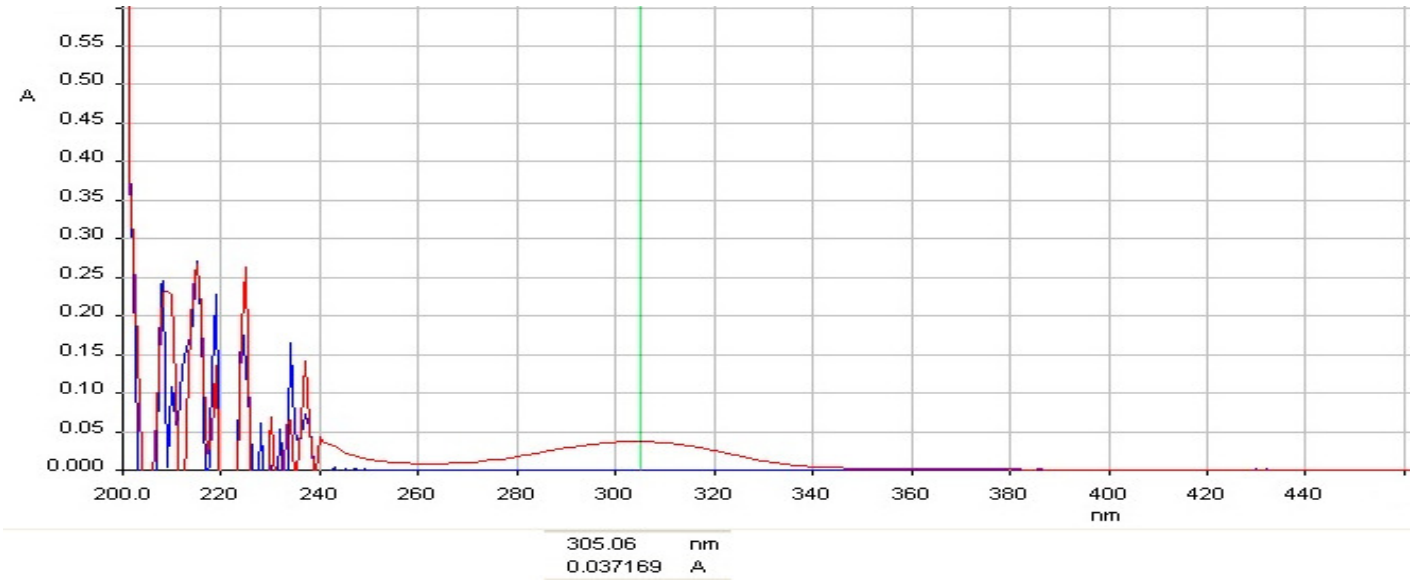


Figure-3
UV-Visible Absorption Spectra of Meropenem: λ max in chloroform

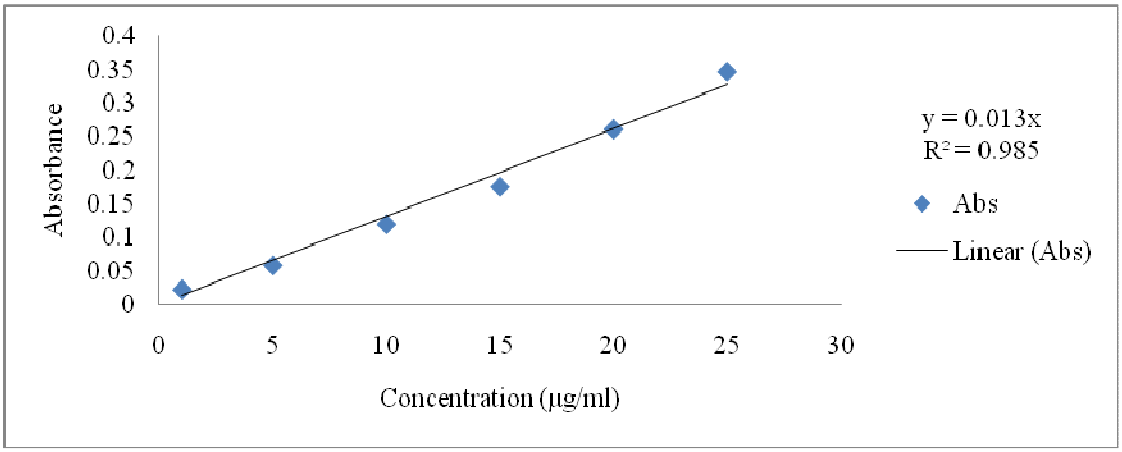


Figure-4
Calibration curve of imipenem

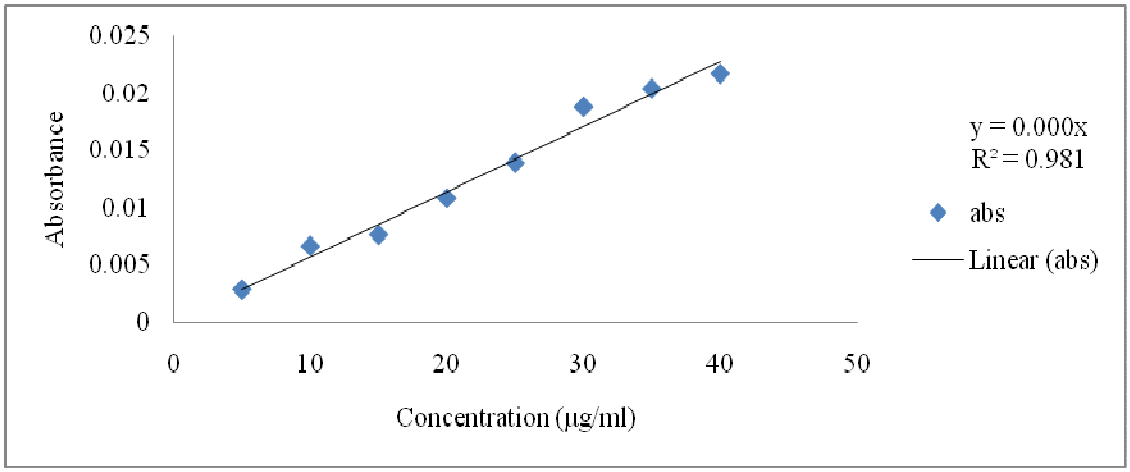


Figure-5
Calibration curve of meropenem

Conclusion

The developed UV spectrophotometric method for both Antibiotics is simple, sensitive and economical over the existing method with chloroform and ethanol as solvents. This method was also validated by checking the Accuracy, concentration, absorbance of the standard as well as extracted sample. This study was to find out effects of carbapenem from synthetic Urine sample. As we know half- life of both antibiotics varies from 1- 10 hrs. (38 approx.) but here we considered a synthetic urine so natural metabolism cannot takes place but it gave a brief idea about at limit UV Spectrophotometer can detect the amount of antibiotic in both standard as well as extracted sample. Quantitative analysis was done by UV spectrophotometer. By doing it was found that UV spectrometer was very suitable for both of the antibiotics as they helped to get λ max as well as % of extracted antibiotics from the sample. Therefore, from above analysis can say that UV spectrophotometer is suitable method for analysis of carbapenems regarding quantification.

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