Stability of meropenem after Reconstitution for Administration by Prolonged Infusion

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Abstract

Objective: Meropenem is a parenteral carbapanem antibiotic which has a broad spectrum of activity against aerobes and aerobes. meropenem's bactericidal activity is determined by the time during which meropenem concentration remains above the minimal inhibition concentration during the dosing interval. Thus, prolonged infusion is the optimal way to maximize the time dependant activity. However, studies to date have shown that carbapanems and in particular, meropenem-, are relatively unstable in solution. The aims of this study were therefore to: (1) establish the effects of temperature on the concentration of a generic brand reconstituted meropenem solution and (2) to determine whether 24 hour continuous infusion is possible without concentrations dropping below the recommended 90%.

Method: Preliminary examination was carried out by the means of nuclear magnetic resonance spectroscopy (NMR). meropenem was subsequently assayed using high performance liquid chromatography (HPLC). The method was developed and validated in compliance with International Council for Harmonisation (ICH) guidelines. meropenem's stability was examined at two temperatures $22^{\circ}C$ and $33^{\circ}C$ to mimic average and high temperature in hospital wards. Solutions were prepared aseptically at the clinically relevant concentration.

Results: The results from the NMR study showed an increase in open ring methyl groups (δ=1.05 and 1.25) peak intensity indicating that meropenem begins to degrade upon dissolution. Results obtained from quantitative HPLC confirm that meropenem concentrations dropped to 90% of initial concentration at 7.4 hours and 5.7 hours at $22^{\circ}C$ and $33^{\circ}C$ respectively.

Conclusion: Although results obtained indicate that meropenem should not be continuously infused over 24 hours, it is possible that meropenem could be continuously infused for at least 7 hours if temperature doesn't exceed $22^{\circ}C$ and for 5 hours if temperature doesn't exceed $33^{\circ}C$.

Keywords: Meropenem; Stability; Continuous-Infusions; Temperature; IV Bags; Normal Saline

Abbreviations: MIC= Minimal Inhibition Concentration; CI= Continuous Infusion; II= Intermittent Infusion; YCD= Yellow Covered Document; NMR= Nuclear Magnetic Resonance; HPLC= High Performance Liquid Chromatography; IV= Intravenous; UV= Ultra-Violet; ICH= International Council for Harmonisation

1 Introduction

Antibiotic resistance is increasing to dangerously high levels worldwide threatening the effective prevention and treatment of an ever-increasing range of infections. The increasing occurrence of infection caused by multidrug-resistant, gram-negative bacterial pathogens, challenges clinicians to adopt new administration strategies with the intent of optimizing bactericidal activity of currently available beta-lactams [1-3].

Meropenem is a parenteral carbapanem that is structurally related to B-lactam antibiotics such as penicillin and cephalosporin. It has an excellent bactericidal activity against a wide range of clinically significant gram-negative and gram-positive aerobic and anaerobic bacteria; thus known as a broad spectrum antibiotic [4- 7]. Meropenem is a time dependant antibiotic, hence bactericidal effects are closely correlated to the time at which concentrations remain above the minimal inhibition concentration (MIC) [8]. Periods at which concentrations are above the MIC (T>MIC) is a major parameter determining efficacy. When concentrations drop lower than the MIC (T<MIC) bacterial growth resumes immediately since meropenem has no significant post antibiotic effect [9-10].

Meropenem is currently administered via intermittent bolus infusion (over ≈15-30 mins) which has numerous advantages including: better utilization of intravenous access, less concern about drug degradation over time and fewer compatibility concerns. This mode of administration however, results in concentrations falling below the MIC [11]. Although administration by continuous infusion maximises the pharmacodynamic properties, carbapanems, in particular meropenem are known to be quite unstable, hence a major parameter that needs to be studied [12]. The stability of meropenem should be maintained throughout the infusion time in order for the patient to receive abundant amounts of

active drug needed to achieve clinical cure while avoiding exposure to harmful degradation products [13].

Various studies have demonstrated that continuous infusion of meropenem is beneficial in terms of efficacy based on pharmacokinetics and pharmacodynamics [14-18]. However to comply with the European and USA Pharmacopeia's, beta lactam antibiotics should always contain at least 90-110% of the initial concentration [13].

One of the major limitations of continuous infusion meropenem is that it would demand frequent drug preparation and administration due to its short stability [16-17]. This presents a challenge in practice as it requires pharmacy staff to produce multiple preparations and nursing staff to coordinate the multiple administrations throughout the day. Moreover, the problem is compounded in hospital wards where temperatures are not controlled therefore exposing the infusion to elevated temperatures that can compromise stability [14]. Therefore the aims of this study were to 1) establish the effects of temperature on the decomposition of reconstituted meropenem solutions and 2) to determine whether 24 hour continuous infusion is possible without concentrations dropping below the recommended 90% of the initial concentration.

2 Methods

Instrumentation: NMR spectroscopy was performed on 600mHz base frequency Bruker Advance lll Two-channel FT-NMR spectrometer. Quantitative analysis was performed by the means of reverse-phase HPLC using an Agilent 1260 HPLC system with single wavelength UV detection and Chemstation Software.

Materials: Meropenem reference standard, Methanol (HPLC grade), acetonitrile (HPLC grade), ammonium acetate, glacial acetic acid, and paracetamol were purchased from Sigma Aldrich. 0.9% saline was bought from Baxter and 100ml 0.9% saline infusion bags were obtained from St George's hospital, London, UK. All of the above materials were used without further purification. Pharmaceutical dosages from meropenem infusion vials (1000mg) were prepared in a fume hood using an aseptic, non-

touch technique. Generic brand, AstraZeneca, IV meropenem was reconstituted with 20ml water for injection and further diluted with 50ml of 0.9% saline.

Preliminary investigation: Preliminary examination of meropenem was carried out by the means of Nuclear Magnetic Resonance (NMR) spectroscopy to provide an indication of how fast meropenem degraded and inform development of the High Performance Liquid Chromatography (HPLC) method. Meropenem solution at the clinically relevant concentration [(1000mg/70ml)*1000=14,285ppm] was placed in an NMR tube and instrument was programmed to periodically analyse throughout the day. The remainder of reconstituted solution was left at room temperature for two weeks and then analysed by HPLC to understand characteristics of meropenem degradation product/s.

Method development: The aim was to develop a HPLC method capable of separating meropenem from its degradation products and measuring its concentration in infusion solutions, precisely and rapidly. Parameters investigated were mobile phase, column type, choice of internal standard, injection volume, flowrate, column temperature, linear range and detection wavelength.

Method validation: The developed method was tested for linearity, range, precision, accuracy, specificity, sensitivity and robustness in compliance with ICH validation guidelines. The method was tested over the period of five days by running three replicates of a standard set of samples once a day for five days.

Reconstituting 1g of meropenem powder for infusion with 20ml water for injection and mixing with 50ml of saline (as in clinical practice) gives an infusion concentration of 14286ppm meropenem. This solution will be diluted 1 in 50 for analysis, which reduces the amount of sample needed, reduces matrix effects and gives a nominal concentration of 286 ppm meropenem, in the usual analytical range for quantitative HPLC with UV detection.

Analytical range was tested by injecting variable volumes (2, 4, 6, 8 10, 15 and 20µl) of a 1000ppm standard solution (equivalent to injecting 10µl of a set of standards, concentrations from 200-2000ppm). Precision was considered at three levels; repeatability, intermediate precision and reproducibility. System and method precision were also considered.

Accuracy was determined by measurement of recovery and evaluated by using QC samples: (1) low (75ppm), (2) medium (250ppm) and, (3) high (450ppm).

Specificity/selectivity was assessed by running diluent blanks. Diluent blanks included running a mobile phase, a saline and water for injection blank that were spiked with 250μl of the internal standard stock solution.

Robustness parameters examined include changes in column temperature (±5°C), changes in flow rate (±0.2ml/min), changes in mobile phase pH (±0.2 units) and changes in the mobile phase composition (95:5: v/v; ammonium acetate: acetonitrile).

Sample preparation: Two 1g meropenem pharmaceutical dose vials were emptied into a 250ml beaker and reconstituted with 40ml water for injection. 100ml 0.9% saline was added and the solution was stirred. Six 100ml 0.9% saline PVC IV bags were emptied completely, dried and injected using a 20ml syringe with same amount (20ml) of reconstituted bulk solution (14285ppm) and incubated at the relevant temperature 22°C and 33°C. The concentration of meropenem was measured every hour for 21 hours after reconstitution and testing was performed in duplicate. The infusion solution was considered stable whilst the percentage of intact molecule remained above 90% (>90%).

Physical Observation: Two responses were measured: physical compatibility and colour change. The solutions stored in IV bags were examined at every sampling interval for cloudiness, crystallisation/precipitation, colour change and signs of interactions.

HPLC assay: The samples were diluted 50-fold with mobile phase (700μl) and paracetamol (250μl; concentration 1000ppm) was used as an internal standard (meropenem concentration=286ppm). The diluted samples were injected (injection volume 10μl) via an auto sampler onto a Synergi 4μ, POLAR-RP-80R, 250x4.6mm column. The mobile phase was composed of 10mM ammonium acetate buffer adjusted to pH 5.4 with acetic acid and acetonitrile (90:10: vol/vol), isocratic elution at a flow rate of 2.8ml/min. the UV detector wavelength was set to 290nm and the column temperature was set at 50+/-1°C.

3 Results

Method Validation

The internal standard corrected calibration curve displayed good linearity over the concentration range of 0 to 500μl/ml. The representative linear equation was y=0.9943x-0.0566, with a correlation coefficient (R^2) of 0.9998 (**Figure.1**). The regression line (R^2=0.9996) in **Figure.2** demonstrates linearity in the range 0- 2000ppm.

Good system precision was obtained where intra-sample precision ranged between %RSD 0.01% and 0.37%. Intra-day precision attained ranged between %RSD 0.25% and 1.90% and inter-day precision obtained ranged between %RSD 1.79% and 5.64%.

The percentage error (%ERROR) determined from all standard QC samples was between 0.29 and 0.68%, therefore %Recovery values obtained were at least 99.32%. For all blanks there was no significant response. meropenem reference standard solution was spiked with internal standard to ensure that peaks are well resolved.

Increasing the column temperature decreased the pressure and retention time. Slight changes in mobile phase pH displayed no significant difference in peak area or peak shape. The robustness study demonstrated that the method can optimally perform reproducibly when parameters change slightly.

The limit of detection was 5.55ppm and the limit of quantitation was 16.82ppm.

Preliminary Results

The results of the preliminary NMR investigation are shown in **Figure.3** below. The solution was analysed periodically by proton NMR with water suppression. The increase in peak intensity in the spectra, most prominently at chemical shifts of 1.05, 1.8, 2.5 and 2.6 ppm, suggests that meropenem begins to degrade upon dissolution. NMR was used as a qualitative method for characterisation rather than a quantitative technique as it would have been difficult to quantify due peak overlap. NMR

provided an estimation of how fast meropenem degraded, however, the instrument did not give an indication of concentration so further testing by HPLC was needed.

Preliminary investigation by the means of HPLC was carried out to give an indication of how many degradants meropenem has and to obtain the retention time of both meropenem and the degradation product/s. Results displayed in **Figure.4** shows the retention time of both meropenem and its degradation product/s. The spectra displays good resolution of meropenem and it degradant/s as no co-elution was observed and the compounds are not distinguished from one another.

Physical Observation Results

Upon preparation the solution was clear and colourless, but as the samples began to age the colour of the solution became tinged yellow. The older the sample the more distinct the colour change became. It was also observed that the change in colour was noticeable for solutions incubated at 33°C (7 hours) before solutions stored 22°C (12 hours).

Quantitative Results

Figure.5 shows the influence of time on meropenem concentrations at 22°C and 33°C, indicating the time at which meropenem concentration drops 10%. All data was drift-corrected and corrected with the internal standard. The slopes of the regression lines shown are statistically significant at the 99% level of confidence, indicating that meropenem concentrations decrease with time.

Results show that meropenem maintained 90% intact molecule for 7.4 hours at 22°C and within 20 hours, 71.2% of the initial concentration remained. meropenem maintained 90% intact molecule for 5.7 hours at 33°C and within 20 hours, 61.4% of initial concentration was maintained.

4 Discussion

In recent years, stability studies have been given significant attention due to their importance in development and quality control of pharmaceutical products. meropenem has no significant post antibiotic effect as bactericidal activity is determined by the time at which the drug concentration

remains 4 or 5 times above the MIC. The interval between intermittent doses may result in meropenem concentrations falling below the MIC as high peak concentrations cannot enhance the bactericidal activity of meropenem [21].

Determining meropenem stability could give an insight as to whether continuous infusions are feasible, thus, increasing patient response to therapy which can theoretically delay development of resistance. Numerous recent studies have assessed the clinical benefits of continuous/extended infusion compared to current intermittent dosing to optimize the bacterial activity of parenteral antibiotics for treating infections [12, 15, 22-26].

Feher *et al* studied groups of patients receiving meropenem to determine whether the administration of meropenem via a 4h extended infusion leads to a more favourable clinical outcome. From this observational study it was concluded that treatment of febrile neutropenia was more successful with extended 4h infusions than 30 minute bolus infusion, with 68.4% and 40.9% of patients clinically cured respectively [P<0.001] [22]. Dulhunty *et al* observed the clinical and pharmacokinetic differences between continuous and intermittent dosing in critically ill patients with severe sepsis. Results obtained from this prospective controlled trial demonstrated that continuous infusion achieved higher antibiotic plasma concentration and also achieved higher rates of clinical cure (70% vs 43%; p=0.0037) [24].

Continuously infusing meropenem will reduce the workload related to multiple administrations; however, the stability of meropenem is an important factor that needed consideration. Our findings confirmed that the stability of meropenem in solution was influenced by the storage temperature. Drug concentrations dropped to 90% of initial concentration after 7.4 hours at 22°C and 5.7 hours at 33°C. The visual colour change observed in meropenem solution is not reported in meropenem stability studies; however it is likely due to the degradation process and the formation of degradation product. Change in colour usually indicates that the beta-lactam ring has been hydrolysed. It is recommended that solutions that have changed colour should not be

administered to patients as changes in physical compatibility occur with loss of potency and an increase in toxicity.

To our knowledge this is one of a few studies that examined the impact of elevated temperatures (experienced on hospital ward) on meropenem's stability. Results obtained in this study suggest that in clinical settings with ambient temperatures below 33°C, extended infusion could be considered. Results from previous studies support our findings as they also suggested that the stability of meropenem reconstituted in solution is influenced by the storage temperature. At lower ambient temperatures meropenem is stable for a longer time than when stored at elevated temperatures [27]. Drug concentration is significantly reduced at high storage temperatures and should not be administered in tropical countries [14]. Smith *et al* looked at the stability of meropenem in different infusion devices at low temperatures and confirmed that meropenem was stable for 5 days at 5°C [26]. However Kuti *el al* suggested that meropenem is stable for only ~4-6 hours at room temperature, and its pharmacokinetic properties, when administered by continuous infusion, are largely unknown [28].

5 Conclusion

Although results obtained determine that meropenem should not be continuously infused over 24 hours it is suggested that patients can receive continuously infused meropenem for at least 7 hours if temperature doesn't exceed 22°C and for 5 hours if temperature doesn't exceed 33°C.

6 Acknowledgements

7 Funding

8 Transparency Declarations

The Authors affirm that this manuscript is an honest, accurate, and transparent representation of the study being reported; and that there are no important aspects of the study that have been left out. Therefore, the authors have nothing to declare.

9 References

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10 Figures

Time (hrs)	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
%R 22ºC	100	105	115	99	91	93	90	90	87	87	84	81	82	82	82	81	76	76	78	78	78	78
%R 33ºC	100	106	107	99	91	91	85	85	82	84	79	74	77	77	77	73	68	66	70	68	67	67

Table.1 Showing the percentage of concentration remaining at each sampling interval for 22ºC and 33ºC
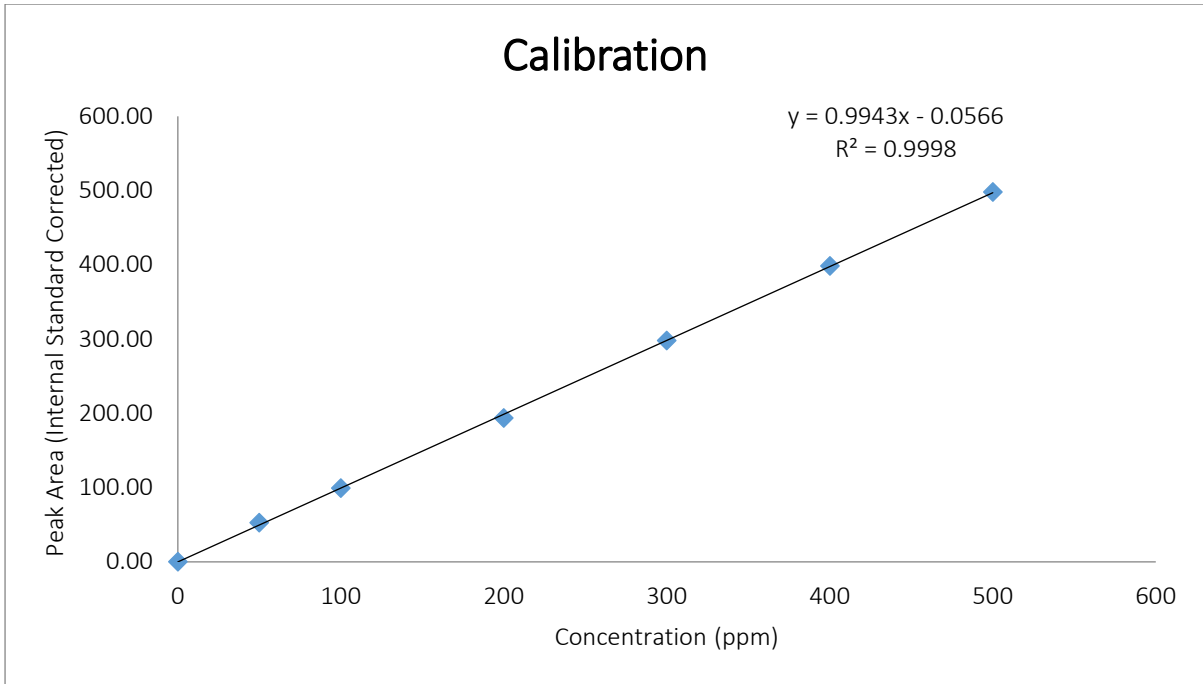
Figure.1. Showing average seven concentration calibration (internal standard corrected) to assess linearity.
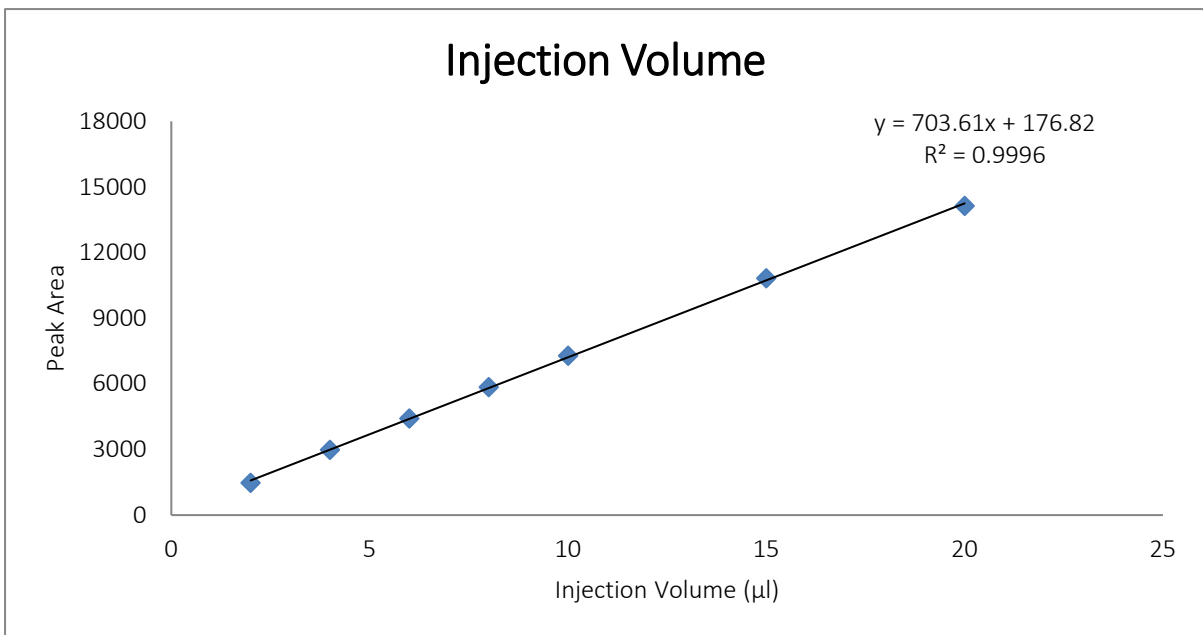
Figure.2 Showing linearity in the range of 0-2000ppm
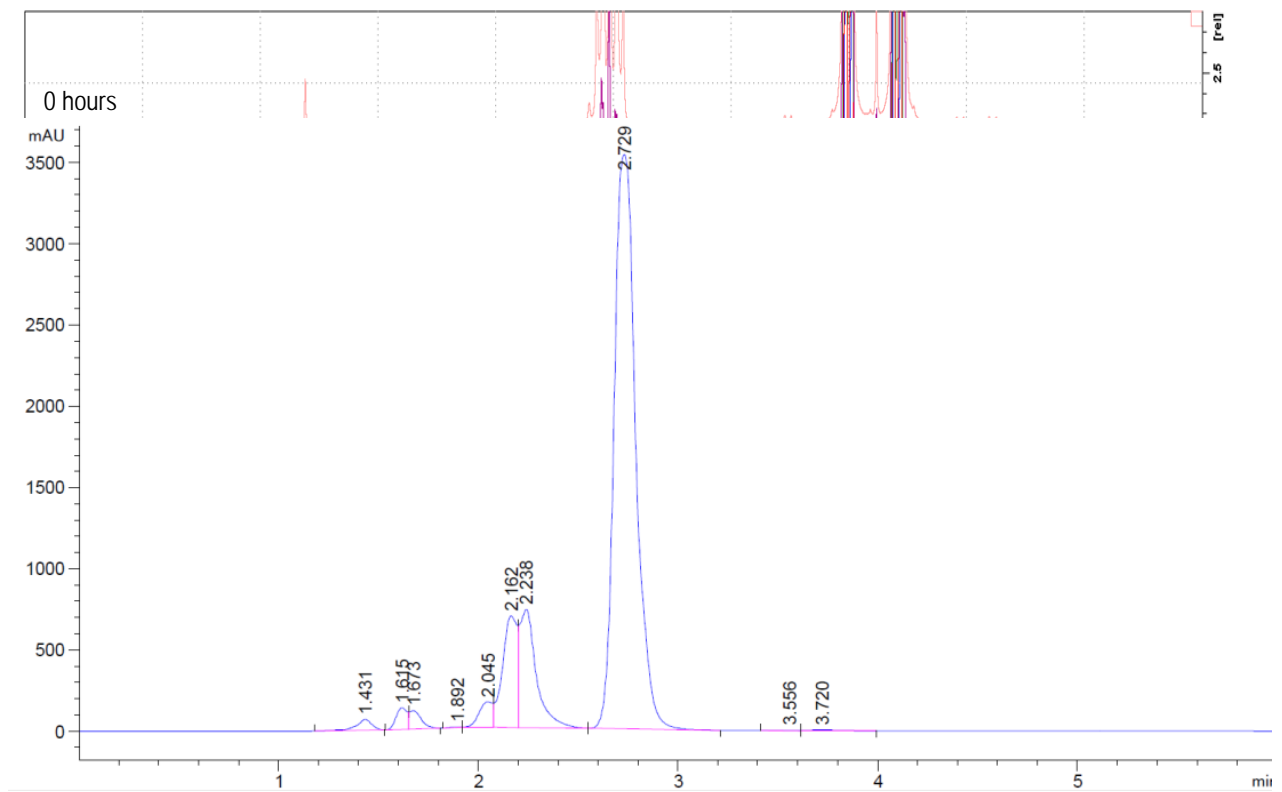
10 hours

12 hours

0 hours

Figure.3 Preliminary NMR spectra of reconstituted meropenem

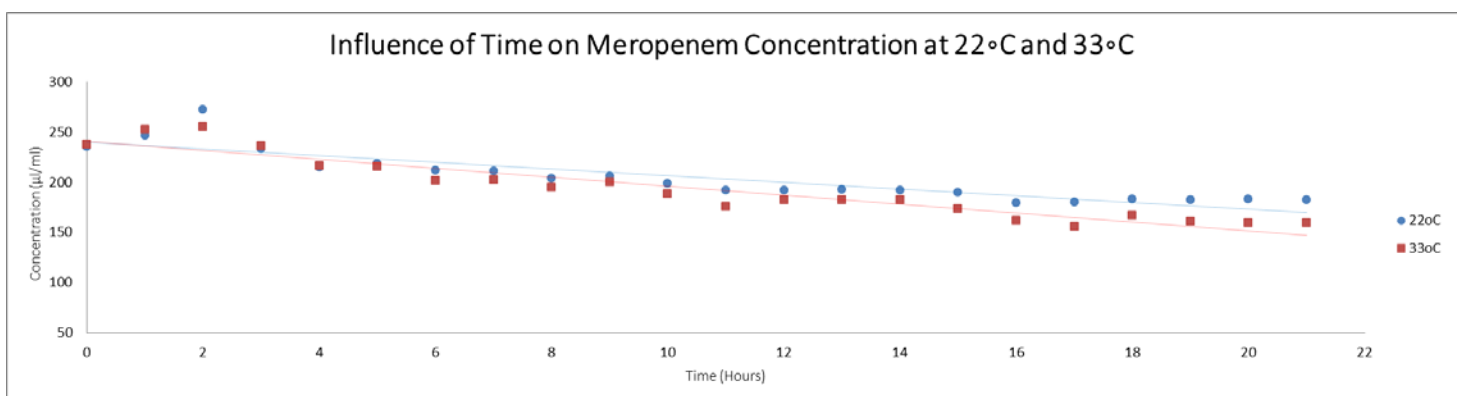**Figure.4** Preliminary HPLC of aged meropenem sample to indicated degradation product/s retention time

Figure.5 Showing the gradual decrease in meropenem concentration at both 22°C and 33°C
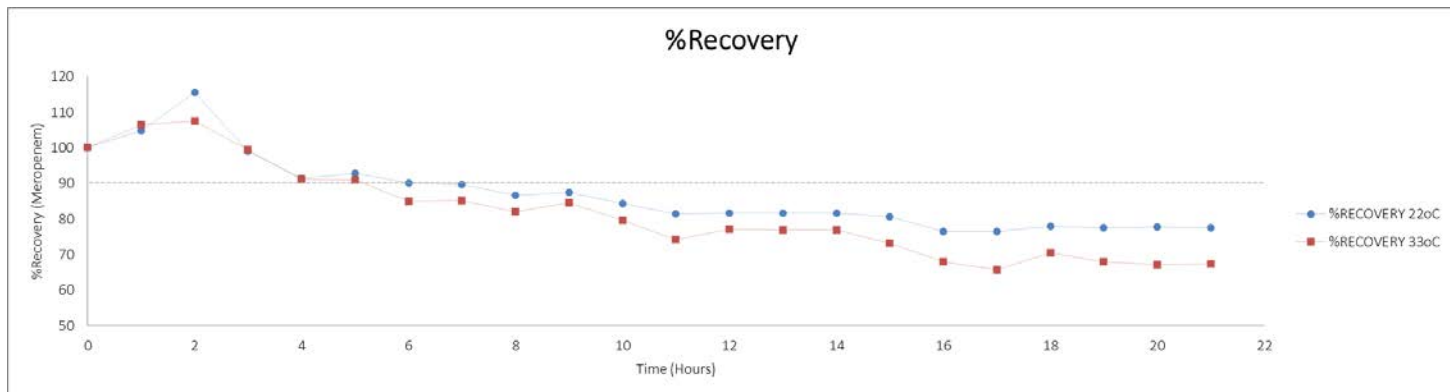
Figure.6 Showing meropenem %Recovery at all sampling intervals