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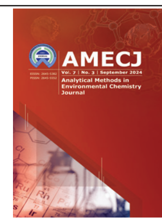

Significant research on meropenem cross-contamination management in a β-Lactam manufacturing unit: A high-performance liquid chromatography approach

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ABSTRACT

Rapid, simple, and sensitive high-performance liquid chromatography with diode-array detection (HPLC-DAD) techniques are described for quantitatively determining meropenem residue from the contact parts of injection filling machines. This involves swab sampling collected after cleaning. The method also addresses the management of meropenem cross-contamination in shared cephalosporin production facilities. Cross-contamination is the product mix-up by which a trace amount of antibiotics can be present in other products that cannot prevent infections but can contribute to initiating antibiotic-resistant pathogens into human microflora. Poor beta-lactam contaminant control can cause residual Meropenem in different dosage forms, resulting in meropenem residue in the human intestinal flora, blood during sepsis, or Environmental wastes. During manufacturing, there should be a validated scientific control with proper monitoring of meropenem contamination. Meropenem residue was determined on the contact parts of production machines using swab sampling collected from surfaces after cleaning. An isocratic chromatographic system used with a mobile phase consisting of acetonitrile: 20% tetrabutylammonium hydroxide adjusted to pH 6.5 ± 0.05 (30:70, v/v) on XTerra RP18 column at a flow rate 1.0 mL min^{-1} with an injection volume, 20 µL and UV (290 nm). HPLC-DAD method developed was found to be linear ($R^2 \geq 0.999$), sensitive, precise (RSD < 2.7%), accurate (recovery between 97% and 109%), and LOD and LOQ were obtained at 0.05 and 0.10 mg $L^{-1,}$ respectively. The area RSD (%) for six replicate injections of LOQ was 7.6. This study validated the Meropenem contaminant controlling procedure for drug manufacturers.

1. Introduction

Meropenem is a parenteral carbapenem that is structurally a B-lactam antibiotic like penicillin and cephalosporin (Fig. 1). It has a broad spectrum Antibiotic with excellent bactericidal activity against clinically significant gram-negative and gram-positive aerobic and anaerobic bacteria [1,2]. Meropenem Antibiotic is manufactured as a β-Lactam group in the same manufacturing unit as other non-penicillin beta-lactam compounds according to cGMP in Bangladesh [3].

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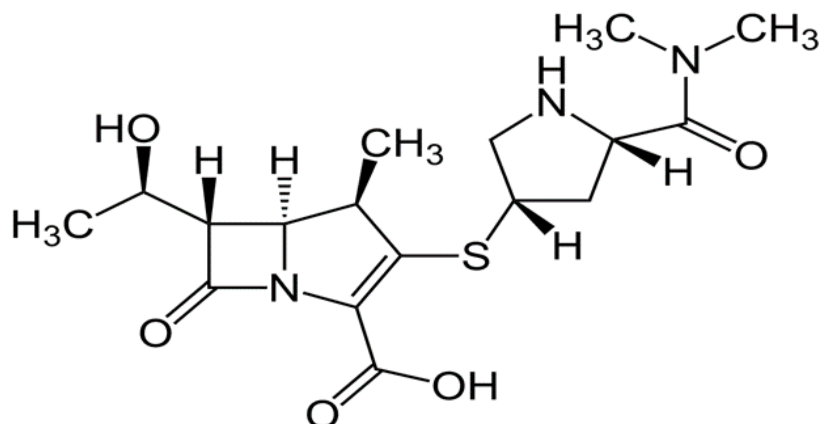

Fig. 1. Structure of Meropenem Trihydrate

Carbapenem, especially Meropenem, is now recognized as the major treatment entity for multi-drug-resistant gram-negative bacteria by WHO, the US CDC, and the ECDC. However, this drug is also becoming resistant among MDR Gram-negative organisms, whereas carbapenem monotherapy may no longer be effective for many patients with severe Gram-negative infections [4]. WHO recommended Carbapenem under the Priority 1 or critical pathogens list for R&D of new antibiotics innovation due to its resistance to several bacteria like Acinetobacter baumannii, Pseudomonas aeruginosa, or Enterobacteriaceae [5]. Meropenem resistance occurs through different mechanisms of action, such as porin-mediated resistance or the overproduction of efflux pumps. Still, Enzyme-mediated resistance to the production of beta-lactamases is, most importantly, the main mechanism by which Bacteria can inactivate carbapenems together with other beta-lactam antibiotics and, therefore, called carbapenemases. This enzyme hydrolyzes almost all beta-lactams [2, 6]. Many sources are found through which bacteria can be resistant, and environmental pollution with residue from carbapenems is one of the main sources of resistant bacteria. Ultimately, all the other pollutions are finally linked to environmental pollution, especially Soil, Water, and Air. Environmental factors, such as lower initial cephalosporin concentration, higher MnO_2 loading, and lower solution pH, promote the oxidative transformation of cephalosporin antibiotics by manganese dioxides [7]. Cephalosporin degradation is accelerated in elevated salt matrices, with chlorine and carbonate radicals involved. High salt-containing hospital wastewater promotes degradation, suggesting thermally activated persulfate as a treatment. However, excessive use of cephalosporin antibiotics in medicine leads to frequent detection in aquatic matrices, posing public health and ecosystem risks [8]. Multiple sources have been identified for this environmental pollution, and the drug manufacturing unit is at the top. Cross-contamination from drug manufacturing exerts a residual amount of Meropenem on other drugs, contributing to this drug's resistance to known pathogens. Meropenem is specially manufactured by injection, and the products whose contamination is likely most significant are those administered by injection [9]. Cross-contamination might cause several organizational risks, like GMP Non-compliance, product recalls, sales loss, or audit failure, which can affect a company's reputation. Besides this, beyond the current concept, this might cause Antibiotic resistance. For example, If Meropenem residue is cross-contaminated with any other dosage forms/products, then it will be available either in the Bloodstream or Intestinal flora where different human pathogens present and the chances of Meropenem-resistant gene or Meropenem-resistant pathogen are purulent [10-15]. When Meropenem products are manufactured combined with other β-Lactms or products, the prevention of cross-contamination becomes one of the major concerns. If residues of Meropenem remain after

cleaning the production equipment, then the product contact surfaces may contaminate the next product manufactured in the same manufacturing equipment. In Bangladesh, around 37 companies are manufacturing the Meropenem injection along with other β-Lactams or products with the same manufacturing machinery [16]. Cleaning validation is the major cGMP tool to control the cross-contamination of pharmaceutical products, and it is performed by considering many factors. The product selection is performed through worse-case determination [9,17], whether Meropenem might be selected. However, considering antibiotic resistance and GMP requirements, pharmaceutical drug manufacturing must have a proper control strategy to manage Meropenem drug residue contamination with other drugs. A literature survey reveals that there are publications on the determination of Meropenem, either alone or in combination with other drugs. These determinations have been carried out using various instruments such as HPLC, UV spectrophotometer, or LC-MS to analyze pharmaceutical dosage forms or human plasma.[18-31]. However, no literature is available on complete control management with residue determination techniques to control the Meropenem residue contamination to the next product manufacturing into a drug manufacturing unit. Several ultra-high performance liquid chromatography with diode-array detection (UHPLC-DAD) methods have been reported for the determination of cephalosporin molecules like cefixime, Cefazoline sodium, ceftazidime, cefotaxime, ceftriaxone, Cephradine dehydrate, Cephapirin sodium, cefepime, Cefuroxime sodium and cephalexin as well as Molecularly imprinted polymers (MIPs) were synthesized for the determination also reported [32], [33], [34]. Only one HPLC method was found to determine the cephalosporin residues on spiked stainless-steel plates and human plasma as a worse-case product for CIP [35]. However, there is still a huge requirement for publication in this area. The analytical technique of choice in this study was high-performance liquid chromatography (HPLC) coupled with a diode array detector (DAD) [36]. This study aims to develop an HPLC method to determine Meropenem in residue levels spiked with different contact parts of the injection production line, including Stainless steel, Teflon, Glass & Silicon, etc. Finally, a complete management procedure is to be established through a validation technique by which it is to be proved that Meropenem residue doesn't contaminate the next manufactured drugs.

2. Experimental

The standard manufacturing process of Meropenem injection starts with sterilizing utensils, Room garments, Flip-off seals, Rubber stoppers, and Product contact parts. The active ingredient of Meropenem for injection is marketed as Meropenem with a mixture of sodium carbonate sterile, and the container is directly transferred to the manufacturing area after weighing in the aseptic filling area and loaded into the hopper of the filling machine. Then, the vials are washed, sterilized, and depyrogenated (at 305^{0}C with 136mm min^{-1} belt speed). Then, the vials are filled with a vial filling machine and sealed with a vial cap sealing machine. Sealed vials are visually checked and then labeled. Labeled vials are blistered with proper diluents. Finally, the blisters are cartooned for marketing.

A protocol for monitoring Meropenem residue on contact parts before changeover needs to be prepared, through which regular monitoring and management will be performed. The meropenem injection is manufactured by filing the ready-mix API into the vial for injection and market with a diluent-like water for injections (WFI) for intravenous injection. A sample manufacturing flowchart for the meropenem injection filling line is shown in Figure 2.

2.1. Chemicals and reagents

Meropenem (potency: 73.2%) working standard and API obtained from The ACME Laboratories, Dhamrai, Dhaka, Bangladesh. HPLC grade acetonitrile (CAS N.: CAS No.: 75-05-8), tetra butyl ammonium hydroxide 40% (CAS N.: 2052-49-5), and phosphoric acid (CAS N.:7664-38-2) were procured from Sigma Aldrich, Germany. HPLC-grade deionized water was used.

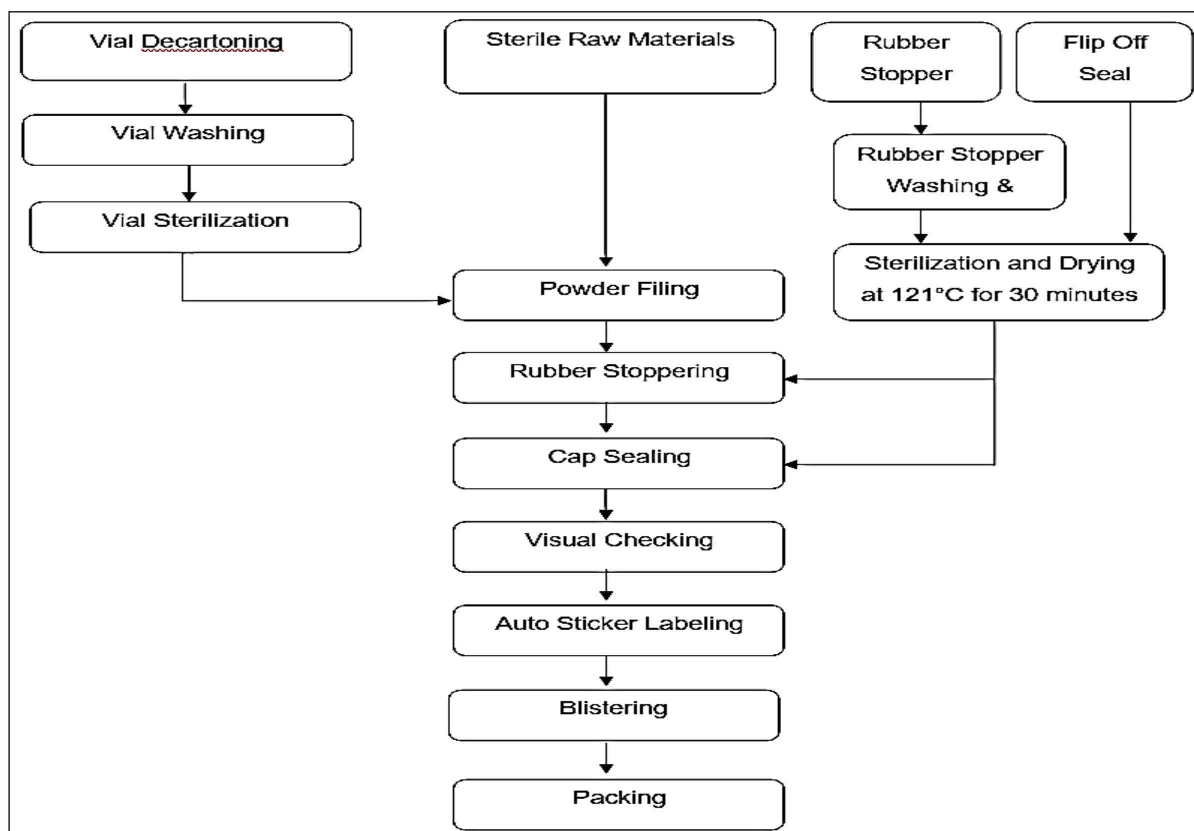

Fig. 2. A sample Manufacturing Flowchart for Meropenem Injection filling line

2.2. Instruments

Waters e2695 HPLC System with 2998 PDA detector (WTR-e2695-2998) includes waters 2695 separations module HPLC system (autosampler, quaternary pump, degasser, and sample heater/ cooler) with waters 2998 photodiode array detector (PDA) (Empower three software), injection volume of 0.1 - 100 µL, the standard of 0.1- 2000 µL, sartorius electronic analytical balance, ultrasonic bath, Mettler Toledo pH meter, and waters XTerra RP18 (4.6 x 250 mm, 5 µm) column was used.

2.3. Mobile Phase & Diluent

Transfer 6.5 mL of Tetra-butyl ammonium hydroxide solution (20%) and dilute with water to obtain 1000 mL solution and mix properly. Adjust the pH of the solution to 6.5 ± 0.05 with phosphoric acid. Dilute 700 mL of this solution with 300 mL acetonitrile and mix the solution. Filter the solution through a 0.22µm membrane filter. This solution has also been used as a diluent to prepare the analytical solutions.

2.4. Wavelength detection

29.7 mg Meropenem sodium carbonate working standard equivalent to 25.0 mg Meropenem was weighed into a 100 mL volumetric flask. 20 mL of mobile phase was added, and the solution was sonicated for 5 minutes until completely dissolved. It made up the volume with the mobile phase and mixed well. Transferred 4 mL of this solution to 100 mL volumetric flask and volume with mobile phase and prepared a solution containing 10 µg/mL concentration of Meropenem. Filtered the solution through a PTFE syringe filter, 0.45 µm, collected the solution in a clean and dry vial, and scanned between 200 and 400 nm with 2998 PDA detector of Waters 2695 HPLC system. The maximum absorbance of each molecule was around 254 nm; thus, the wavelength detection was set at 290 nm.

2.5. Chromatographic conditions

Chromatographic conditions were finalized as ambient column oven temperature with a 290 nm UV detection at a flow rate of 1.0 mL per min at isocratic elution, and the run time was only 6 minutes as the retention time of Meropenem is only 3 minutes. Before injection, the column needs to be equalized with the Mobile phase for 60 minutes. The injection volume was set to 20 µL.

2.6. Pretreatment of swabs & Swab blank solution

Swab Stick (TX714 or TX715, Tex wipe swabs) was used, and the mobile phase was used to swab diluent. The swab sticks were dipped into a sufficient amount of swabbing diluent in a beaker sonicate for 10 minutes, and kept the sticks on a watch glass for drying. A pretreated swab stick was taken into a test tube containing 10 mL of swabbing diluent sonicated for 5 minutes and mixed well to see the blank interference.

2.7. System suitability solution preparation

Weighed accurately and transferred about 29.7 mg of Meropenem with sodium carbonate equivalent to 25.0 mg Meropenem working standard into a 100 mL clean and dried volumetric flask and added 60 mL mobile phase and sonicated about 5 minutes to dissolve, made volume up to 100 mL with mobile phase and mix well. Transfer 4 mL of this solution to a 100 mL volumetric flask and volume with mobile phase. 10.0 µg mL^{-1} solution was prepared through further dilution of this solution with diluent. Filtered the solution through a PTFE syringe filter, 0.45 µm, and collected the solution in a clean and dry vial.

2.8. Selection of Cleaning Level and Cleaning Procedure Identification

Meropenem with sodium carbonate is a salt-form API administered directly after reconstitution. If Meropenem injection is manufactured in the same production line with other β-Lactams/products, then the production of Meropenem injections should be on a Campaign basis. That means the production planning should be done during Meropenem injection manufacturing. Other products will not be manufactured at all into the injection line. So, it will be easy to control the cross-contamination due to manufacturing on a campaign basis. After completion of Meropenem campaign manufacturing, A-type general cleaning needs to be conducted to execute the changeover. Meropenem with sodium carbonate is very soluble in water [37]; thus, cleaning the machine, including contact parts, has been developed with purified water. Finally, the machines and contact parts are rinsed with WFI & Isopropyl alcohol to make them ready by keeping them safe from Microbial contamination.

2.9. Selection of Machine Parts for Meropenem API contacts

After evaluating the contact parts of the Meropenem injection line, it is identified that the injection filling unit has direct contact with the Meropenem API as the API containers are kept on the machine’s hopper, and then the automatic filling line starts. Several contact parts of this filling line are available and undergo contact with the Meropenem API, which depends on the brand/model of different manufacturers’ injection filling machines. Some common contact parts are the container holder/adapter, stainless steel (S.S) channel, silicon channel, upper hopper, lower hopper made of glass, S. S. powder support, side cover, dosing disc, etc. (Fig. 3). Four types of material of construction for this contact parts have been identified and found as stainless steel, Teflon, glass, and silicon (Fig. 4).

During the sampling exercise, the change parts were visually examined, and a visual inspection was performed before and after the swab sampling. These visual check results were also recorded to validate the cleaning procedure. The sample will be taken from the areas that are most difficult to clean visually. All sampling locations and sample

Fig. 3. A sample powder for injection filling machine from Macofer (Romaco Holding GmbH, sampling Location is shown in Table 1) [38]

Fig. 4. The contact parts items used for swabbing method validation

Table 1. Sampling location and Sample quantity for residue determination

Equipment Name	Sampling Location		Swab Sample quantity
Vial filling and rubber stoppering machine	A	Container Holder/ Adapter	S-1: Lower side of Adapter S-2: Inner Wall of Adapter
	B	S.S Channel	S-3: Inner wall of S.S Channel S-4:Inner wall of S.S Channel
	C	Silicon Channel	S-5: Inner Side of Silicon Connector S-6: Front Side of Silicon Connector
	D	Upper Hopper	S-7: Inner Wall Corner of Upper Hopper S-8: Inner Middle Side of Upper Hopper S-9: Lower side of Upper Hopper
	E	Lower Hopper (glass)	S-10: Glass of Lower Hopper
	F	S. S Powder support	S-11: Inner side of Lower Hopper
	G	Side Cover	S-12: Glass of Lower Hopper
	H	Dosing disc with Niddle	S-13: Inner side of Dosing Disc S-14: Niddle
Total Samples			14

quantities were determined in Table 1.

2.10. Swabbing Procedure and Preparation for Swabbing Sample

Corresponding to each swab location, one pretreated swab stick with mobile phase as diluent taken containing 10 mL swabbing diluent by dipping and removing the excess swabbing diluent by pressing the wet swab stick head against the walls of the test tube, swabbing the designated cleaned equipment location of size 5×5 cm (25sq.cm) as per the following procedures (Fig. 5). The first side of the swab is swiped horizontally over the designated location, then the swab is flipped over and the second side is swiped vertically downwards over the same surface, dip the swab stick into the diluent, squeeze the swab, and take it out. Now, swab the wet surface area with a dry pretreated swab using the same procedure described above. Immediately after swabbing, transfer the sticks into the test tube containing 10 mL of swabbing diluent. Sonicate the solution for about 5 minutes, squeeze the swab stick, and take it out. Filtered the solution through a PTFE syringe filter, 0.45 µm, and collected the solution in a clean and dry vial.

3. Results and Discussion

3.1. Method validation study

A cost-effective HPLC method has been developed to test the Meropenem residue on the contact parts. The test method has been validated for the analysis of the residue of Meropenem to evaluate the cleaning procedure of the drug product manufacturing equipment. So, the method validation for residue determination of Meropenem has been performed on 5 X 5 cm^2 of 316 stainless steel, Teflon, glass, and silicon. During the validation process, several experiments have been performed to demonstrate that the method used for the residue analysis of Meropenem injection is specific and consistently generates reproducible data. System suitability, specificity, LOD, LOQ, linearity, recovery efficiency/recovery factor, precision, accuracy, stability of the analytical solution and mobile phase, filter evaluation, and robustness have been obtained to confirm the method as validated.

3.2. Validation parameter

3.2.1.Specificity

After evaluating all the materials for the construction of the injection line, we contacted Meropenem and found stainless steel, basically 316 stainless steel, Teflon, Glass, & Silicon. Thus, we collected these items from the same construction material as contact parts of the Injection filling machine and checked the interference of the Diluent used for swabbing diluent. This method can also test the wastewater from the drug manufacturing unit and the Exhaust Air sample. Waste water was checked for specificity by seeing the interference with water, and for the Exhaust Air sample, swabbing from stainless steel was practically done. Thus, the specificity of 316 stainless steel will work for the same. There was no interference peak at the retention time of Meropenem.

3.2.2.Linearity, LOD and LOQ

LOD & LOQ are two of the main parameters that will be determined to ensure the lowest detectability of Meropenem by this method. Thus, to determine the LOD & LOQ, the linearity of the Meropenem

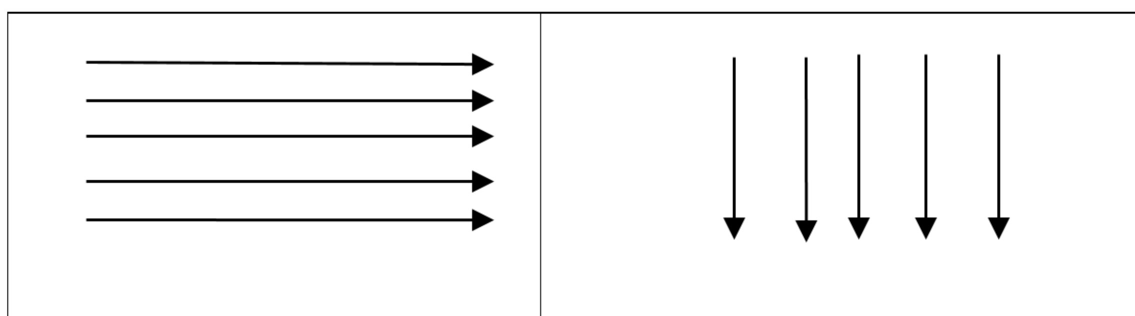

Fig. 5. Swabbing Technique, Total 10 Strokes (5 strokes in each direction)

working standard has been performed individually at concentration levels 0.1 – 15 µg mL^{-1}. The statistical data for linearity has been calculated as per the linear regression line, and the linearity of individual cephalosporin compounds with a correlation coefficient (r) was found to be equal to or more than 1.000. LOD means the lowest detectable amount of a sample solution, which cannot be quantified but can be detected using the analytical procedure. Standard deviation (SD) and slope values (S) were taken to calculate the LOD & LOQ calculated by using the calibration curve with the formula LOD = 3.3 (SD/S) and LOQ = 10 (SD/S).

3.2.3.Determination of recovery efficiency and recovery factor

Evaluation of recovery efficiency is the most critical part of swab recovery. Here, we recovered at LOQ, 50%, 100%, and 150% levels of the nominal concentration of 10 ppm Meropenem. To determine the recovery factor, we used Equation 1 if the mean recovery is below 95%.

$$\textbf{Recovery factor} = \frac{\text{Theoretical result (100 \%)}}{\text{Mean recovery (\%)}} \quad \text{(Eq.1)}$$

3.2.4.Method precision and intermediate precision

Method precision and Intermediate precision were carried out for the swabbing samples injection into two different HPLC systems (Waters and Shimadzu). Prepared the swabbing spiked sample with known concentration at 100% Level (10 mg L^{-1}) of Meropenem onto different contact parts like 316 Stainless steel, Teflon, Glass, & Silicon. The samples were dried, and took swab samples according to the method. Then, the samples were injected, and the peak area was measured. The peak area of Meropenem of six swab sample solutions for different items was calculated and reported along with the standard deviation and RSD% of the peak area of these solutions for Meropenem.

3.2.5.Solution Stability

The stability of the standard solution and swabbing sample solutions from different machine contact parts (316 stainless steel, Teflon, glass, and silicon) has been determined by keeping the samples in an ambient condition and 2-8°C condition.

3.2.6.Filter validation

To demonstrate filter compatibility in standard solution and sample solution by discarding different volumes as well as 2 mL, 4 mL, and 6 mL by using PVDF / PTFE 0.45 µm cartridge filter, swab recovery sample solutions for glass plate, Teflon, SS plate, silicon plate has been considered for filter evaluation study.

3.2.7.Robustness

The robustness has been done with different chromatographic parameter changes like wavelength variation (± 5 nm), Flow rare variation (± 0.1 mL/min), and column oven temperature variation (25°C ± 5°C).

3.2.8.Establishment of acceptance criteria for Meropenem residue

The acceptance criteria of Meropenem residue on each machine contact part (Adapter, S.S Channel, Silicon Channel, Upper Hopper, Lower Hopper, S.S Powder support, side cover, and dosing disc) has been set as No residue will be present / if present, should be less than LOQ level (0.10 mg L^{-1}).

3.3. Discussion

Meropenem residue might cause selective pressure on the pathogens to produce carbapenem, which is difficult to treat and has high mortality rates due to its appearance in multidrug-resistant pathogens such as K. pneumoniae, p. aeruginosa, and Acinetobacter spp. [39]. There are several ways to initiate this contamination. Pharmaceutical drug manufacturing is one of the main sources of this. Meropenem drugs are being manufactured in Bangladesh in the same manufacturing facilities as other beta-lactams/ drugs. Thus, it is a must to ensure the lowest level

of residue contamination with other drugs during the changeover. To facilitate this management technique, Meropenem should be controlled from entry to drug manufacturing to the final dispatch. The management initiated everything from procedural development to the implementation of practices in the drug manufacturing unit. The protocol-based studies represent the total control mechanism of Meropenem residue during manufacturing, and the residue determination method details the capability of this method to identify and quantify the Meropenem residue remaining on contact parts after cleaning. The responsibilities, cleaning procedure, sampling procedure, testing procedure, testing method, acceptance criteria, and reporting have been done according to a protocol-based study. Meropenem residue of machine contact parts can be identified and quantified to a minimum level (0.05 mgL^{-1} LOD) by the developed method with an isocratic mobile phase consisting of TBAH buffer, pH 6.5, and acetonitrile at a ratio of 70:30 with a 250 cm x 4.6 mm, five µ column as the stationary phase at ambient oven temperature and a flow rate of 1.0 mL min^{-1}. The developed method demonstrated no interference with a unique chromatogram for diluent, standard, swabbing diluent, and swab samples with contact parts. The stability of the analytical solution was determined, and the solutions remained stable for up to 24 hours under ambient conditions.

All System Suitability criteria were met as per the requirements. The theoretical plat count for column efficiency was more than 2000 USP, the USP Tailing factor was less than 2.0, and the peak area percentage relative standard deviation (RSD) of six standard solutions was less than 10.0%; the recovery of standard (1) and Standard (2) solution was within 98.0%-102.0%, Overall % RSD of all standard solution (1) and bracketing standard (standard solution1) was within 10.0 %. The solvent interference was checked with a diluent. The retention times of the analyte peak in a spiked sample and the standard are eluted at the same retention times. Therefore, this method was specific. The swabbing diluent/bank and the

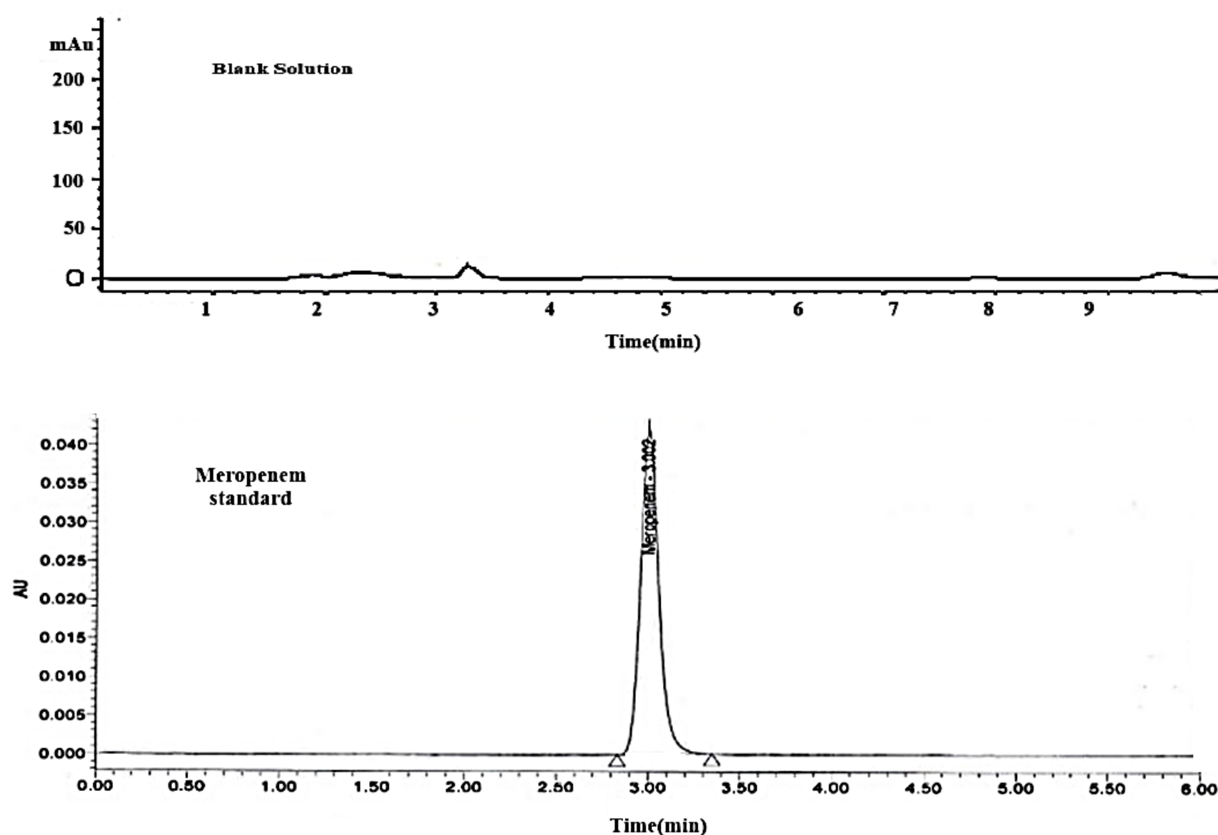

Fig. 6. Chromatogram of swabbing blank and standard

standard chromatogram are shown in Figure 6.
The % recovery for Meropenem was determined for LOQ, 50%, 100%, and 150% levels and found the % recovery within 97.0% to 109.0% that were within the limit, and the recovery factor was determined to be 1.0 as there was no mean recovery for any contact parts of the machine under 95%. The method was found to be precise during method precision and intermediate precision with different analysts and different instruments. The method is linear for the Meropenem compound by giving a correlation coefficient (r) value of 1.000 at concentration levels of 0.1–15.0 µg mL^{-1} for Meropenem. The concentrations of LOD and LOQ based on signal-to-noise ratio (S/N) are 0.05 and 0.1 µg mL^{-1}. LOQ precision was performed by injecting six replicate injections of LOQ solution. Based on the results, the s/n ratio was greater than 10 for LOQ solutions. The area RSD (%) for six replicate injections of LOQ precision was 7.6 for Meropenem. The linearity graph peak responses plotted against peak concentrations of Meropenem evaluated the square of the correlation coefficient (r)

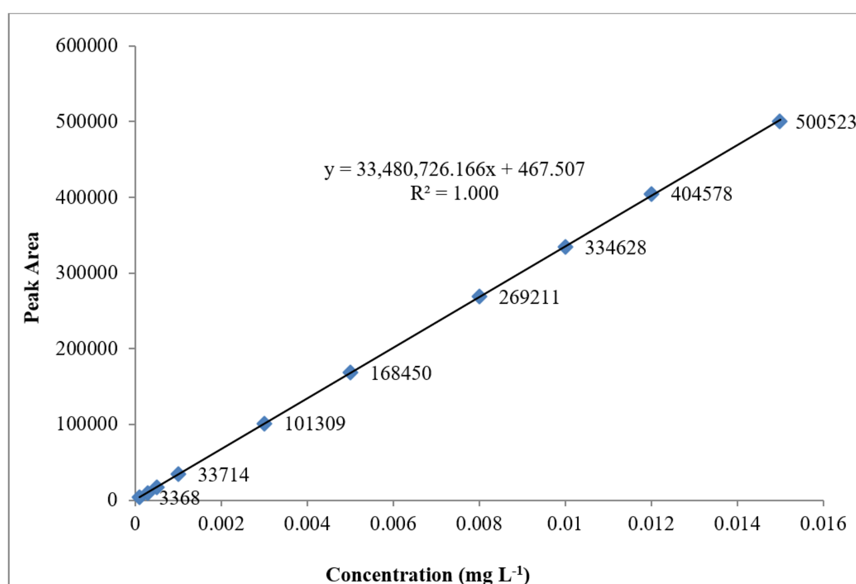

Fig. 7. Linearity curve of Meropenem

Table 2. Analysis of results for LOD and LOQ of Meropenem

Levels	Conc. (µg mL^{-1})	Area	Final LOD	Final LOQ
Level 1	0.1	3368	Based on S/N Ration LOD 0.05 µg mL^{-1}	Based on S/N Ration LOQ 0.1 µg mL^{-1}
Level 2	0.3	10145		
Level 3	0.5	16841		
Level 4	1.0	33714		
Level 5	3.0	101309		
Level 6	5.0	168450		
Level 7	8.0	269211		
Level 8	10.0	334628		
Level 9	12.0	404578		
Level 10	15.0	500523		
Correlation Co-efficient, R		1.000		
STD Response		1243.626		
Y-intercept		467.507		
Slope		33480726.200		
%RSD of LOD and LOQ Precision (6 Injections)			5.1	7.6
S/N Ratio of last injection			12.12	32.54

and found 1.000, Figure 7, Table 2.

After evaluation of data, we found that the system suitability parameters, as cited as Theoretical plates, tailing factor, % RSD of cumulative data, and % Recovery, are within specified limits for original and changed conditions. Thus, the results revealed that the system meets the required system suitability criteria, and the method is robust for wavelength variation and column oven variation but not for flow rate variation (Table 3). The peak area of Meropenem of six swab sample solutions for different items was calculated and reported along with the standard deviation and RSD% of the peak area of these solutions for Meropenem. This shows that the precision of the swabbing method is satisfactory as RSD is not more than 10%, as shown in Tables 4a and b. The spiked sample solutions (n = 6) having RSD (%) were 0.1, 0.16, 0.37, and 0.24 respectively. RSD (%) for preparations (n = 12) of MP and IP spiked samples at specification levels were 2.55, 2.28, 2.38, 2.15, and less than 10.0%. The results show that the method was rugged, as shown in Tables 4a and b. The standard solution and swabbing solution were stable for at least 24 hours at room temperature. So, the solution can be used up to 24 hours. The results revealed that both PTFE and PVDF (0.45 µm) are suitable to use by discarding at least 2 mL for the preparation of sample solution as well as standard solution. The results are summarized in Tables 4a and b.

This method was used to test the validation samples for three (03) consecutive batches after cleaning. The selection of sampling location and sample quantity was based on the direct contact between the Meropenem and the machine, their difficulty in cleaning, and the accumulation of residue or product. This testing will be continued after each Meropenem campaign batch is manufactured according to the procedure. This way, chances of cross-contamination

Table 3. Robustness Data for Meropenem

Parameters	Standard Solution					Sample Solution					
	Theoretical plate	**Tailing Factor**	**Recovery (%)**	**RSD (%)**	**RSD (%) with bracketing standard**	**Result (% content) on SS plate**	**Result (% content) on Teflon plate**	**Result (% content) on Silicon plate**	**Result (% content) on Glass plate**	**% Difference**	**Remark**
Wavelength Variation (290 nm ± 5 nm)											
Original Condition	4249	1.15	100.9	0.3	0.3	99.9	100.0	99.9	100.2	N/A	N/A
285 nm	4461	1.15	100.3	0.1	0.1	99.2	99.4	99.2	99.6	0.6	Robust
295 nm	4534	1.15	99.9	0.1	0.2	98.8	98.6	99.2	99.4	0.8	
Column Oven (25 ± 50C)											
Original Condition	4249	1.15	100.9	0.3	0.3	99.9	100.0	99.9	100.2	N/A	N/A
20^0C	4181	1.15	99.5	0.2	0.2	98.6	98.6	98.4	98.9	1.3	Robust
30^0C	5145	1.15	98.7	0.1	0.1	97.8	97.9	97.6	98.1	1.1	
Flow Rate (1.0mL ± 0.1mL)											
Original Condition	4249	1.15	100.9	0.3	0.3	99.9	100.0	99.9	100.2	N/A	N/A
0.9	5291	1.16	97.6	0.2	0.2	96.6	96.9	96.9	97.4	2.8	Not Robust
1.1	4641	1.13	97.1	0.2	0.2	95.9	95.7	95.8	96.3	3.9	

Table 4a. Summary of method validation results.

Validation parameters	Acceptance criteria	Results obtained				
System Suitability	NMT 10.0 % from six replicate injection	Standard Solution-1	%RSD	0.1		
	Not more than 2.0		Tailing Factor	1.21		
	Not less than 2000		Theoretical plate	4533		
	98.0%-102.0%	Standard Solution- 1& 2	% Recovery	100.7%		
	NMT 10.0 %	Standard Solution-1& Bracketing Standard	RSD (%) of peak area	0.3%		
	No interfering peak	Blank & Swabbing Blank Solution	No interfering peak was observed			
Specificity	There should not be any interfering peak in the chromatogram obtained from the diluent and swab stick solution at the retention time corresponding to Meropenem.	No interfering peak was observed in the diluent and swab stick solution.				
		Sample Name	**Analyte Name**	**Retention Time (min.)**		
		Standard Solution	Meropenem	3.00		
		Swab_SS_Plate	Meropenem	3.01		
		Swab_Teflon_Plate	Meropenem	3.00		
		Swab_Glass_Plate	Meropenem	3.00		
		Swab_Silicon_Plate	Meropenem	2.99		
Linearity	The squared correlation coefficient (r^2) should be not less than 0.99.	**Analyte**	r^2			
		Meropenem	1.00			
LOQ & LOD determination	Reported value Concentration (mg mL^{-1})	**LOD**	**LOQ**			
		0.000122577	0.000371445			
LOQ precision	For the LOQ solution, the Signal-to-noise ratio should be NLT 10, and RSD (%) for the six replicate responses should be NMT 25%.	**S/N ratio**		**% RSD**		
		LOD	**LOQ**	**LOD**	**LOQ**	
		12.12	36.54	5.1	7.6	
Swab Recovery	**LOQ Recovery solution:** The recovery should be between 70.0%-130.0% **50%-150% Recovery solution:** The recovery should be between 80.0%-120.0%	**Recovery**				
		Level	**LOQ**	**50%**	**100%**	**150%**
		Glass_Plate	109.4	101.7	104.5	100.2
		Teflon_Plate	109.0	101.6	103.6	100.0
		SS_Plate	100.5	101.6	103.6	97.6
		Silicon_Plate	99.5	101.7	103.3	97.3
Filter Evaluation	% Difference between the initial result and after the time interval should be NMT 10.0%.	**Names**	**Area**	**Meropenem**		
				RSD (%)	**Results (%content)**	**% Difference**
		Centrifuged	306191	N/A	99.7	N/A
		PTFE0.45 µm dis. 2 mL	306079	0.14	100.1	0.4
		PTFE0.45 µm dis. 4 mL	306025	0.16	100.1	0.4
		PTFE0.45 µm dis. 6 mL	307196	0.18	100.1	0.4
		PVDF0.45 µm dis. 2 mL	306217	0.09	100.1	0.4
		PVDF0.45 µm dis. 4 mL	306362	0.13	99.9	0.2
		PVDF0.45 µm dis. 6 mL	306191	0.10	100.1	0.4

Table 4b. Continue summary of method validation results.

Validation parameters	Acceptance criteria	Results obtained				
		Test solution	Results (% content)			
			SS Plate	Teflon Plate	Glass Plate	Silicon Plate
Method Precision (MP) (n = 6)	The Recovery should be between 80.0%-120.0%. RSD of recovery results should not be more than 10.0%.	Sample-1	99.2	98.0	99.1	98.5
		Sample -2	99.0	98.2	98.5	97.8
		Sample -3	99.2	98.3	98.1	98.3
		Sample -4	99.2	98.0	98.2	98.2
		Sample -5	99.1	98.2	98.2	98.1
		Sample -6	99.0	98.4	98.4	98.3
		Mean	**99.1**	**98.2**	**98.4**	**98.2**
		RSD (%)	**0.10**	**0.16**	**0.37**	**0.24**
Intermediate Precision(IP) (n = 6, MP+IP, n = 12)	The Recovery should be between 80.0%-120.0%. RSD of recovery results should not be more than 10.0%.	Sample -1	94.2	93.5	94.2	93.8
		Sample -2	93.6	94.3	93.5	94.2
		Sample -3	94.5	99.3	99.3	97.1
		Sample -4	99.3	94.1	94.3	94.2
		Sample -5	94.2	94.3	99.4	99.1
		Sample -6	99.3	99.2	94.2	94.0
		Mean	**95.9**	**95.8**	**95.8**	**95.4**
		RSD (%)	**2.80**	**2.82**	**2.87**	**2.30**
		Mean (%) (MP+IP, n = 12)	97.48	96.98	97.17	96.80
		Cumulative RSD (%) (MP+IP, n = 12)	2.55	2.28	2.38	2.15
Robustness	Results should be within the specification limit.	**Variations** i. Wavelength variation (290 ± 5 nm) ii. Flow Rate variation (±0.1 mL) iii. Oven Temp.(25 ± 5^{0}C)			The method was found robust for the Wavelength variation & Oven Temp variation.	

Validation parameters	Acceptance criteria	Swab Samples	Meropenem		
			% Recovery		Difference (%)
			Initial	24H	
Solution Stability	% RSD between the initial result and after the time interval should be NMT 10.0%.	Sample SS plate	99.9	97.6	2.3
		Sample Teflon plate	100.0	97.3	2.7
		Sample Glass plate	100.2	97.2	3.0
		Sample Silicon plate	99.9	97.1	2.8

will be drastically reduced.

The production department has performed the cleaning procedure for the injection filling line. A visual test and a chemical evaluation of the equipment need to be performed after cleaning to demonstrate that Meropenem residue has been removed & cleaned as per the designated cleaning method of the production unit. In many cases, the surface of production equipment will not be made of flat stainless steel. Therefore, the swab must be done as close as practically possible to the validated swab procedure. Besides stainless steel, there are some other materials of construction also involved, like container holder/Adapter, which is made of Teflon, and Silicon channel, which is made of silicon; there is a glass on the lower hopper and side cover. Thus, sampling was done using swab samples according to the analysis method and location (Table 1). The area swabbed area was always 25 cm^2 (5 cm x 5 cm), where it is not possible to swab this size area; swabs were taken from several locations distributed across the equipment product contact surface such that the total area is close to 25 cm^2 (the actual surface area swabbed was calculated and used

for carryover calculations). After establishing the cleaning procedure through this validation, the manufacturing department will clean the machine contact parts according to this established and validated cleaning procedure. After each campaign production of Meropenem injections, the sample will have to be sent to QC for analysis according to the validated analytical method for routine review of the cleaning procedure established to clean the Meropenem residue before changeover to other drug manufacturing as a routine management for prevention of Cross-contamination. If any changes occur in the production machine, such as critical equipment modifications or cleaning procedures, a change control system is in place to ensure that the cleaning process is reassessed at defined intervals and re-validation as necessary.

4. Conclusion

This protocol-based study for the Meropenem residue determination is a must to do by every drug manufacturing unit that manufactures Meropenem drug with other drugs into the same premise. Meropenem cross-contamination is more vital to control than other drugs, whereas this drug is the last resort of antibiotic treatment. To control cross-contamination, residue determination of Machine contact parts is essential to monitor the machine cleaning efficiency during the changeover to other drug manufacturing. A study protocol has been established to control and monitor the cleaning procedure developed for manufacturing Meropenem formulations, especially powder for injections. Besides the cleaning method, the analytical method with swab sampling is a vital tool that needs to be developed and validated to determine the Meropenem residue. In this method, an HPLC method was developed and validated to quantify the Meropenem residue on the machine contact parts selected, such as 316 stainless steel, Teflon, glass, and silicon. The method developed and validated for analyzing swab samples is simple to execute. This HPLC method is very cost-effective as the run time is only 6 minutes with a simple Mobile phase, stationary phase, and chromatographic conditions (recovery of more than 95%). The validation results indicated that the developed HPLC method for determining Meropenem residue is precise, accurate, linear over the test concentration range, rugged, and specific. The isocratic HPLC method to determine Meropenem will not only save cost and time but also it will be a unique method to determine the Meropenem residue from Machine contact parts by swabbing and also the wastes discharged from the manufacturing unit to the environment and because there is only water interference into that samples. Thus, it will be a better option for the analytical & quality control labs to use to control Meropenem cross-contamination in the same manufacturing plant with other drugs manufactured.

5. Abbreviations

HPLC: High-performance liquid chromatography
CIP: Cleaning in process
WHO: World Health Organization
US CDC: United States Centers for Disease Control and Prevention
ECDC: European Center for Disease prevention & control
PDA: Photodiode array detector
CGMP: Current good manufacturing practices
ICH: International Council for Harmonization
RSD: Relative standard Deviation
LOD: Limit of detection
LOQ: Limit of quantification
COT: Column oven temperature
FR: Flow rate
TBAH: Tetra-butyl Ammonium Hydroxide

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