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ORIGINAL ARTICLE **OPEN ACCESS**

Development and Validation of RP-HPLC Method for the Estimation of Meropenem and Tazobactam in Pure Drug and Formulation

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ABSTRACT
A new, simple, accurate and reproducible RP-HPLC method was developed and validate for the estimation of meropenem and tazobactam in Pure drug and pharmaceutical formulations. The UV spectrum recorded between 200-400 nm and the wavelength 230 nm was selected for the estimation of both the drugs. RP-HPLC analysis was carried out using Column – Inertsil C-18 (250 mm x 4.6 mm, 20 µL) and mobile phase composed of Buffer: Acetonitrile: Water (75: 15: 10) at a flow rate of 1.0 mi/min. Linearity was established in the range of 50-250 µg/ml with a coefficient correlation of 0.9996 for Meropenem and 5-25 µg/ml for Tazobactam with a coefficient correlation of 0.9999. The regression equation for the calibration plot was Y = 10592 X - 8063 for Meropenem and Y = Y = 4299 X – 21.1 for Tazobactam. Accuracy values were (99.75%-100.02%) for Meropenem and (98.90% to 101.56%) for Tazobactam. From degradation study, it was found that Meropenem degradation in alkaline and oxidative condition but no degradation was observed at room temperature. The drug was exposed to 1% H_2O_2 at room temperature for 1 hr. degradation product at RT: 4.41 and 2.68 min. The LOD and LOQ were found to be 0.820 and 1.059 for Meropenem and 2.487 and 3.210 for Tazobactam respectively. For both drugs Intra-day and Inter-day precision, % Assay and % Recovery was determined. Robustness and ruggedness were observed that results were well within acceptance limits of 98–102%, with %RSD ±2.0%, indicating the method is rugged and provides consistent and reliable results which are not affected by small changes in experimental conditions. The present method can be recommended for estimation of Meropenem and Tazobactam in routine control analysis of drug. The proposed RP-HPLC method was validated according to ICH guidelines for linearity, precision, accuracy, specificity, LOD and LOQ.
Keywords: *HPLC, Meropenem, Tazobactam, LOD, LOQ, ICH guidelines.*

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INTRODUCTION

High Performance Liquid Chromatograph was derived from the classical column chromatography and most important tools of analytical chemistry today. HPLC methods development and validation play important roles in new discovery, development, manufacture of pharmaceutical drugs. [1,2,3] HPLC is the most accurate analytical methods widely used for the quantitative as well as qualitative analysis of drug product and used for determining drug product stability [4,5]. validation is a process of establishing documentary evidence demonstrating that a procedure, process, or activity carried out in production or testing maintains the desired level of compliance at all stages. In Pharma Industry it is very important apart from final testing and compliance of product with standard that the process adapted to produce itself must assure that process will consistently produce the expected results. The objective of validation of an analytical procedure is to demonstrate that it is suitable for its intended purpose. A tabular summation of the characteristics applicable to identification, control of impurities and assay procedures is included. We use Tazobactam in place of Sulbactam because both the drugs come under same category and have similar pharmacological activity. This drug is given in combination with β-lactam antibiotics to inhibit β-lactamase, an enzyme produced by bacteria that destroys the antibiotics. There is urgent need to develop a simple, sensitive, accurate and precise HPLC method of Meropenem and Tazobactam in pure and pharmaceutical dosage form and compare both the method. The results of the analysis were validated by latest guidelines set by International Conference on Harmonization (ICH) [7,8].

Drug Profile: Meropenem is a broad-spectrum antibiotic used to treat a variety of bacterial infections. Some of these include meningitis, intra-abdominal infection, pneumonia, sepsis, and anthrax. It is given by

injection into a vein. Common side effects include nausea, diarrhea, constipation, headache. Serious side effects include *Clostridium difficile* infection and allergic reactions including anaphylaxis. Those who are allergic to other β-lactam antibiotics are more likely to be allergic to meropenem. Use in pregnancy appears to be safe. It is in the carbapenem family of medications. Tazobactam is a pharmaceutical drug that inhibits the action of bacterial β-lactamases, especially those belonging to the SHV-1 and TEM groups. It is commonly used as its sodium salt, tazobactam sodium. This drug is used in conjunction with beta-lactamase susceptible penicillin to treat infections caused by beta-lactamase producing organisms. Chemical name and empirical formula of Meropenem and Tazobactam are: 3- [5-(dimethyl carbamoyl) pyrrolidin-2-yl] sulfanyl-6-(1-hydroxyethyl)-4-methyl-7-oxo-1-azabicyclo [3.2.0] hept-2-ene-2-carboxylic acid and (2S,3S,5R)-3-methyl-7-oxo-3- (1H-1,2,3-triazol-1-ylmethyl)-4-thia-1-azabicyclo [3.2.0] heptane-2-carboxylic acid 4,4-dioxide respectively.Fig.1,2

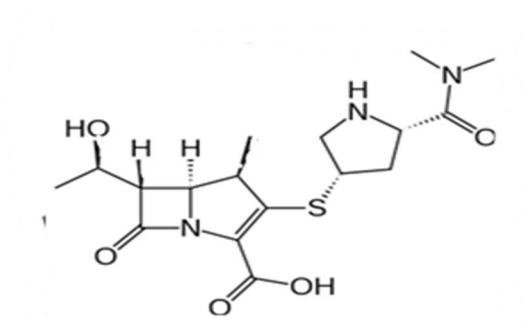

Fig1. Chemical structure of Meropenem Sodium

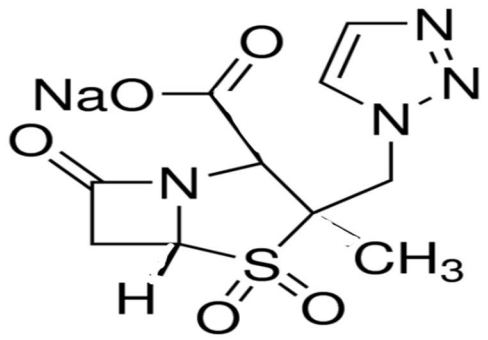

Fig2. Chemical structure of Tazobactam

Physicochemical Characterization of the Drug:

Meropenem and Tazobactam Sodium both are white to pale yellow powder freely soluble in water. Physiochemical properties shown in Table 1.

Table 1. Physicochemical properties of used drug.

Drug	Meropenem	Tazobactam Sodium
Category	Carbapenem Antibiotic β-lactam Antibiotic	β-Lactamase inhibitor Antibiotic agent
Appearance	White to pale yellow powder	White to pale yellow powder
Chemical Formula	$C_{17}H_{25}N_3OS.3H_2O$	$C_{10}H_{12}N_4O_5S$
Molecular weight	437.52 g/mol	322.27 g/mol
pH	7.3 – 8.3	2.0 – 2.5
M.P	191-201°C	115 - 145° C

MATERIAL AND METHODS

Meropenem and Tazobactam both substances were obtained as gift sample from Local API manufacturing unit. Tablet dosage forms of Meropenem and Tazobactam such as Temero-1 gm (1.0 gm/Injection, Arion Healthcare Pharmaceutical Ltd), Merobax-1 (1.0 gm/Injection, Aristo Pharma) and Meropenem and Tazobactam Injection USP, 1-125 gm (PCD Pharma, India) were purchased from local pharmacy market. The mobile was freshly prepared and filtered through a 0.45µm Millipore filter made of polyamide and degassed in an ultrasonic bath. All the chemicals used for mobile phase were of HPLC grade.

Table 2. Instrumentation & Chromatographic Condition.

INSTRUMENTS	CHROMATOGRAPHIC CONDITION
HPLC System: Shimadzu (model-LC-20AT)	Flow Rate: 1ml/min.
Shimadzu UV spectrophotometer (Model-UV-1700)	UV range: 200 to 400 nm
Colum: Inertsil C18 column	Wavelength:230 nm
Pump: Shimadzu (Model- LC-20AT)	Injection Volume: 20µL
Detector: UV-Visible Spectrophotometer (SPD-20A)	Column Temperature: Ambient
PH Meter: Digital pH meter (Cyber Labs, USA)	Mobile Phase: Buffer: ACN: Methanol

Preparation of Standard solution

Stock Solution of drug: For the creation of the calibration curve, Stock solution of Meropenem and Tazobactam was prepared in mobile phase. Meropenem (100mg) and Tazobactam (100mg) were weighed accurately and transferred to the 100 ml volumetric flask quantitatively. It was dissolved in 75 ml of mobile

phase with the aid of sonication. The final volume was made up to 100 ml with mobile phase. Different working solutions of Meropenem and Tazobactam were prepared from the stock solution using appropriate dilutions.

Preparation of standard sample: 10 mg of each drug were transferred to a 100 ml calibrated flask and dissolved in 75 ml of mobile phase. The content was shaken for 10 min. Final volume was made up to 100 ml with mobile phase. Different working solution of Meropenem and Tazobactam were prepared from the sample solution using appropriate dilutions. The final solution has been sonicated and filtered through 0.45-µm Millipore filter.

Preparation of sample for different test: Aliquots of the sample solution were transferred to the 10ml volumetric flask containing 50 µg/ml to 250µg/ml of Meropenem and 5 µg/ml to 25µg/ml for Tazobactam.

Method Development and Validation of Dutasteride in Bulk and Dosage form

By U.V Scan: showing wavelength maxima at 230 nm From the UV spectra the wavelengths 230 nm was selected for monitoring of the drugs. Fig. 3 After trying different mobile phase, the final choice of the mobile please giving satisfactory resolution and run time was Buffer pH 7.52 ± 0.1 with 10%v/v phosphoric acid, acetonitrile and methanol in composition with (75: 15: 10). From the UV spectra the wavelengths 230 nm was selected for monitoring of the drugs.

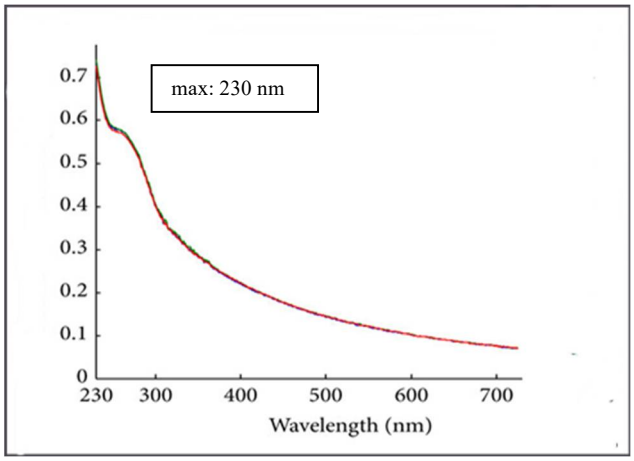

Fig. 3. UV spectra of Drug showing wavelength at 230 nm

Table 3. Chromatographic parameters in different mobile phase compositions.

	TRIAL 1 (λ: 230 nm) (Flow Rate: 1.0 ml/min.)		**TRIAL 2 (λ: 230 nm) (Flow Rate: 1.5ml/min.)**		
Parameter	(Buffer: ACN: Methanol)		(Water: Methanol: Phosphate Buffer)		
(Mobile Phase)	80: 15: 5	75:15:10	80:15:05	75:20:05	60:20:10
Retention Time	10.21	9.35	7.5	7.4	6.8
Tailing Factor	0.24	1.28	1.27	0.88	0.79
No. of Theo Pt.	4416	5451	2854	2737	2488

System suitability

System suitability was performed by injecting repetitive injection (n=6) of Meropenem (1mg/ml) and Tazobactam(0.125mg/ml) to the chromatograph, acceptance criteria: - % CV should be less than 2%. and the parameters were reported. Based on the observation that the column efficiency as determined for Meropenem and Tazobactam tailing factor was not more than 2 respectively. The % RSD of the peak area is not more than 1. The data were represented in Table 4 and the chromatogram were represented in Fig.6

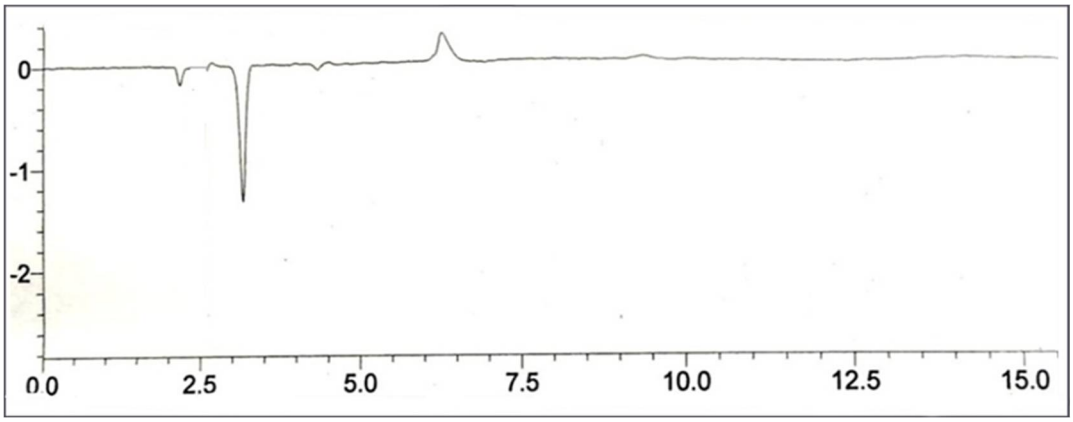

Fig.4. Chromatogram of mobile phase used for the preparation of sample

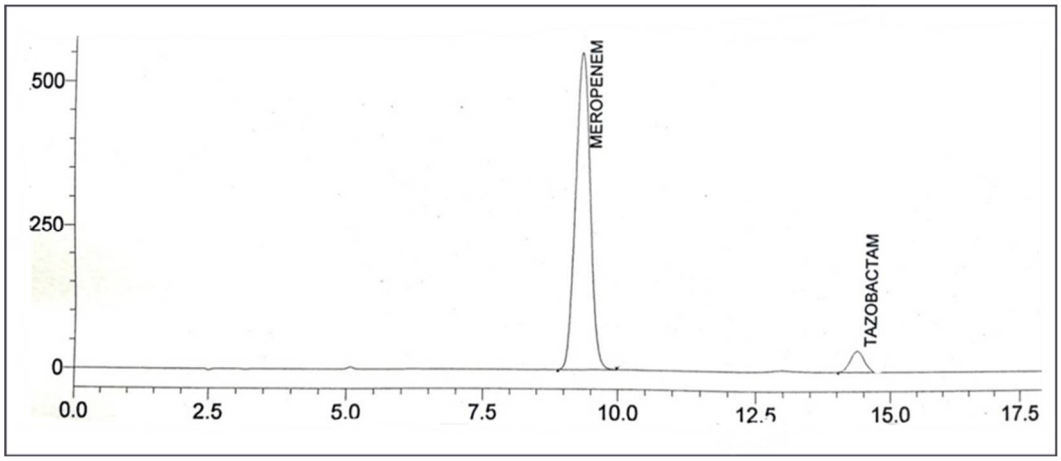

Fig. 5. System Suitability Curve showing SST result for meropenem and Tazobactam (n*=6)

Table 4. SST Result for Meropenem and Tazobactam.

		Retention time	Area	Height	Tailing factor	Theoretical plates
Meropenem	Mean (n*=6)	9.35	10579286	559517	1.28	5451
	%RSD	0.087	1.346	0.107	0.809	0.482
Tazobactam	Mean (n*=6)	14.37	644068	36023	1.37	13957
	%RSD	0.057	10274	0.088	1.20	0.140
	Limit (%RSD)	2.0 %	2.0 %	2.0 %	2.0 %	2.0 %
	Result	Pass	Pass	Pass	Pass	Pass

Table 5. Validation parameters of method development.

Parameter	Meropenem	Tazobactam
Wavelength	230 nm	230 nm
Linearity Range (µg/mL)	50-250	5-25
Standard Regression Equation	Y = 10592 X - 8063	Y = 4299 X – 21.1
Regression coefficient (r^2)	0.9996	0.9999
Accuracy (% Recovery ±SD)	99.75 to 100.023	98.907 to 101.560
Precision (Intra-day)	0.73 to 1.914	0.505 to 2.068
Precision (Inter-day)	0.163 to 1.914	0.176 to 2.068
LOD µg/ml	0.820 µg/ml	2.487 µg/ml
LOQ µg/mL	1.059 µg/ml	3.210 µg/ml

Linearity

The calibration curves were plotted over the concentration range of 50 to 250 µg/ml for Meropenem and 5 to 25 µg/ml for Tazobactam were prepared in triplicate. The regression equation for the calibration plot of Meropenem and Tazobactam were Y = 10592 X – 8086.1 (regression coefficient (r^2) 0.9996) and Y = 4299 X + 21.1 (regression coefficient (r^2) 0.9999) respectively as shown in Table **6** and Figure **7**

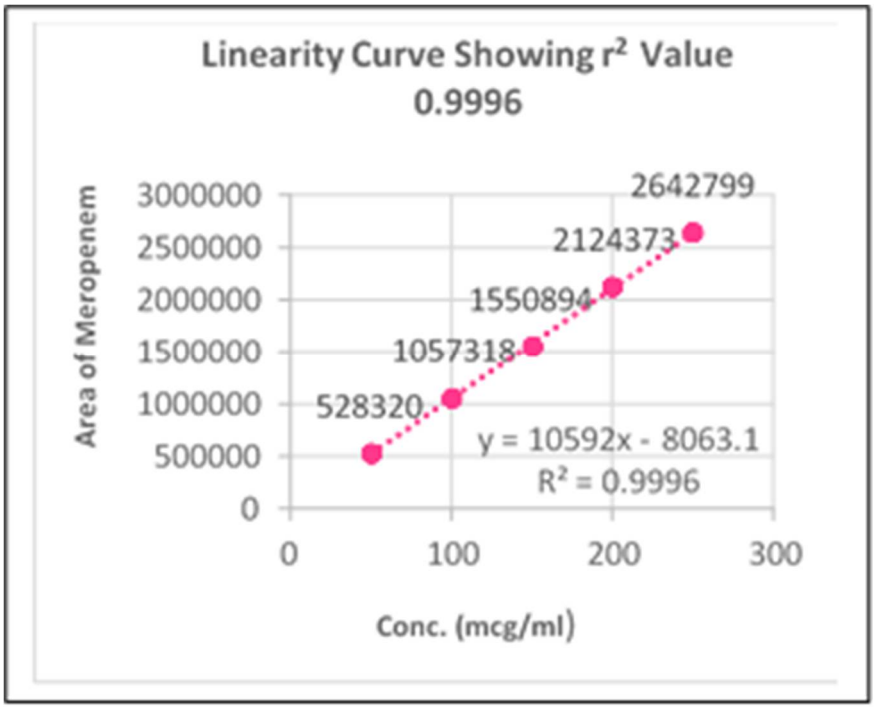

Fig. 6. Linearity curve showing r² value 0.9996 (Meropenem)

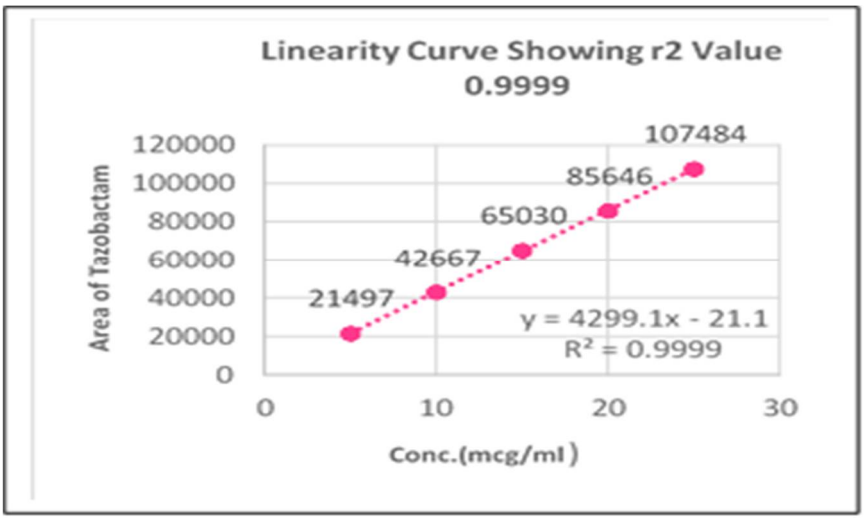

Fig. 7. Linearity curve showing r² value 0.9999 (Tazobactam)

Table 6. Calibration curve data

Meropenem: Conc. (50-250 µg/mL)						
	CC1	**CC2**	**CC3**	**CC4**	**CC5**	**CC6**
r²	0.9999	0.9998	0.9998	0.9979	0.9998	0.9966
Slope	10609	10640	10635	10555	10550	10564
Intercept	-594	-445	-349	-155	-340	-218
Tazobactam: Conc. (5-25 µg/mL)						
r²	0.9997	0.9970	0.9999	0.9999	0.9998	0.9999
Slope	4309	4326	4297	4292	4285	4289
Intercept	-167	+154	-510	-223	+174	-120
	1st Day		2nd Day		3rd Day	

Accuracy

A known quantity of standard solution has been added to the sample solution previously analyzed at three different levels (50, 100,150 µg/ml) for meropenem and (5, 10, 15 µg/ml) for Tazobactam. Percentage recovery was calculated for the intra-day assay experiments. Standard addition and recovery experiments

were also conducted to determine the accuracy of the method. The calculated recovery and percentage recovery was within 100±2.0% the acceptable range which indicated method was found to be accurate.

Table 7. The accuracy of the method development by the measurement of recovery:

Meropenem Recovery Data					
Theoretical (Conc.)	Measured (Conc.)	Area (Drugs)	SD	% RSD	% Accuracy
50*	49.935	524495	3792	0.723	99.871
100*	100.023	1058863	1653	0.156	100.023
150*	149.761	1571459	2185	1.39	99.757
Tazobactam Recovery Data					
5*	4.945	21673	279	1.288	98.907
10*	9.984	43298	530	1.225	100.15
15*	15.070	65551	653	0.997	101.560

*Every value is the mean of three analyses parameters. All concentration measured in µg/ml

Precision

The precision of the method was assessed by study of repeatability and intermediate precision. Repeatability (intra-day variation) of the assay measured for different concentrations (100, 200, and 300 µg/ml) for meropenem and (25, 50, and 75 µg/ml) for Tazobactam was expressed as RSD calculated from results from analysis on each of three days. Intermediate precision (inter-day variation) at the same concentrations was determined on successive days. For study of intra-day precision, the concentration of both drugs calculated three times on the same day at interval of 3 hrs. In the inter-day study, the drug concentration was calculated on three different days. Intra- day and Inter-day precision for both the methods were within the acceptable range ± 2, indicative of good method precision.

Table 8. Intra-day precision for the determination of Meropenem and Tazobactam:

Meropenem: Intra-day precision data									
Conc.	00 Hrs.			3 Hrs.			6 Hrs.		
(µg/mL)	Area	SD	%RSD	Area	SD	%RSD	Area	SD	%RSD
100*	1059510	1643	0.155	1054493	1932	0.183	1048765	1274	0.122
200*	2149129	1185	0.552	2112605	1543	0.073	2035185	3100	1.523
300*	3160175	6048	1.914	3007379	5824	0.194	2990136	1738	0.582
Tazobactam: Intra-day precision data									
25*	109164	1639	1.502	101628	2194	2.159	99151	763	0.770
50*	206964	4280	2.068	200452	1011	0.505	192133	3769	1.962
75*	308963	6244	2.021	288088	3208	1.114	271090	2033	0.750

*Every value is the mean of three analysis parameters

Table 9. Inter-day precision for the determination of Meropenem and Tazobactam

Meropenem: Inter-day precision data									
Conc.	DAY 1st			DAY 2nd			DAY 3rd		
(µg/mL)	Area	SD	%RSD	Area	SD	%RSD	Area	SD	%RSD
100*	1059510	1643	0.155	1043200	2142	0.205	1032804	1688	0.163
200*	2149129	1185	0.552	2013533	5301	0.263	1993657	5645	0.283
300*	3160175	6048	1.914	2917916	2169	0.744	2842609	3812	1.341
Tazobactam: Inter-day precision data									
25*	109164	1639	1.502	99389	356	0.359	97996	172	0.176
50*	206964	4280	2.068	193117	206	1.070	182342	356	1.956
75*	308963	6244	2.021	278588	2447	0.879	266412	284	1.067

*Every value is the mean of three analysis parameters

Assay of Tablet Formulation

Average weight of the 20 tablets was determined. These tablets were crushed to a fine powder. Powder equivalent to 10 mg was weighed and transferred to a 100 mL volumetric flask. It was dissolved in mobile phase. Six replicates of the required dilution were prepared from tablet stock solution and sonicated for 10 min. These solutions (50 µg/mL) for meropenem and (25 µg/mL) for Tazobactam were analyzed and mean, standard deviation and relative standard deviation (RSD) were calculated for both meropenem and tazobactam.

Table 10. Recovery for the assay of Method development of stock and dosage form.

		Theoretical (µg/mL)	Measured (µg/mL)	Area of drug	S.D.	% Recovery	%RSD
Meropenem	Stock	50*	49.90	526046	5185	99.79	0.550
	Dosage	50*	49.71	505367	8642	99.69	0.240
Tazobactam	Stock	25*	24.84	103783	1323	99.33	1.059
	Dosage	25*	24.56	99680	471	98.45	1.106

*Every value is the mean of three analyses parameters

Recovery Studies

Recovery study carried out for the drug was performed by spiking the standard drug in powder formulation. Recovery was calculated by use of the regression equation and a regression line graph was drawn using the amount added on the x-axis and the amount found on the y-axis. The calculated recovery and percentage recovery values listed in Table No.11 are within ±2.0 of the true values in intra-day assay experiments for both meropenem and tazobactam.

Recovery level1 (50% level): Accurately pipette and transfer the stock solution (2.0ml, 100 µg/ml), sample solution (4.0ml, 100 µg/ml) and mix.

Recovery level 2 (100% level): Accurately pipette and transfer the stock solution (4.0ml, 100 µg/ml), sample solution (4.0ml, 100 µg/ml) and mix.

Recovery level 3 (150% level): Accurately pipette and transfer the stock solution (6.0ml, 100 µg/ml), sample solution (4.0ml, 100 µg/ml) to a 10ml volumetric flask and dilute with mobile phase to volume and mix.

Table 11. Recovery for the analysis of drugs

Meropenem: Recovery data:						
Recovery Level	Concentration (µg/mL)				Area (drugs)	% Recovery
	Taken*	Labeled*	Added*	Found*		
50%	120	80	40	119.17	1195568	99.31
100%	160	80	80	159.23	1560856	99.53
150%	200	80	120	198.05	1946130	99.02
Tazobactam: Recovery data						
50%	60	40	20	59.65	293211	99.42
100%	80	40	40	80.06	392479	100.07
150%	100	40	60	99.22	482925	99.22

Specificity (Degradation Studies):

From degradation study, it was found that Meropenem degradation in alkaline and oxidative condition but no degradation was observed at room temperature.

Photodegradation

The dry drug (10 mg) was subjected to UV irradiation for three Hrs. the drug was than dissolved in methanol (10 ml). to give 1000 µg/ml. Different working solutions of meropenem were prepared from the stock solution using appropriate dilutions. (200µg/mL). This solution was filtered through a 0.45-µm syringe filter and analyzed by HPLC. Sample did not produce any other signal than meropenem indicating that the drug was stable under UV irradiation. (as shown in Fig. 9,10)

Oxidative Condition

Two degradation products were found under peroxide condition; The drug was exposed to 1% H_2O_2 at room temperature for 1 hr. degradation product at RT: 4.41 and 2.68 min. (as shown in Fig. 9,10)

Table 12. Results of forced degradation study of Meropenem

Sr. No.	Stress type	Condition	No. of degradation peak	Retention time (min.)
1	Acid hydrolysis	0.1 N HCl at room temperature for 60 min.	MER-1	3.31
2	Alkali hydrolysis	0.1 N NaOH at room temperature for 60 min	MER-1	4.46
3	Oxidative degradation	1% H_2O_2 at room temperature for 60 min	MER-1 MER-2	4.41 2.68

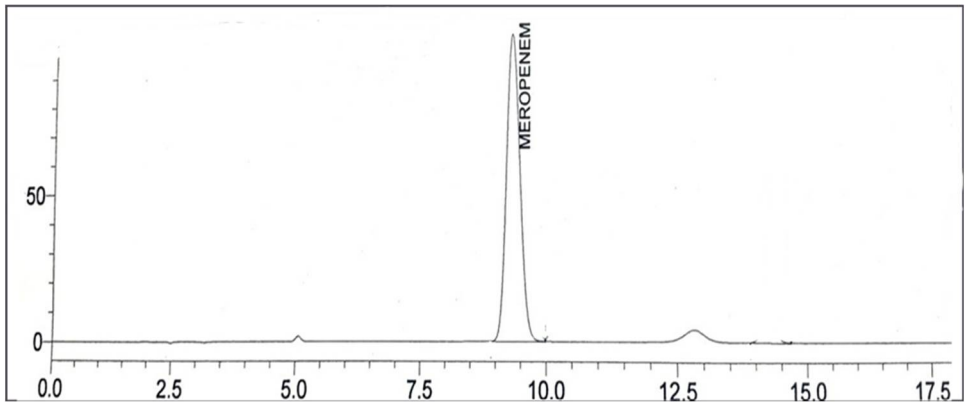

Fig 8. Chromatogram of meropenem before stress condition

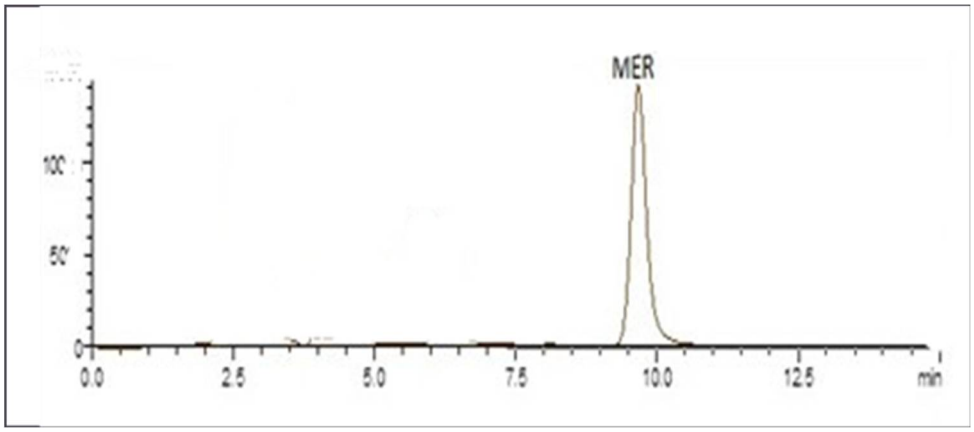

Fig 9. Chromatogram of meropenem obtained after UV irradiation.

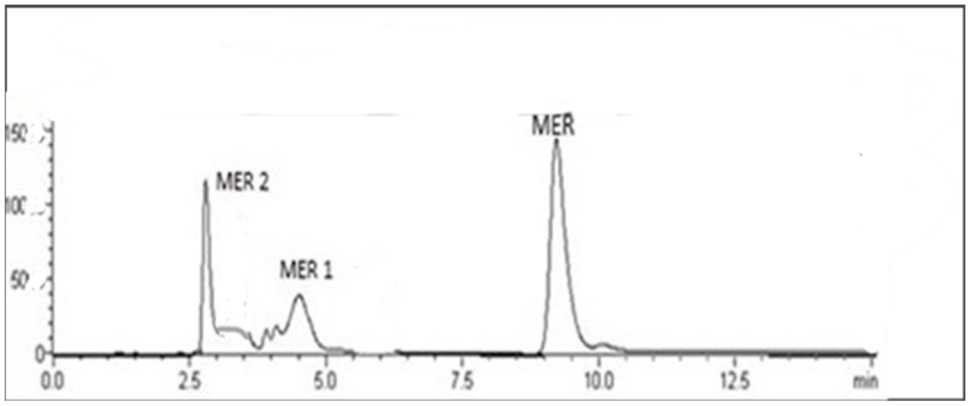

Fig. 10. Chromatogram of meropenem obtained after oxidative degradation.

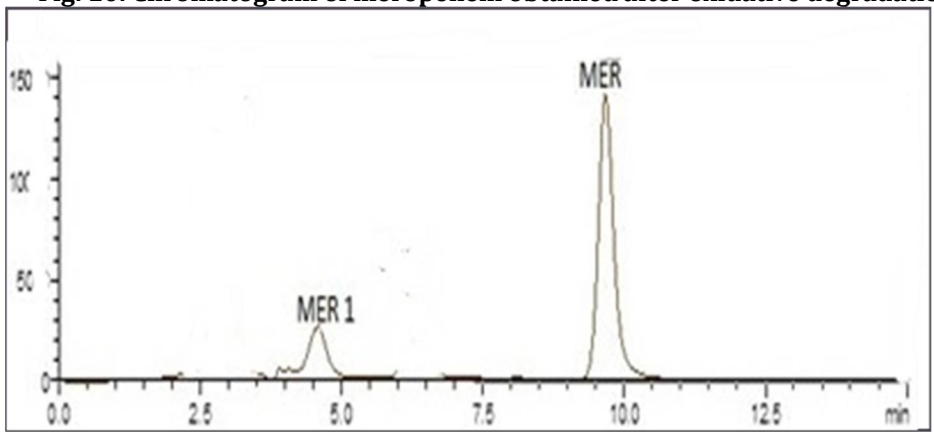

Fig. 11. Chromatogram of meropenem obtained after alkali hydrolysis

Limit of Detection and Limit of Quantitation (Sensitivity)

For both the Method a series of solutions in the range 0.2-1.0 % of the assay concentration (10 µg /mL) were prepared by dilution of the standard solutions. Each solution (5 µg/mL) (n = 5) injected five times. The area was measured for the drug solution, Both the method was based on the SD of response and slope. The data were represented in Table.12

Table 13. LOD and LOQ determination.

	Concentration (µg/mL)		Slope of Drug	Area (Drug)	S.D.	
	Theoretical	Measured				
Meropenem	5*	4.810	10592	55320	2634	LOD =0.820 µg/mL LOQ =2.487 µg/mL
Tazobactam	5*	4.769	4299	23925	1380	LOD =1.059 µg/mL LOQ =3.210 µg/mL

Ruggedness

The ruggedness of a method is its ability to remain unaffected by small, unintentional changes in experimental conditions. *The* method was assessed by analyst-to-analyst variation by use of a matrix design involving the estimation on two different days using two different analysts on two different days, with a total of four analyses. Under each of the conditions, samples were analyzed including a duplicate injection for each estimate.

Table 14. Data for Ruggedness and Robustness Test

	Meropenem			**Tazobactam**		
	S. D	%RSD	Recovery (%)	S. D	%RSD	Recovery (%)
Developer	3079	1.207	99.84	846	1.320	99.71
analyst #2	5033	2.050	99.04	644	1.012	98.20

Robustness

For the robustness of the analytical method at same Chromatographic Condition (Mobile Phase: ACN: Water: 80:20) and pH, changed the flow rate, p^H and wavelength. To study the effect of the flow rate, it was changed to 0.1 unit i.e. 0.9 and 1.1 ml/min. The change in wavelength 228 to 232. Data were shown in Table 14.

Table 15. Data for Robustness Test: Meropenem

Chromatographic Condition: Mobile Phase; ACN: Water (80:20), pH: 2.7					
Wave Length	Retention Time(min.)	Area of drug	Flow Rate (ml/min.)	Retention Time(min.)	Area of drug
228	9.30	1011121	0.9	11.42	1310366
230	9.35	1056822	1.0	9.35	1057928
232	9.32	952132	1.1	8.58	991365

Table 16. Data for Robustness Test: Tazobactam

Chromatographic Condition: Mobile Phase; ACN: Water (80:20), pH: 2.7					
Wave Length	Retention Time(min.)	Area of drug	Flow Rate (ml/min.)	Retention Time(min.)	Area of drug
228	14.15	63813	0.9	17.36	79538
230	14.37	63983	1.0	14.37	64406
232	14.14	49443	1.1	13.00	59995

Robustness and ruggedness were observed that results were well within acceptance limits of 98–102%, with %RSD ±2.0%, indicating the method is rugged and provides consistent and reliable results which are not affected by small changes in experimental conditions

RESULT AND DISCUSSION

System Suitability

The tailing factor for the peak due to Meropenem and Tazobactam in stock standard solution not be more than 1.5. The system suitability of the method was checked by injecting six different preparations of same concentration of the meropenem and Tazobactam standard. The peak area and retention time for the drug were within 2% indicating the suitability of the system. The data were represented in **Table 4** and the chromatogram were represented in **Fig.4, 5**

Linearity

The regression equation for the calibration plot was Y = 10592 X - 8063 and Regression Coefficient (R^2) 0.9996 for Meropenem and Y = 4299 X – 21.1 and Coefficient (R^2) 0.9999 for Tazobactam. the linearity was established in the range of 50 to 250 µg/mL for meropenem and 5 to 25 µg/mL for Tazobactam. These results showed there was a good linear relationship between area of drugs and the amount of analyte in the range studied. The data were represented in **Table 6** and the chromatogram were represented in **Fig. 6.**

Accuracy: The calculated recovery and percentage recovery values were (99.76%-100.02%) for Meropenem and (98.91% to 101.56%) for Tazobactam. Percent recovery was within 100±2.0% the acceptable range which indicated method was found to be accurate. The calculated recovery and percentage recovery values listed in **Table 7**.

Precision

The result was found to be Intra-day precision from 0.073 to 1.914 and Inter-day precision from 0.155 to 1.914 for Meropenem and 0.505 to 2.068 and 0.176 to 0.2068 for Tazobactam respectively. Intra- day and Inter-day precision within the acceptable range ± 2, indicative of good method precision. The data were represented in **Table 9**.

Assay

In assay studies the % recovery of dutasteride from API and dosage form were 99.79 % and 99.69 % for Meropenem and 99.33 % and 98.45 %. for Tazobactam. The data were represented in **Table 10.**

Recovery Studies

In recovery studies results: data were represented in **Table.11**

Recovery level1 (50% level)

For meropenem 99.31% and For Tazobactam 99.42%

Recovery level 2 (100% level)

For meropenem 99.53 % and For Tazobactam 100.07%

Recovery level 3 (150% level)

For meropenem 99.02 % and For Tazobactam 99.22%The

Degradation studies

Two degradation products were found under peroxide condition; The drug was exposed to 1% H_2O_2 at room temperature for 1 hr. degradation product at RT: 4.41 and 2.68 min. The dry sample of drug (1 mg) was subjected to UV irradiation for one Hrs. The drug was than dissolved in methanol. This solution was filter through a 0.45-µm syringe filter and analyzed by HPLC. It did not produce any signal for degraded products; indicate that the drug is stable under UV irradiation. The data were represented in **Table 12** and the chromatogram were represented in **Fig. 8-11.**

Limit of detection and Limit of quantitation

LOD and LOQ of described method were observed as 0.820 µg/ml and 2.487 µg/ml for Meropenem and 1.059 µg/ml and 3.210 µg/ml for Tazobactam based on the SD of response and slope. The data were represented in **Table 13.**

Ruggedness

The assay result with analyst #1 and Analyst #2 were % Assay = 99.84% (%RSD = 1.20%) and % Assay = 99.04% (%RSD = 2.05) respectively for Meropenem and % Assay = 99.71% (%RSD = 1.32%) and % Assay = 98.20% (%RSD = 1.01) respectively for Tazobactam. The data were represented in **Table 14**.

Robustness

When the wave length was adjusted to 228 and 232 the retention time of Meropenem and Tazobactam were 9.30 & 9.32 min. and 14.15 & 14.14 respectively. When the flow rate was changed ± 0.1 unit the retention time of Meropenem and Tazobactam were 8.58 & 11.42 min. and 13.00 & 17.36 respectively. The data were represented in **Table 15.**

CONCLUSION

The developed HPLC method was found to be stability indicating as it achieved separation of both the drug combination products from their degraded products formed under stress conditions. The methods could be considered superior in comparison with the previously reported methods. The apparatus and reagents used are easily accessible even for the simple laboratories and the procedures do not involve any critical reaction. This method may be successfully applied for routine and quality control analysis of combined formulations. the proposed method is simple accurate and precise for the simultaneous estimation of Meropenem and Tazobactam was developed and validated as per ICH guidelines.

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REFERENCES

1. Gupta V., Jain K., et. al. (2012). Development and validation of HPLC method, Int. Res J Pharm, App Sci., 17-25.
2. Kazakevich Y., Lobrutto R., (2007). HPLC for Pharmaceutical Scientists, John Wiley & Sons, New Jersey.
3. Ahuja H., Rasmussen H., (2007). Pharmaceutical separation science and Technology. Elsevier, New York
4. Rao B.V., Sowjanya G.N., (2015). Review on stability indicating HPLC method development. WJPAPS, 405-423,
5. Shinde G., Goodge R.K., et al.(2020). A Review on Advance in UV Spectroscopy, Research journal of Science and Technology, ISSN: 2349-2988.
6. More D., Ways' S., Zan Zane N., et al. (2023). A Review paper on UV Visible spectroscopy & It's pharmaceutical application, ISSN: 2320-2882.
7. International Conference on Harmonization, Q1A (R2), Stability testing of new drug substances and products, Geneva; **2003**
8. International Conference on Harmonization of Technical Requirement for Human use, Validation of analytical procedure; Text and Methodology, Q2 (R1)- **2005**
9. Patel S.A. & Patel S.R. (2013). Development and Validation of RP-HPLC Method for Simultaneous Estimation of Meropenem and Sulbactam Sodium in Combined Dosage Form. International Journal of Pharma Medix India. Page No. 336-346.
10. Patel BA, Singh K & Nilak PB. (2014). International Journal of Biological & Pharmaceutical Research. Page No. 389-395.
11. Sharma S, Agrawal N, Singh J and Shukla SK. (2015). Qualitative analysis of 5th Generation of Carbapenem Antibiotics by UV Spectrophotometric Method. Research Journal of Chemical Sciences. Page No. 35-39.
12. Ramona K, Kavitha M and Rathbone JM et al. (2014). 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. Int. J. Pharm. Sci. Page No. 149-152.
13. Judyta CP, Magdalena P., et al. (2013). Solid-state stability study of meropenem – solutions based on spectrophotometric analysis. Chemistry Central Journal. Page No. 1-9.
14. Patel JB, Patel NS, Patel BD, and Patel. (2014). HPLC for simultaneous estimation of meropenem and sulbactam sodium in pharmaceutical dosage form. Spectrochemical Acta Part A: Molecular and Biomolecular Spectroscopy, Page No. 292–299.
15. Judyta CP, Daria SP, Magdalena P, Piotr L, Przemysław Z and Piotr G. (2015). Stability, compatibility and microbiological activity studies of meropenem–clavulanate potassium The Journal of Antibiotics, Page No. 68: 35–39.
16. Srinivasa R N., Saraswathi K., et al. (2011). RP- HPLC and Visible Spectrophotometric methods for the Estimation of Meropenem in Pure and Pharmaceutical Formulations. Vol.3. Page No. 605-609.
17. Mendez S L., Steppe M., Shapoval E S. (2003). Validation of UV-HPLC method for the determination of meropenem in pharmaceutical dosage form. J Pharm Biomed Anal. Page no. 947-54.
18. Chang Y., Chou M.H., M.F. Lin. (2002). Determination and pharmacokinetic study of meropenem in rat bile using on-line micro dialysis and liquid chromatography. 961 119–124.
19. Utpal., Ayan D., Sitesh C. B., Tapan K. P. (2011). Estimation of Meropenem in Human Plasma by HPLC-UV and its Application in Comparative Bioavailability Study. ISSN 2229 – 6867, Vol 1:6
20. Narola B., Singh A., Mitra A. (2011). Validated Reverse Phase HPLC Method for the Determination of Disodium EDTA in Meropenem Drug Substance with UV-Detection using Precolumn Derivatization Technique: Volume 6 Page No. 7–14.
21. Keiko K., Kazuo K., Kayo K. (2010). HPLC Method for Measuring Meropenem and Biapenem Concentrations in Human Peritoneal Fluid; Vol. 48, 406- 411.
22. Toshihiro K., Itaú F., (2005). Determination of Meropenem by Capillary Electrophoresis Using Direct Injection of Serum. Vol. 43; 430- 433.
23. Venkateswara R., Ramu G., Sravan M. K., Rambabu C. (2012). Reverse Phase HPLC and Visible Spectrophotometric Methods for the Determination of Meropenem in Pure and Pharmaceutical Dosage Form. Vol.4, Page No. 957-962.
24. Vaishali A. Shah, Sapna M. Rathod, Rajesh R. Parmar, Dushyant A. Shah (2012). Development and Validation of Analytical Method for Meropenem in Pharmaceutical Dosage Form. (ISSN): 2249-6807 503-509.
25. Lee H., Shim O., et al (1996). HPLC Determination of Meropenem in Rat Plasma Using Column-Switching. Vol. 42, Page No. 405.

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