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STABILITY AND ANALYSIS OF MEROPENEM AND β-LACTAMASE INHIBITORS IN INJECTABLE PREPARATIONS

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ABSTRACT

The aim of present work was to develop and validate a common RP-HPLC method that can be employed both for fixed dose combination of Meropenem with Sulbactam or Tazobactam that are vital in the treatment of resistant infections. The objective was to study stability of Meropenem injection in combination with Sulbactam or Tazobactam in aqueous reconstituted solution at room temperature and to study stability of marketed formulation of Meropenem and Sulbactam injection in two intravenous fluids at room temperature. In RP-HPLC method, chromatographic separation was achieved on Phenomenex, Phenyl Hexyl (250×4.6 mm, 5µm) column using Acetonitrile: Phosphate buffer solution pH 3.5 (7.5+92.5, v/v) as the mobile phase with UV detection at 220 nm. Both the drugs were subjected to acidic and alkaline hydrolysis and oxidative stress individually and in combination. RP-HPLC method showed adequate linearity in the concentration range of 0.072-0.168 mg/mL for Meropenem, 0.036-0.084 mg/mL for Sulbactam and 0.018-0.042 mg/mL for Tazobactam. The method successfully separated the Meropenem and Sulbactam or Tazobactam from their potential degradation products formed under stress conditions. Stability of reconstituted aqueous solution of Meropenem and Sulbactam as well as Meropenem and Tazobactam was studied and found good for about 4 h Stability of reconstituted solution of Meropenem and Sulbactam injection in normal saline and 5%dextrose intravenous fluid was also studied. The stability of Meropenem was higher in normal saline than dextrose injection.

KEYWORDS: Meropenem, Sulbactam, Tazobactam, High performance liquid chromatography, Validation, Stability study.

INTRODUCTION

Meropenem is chemically (4R,5S,6S)-3-[[(3S,5S)-5-(dimethylcarbamoyl)-3-pyrrolidinyl]thio]-6-[(1R)-1-hydroxyethyl]-4-methyl-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid[1] (as shown in Fig. 1), It is available as Meropenem trihydrate and belongs to a group of carbapenem, β-lactam antibiotic. It causes rapid bacterial cell death by covalently binding to penicillin-binding proteins (PBPs) involved in the biosynthesis of mucopeptides in bacterial cell walls. Meropenem has extended antibacterial spectrum, but with the widespread use of the drug, Acinetobacter baumannii has developed resistance against the drug. Meropenem is therefore combined with Sulbactam, an irreversible β – lactamase inhibitor to expand the activity of Meropenem against resistant strains.[2] Sulbactam is chemically (2S, 5R)-3, 3-dimethyl-7-oxo-4-thia-1 sazabicyclo[3.2.0]heptane-2-carboxylic acid 4,4-dioxide[1] (as shown in Fig. 2). It is available as Sulbactam sodium. Likewise, Meropenem is also combined with Tazobactam, other irreversible β – lactamase inhibitor.[3] Tazobactam is chemically (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[1] (as shown in Fig. 3). It is available as Tazobactam sodium.

Meropenem and Sulbactam powder for injection is approved for marketing in India by CDSCO in 2011. Meropenem and Tazobactam powder for injection is under review of new drugs advisory committee of CDSCO for marketing in India. Meropenem injection is official in all pharmacopoeias[4-8] (IP, BP, USP, EP, JP) whereas combination of Meropenem with Sulbactam/Tazobactam is not official in any pharmacopoeia. Literature survey reveals UV and HPLC methods for estimation of Meropenem singly[9-14] and in combination with Sulbactam[15-18] and no method for estimation of Meropenem in combination with Tazobactam.

Meropenem injection is used as slow intravenous injection as such and diluted in intravenous infusions.[19] This necessitates stability study of combination injections as reconstituted injection and diluted in usual intravenous fluids. Stress testing was also done with a view to form potential degradation products and develops stability indicating assay method that can be employed for both Meropenem and Sulbactam/Tazobactam combination preparations that are vital in the treatment of resistant infections.

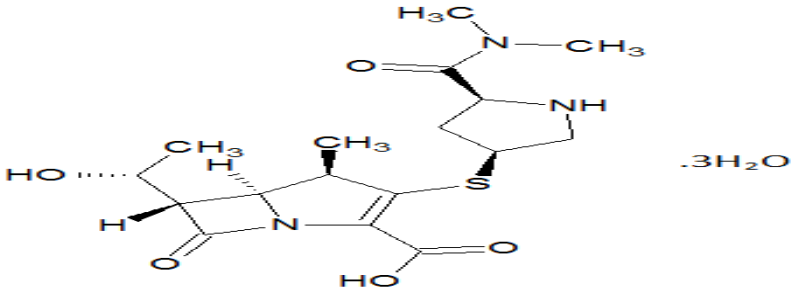

Fig. 1: Meropenem trihydrate.

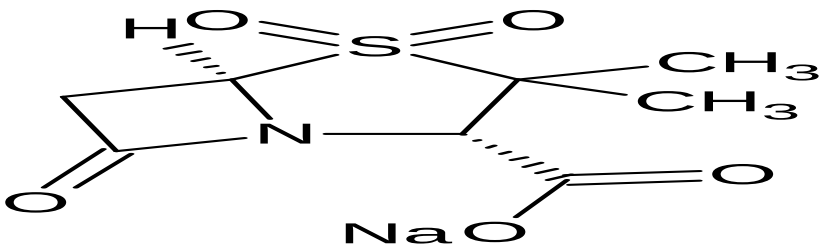

Fig. 2: Sulbactam sodium.

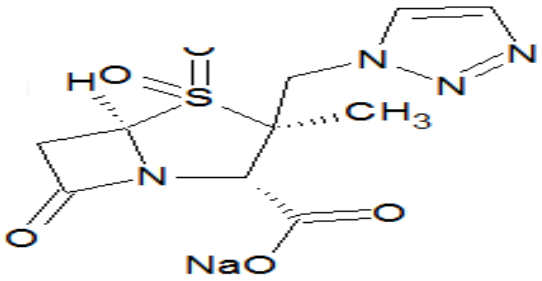

Fig. 3: Tazobactam sodium.

MATERIALS AND METHODS

Reagents

a) Meropenem (Gift sample from Montage laboratory Ltd., Himatnagar, India).

b) Tazobactam sodium and Sulbactam sodium (Gift sample from Akums drugs and pharmaceuticals Ltd., Haridwar, India).

c) Merotec-XP (1.5 g) dry powder injection procured from Indian market.

Apparatus

a) HPLC.- HPLC was performed onShimadzu LC-2010 CHT with a variable wavelength detector (VWD) at 220 nm.

b) Analytical balance.-AcculabALC-210.4 (Huntingdon Valley, PA).

c) Ultrasonicator.-EN 0.03US, Enertech Fast Clean (Mumbai, India).

d) pH meter.-Thermo Electron Crop.(Pune, India).

Reagents

a) Phosphate buffer solution (PBS), 0.01M, pH 3.5.-Dissolve 1.36 g anhydrous KH_2PO_4 in water, then adjust the pH with 1% H_3PO_4 and dilute to 1 L. Filter through 0.45µfilter under vacuum and sonicate for 15 min.

b) Standard solution of Meropenem.-Stock solution of Meropenem was prepared in water to give 12 mg/mL concentration. This was diluted 1 in 100 to give standard solution of 0.12 mg/mL concentration.

c) Standard solution of Sulbactam.-Stock solution of Sulbactam was prepared in water to give 6 mg/mL concentration. This was diluted 1 in 100 to give standard solution of 0.06mg/mL concentration.

d) Standard solution of Tazobactam.-Stock solution of Tazobactam was prepared in water to give 3 mg/mL concentration. This was diluted 1 in 100 to give standard solution of 0.03 mg/mL concentration.

e) Standard solution of Meropenem and Sulbactam.-1 mL stock solution of Meropenem and 1 mL stock solution of Sulbactam prepared as above were transferred to 100 mL volumetric flask and diluted up to the mark to give0.120 mg/mL of Meropenem and 0.06 mg/mL of Sulbactam concentration.

f) Standard solution of Meropenem and Tazobactam.-1 mL stock solution of Meropenem and 1 mL stock solution of Tazobactam prepared as above were transferred to 100 mL volumetric flask and diluted up to the mark to give 0.120 mg/mL of Meropenem and 0.03 mg/mL of Tazobactam concentration.

g) Simulated preparation of dry powder injection.-Required quantity of Meropenem trihydrate equivalent to 1g Meropenem and Tazobactam sodium equivalent to 250 mg of Tazobactam were taken and blended properly.

Preparation of samples for forced degradation study

a) Acid Hydrolysis.-Forced degradation was performed by taking 1 mL stock solution of respective drug/standard drug combination. To this was added 1mL of 0.1 N HCL and kept for 1 h. This was neutralized with 0.1N NaOH and diluted to 100 mL with water to yield 0.120 mg/mL of Meropenem and 0.06 mg/mL of Sulbactam or 0.03 mg/mL of Tazobactam.

b) Alkali Hydrolysis.-Forced degradation was performed by taking 1 mL stock solution of respective drug/standard drug combination. To this was added 0.5 mL of 0.1 N NaOH and kept for 0.03 min. This was neutralized with 0.1N HCL and diluted to 100 mL with water to

yield 0.120 mg/mL of Meropenem and 0.06 mg/mL of Sulbactam or0.03 mg/mL of Tazobactam.

c) Oxidative Degradation.-Forced degradation was performed by taking 1 mL stock solution of respective drug/standard drug combination. To this was added 1mL of 1% H_2O_2 and kept for 0.03 min. This was diluted to 100 mL with water to yield 0.120 mg/mL of Meropenem and 0.06 mg/mL of Sulbactam or 0.03 mg/mL of Tazobactam.

Preparation of reconstituted injection for assay and stability study

a) Reconstituted injection of Meropenem and Sulbactam for assay and stability study.-Content of the injection of one vial (1000+500 mg) were diluted in 20 mL of water for injection (to give 50+25 mg/mL). This reconstituted injection was diluted at time interval of 0, 1, 2, 3 and 4 h with water to yield 0.120+0.06 mg/mL concentration of Meropenem and Sulbactam.

b) Reconstituted injection of Meropenem and Tazobactam for assay and stability study.-Content of the injection of one vial (1000+250 mg) were diluted in 20 mL of water for injection (to give 50+12.5 mg/mL). This reconstituted injection was diluted at time.

Optimized Chromatographic Conditions

a) HPLC system.- LC 2010CHT, Shimadzu UV detector

c) Stationary phase.-Phenomenex interval of 0, 1, 2, 3 and 4 h with water to yield 0.120+0.03 mg/mL concentration of Meropenem and Tazobactam.

d) Solution of Meropenem for stability study.-Required quantity of Meropenem trihydrate equivalent to 1000 mg of Meropenem was diluted in 20 mL of water for injection to give 50 mg/mL concentration. This was diluted at time interval of 0, 1, 2, 3 and 4 with water to yield 0.120 mg/mL concentration of Meropenem.

Dilution of reconstituted injection into intravenous fluids

a) Dilution of reconstituted injection into normal saline solution.-Required quantity of powder injectable powder preparation equivalent to 250 mg Meropenem and 125 mg Sulbactam (1/4 average weight) was transferred to 250 mL volumetric flask. This dissolved and diluted with normal saline to make 250 mL to give 1.0+0.5 mg/mL concentration. This was diluted at time interval of 0, 1, 2, 3, 5 and 7 h with water to give 0.120+0.06 mg/mL concentration of Meropenem and Sulbactam.

b) Dilution of reconstituted injection into 5% Dextrose solution.-Required quantity of powder injectable powder preparation equivalent to 250 mg Meropenem and 125 mg

Sulbactam (1/4 average weight) was transferred to 250 mL volumetric flask. This was dissolved and diluted with 5% Dextrose to make 250 mL to give 1.0+0.5 mg/mL concentration. This was diluted at time interval of 0, 1, 2, 3, 5 and 7 h with water to give 0.120+0.06 mg/mL concentration of Meropenem and Sulbactam.

RESULTS AND DISCUSSION

a) Luna phenyl hexyl (250 mm × 4.6 mm,5 µm)

b) Mobile phase.-Acetonitrile: PBS pH 3.5 (7.5+92.5, v/v)

c) Flow rate.-1.0 mL/min

d) Detection wavelength.-220 nm

e) Column temperature.-room temperature

f) Total run time.-20 min.

g) Injection volume.- 10 µl

h) Diluent.-All Final Solution of Test and Standard were done with HPLC grade water

Chromatograms of standard drug mixture of Meropenem and Sulbactam is shown in Fig. 4 and Chromatograms of standard drug mixture of Meropenem and Tazobactam is shown in Fig. 5. System suitability parameters for chromatogram of standard mixtures are shown in table 1.

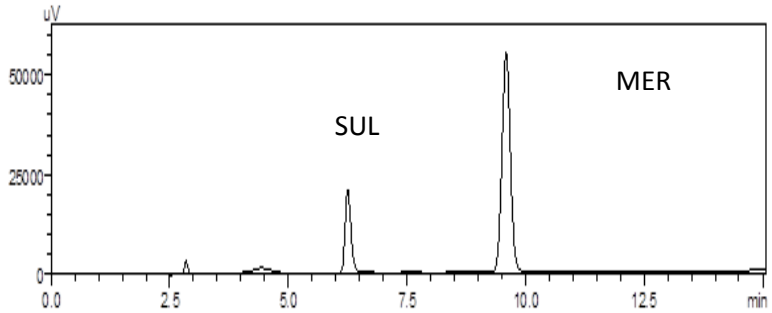

Fig. 4: Chromatogram of Meropenem (120 µg/mL) and Sulbactam (60 µg/mL).

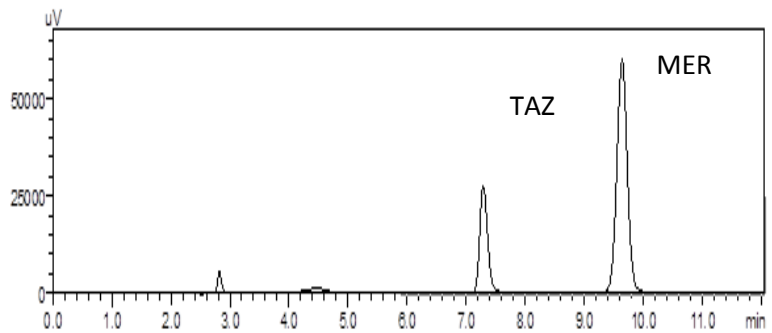

Fig. 5. Chromatogram of Meropenem (120 µg/mL) and Tazobactam (30µg/mL).

Table. 1: System suitability parameters for chromatogram of standard mixture.

Parameters	MER and TAZ		MER and SUL	
	MER	TAZ	MER	SUL
Retention time	9.36±0.07	7.21±0.08	9.35±0.07	6.27±0.04
Theoretical plates	13584.8±14.67	13481.2±20.02	13459.6±15.01	11612.3±24.65
Area	736297.0±2566.5	255087.7±1323.9	735362.3±2498.4	201679.7±991.4
Tailing factor	1.04±0.015	1.24±0.020	1.03±0.014	1.34±0.016
Capacity factor	2.63±0.003	1.74±0.009	2.62±0.003	1.33±0.005
Resolution	8.07±0.060	6.14±0.016	8.08±0.059	3.72±0.012

**Average of three determination.*

Results of forced degradation study

From degradation study, it was found that Meropenem degraded extensively in alkaline and oxidative conditions and degraded significantly in acidic condition (as shown in table 2, 3). Hydrolytic impurity of Meropenem was observed at same relative retention time for acid, alkali degradation and this impurity was also found in marketed formulation (as shown in Fig. 6, 7, 8). Two degradation products were found under peroxide oxidation condition; one of these degradation products showed same relative retention time as hydrolytic degradation product (as shown in Fig. 9, 10, 11).

Sulbactam was extensively degraded in alkali and oxidative conditions and significantly degraded in acidic condition (as shown in table 2). Sulbactam gave degradation product/s under acidic and alkaline hydrolysis that eluted very early and coincided with the blank peak (as shown in Fig. 6, 7).The degradation products of Sulbactam under oxidative condition could not be judged properly as the blank gave heightened response and this could possibly hide the oxidative impurities that eluted early with blank (as shown in Fig. 9, 10).

Tazobactam was extensively degraded in alkali and oxidative condition and significantly degraded in acidic condition (as shown in table 3). Tazobactam gave degradation product/s under acidic and alkaline hydrolysis that eluted very early and coincided with the blank peak (as shown in Fig. no 6, 8). When the attempts were made to separate these peaks by using gradient method, the sharpness of Tazobactam peak was distorted. The degradation products of Tazobactam under oxidative condition could not be judged properly as the blank gave heightened response and this could possibly hide the oxidative impurities that eluted early with the blank (as shown in Fig. 9, 11).

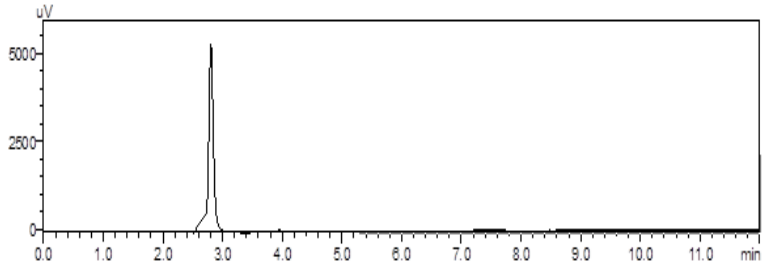

Fig. 6. Chromatogram of blank of 0.1N NaOH

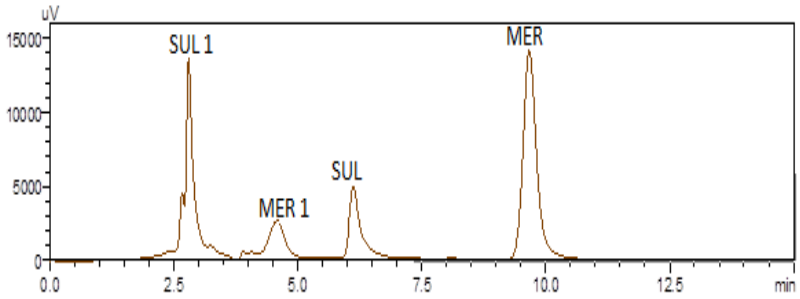

Fig. 7. Chromatogram of Meropenem (120 µg/mL) and Sulbactam (60 µg/mL) in alkali hydrolysis.

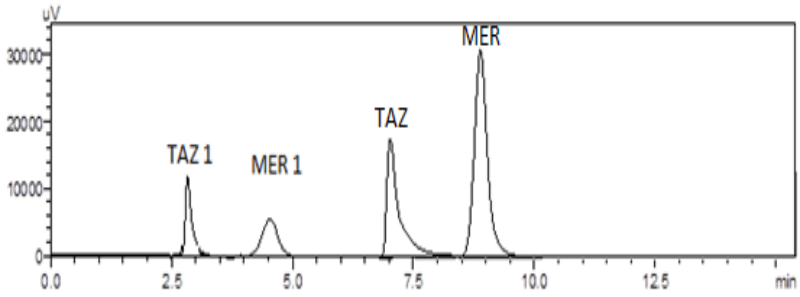

Fig. 8. Chromatogram of Meropenem (120 µg/mL) and Tazobactam (30 µg/mL) in alkali hydrolysis.

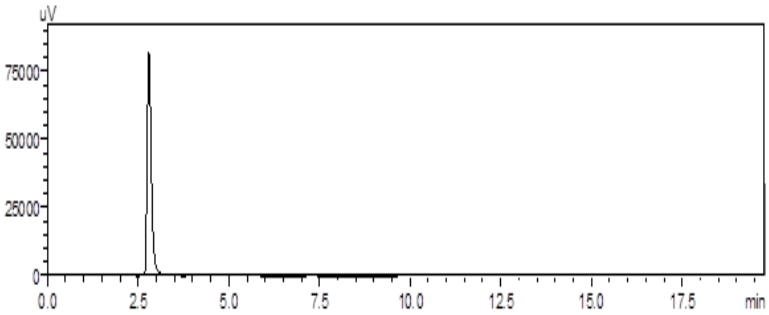

Fig. 9. Chromatogram of blank of 1% H_2O_2.

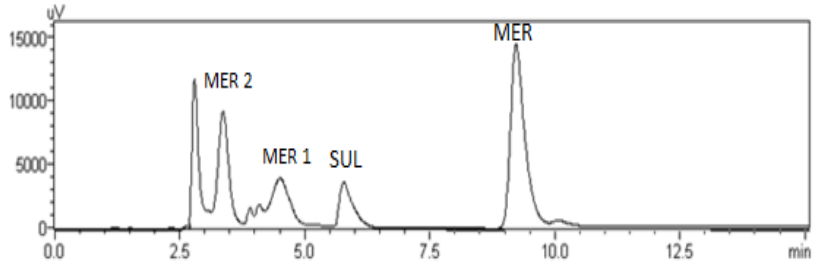

Fig. 10. Chromatogram of Meropenem (120 µg/mL) and Sulbactam (60 µg/mL) in oxidative degradation.

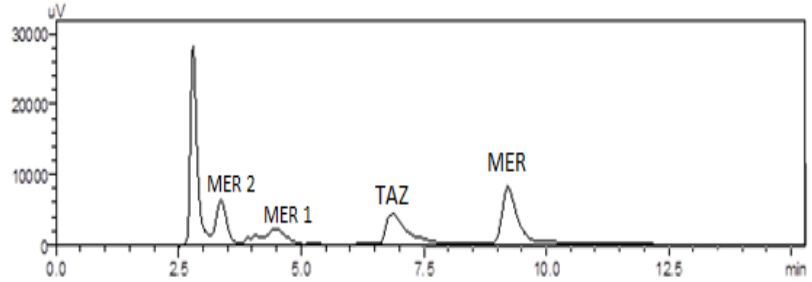

Fig. 11. Chromatogram of Meropenem (120 µg/mL) and Tazobactam (30 µg/mL) in oxidative degradation.

Table. 2: Results of forced degradation study of meropenem and sulbactam.

Sr. No.	Stress type	Condition	No. of degradation peaks and RRT*		% degradation			
					Individual		Combination	
			MER	SUL	MER	SUL	MER	SUL
1	Acid hydrolysis	0.1 N HCl at room temperature for 1 h	MER 1 (2.06)	SUL 1 (3.45)	21.67	10.69	22.57	12.98
2	Alkali hydrolysis	0.05 N NaOH at room temperature for 30 min	MER 1 (2.10)	SUL 1 (3.38)	47.29	43.94	49.12	45.82
3	Oxidative degradation	1% H_2O_2 at room temperature for 30 min	MER 1 (2.07) MER 2 (2.78)	-	45.35	41.51	48.12	39.86

Table. 3: results of forced degradation study of meropenem and tazobactam.

Sr. No.	Stress type	Condition	No. of degradation peaks and RRT*		% Degradation			
					Individual		Combination	
			MER	TAZ	MER	TAZ	MER	TAZ
1	Acid hydrolysis	0.1 N HCl at room temperature for 1 h	MER 1 (2.065)	TAZ 1 (3.487)	23.2	11.3	25.7	12.7
2	Alkali hydrolysis	0.05 N NaOH at room temperature for 30 min	MER 1 (2.107)	TAZ 1 (3.493)	51.03	44.60	56.16	48.16
3	Oxidative degradation	1% H_2O_2 at room temperature for 30 min	MER 1 (2.07) MER 2 (2.78)	-	47.26	44.12	48.59	41.25

** Relative retention time (RRT) reported with respect to farthest eluted Meropenem peak.*

Method Validation

Specificity.-The method was found to be specific with respect to excipients and potential degradation products all the drugs were adequately separated from one another and potential degradation products as indicated by good resolution factor for all analyte peaks.

Method Validation for Meropenem and Sulbactam

a) Linearity and Range.-The method was linear at range of 0.072-0.168 mg/mL of Meropenem and 0.036-0.084 mg/mL of Sulbactam (as shown in Fig. 12, 13).

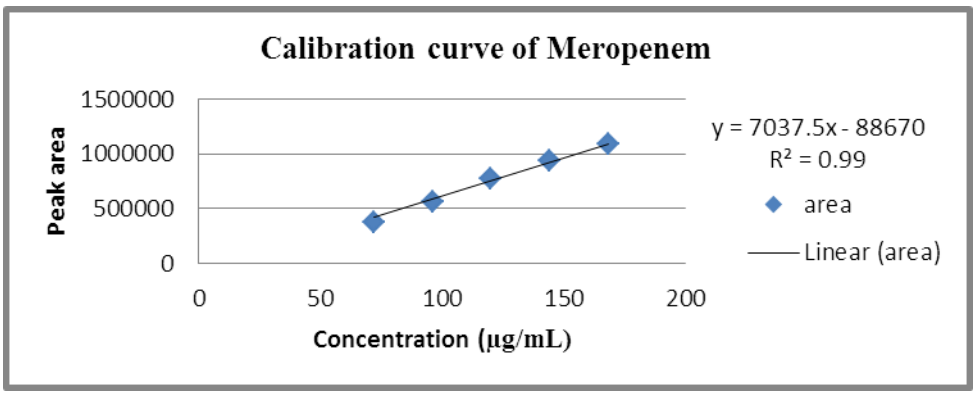

Fig. 12. Calibration curve of Meropenem in combination with Sulbactam.

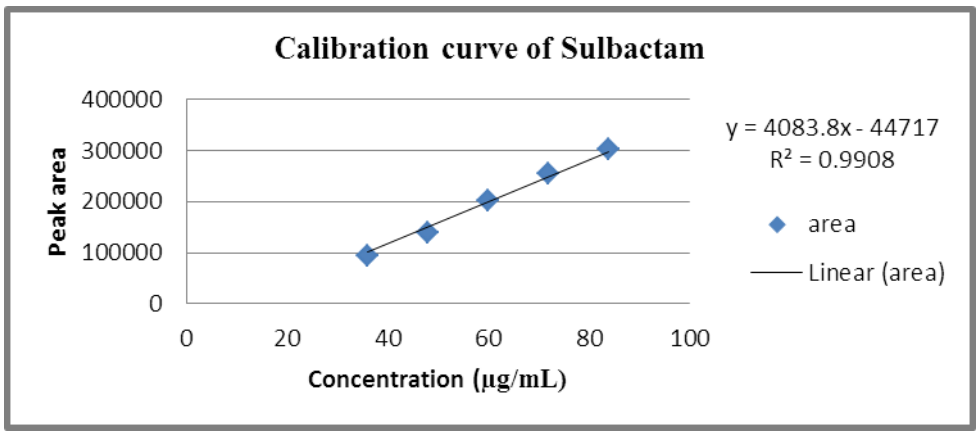

Fig. 13. Calibration curve of Sulbactam.

Table. 4: Linearity results for meropenem and sulbactam.

Regression analysis	Meropenem	Sulbactam
Regression equation	y = 7037.5x-88670	y = 4083.8x-44717
Correlation co-efficient	0.99	0.9908

b) Precision.-The % RSD for intraday and inter-day precision was found to be 0.31-0.57% and 0.75-1.08% for Meropenem and 0.39-0.73% and 0.62-1.16% for Sulbactam.

c) Accuracy.-The range of the method was established at 80 to 120% by determining linearity, precision and accuracy at three concentration level of 0.096, 0.120 and 0.144 mg/mL of Meropenem and 0.048, 0.06 and 0.072 mg/mL of Sulbactam corresponding to 80, 100 and 120% of target concentration. Accuracy was found between 98 to 102% by standard spiking method. Percentage recovery for Meropenem was 98.04-101.87%, while for Sulbactam, it was found to be in range of 98.14-99.65%.

d) Limit of Detection and Limit of Quantification.-The Limit of Detection (LOD) was found to be 1.19 and 1.52µg/mL, while the Limit of Quantification (LOQ) was found to be 3.61 and 4.61µg/mL for Meropenem and Sulbactam respectively.

e) Analysis of Marketed Formulation (Merotec-XP) by proposed method.-Applicability of the proposed method was tested by analyzing the commercially available marketed formulation (Merotec-XP). % Assay was found to be 98.38%±0.96 and 98.46%±0.89 for Meropenem and Sulbactam (as shown in Table 5).

Table. 5: Analysis of marketed formulation of meropenem and sulbactam.

Injection formulation	**Lable claim (mg)**		**% Assay*± RSD**	
Merotec –XP dry powder injection of MER and SUL	MER	SUL	MER	SUL
	1000	500	98.38±0.96	98.46±0.89

**Average of six determinations*

f) Robustness.-The method was found to be robust as assay result was not affected much by changing the flow rate ±10%, Mobile phase organic modifier ±7%, pH ±0.5% as measured by relative difference with unmodified conditions and original assay as well as overall RSD. Overall %RSD was found to be 0.54-1.59 and 0.61-1.45 for Meropenem and Sulbactam respectively.

Method Validation for Meropenem and Tazobactam

a) Linearity and range.-The method was linear at range of 0.072-0.168 mg/mL of Meropenem and 0.018-0.042 mg/mL of Tazobactam (as shown in Fig. 14, 15).

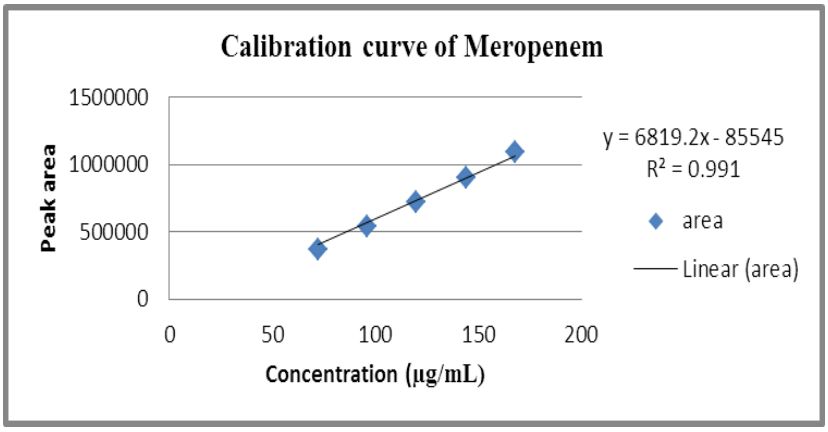

Fig. 14. Calibration curve of Meropenem in combination with Tazobactam.

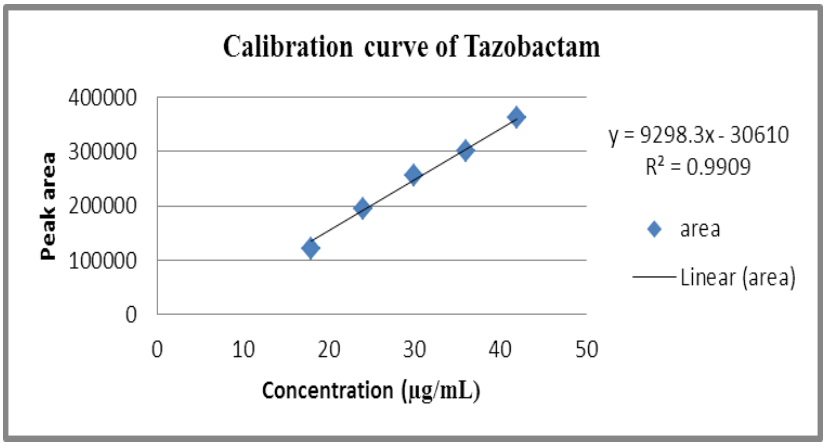

Fig. 15. Calibration curve of Tazobactam.

Table 6: Linearity results for meropenem and tazobactam

Regression analysis	Meropenem	Tazobactam
Regression equation	y = 6819.2x-85545	y = 9298.3x-30610
Correlation co-efficient	0.991	0.9909

b) Precision.-The % RSD for intraday and inter-day precision was found to be 0.34-0.48% and 0.63-0.84% for Meropenem and 0.41-0.79% and 0.71-0.97% for Tazobactam.

c) Accuracy.-The range of the method was established at 80 to 120% by determining linearity, precision and accuracy at three concentration level of 0.096, 0.120 and 0.144 mg/mL of Meropenem and 0.024, 0.03 and 0.0 36 mg/mL of Tazobactam corresponding to 80, 100 and 120% of target concentration. Accuracy was found between 98 to 102%. Percentage recovery for Meropenem was 98.04-101.87%, while for Tazobactam, it was found to be in range of 98.14-99.65%.

d) Limit of Detection and Limit of Quantification.-The Limit of Detection (LOD) was found to be 1.42 and 0.53µg/mL, while the Limit of Quantification (LOQ) was found to be 4.32 and 1.60µg/mL for Meropenem and Tazobactam respectively.

e) Analysis of synthetic mixture by proposed method.-Applicability of the proposed method was tested by analyzing the synthetic mixture of Meropenem and Tazobactam. Results as % Assay was found to be 100.57%±0.81 and 99.44%±0.96 for Meropenem and Tazobactam (as shown in Table 7).

Table. 7: Analysis of synthetic mixture of meropenem and tazobactam.

Injection formulation	Lable claim (mg)		% Assay*± RSD	
Synthetic mixture of MER and TAZ	MER	TAZ	MER	TAZ
	1000	250	100.57±0.81	99.44±0.96

**Average of six determinations*

f) Robustness.-The method was found to be robust as assay result was not affected much by changing the method parameter as above under section 3.4.2.6. Overall %RSD was found to be 0.64-1.91 and 0.79-1.19 for Meropenem and Tazobactam respectively.

Results of stability study of reconstituted solution

Results of stability study for Meropenem and Sulbactam reconstituted solution reveals that the shelf life found to be 5.25 and 3.248 h for Meropenem and Sulbactam respectively (as shown in table 8 and 9) (log C vs Time plot shown in Fig. 16, 17).

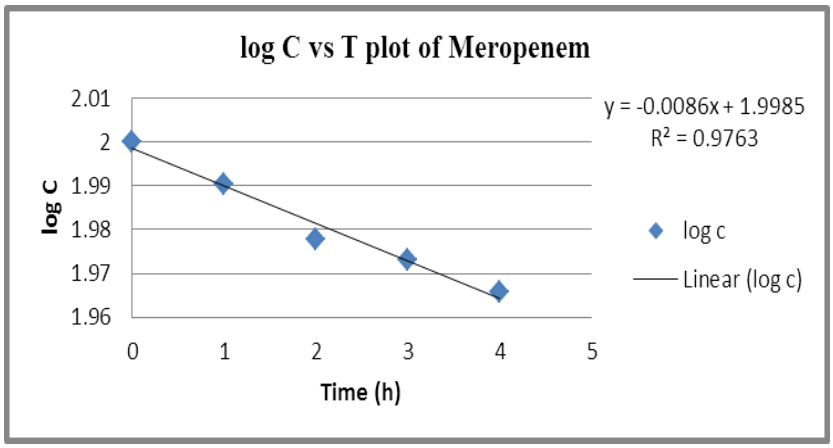

Fig. 16: log C vs T plot of Meropenem in combination with Sulbactam in HPLC grade water

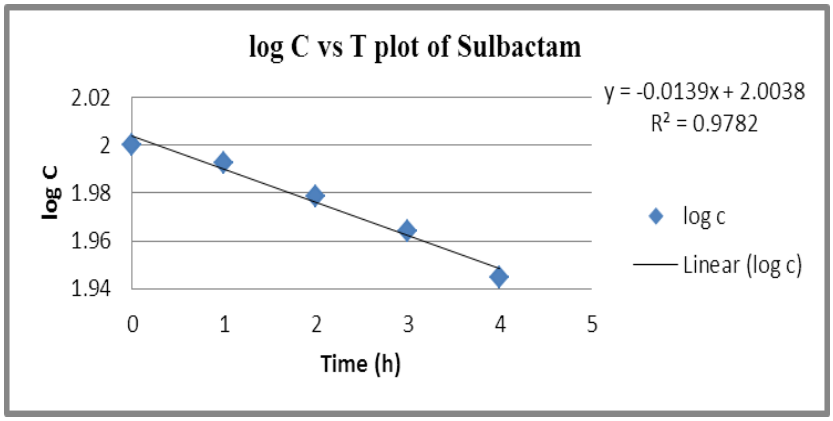

Fig. 17: log C vs T plot of Sulbactam in HPLC grade water.

Table. 8: Results of stability study for meropenem and sulbactam reconstituted solution.

Time (h)	Meropenem			Sulbactam		
	Peak Area	% Assay (C)	log C	Peak Area	% Assay (C)	log C
0	890001	100	2.000000	245241	100	2.0000000
1	871005	97.80	1.990339	241093	98.30	1.9925535
2	846860	95.00	1.977724	233503	95.21	1.9786826
3	836741	94.01	1.973174	225744	92.05	1.9640238
4	822457	92.40	1.965672	216058	88.10	1.9449759

Table. 9: Degradation rate constant and shelf life meropenem and sulbactam reconstituted solution.

Diluent	Meropenem		Sulbactam	
	k value (h^{-1})	$t_{0.9}$ (h)	k value	$t_{0.9}$ (h)
HPLC grade water	0.0198	5.25	0.032	3.248

Equation of first order chemical reaction.

Eq. 1.log C = log C_0 –kt/2.0.033

From plot of log C vs Time.- Eq. 2.k= -slope×2.0.033

Eq. 3. $t_{0.9}$ = 0.104/k

Results of stability study for Meropenem and Tazobactam reconstituted solution

Results of stability study for Meropenem and Tazobactam reconstituted solution reveals that the Shelf life found to be 9.03 and 4.068 h for Meropenem and Tazobactam respectively (as shown in table 10 and 11) (log C vs Time plot shown in Fig. 18, 19).

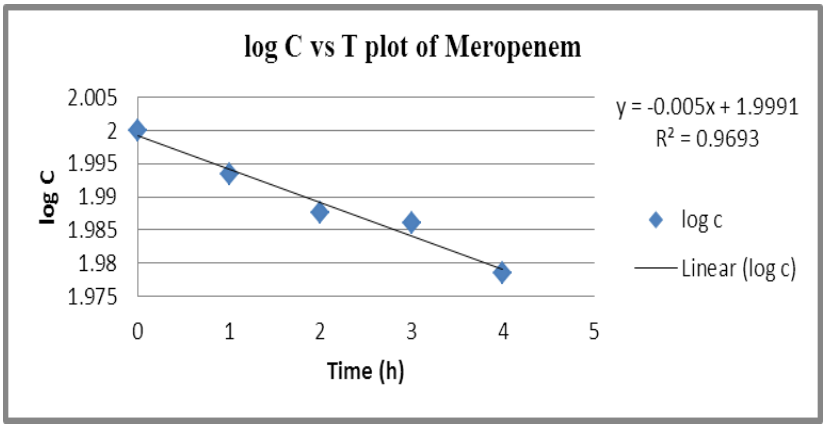

Fig. 18: log C vs T plot of Meropenem in combination with Tazobactam in HPLC grade water.

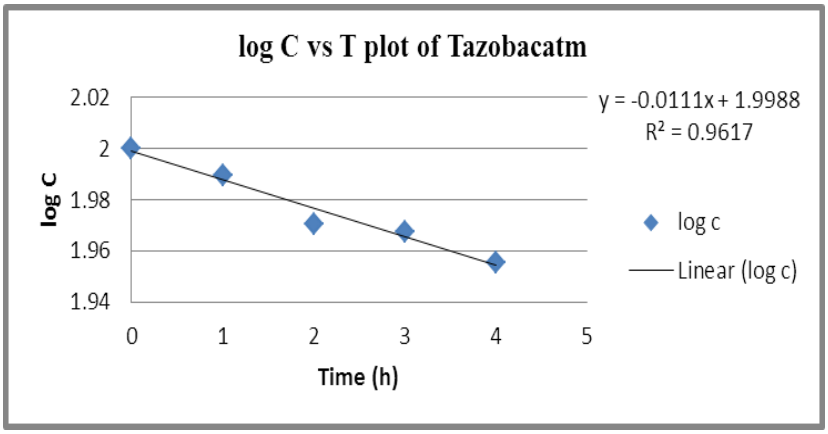

Fig. 19: log C vs T plot of Tazobactam in HPLC grade water.

Table. 10: Results of stability study for meropenem and tazobactam reconstituted solution.

Time (h)	Meropenem			Tazobactam		
	Peak Area	% Assay (C)	log C	Peak Area	% Assay (C)	log C
0	816477	100	2.000000	260482	100	2.0000000
1	804066	98.48	1.993348	254334	97.62	1.9896278
2	793371	97.17	1.987532	243264	93.39	1.9703004
3	790676	96.84	1.986055	241466	92.70	1.9673139
4	777041	95.17	1.978500	235111	90.26	1.9554953

Table. 11: Degradation rate constant and shelf life of meropenem and tazobactam reconstituted solution.

Diluent	Meropenem		Tazobactam	
	k value	$t_{0.9}$ (h)	k value	$t_{0.9}$ (h)
HPLC grade water	0.0115	9.03	0.0255	4.068

Results of stability study for Meropenem aqueous solution (HPLC grade water)

Results of stability study for Meropenem in aqueous solution (HPLC grade water) reveals that the shelf life of Meropenem found to be 7.05 h in aqueous solution (as shown in table 12 and 13) (log C vs Time plot shown in Fig. 20).

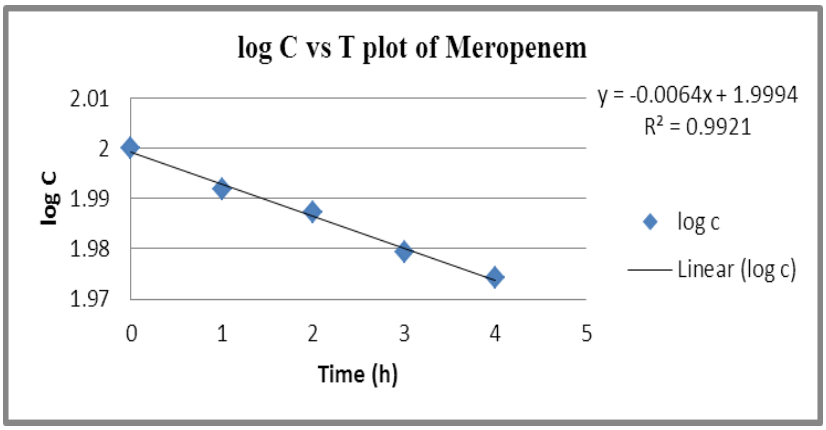

Fig. 20: log C vs T plot of Meropenem in HPLC grade water.

Table. 12: Results of stability study for meropenem in aqueous solution (HPLC grade water).

Time (h)	Meropenem		
	Peak Area	% Assay (C)	log C
0	843435	100	2.000000
1	828000	98.17	1.991979
2	819329	97.14	1.987398
3	804050	95.33	1.979230
4	795021	94.26	1.974327

Table. 13: Degradation rate constant and shelf life of meropenem in aqueous solution.

Diluent	Meropenem	
	k value	$t_{0.9}$ (h)
HPLC grade water	0.0147	7.05

Results of stability study of marketed formulation in intravenous fluids.

Results of stability study reveals that Meropenem degraded significantly in 5% Dextrose ($t_{0.9}$ 2.65 h) than normal saline ($t_{0.9}$ 5.19 h) and Sulbactam degraded somewhat higher in 5% Dextrose ($t_{0.9}$ 2.25 h) than normal saline ($t_{0.9}$ 2.78 h) (as shown in table. 14, 15, 16) (log C vs Time plot shown in Fig. 21, 22, 23, 24).

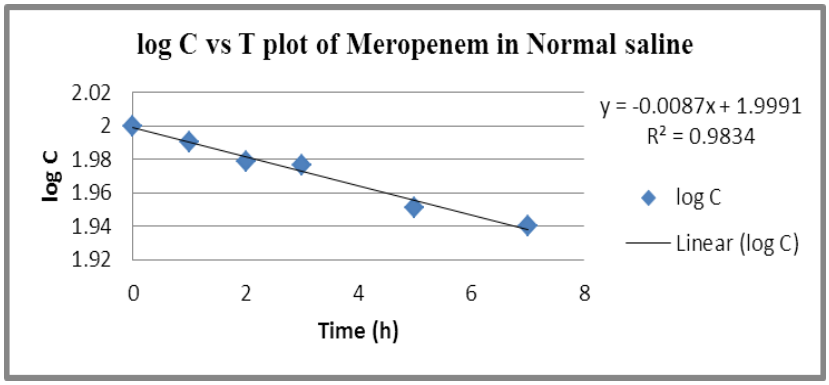

Fig. 21: log C vs T plot of Meropenem in Normal saline.

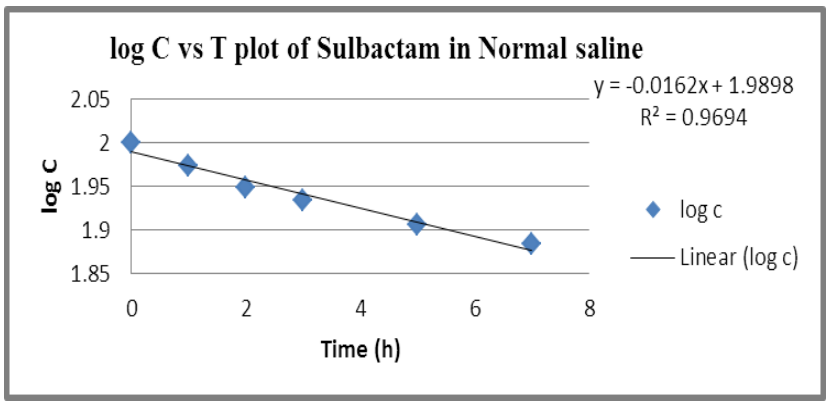

Fig. 22: log C vs T plot of Sulbactam in Normal saline.

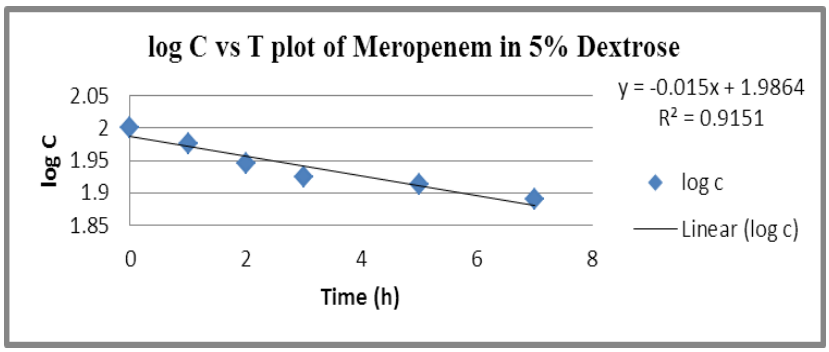

Fig. 23: log C vs T plot of Meropenem in 5% Dextrose.

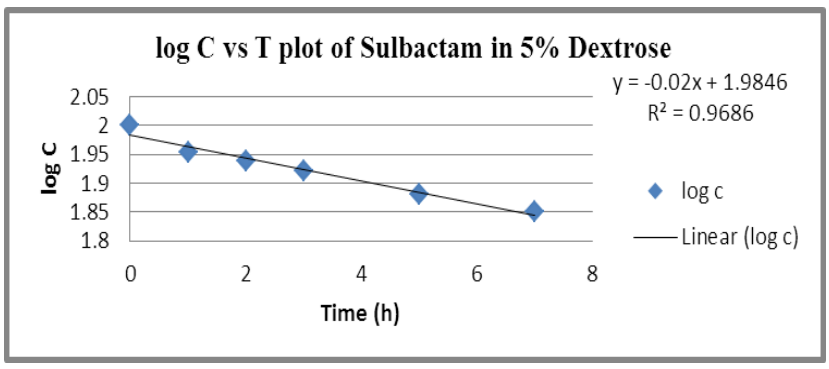

Fig. 24: log C vs T plot of Sulbactam in 5% Dextrose.

Table. 14: Results of stability study of marketed formulation in normal saline.

Time (h)	Meropenem			Sulbactam		
	Peak Area	% Assay (C)	log C	Peak Area	% Assay (C)	log C
0	1018541	100	2.000000	198268	100	2.000000
1	996540	97.84	1.9905164	186827	94.23	1.974189
2	970160	95.25	1.978865	176478	89.01	1.949439
3	965067	94.75	1.9765792	170173	85.83	1.933639
5	910677	89.41	1.9513861	159942	80.67	1.906712
7	887556	87.14	1.9402176	151655	76.49	1.883605

Table 15: Results of stability study of marketed formulation in 5% dextrose.

Time (h)	Meropenem			Sulbactam		
	Peak Area	% Assay (C)	log C	Peak Area	% Assay (C)	log C
0	987786	100	2.000000	215335	100	2.000000
1	934840	94.64	1.976075	189811	90.00	1.954243
2	869646	88.04	1.944680	183056	87.00	1.939519
3	830332	84.06	1.924589	179438	83.33	1.920801
5	802872	81.28	1.912753	163719	76.03	1.880985
7	767411	77.69	1.873262	152952	71.03	1.851258

Table. 16: Degradation rate constant and shelf life of meropenem and sulbactam in intravenous fluids.

Diluent	Meropenem		Sulbactam	
	k value	$t_{0.9}$ (h)	k value	$t_{0.9}$ (h)
Normal Saline solution	0.0200	5.19	0.0373	2.78
5% Dextrose solution	0.0391	2.65	0.0460	2.25

CONCLUSION

The suitable chromatographic (RP-HPLC) method has been developed and validated for the estimation of fixed dose combinations of Meropenem with β-lactamase inhibitors in bulk and in injectable preparations. The method worked well for combination of drug with both Sulbactam and Tazobactam. 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. All method validation parameters were found within the acceptance criteria as per ICH Q2 (R1) guidelines.

Stability of Meropenem in reconstituted injection was adequately good (**t0.9** 7.05 h). This increased somewhat in presence of Tazobactam (**t0.9** 9.03 h) and decreased somewhat in presence of Sulbactam (**t0.9** 5.25 h) Meropenem degraded significantly in 5% Dextrose (**t0.9** 2.65 h) than normal saline (**t0.9** 5.19 h) This is in agreement with published data of

recommended usage period of 4 hand 1 h in normal saline and dextrose respectively. Sulbactam degraded somewhat higher in 5% Dextrose (**t0.9** 2.25 h) than normal saline (**t0.9** 2.78 h). The difference in stability between dextrose and normal saline is less pronounced than that observed in case of Meropenem. Meropenem was found far more stable than Sulbactam in normal saline whereas stability of Meropenem and Sulbactam was almost comparable in dextrose.

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