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“Development and Validation of RP-HPLC Method for Simultaneous Estimation of Meropenem and Sulbactam Sodium in Combined Dosage Form”.

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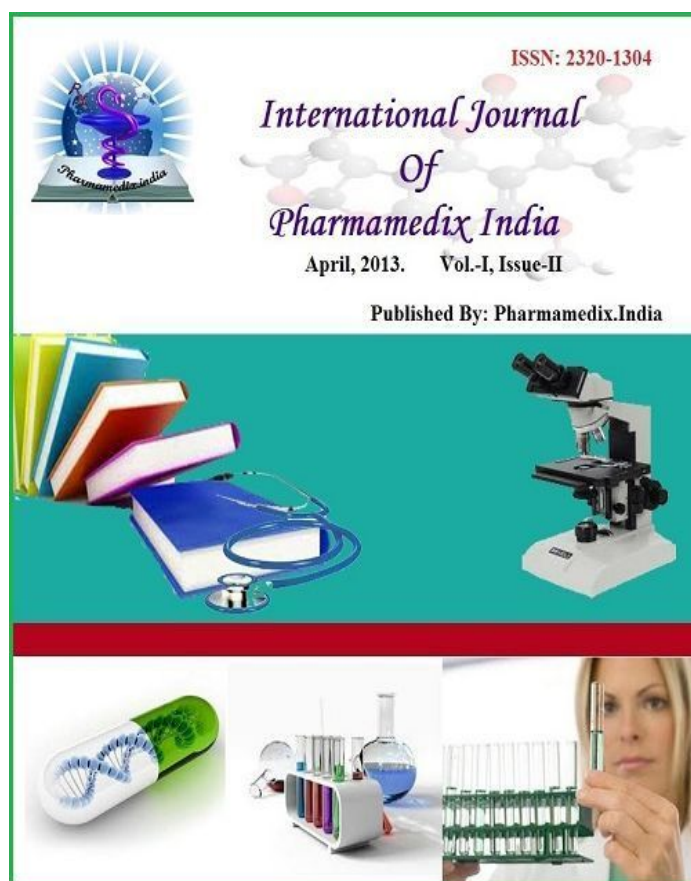
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Abstract:

A simple, precise and accurate reverse phase high performance liquid chromatographic (RP-HPLC) method has been developed and validated for the simultaneous determination of Meropenem and Sulbactam Sodium from formulation. The chromatographic separation was performed on Hypersil C18 column (250 mm × 4.6 mm i.d, 5 µm particle size). Mobile phase consisted of a mixture of phosphate buffer (0.1 M KH₂PO₄, pH : 5), acetonitrile and methanol in the ratio of 90: 05: 05, v/v/v at a flow rate of 1.0 ml/min. The detection wavelength was set at 230 nm. The proposed method was validated for linearity, accuracy, precision, LOD and LOQ. The calibration was linear over the range of 5-50 µg/ml for Meropenem and 5-50 µg/ml for Sulbactam Sodium. The retention times were 4.5 ± 0.004 min for Sulbactam sodium and 6.6 ± 0.008 min for Meropenem. The mean recoveries were 100.4 ± 0.61 and 98.5 ± 0.46 for Meropenem and Sulbactam sodium, respectively. The method can be easily adopted for quality control analysis.

Keywords: Meropenem, Sulbactam sodium, Accuracy, RP-HPLC, Validation.

Introduction:

Meropenem, chemically 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, is an anti-bacterial agent for systemic use^[1]. The bactericidal activity of Meropenem results from the inhibition of cell wall synthesis. Meropenem readily penetrates the cell wall of most gram-positive and gram-negative bacteria to reach penicillin-binding- protein (PBP) targets. It is official in Indian Pharmacopoeia (IP)^[2]. Literature survey reveals UV and HPLC methods for the

estimation of Meropenem in pharmaceutical formulations^[5-10]. Sulbactam is (R)-3, 3-dimethyl-7-oxo-4-thia-1-azabicyclo [3.2.0] heptane-2-carboxylic acid 4, 4-dioxide is a beta-lactamase inhibitor. Sulbactam is an irreversible inhibitor of beta-lactamase; it binds the enzyme and does not allow it to interact with the antibiotic. It is official in United States Pharmacopoeia (USP)^[3]. Literature survey reveals UV and HPLC methods for the estimation of Sulbactam with other drugs in pharmaceutical formulations^[11-20]. Literature survey also reveals spectrophotometric method based

simultaneous equations for simultaneous estimation of Meropenem and Sulbactam in mixture^[4]. Literature survey does not reveals any simple HPLC method for estimation of Meropenem and Sulbactam sodium in mixture. The present manuscript describes simple and precise RP-HPLC method for the estimation of above drugs in combined dosage form.

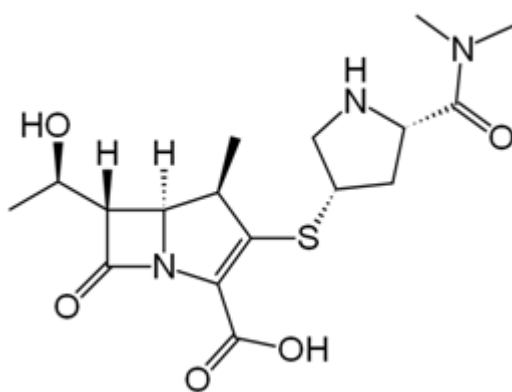


Figure 1: Meropenem

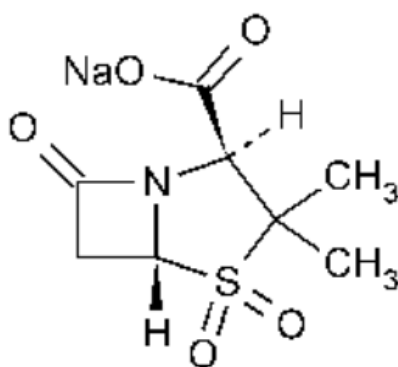


Figure 2: Sulbactam Sodium

MATERIALS AND METHODS:

Apparatus

RP-HPLC instrument (Shimadzu, LC-2010CHT, Japan) equipped with a UV-

Visible detector and a photodiode array detector, auto sampler, Hypersil C18 column (250 mm × 4.6 mm i.d, 5 µm particle size) was used. Chromatograms were automatically obtained by LC-Solution system software. A Sartorius CP224S analytical balance (Gottingen, Germany), an ultrasonic bath (Frontline FS 4, Mumbai, India), Nylon 0.45 µm – 0.47 mm membrane filter (Gelman Laboratory, Mumbai, India). Whatman filter paper no. 41. (Whatman International Ltd., England) were used in the study.

Reagent and materials

Meropenem (MERO) and Sulbactam Sodium (SUL) were kindly supplied as a gift samples from Montage Laboratories, Himmatnagar, Gujarat, India. The pharmaceutical formulation containing 1000 mg MERO and 500 mg SUL was kindly purchased from local market. HPLC grade methanol (Merck Ltd., Mumbai, India) and HPLC grade acetonitrile (Finar Chemicals Ltd., Mumbai, India) were used in the study. Water for RP-HPLC was prepared by triple glass distillation and filtered through a nylon 0.45 µm – 47 mm membrane filter.

Preparation of phosphate buffer solution (0.1 M KH₂PO₄, pH 5.0)

Phosphate buffer (0.1 M KH₂PO₄, pH 5.0) was prepared by dissolving 13.61 g potassium dihydrogen ortho-phosphate in 1000 ml HPLC-grade water.

Preparation of mobile phase

Mobile phase was prepared by mixing 90 ml of the above prepared solution of buffer, pH 5.0, 5 ml of methanol and 5 ml acetonitrile. (buffer: methanol: acetonitrile, 90:05:05, v/v/v).

Preparation of standard stock solutions (100 µg/ml MERO and 100 µg/ml SUL)

A mixed standard solution of MERO (100 µg/ml) and SUL (100 µg/ml) was prepared by accurately weighing MERO (10 mg) and SUL (10 mg) and dissolving in water and diluted to 100 ml with water in the same volumetric flask.

Preparation of working standard solutions

The working standard solutions of MERO and SUL were prepared by accurately transferring (0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5 and 5.0 ml) aliquots to separate 10 ml volumetric flask and diluted up to mark with water.

Preparation of sample solution (100 µg/ml MERO and 50 µg/ml SUL)

The injectable powder equivalent to 10 mg MERO and 5 mg SUL was transferred to 100 ml volumetric flask. Water (50 ml) was added to it and sonicated for 20 min. The solution was filtered through Whatman filter paper No. 41 and the volume was adjusted up to the mark with water to prepare 100 µg/ml MERO and 50 µg/ml SUL.

Methodology

To optimize the RP-HPLC parameters, several mobile phase compositions were tried. A satisfactory separation and good peak symmetry for MERO and SUL was obtained with a phosphate buffer (0.1 M KH_2PO_4 , pH: 5), acetonitrile and methanol in the ratio of 90: 05: 05 at a flow rate 1.0 ml/min to get better reproducibility and repeatability. Quantification was carried out at 230 nm because both the drugs show reasonable absorbance at 230 nm (Figure 4). Complete resolution of the peaks with clear baseline was obtained (Figure 3). System suitability test parameters for MERO and SUL for the proposed method are reported in Table 1.

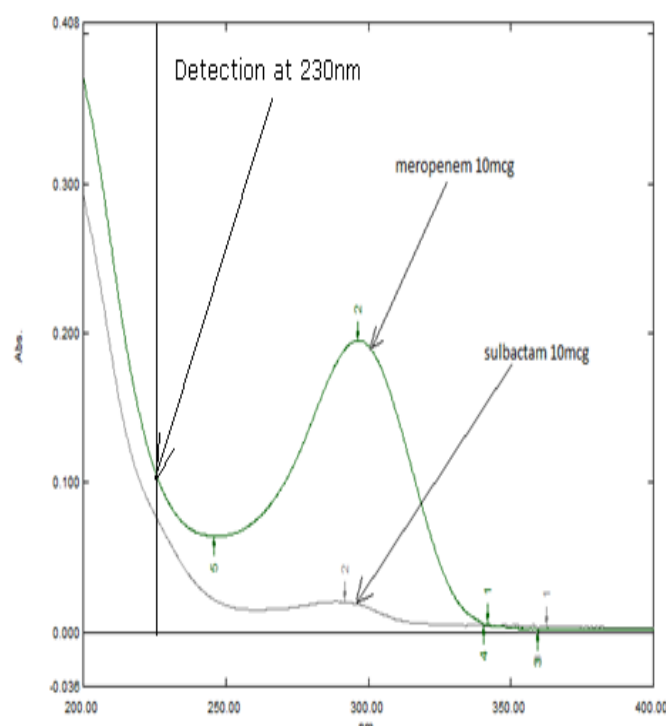


Figure 4: Overlain UV spectra of MERO (10 µg/ml) and SUL (10 µg/ml) in water

Table 1: System suitability parameters of chromatogram for

Parameters	MERO $\pm$ RSD (n = 6)	SUL $\pm$ RSD (n = 6)
Retention time (min)	4.49 $\pm$ 0.01	6.63 $\pm$ 0.01
Tailing factor	0.855 $\pm$ 0.006	1.128 $\pm$ 0.005
Theoretical plates	8436 $\pm$ 0.01	7893 $\pm$ 0.01
Resolution	8.53 $\pm$ 0.01	

Validation of proposed method

The proposed method was validated according to the International Conference on Harmonization (ICH) guidelines^[21].

Calibration curve (Linearity)

Calibration curves were constructed by plotting peak areas versus concentrations of MERO and SUL, and the regression equations were calculated. The calibration curves were plotted over the concentration range 5-50 $\mu\text{g/ml}$ for each MERO and SUL. Accurately measured standard working solutions of MERO and SUL (0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5 and 5.0 ml) were transferred to a series of 10 ml of volumetric flasks and diluted to the mark with mobile phase. Aliquots (10 μl) of each solution were injected under the operating chromatographic conditions and peak area was determined.

Method precision (Repeatability)

The precision of the instrument was checked by repeatedly injecting six sample solutions

of MERO and SUL (20 $\mu\text{g/ml}$) under the same chromatographic condition and measurements of peak area, retention time and tailing factor. Percentage relative standard deviation (RSD) or coefficient of variation (CV) should not be more than 2 %.

Intermediate precision (Reproducibility)

The intraday and interday precision of the proposed method was determined by analyzing the corresponding responses 3 times on the same day and on 3 different days over a period of 1 week.

for 3 different concentrations of sample solutions of MERO and SUL (10, 15, and 20 $\mu\text{g/ml}$). The results were reported in terms of relative standard deviation (RSD).

Limit of detection and Limit of quantification

LOD and LOQ of dug calculated using the following equations designated by International Conference on Harmonization (ICH) guidelines.

$$\text{LOD} = 3.3 \times \sigma/S$$

$$\text{LOQ} = 10 \times \sigma/S$$

Where, σ = the standard deviation of the response and S = slope of the calibration curve.

Accuracy (recovery study)

The accuracy of the method was determined by calculating recovery of MERO and SUL by the standard addition method. Known amounts of standard solutions of MERO and SUL were added at 50, 100 and 150 % level to prequantified sample solutions of MERO and SUL. The experiment was repeated for three times. MERO and SUL were estimated by applying obtained values to the respective regression equations of calibration curve.

Assay of pharmaceutical formulation

The proposed validated method was successfully applied to determine MERO and SUL in their combined dosage form. The result obtained for MERO and SUL was comparable with the corresponding labeled amounts (Table 3). The RP-HPLC chromatogram for MERO and SUL in sample was recorded and is shown in Fig. 6.

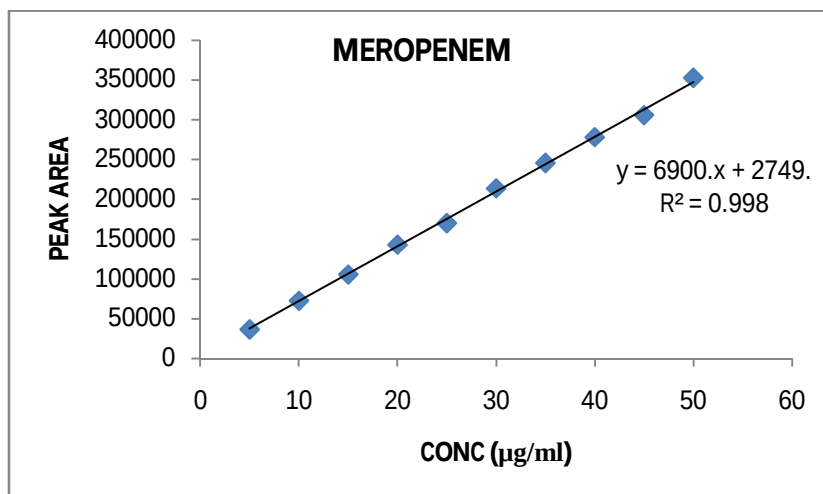


Figure 4: Calibration curve of MERO at 230 nm

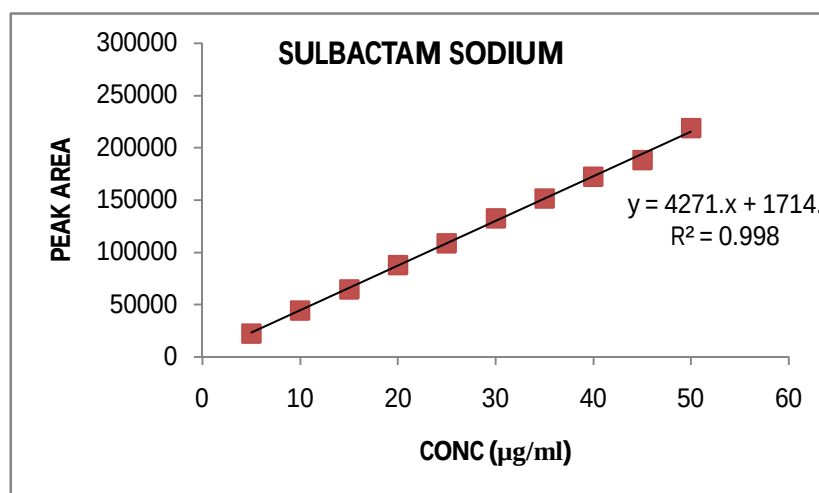


Figure 5: Calibration curve of SUL at 230 nm

Table 2: Recovery data for the proposed method

Drug	Level	Amount of sample taken (µg/ml)	Amount of standard spiked (%)	Mean % Recovery ± RSD
MERO	I	20	50 %	100.6 ± 0.35
	II	20	100 %	99.64 ± 0.37
	III	20	150 %	100.7 ± 0.35
SUL	I	10	50 %	98.22 ± 0.13
	II	10	100 %	99.09 ± 0.14
	III	10	150 %	98.27 ± 0.11

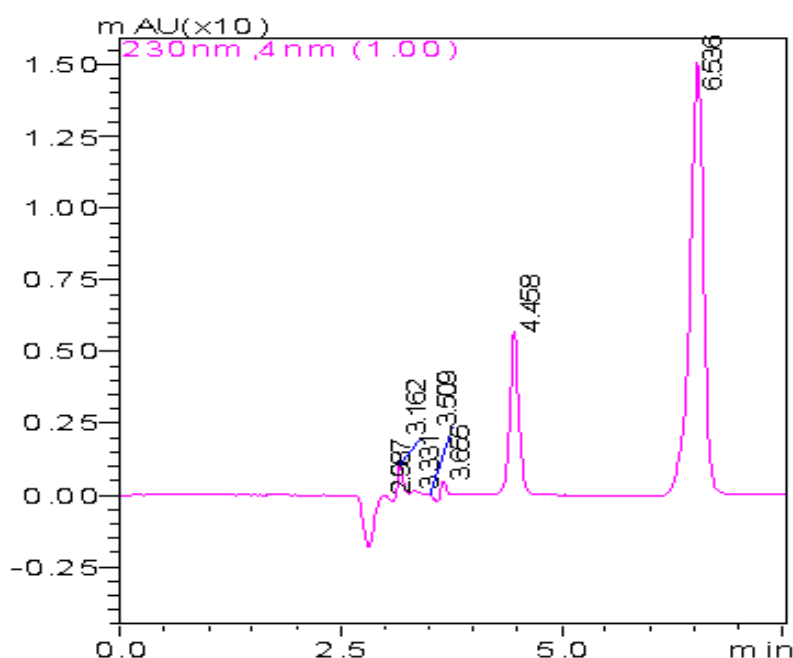


Figure 6: Chromatogram of sample solution of SUL (10 µg/ml) (R_T 4.458 min) and MERO (20 µg/ml) (R_T 6.536 min) at 230 nm

Table 3: Analysis of formulation of MERO and SUL by proposed method (n=5)

Sample No.	Label Claim		Amount Found		% Label Claim	
	MERO (mg)	SUL (mg)	MERO (mg)	SUL (mg)	MERO (%)	SUL (%)
1	20	10	20.27	9.86	101.37	98.60
2	20	10	20.30	9.82	101.50	98.19

3	20	10	20.35	9.90	101.74	99.01
4	20	10	20.22	9.88	101.12	98.78
5	20	10	20.24	9.87	101.20	98.67
Mean			20.29	9.86	101.44	98.65
S.D.			0.05	0.03	0.26	0.35
RSD%			0.26	0.35	0.26	0.35

RESULTS AND DISCUSSION:

A RP-HPLC method was developed and validated for the determination of MERO and SUL in combined dosage forms on a Hypersil C18 column (250 mm × 4.6 mm i.d, 5 µm particle size) with wavelength detection at 230 nm. The retention time of MERO and SUL was 6.63 ± 0.01 min and 4.49 ± 0.01 min, respectively. Linear correlation was obtained between area and concentration of MERO and SUL in the concentration range of 5-50 µg/ml for both drugs. The low RSD value of interday (0.14 - 0.23% for MERO and 0.05 - 0.2 % for SUL) and intraday (0.14 - 0.19% for MERO and 0.12 - 0.26% for SUL) at 230 nm, reveal that proposed method is precise. The limit of detection (LOD) and limit of quantification (LOQ) for MERO and SUL were found to be 0.1467 µg/ml and 0.6616 µg/ml and 0.4446 µg/ml and 2.0048 µg/ml,

respectively. These data show that method is sensitive for the determination of MERO and SUL.

The recovery experiment was performed by the standard addition method. The mean recoveries were 100.4 ± 0.61 and 98.5 ± 0.46 for MERO and SUL, respectively (Table 2). The results of recovery studies indicate that the proposed method is highly accurate. The proposed validated method was successfully applied to determine MERO and SUL in the combined dosage form. The results obtained for MERO and SUL were comparable with the corresponding labeled amounts (Table 3). No interference of the excipients with the absorbance of interest appeared; hence the proposed method is applicable for the routine simultaneous estimation of MERO and SUL in pharmaceutical dosage form.

Table 4: Regression analysis data and summary of validated parameter for the proposed method

Parameters		RP-HPLC method	
		MERO	SUL
Detection wavelength(nm)		230	230
Concentration range (µg/ml)		5-50	5-50
Regression equation $y = mx + c$		$y = 6900x + 2749$	$y = 4271x - 1714$
Slope		6900	4271
Intercept		2749	1714
Correlation coefficient		0.9987	0.9985
Repeatability (% RSD, n = 6)		0.31	0.18
Precision (%RSD)	Interday(%RSD)	0.14 - 0.23	0.05 - 0.2
	Intraday(%RSD)	0.14 - 0.19	0.12 - 0.26
LOD (µg/ml)		0.1467	0.6619
LOQ (µg/ml)		0.4446	2.0048
% Recovery (Accuracy, n = 6)		100.4 ± 0.61	98.50 ± 0.46
% Assay ± RSD (n = 5)		101.4 ± 0.35	98.65 ± 0.26

CONCLUSION:

In this proposed method the linearity is observed in the concentration range of 5-50 µg/ml and 5-50 µg/ml with co-efficient of correlation, (r^2) = 0.9987 and (r^2) = 0.9985 for MERO and SUL, respectively at 230 nm.

The results of the analysis of pharmaceutical formulation by the proposed method are highly reproducible and reliable and it is in good agreement with the label claim of the

drug. The method can be used for the routine analysis of the MERO and SUL in combined dosage form without any interference of excipients.

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REFERENCES :

1. Maryadele. J. O' Neil. The Merck Index: An Encyclopedia of chemicals, Drugs and Biologicals, 14th edition, Merck and Co., Inc. Whitehouse station. NJ; 2006: 1124.
2. Indian Pharmacopoeia 2010, volume II, 6th edition , Govt of India , New Delhi: The Controler of publication 2010 : 1655.
3. U.S.Pharmacopoeia / NF 2009 volume II , Twinbrook Pathway Rockwillae : The United State Pharmacopoieal Convention;2009 : 2714.
4. Patel N; "Method Development and Validation for the Simultaneous Estimation of Meropenem and Sulbactam Sodium". *CPR* 2012 (2)2: 480-486.
5. Srinivasa R.N; "RP- HPLC and Visible Spectrophotometric methods for the Estimation of Meropenem in Pure and Pharmaceutical Formulations". *International Journal of ChemTech Research* 2011 (3)2 : 605-609.
6. Rao L. V; "Reverse Phase HPLC and Visible Spectrophotometric Methods for the Determination of Meropenem in Pure and Pharmaceutical Dosage Form". *International Journal of PharmTech Research* 2012 (4)3: 957-962.
7. Andreas S. L; "Validation of HPLC and UV spectrophotometric methods for the determination of meropenem in pharmaceutical dosage form". *Journal of Pharmaceutical and Biomedical Analysis* 2004 33(5): 947-954.
8. Utpal N; " Estimation of Meropenem in Human Plasma by HPLC-UV and its Application in Comparative Bioavailability Study", *IJPI's Journal of Analytical Chemistry* 2011 (2)3 : 125-132.
9. Kameda K; "HPLC Method for Measuring Meropenem and Biapenem Concentrations in Human Peritoneal Fluid and Bile: Application to Comparative Pharmacokinetic Investigations". *Journal of Chromatographic Science* 2010 (48)5: 532-545.
10. John N, van den A; "Meropenem Pharmacokinetics in the Newborn". *Antimicrob Agents Chemother* 2009 (53)9 : 3871-3879.
11. Roger E; "High-Pressure Liquid Chromatographic Assay of Sulbactam in Plasma, Urine, and Tissue". *Antimicrobial agents and chemotherapy* 1986; (30)2: 231-233.
12. Kumar K.V, Dharuman J; "RP-HPLC method development and validation

- for simultaneous estimation of sulbactam and cefoperazone in dosage form and in plasma". *International Journal of pharmaceutical and biological sciences* 2010 **(1)**4: 87-92.
13. Shrivasta S.M, Singh R.K; "A novel hplc method for simultaneous determination of ceftriaxone and sulbactam in sulbactomax". *International journal of biomedical science* 2009 **(5)**1: 37-43.
 14. Ling M.Q; "A Validated Method for Simultaneous Determination of Piperacillin Sodium and Sulbactam Sodium in Sterile Powder for Injection by HPLC". *Journal of Liquid Chromatography & Related Technologies* 2003 **(26)**4: 665- 676.
 15. Bello A, Callejon M.O; "Simultaneous determination of rifampicin and sulbactam in mouse plasma by high-performance liquid chromatography". *Biomedical Chromatography* 2006 **(20)**8: 748-752.
 16. Siddiqui M.R, Tariq A; "HPLC method for simultaneous determination of cefipime and Sulbactam in supime and biological samples", *International journal of pharmacology* 2010 **(6)**3: 271-277.
 17. Sulochana K.N; "HPLC method for simultaneous determination of ampicillin and Sulbactam in biological samples". *Indian Journal of Pharmacology* 1995 **(27)**3: 189-192.
 18. Pei Q, Yang G.P, Li Z.J; "Simultaneous analysis of amoxicillin and sulbactam in human plasma by HPLC-DAD for assessment of bioequivalence". *Biomed Life Science* 2011**(879)**21: 2000-2004.
 19. Siddiqui M.R, Abu Tariq; "Development and validation of high performance liquid chromatographic method for the simultaneous determination of Ceftazidime and sulbactam in spiked plasma and combined dosage form". *Zydotam American Journal of Applied Sciences* 2009 **(6)**10: 1781-1787.
 20. Patel F.M, Dave J.B; "A validated stability indicating high-performance liquid chromatographic method for simultaneous estimation of cefuroxime sodium and sulbactam sodium in injection dosage form". *Chron Young Sci* 2012 **(3)**4: 279-285.
 21. The International Conference on Harmonization, Q2 (R1), Validation of Analytical Procedure, Text and Methodology, 2005.