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SIMULTANEOUS ESTIMATION OF NEW ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF SULBACTAM, DURLOBACTAM BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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Abstract:

A simple, specific, precise, and efficient method for the Simultaneous estimation of Sulbactam and Durlobactam in pure and pharmaceutical dosage forms by a Reverse Phase-High Performance Liquid Chromatography method is developed and validated. Selected mobile phase were in a combination of Acetonitrile and Acetate buffer (pH-4.3) (35:65% v/v). Optimized column is a Develosil C18 (4.6mm×250mm) 5µm particle size and at a flow rate of 1.0mL/min with detection wavelength at 238nm for Sulbactam and Durlobactam. In our study, the validation of analytical method for determination of Sulbactam and Durlobactam in pure and pharmaceutical dosage forms was performed in accordance the parameters including-system suitability, specificity, linearity of response, accuracy, precision (reproducibility & repeatability), robustness (change of wave length±2 nm). The method is validated according to ICH guidelines. In RP-HPLC method, the calibration graphs were linear in the concentration range of 10-30µg/ml for Sulbactam and 30-90µg/ml for Durlobactam with percentage recoveries are within the limits. The results obtained by RP-HPLC methods are rapid, accurate and precise. Therefore, proposed method can be used for routine analysis of Sulbactam and Durlobactam in the pure form as well as in combined pharmaceutical dosage form.

Keywords: *Sulbactam and Durlobactam, HPLC, Method Development, Validation.*

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INTRODUCTION:

Analysis may be defined as the science and art of determining the composition of materials in terms of the elements or compounds contained in them. In fact, analytical chemistry is the science of chemical identification and determination of the composition (atomic, molecular) of substances, materials and their chemical structure.

Chemical compounds and metallic ions are the basic building blocks of all biological structures and processes which are the basis of life. Some of these naturally occurring compounds and ions (endogenous species) are present only in very small amounts in specific regions of the body, while others such as peptides, proteins, carbohydrates, lipids and nucleic acids are found in all parts of the body. The main object of analytical chemistry is to develop scientifically substantiated methods that allow the qualitative and quantitative evaluation of materials with certain accuracy. Analytical chemistry derives its principles from various branches of science like chemistry, physics, microbiology, nuclear science and electronics. This method provides information about the relative amount of one or more of these components. [1]

DIFFERENT METHODS OF ANALYSIS

The following techniques are available for separation and analysis of components of interest.

Spectral methods

The spectral techniques are used to measure electromagnetic radiation which is either absorbed or emitted by the sample.

E.g. UV-Visible spectroscopy, IR spectroscopy, NMR, ESR spectroscopy, Flame photometry, Fluorimetry.2

Electro analytical methods

Electro analytical methods involved in the measurement of current voltage or resistanceas a property of concentration of the component in solution mixture.

E.g. Potentiometry, Conductometry, Amperometry.[2]

Chromatographic methods

Chromatography is a technique in which chemicals in solutions travel down columns or over surface by means of liquids or gases and are separated from each other due to their molecular characteristics.

E.g. Paper chromatography, thin layer chromatography (TLC), High performance thin layer chromatography (HPTLC), High performance liquid chromatography (HPLC), Gas chromatography (GC). [2]

Miscellaneous Techniques

Mass Spectrometry, Thermal Analysis.

Hyphenated Techniques

GC-MS (Gas Chromatography – Mass Spectrometry), LC-MS (Liquid Chromatography – Mass Spectrometry), ICP-MS (Inductivity Coupled Plasma- Mass Spectrometry), GC-IR (Gas Chromatography – Infrared Spectroscopy), MS-MS (Mass Spectrometry – Mass Spectrometry).

Analytical techniques that are generally used for drug analysis also include biological and microbiological methods, radioactive methods and physical methods etc. are mentioned in Table 1.[2]

Table-1: Summary of Hyphenated separation techniques.[2]

Separation technique	Hyphenated mode
Liquid chromatography	Liquid chromatography-mass spectrometry(LC/MS) Liquid chromatography-Fourier-transform infrared Spectrometry(LC-FTIR) Liquid chromatography-nuclear magnetic resonance spectroscopy(LC/NMR) Liquid chromatography-inductively coupled plasma mass spectrometry(LC-ICPMS)
Gas chromatography	Gas chromatography-mass spectrometry(GC/MS) Gas chromatography-Fourier-transform infrared(GC-FTIR) Gas chromatography-FTIR-MS(GC-FTIR-MS)
Capillary electrophoresis	Capillary electrophoresis-mass spectrometry(CE/MS) Capillary electrophoresis- nuclear magnetic resonance spectroscopy(CE/NMR) Capillary electrophoresis-surface enhanced Raman spectrometry (TLC-SERS)

Thin layer chromatography(TLC)	Thin layer chromatography- mass spectrometry(TLC/MS) Thin layer chromatography- surface enhanced Raman spectrometry(TLC-SERS)
Superficial fluid chromatography/ extraction(SFC/SFE)	Superficial fluid extraction-capillary gas chromatography-mass spectrometry(SFE-CGC-MS) Superficial fluid-Fourier-transform infrared(SFC-FTIR)

INTRODUCTION TO HPLC

HPLC is also called as high pressure liquid chromatography since high pressure is used to increase the flow rate and efficient separation by forcing the mobile phase through at much higher rate. The pressure is applied using a pumping system. The development of HPLC from classical column chromatography can be attributed to the development of smaller particle sizes. Smaller particle size is important since they offer more surface area over the conventional large particle sizes. The HPLC is the method of choice in the field of analytical chemistry, since this method is specific, robust, linear, precise and accurate and the limit of detection is low and also it offers the following advantages.

1. Improved resolution of separated substances
2. column packing with very small (3,5 and 10 µm) particles
3. Faster separation times (minutes)
4. Sensitivity
5. Reproducibility
6. continuous flow detectors capable of handling small flow rates
7. Easy sample recovery, handling and maintenance. [6]

Types of HPLC Techniques

Based on Modes of Chromatography

These distinctions are based on relative polarities of stationary and mobile phases

Reverse phase chromatography: In this the stationary phase is non-polar and mobile phase is polar. In this technique the polar compounds are eluted first and non polar compounds are retained in the column and eluted slowly. Therefore it is widely used technique.

Normal phase chromatography: In this the stationary phase is polar and mobile phase is non-polar. In this technique least polar compounds travel faster and are eluted first where as the polar compounds are retained in the column for longer time and eluted.[4]

INSTRUMENTATION OF HPLC

The basic liquid chromatograph consists of six basic units. The mobile phase supply system, the pump and programmer, the sample valve, the column, the detector and finally a means of presenting and processing the results.

Mobile phase (solvent) reservoirs and solvent degassing

The mobile phase supply system consists of number of reservoirs (200 mL to 1,000 mL in capacity). They are usually constructed of glass or stainless steel materials which are chemically resistant to mobile phase**.**

Mobile phase

Mobile phases in HPLC are usually mixtures of two or more individual solvents. The usual approach is to choose what appears to be the most appropriate column, and then to design a mobile phase that will optimize the retention and selectivity of the system. The two most critical parameters for non-ionic mobile phases are strength and selectivity. [8,24]

Mobile phase preparation

Mobile phases must be prepared from high purity solvents, including water that must be highly purified. Mobile phases must be filtered through ≤1 µm pore size filters and be degassed before use.

Degassing of solvents

Many solvents and solvent mixtures (particularly aqueous mixtures) contain significant amounts of dissolved nitrogen and oxygen from the air. These gasses can form bubbles in the chromatographic system that cause both serious detector noise and loss of column efficiency. These dissolved gases in solvent can be removed by the process of degassing. Every solvent must be degassed before introduction into pump as it alter the resolution of column and interfere with monitoring of the column effluent.

Degassing is done in many ways:

- By warming the solvents
- By stirring vigorously with a magnetic stirrer
- By subjecting to vaccum filtration
- By ultra sonication (using ultra sonicator)
- By bubbling He gas through the solvent reservoir. [8]

Table-2: Physical properties of common HPLC solvents.[8]

Solvent	MW	BP	RI (25°C)	UV Cutoff (nm)	Density g/Ml (25°C)	Viscosity (25°C)	Dielectric Constant
Acetonitrile	41.0	82	1.342	190	0.787	0.358	38.8
Dioxane	88.1	101	1.420	215	1.034	1.26	2.21
Ethanol	46.1	78	1.359	205	0.789	1.19	24.5
Ethyl acetate	88.1	77	1.372	256	0.901	0.450	6.02
Methanol	32.0	65	1.326	205	0.792	0.584	32.7
CH_2Cl_2	84.9	40	1.424	233	1.326	0.44	8.93
Isopropanol	60.1	82	1.375	205	0.785	2.39	19.9
n-Propanol	60.1	97	1.383	205	0.804	2.20	20.3
THF	72.1	66	1.404	210	0.889	0.51	7.58
Water	18.0	100	1.333	170	0.998	1.00	78.5

1.3.2 Pumping systems

The pumping system is one of the most important features of an HPLC system. There is a high resistance to solvent flow due to the narrow columns packed with small particles and high pressures are therefore required to achieve satisfactory flow rate.

The main requirements of pumping systems are:

1. Generation of pressures up to 6000 psi.
2. Pulse free output
3. Flow rates ranging from 0.01 to 10 mL/min
4. Flow control and flow reproducibility of ± 0.5%
5. Corrosion resistant components (seals of Teflon and stainless steel

MATERIALS AND METHODS

INSTRUMENTS USED

HPLC, pH meter, Weighing machine, Volumetric flasks, Pipettes and Burettes, Beakers, Digital ultra sonicator.

HPLC METHOD DEVELOPMENT:

TRAILS

Preparation of standard solution:

Accurately weigh and transfer 10 mg of Sulbactam and Durlobactam working standard into a 10ml of clean dry volumetric flasks add about 7ml of Methanol and sonicated to dissolve and removal of air completely and make volume up to the mark with the same Methanol.

Further pipette 0.2ml of Sulbactam and 0.6ml of Durlobactam from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with Methanol.

Procedure:

Inject the samples by changing the chromatographic conditions and record the chromatograms, note the conditions of proper peak elution for performing validation parameters as per ICH guidelines.

Mobile Phase Optimization:

Initially the mobile phase tried was Methanol: Water and ACN: Water with varying proportions. Finally, the mobile phase was optimized to Acetonitrile and Acetate buffer (pH-4.3) in proportion 35:65 v/v respectively.

Optimization of Column:

The method was performed with various C18 columns like Symmetry, X terra and ODS column. Develosil C18 (4.6mm×250mm) 5µm particle size Column was found to be ideal as it gave good peak shape and resolution at 1ml/min flow.

OPTIMIZED CHROMATOGRAPHIC CONDITIONS:

Instrument used : Waters Alliance 2695 HPLC with PDA Detector 996 model.
Temperature : Ambient
Column : Develosil C18 (4.6mm×250mm) 5µm particle size Column
Mobile phase : Acetonitrile and Acetate buffer (pH-4.3) (35:65% v/v)
Flow rate : 1ml/min
Wavelength : 238nm
Injection volume : 20µl
Run time : 6minutes

VALIDATION

PREPARATION OF MOBILE PHASE:

Preparation of mobile phase:

Accurately measured 350ml of Acetonitrile (35%) of and 650ml of Acetate buffer (65%) were mixed and degassed in a digital ultra sonicated for 20 minutes and then filtered through 0.45 µ filter under vacuum filtration.

Diluent Preparation:

The Mobile phase was used as the diluent.

RESULTS AND DISCUSSION:

Optimized Chromatogram (Standard)

Mobile phase ratio : Acetonitrile and Acetate buffer (pH-4.3) (35:65% v/v)
Column : Develosil C18 (4.6mm×250mm) 5µm particle size Column
Column temperature : Ambient
Wavelength : 238nm
Flow rate : 1ml/min
Injection volume : 20µl
Run time : 6minutes

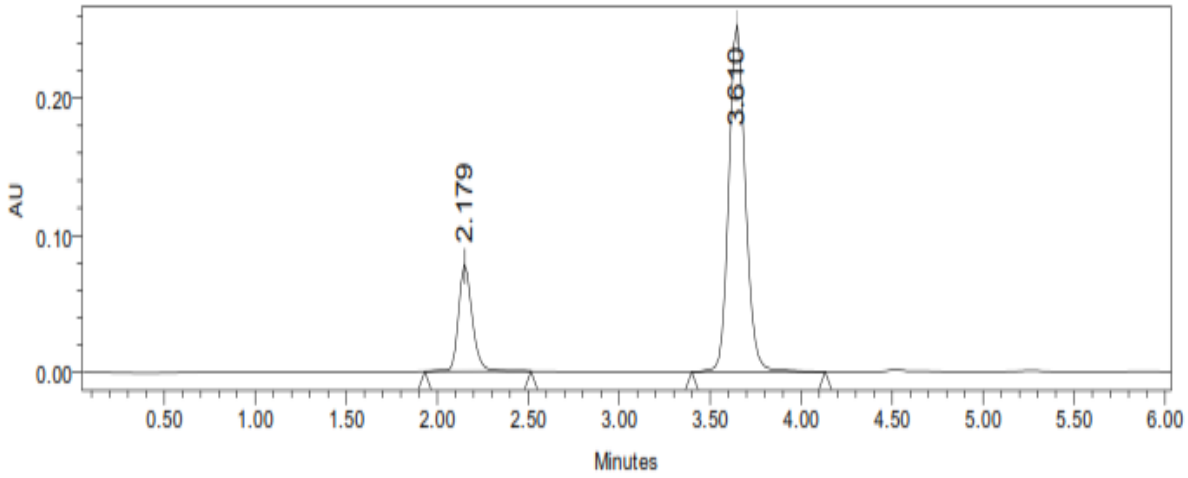

Figure-: Optimized Chromatogram (Standard)

Table-: Optimized Chromatogram (Standard)

S.No	Name	RT	Area	Height	USP Tailing	USP Plate Count	Resolution
1	Sulbactam	2.179	513567	78659	1.2	4536	
2	Durlobactam	3.610	1625892	265321	1.1	7985	9.8

Observation: From the above chromatogram it was observed that the Sulbactam and Durlobactam peaks are well separated and they shows proper retention time, resolution, peak tail and plate count. So it's optimized trial.

Optimized Chromatogram

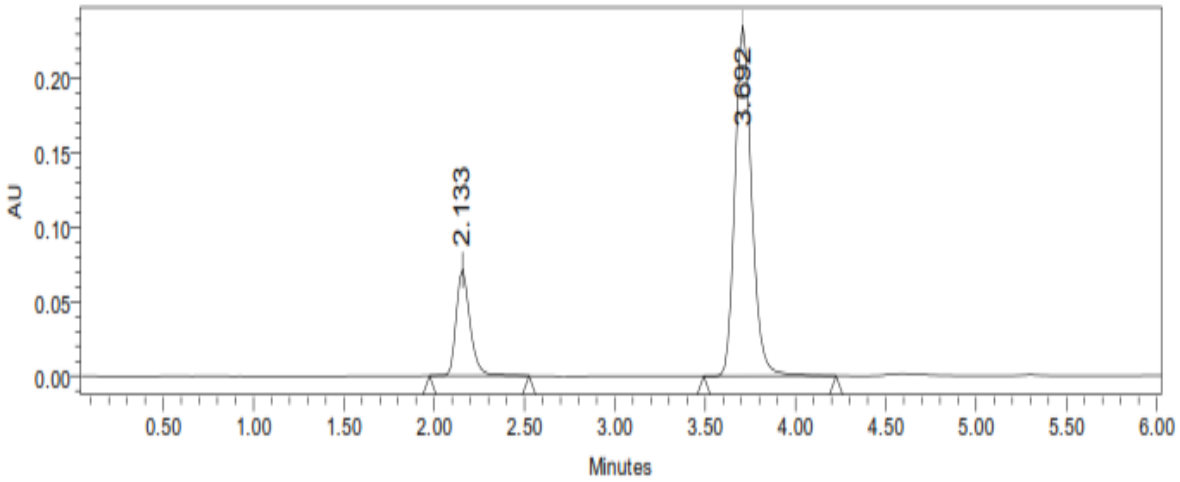

Figure: Optimized Chromatogram (Sample)

Table: Optimized Chromatogram (Sample)

S.No	Name	Rt	Area	Height	USP Tailing	USP Plate Count	Resolution
1	Sulbactam	2.133	512659	78956	1.2	4652	
2	Durlobactam	3.692	1615985	263587	1.1	7982	10.3

Acceptance criteria:

- Resolution between two drugs must be not less than 2.
- Theoretical plates must be not less than 2000.
- Tailing factor must be not less than 0.9 and not more than 2.
- It was found from above data that all the system suitability parameters for developed method were within the limit.

Assay (Standard):

Table-: Peak results for assay standard of Sulbactam

S.No	Name	RT	Area	Height	USP Tailing	USP Plate Count	Injection
1	Sulbactam	2.152	513538	78074	1.2	4562	1
2	Sulbactam	2.198	513975	79001	1.2	4620	2
3	Sulbactam	2.179	513283	78048	1.2	4652	3

Table-: Peak results for assay standard of Durlobactam

S.No	Name	RT	Area	Height	USP Tailing	USP Plate Count	Injection
1	Durlobactam	3.646	1625632	265325	1.1	7949	1
2	Durlobactam	3.604	1635458	265423	1.1	7919	2
3	Durlobactam	3.610	1635241	265874	1.1	7926	3

Assay (Sample):

Table-: Peak results for Assay sample of Sulbactam

S.No	Name	RT	Area	Height	USP Tailing	USP Plate Count	Injection	% of Assay
1	Sulbactam	3.651	513265	78548	1.2	4582	1	100.1
2	Sulbactam	2.150	513254	78547	1.2	4658	2	100.1
3	Sulbactam	2.187	513876	78498	1.2	4597	3	99.9

Table-: Peak results for Assay sample of Durlobactam

S.No	Name	RT	Area	Height	USP Tailing	USP Plate Count	Injection	% of Assay
1	Durlobactam	3.646	1625284	78569	1.1	7985	1	100.0
2	Durlobactam	3.651	1624613	78547	1.1	7898	2	100.7
3	Durlobactam	3.601	1625874	78462	1.1	7854	3	100.6

$$\%ASSAY = \frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Weight of standard}}{\text{Dilution of standard}} \times \frac{\text{Dilution of sample}}{\text{Weight of sample}} \times \frac{\text{Purity}}{100} \times \frac{\text{Weight of tablet}}{\text{Label claim}} \times 100$$

The % purity of Sulbactam and Durlobactam in pharmaceutical dosage form was found to be 99.57%

LINEARITY

CHROMATOGRAPHIC DATA FOR LINEARITY STUDY OF SULBACTAM:

Concentration µg/ml	Average Peak Area
10	245899
15	365687
20	481526
25	589854
30	705882

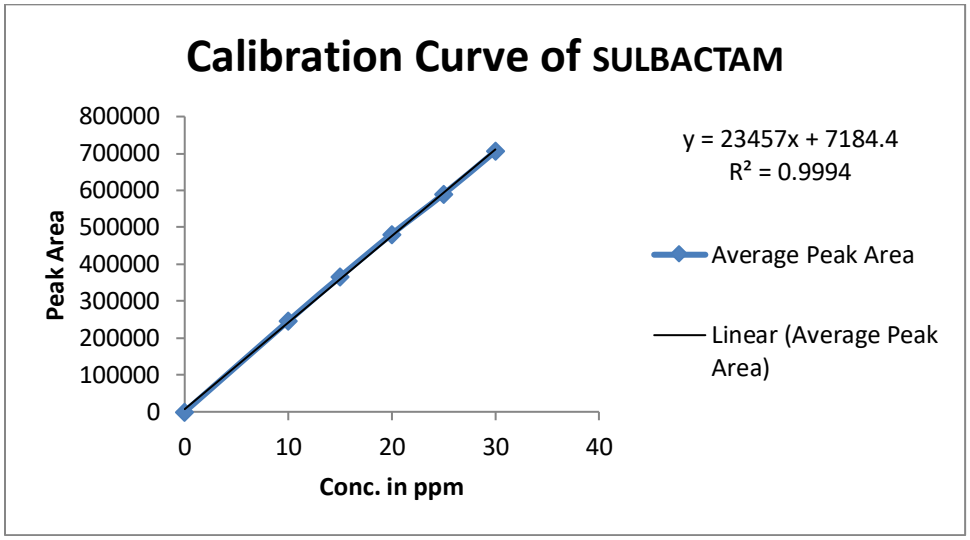

Fig-: Calibration Graph of Sulbactam

CHROMATOGRAPHIC DATA FOR LINEARITY STUDY OF DURLOBACTAM:

Concentration µg/ml	Average Peak Area
30	863094
45	1249397
60	1678592
75	2050412
90	2468444

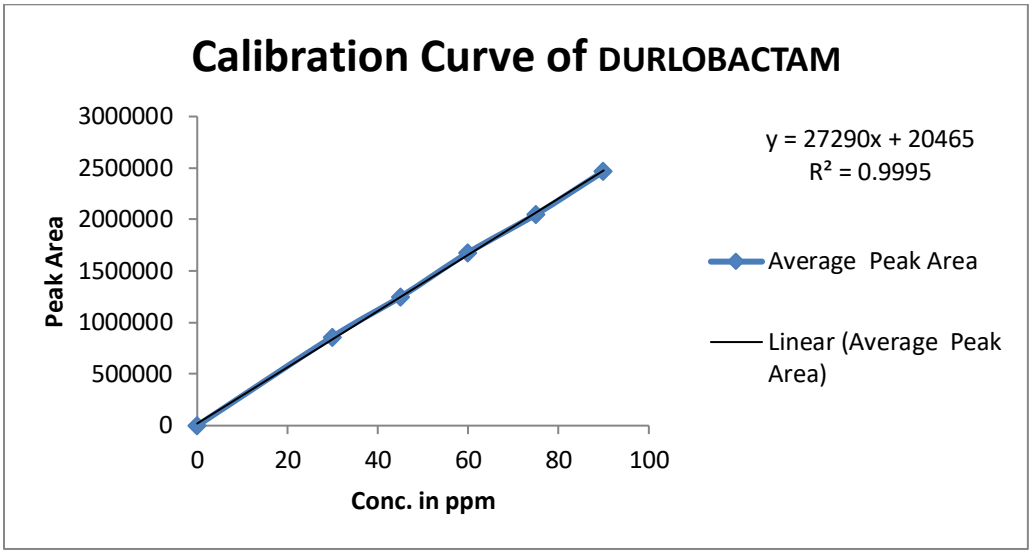

Fig-: Calibration Curve of Durlobactam

REPEATABILITY

Table-: Results of repeatability for Sulbactam:

S. No	Peak name	Retention time	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Sulbactam	2.157	513568	78546	1.2	4528
2	Sulbactam	2.159	513685	78541	1.2	4572
3	Sulbactam	2.186	513659	79852	1.2	4598
4	Sulbactam	2.160	513254	78498	1.3	4529
5	Sulbactam	2.170	513647	77898	1.2	4572
Mean			513562.6			
Std.dev			177.9475			
%RSD			0.03465			

Acceptance criteria:

- %RSD for sample should be NMT 2.
- The %RSD for the standard solution is below 1, which is within the limits hence method is precise.

Table-: Results of repeatability for Durlobactam:

S. No	Peak name	Retention time	Area(µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Durlobactam	3.603	1635625	265325	1.1	7985
2	Durlobactam	3.608	1658744	264588	1.1	7859
3	Durlobactam	3.600	1652985	265985	1.2	7845
4	Durlobactam	3.696	1645898	264898	1.1	7969

5	Durlobactam	3.629	1652364	268489	1.1	7846
Mean			1649123			
Std.dev			8811.631			
%RSD			0.534322			

Intermediate precision:

Table-: Results of Intermediate precision for Sulbactam

S.No.	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate count	USP Tailing
1	Sulbactam	2.198	514658	78698	4658	1.2
2	Sulbactam	2.196	514354	78599	4598	1.2
3	Sulbactam	2.160	513985	79854	4652	1.2
4	Sulbactam	2.160	514875	79879	4561	1.2
5	Sulbactam	2.160	514658	79865	4659	1.2
6	Sulbactam	2.186	516452	79854	4589	1.2
Mean			514830.3			
Std. Dev.			852.3705			
% RSD			0.165563			

Acceptance criteria:

- %RSD of five different sample solutions should not more than 2.

Table-: Results of Intermediate precision for Durlobactam

S.No	Peak Name	Rt	Area (µV*sec)	Height (µV)	USP Plate count	USP Tailing	Resolution
1	Durlobactam	3.623	1645875	266589	7985	1.1	10.1
2	Durlobactam	3.611	1658554	265898	8001	1.1	10.1
3	Durlobactam	3.696	1649854	265415	7985	1.1	10.1
4	Durlobactam	3.696	1659842	265154	7956	1.1	10.1
5	Durlobactam	3.696	1645985	266598	7985	1.1	10.1
6	Durlobactam	3.642	1659852	265341	8002	1.1	10.1
Mean			1653327				
Std. Dev.			6838.733				
% RSD			0.413635				

Acceptance criteria:

- %RSD of five different sample solutions should not more than 2.

Table-: Results of Intermediate precision Day 2 for Sulbactam

S.No	Peak Name	RT	Area (µV*sec)	Height (µV)	US Plate count	USP Tailing
1	Sulbactam	2.198	514658	78572	4672	1.2
2	Sulbactam	2.196	514895	78516	4639	1.2
3	Sulbactam	2.178	514658	78572	4783	1.2
4	Sulbactam	2.142	514784	78372	4623	1.2
5	Sulbactam	2.177	515268	78592	4639	1.2
6	Sulbactam	2.177	514598	78526	4737	1.2
Mean			514810.2			
Std. Dev.			248.5224			
% RSD			0.048275			

Acceptance criteria:

- %RSD of five different sample solutions should not more than 2.

Table-: Results of Intermediate precision Day 2 for Durlobactam

S.No	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate count	USP Tailing	Resolution
1	Durlobactam	3.611	1638732	264384	7985	1.1	10.1
2	Durlobactam	3.623	1637438	265827	7946	1.1	10.1
3	Durlobactam	3.684	1638474	266382	7943	1.1	10.1
4	Durlobactam	3.697	1634273	269183	7964	1.1	10.1
5	Durlobactam	3.684	1636372	261931	7968	1.1	10.1
6	Durlobactam	3.684	1639283	264356	7982	1.1	10.1
Mean			1637429				
Std. Dev.			1860.366				
% RSD			0.113615				

Acceptance criteria:

- %RSD of five different sample solutions should not more than 2.

ACCURACY:

Table-: The accuracy results for Sulbactam

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	245954	10	10.179	101.79%	101.36%
100%	483747	20	20.316	101.58%	
150%	715961	30	30.	100.72%	

Acceptance Criteria:

- The percentage recovery was found to be within the limit (98-102%).

Table-: The accuracy results for Durlobactam

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	842287	30	30.114	100.38%	100.26%
100%	1659744	60	60.068	100.113%	
150%	2483885	90	90.268	100.297%	

The results obtained for recovery at 50%, 100%, 150% are within the limits. Hence method is accurate.

Robustness

Table-: Results for Robustness

SULBACTAM

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 mL/min	513567	2.179	4536	1.2
Less Flow rate of 0.9 mL/min	523652	2.210	4462.3	0.9
More Flow rate of 1.1 mL/min	502146	2.184	4325.1	1.0
Less organic phase	521574	2.200	4632.4	0.9
More Organic phase	502416	2.172	4190.8	0.8

Acceptance criteria:

The tailing factor should be less than 2.0 and the number of theoretical plates (N) should be more than 2000.

DURLOBACTAM

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 mL/min	1625892	3.610	4536	1.1
Less Flow rate of 0.9 mL/min	1758455	4.498	4426.4	0.9
More Flow rate of 1.1 mL/min	1742514	3.505	4421.5	0.8
Less organic phase	1726451	4.504	4355.1	0.9
More organic phase	1725466	3.512	4426.6	0.9

Acceptance criteria:
The tailing factor should be less than 2.0 and the number of theoretical plates (N) should be more than 2000.

CONCLUSION:

In the present investigation, a simple, sensitive, precise and accurate RP-HPLC method was developed for the quantitative estimation of Sulbactam and Durlobactam in bulk drug and pharmaceutical dosage forms.

Sulbactam is soluble in water, alcohol, chloroform or ether, and in alkaline solutions and soluble in dimethyl formamide, dimethyl sulfoxide, slightly soluble in methanol, ethanol and Durlobactam is very slightly soluble in water, ethanol, and chloroform. It is practically insoluble in ether and soluble in formic acid. Very slightly soluble in water and in ethanol (96%). Soluble in DMSO, it is insoluble in water.

Acetonitrile and Acetate buffer (pH-4.3) (35:65% v/v) was chosen as the mobile phase. The solvent system used in this method was economical.
The %RSD values were within 2 and the method was found to be precise.

The results expressed in Tables for RP-HPLC method was promising. The RP-HPLC method is more sensitive, accurate and precise compared to the Spectrophotometric methods.

This method can be used for the routine determination of Sulbactam and Durlobactam in bulk drug and in pharmaceutical dosage forms.
This method can be used for the routine determination of Trihexyphenidyl HCL and Trifluoperazine HCL in bulk drug and in Pharmaceutical dosage forms.

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