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Research

A new rp-hplc method for the simultaneous estimation of levodopa, benserazidein it's pure and pharmaceutical dosage form as per ich guidelines

Gubbala Haritha*[1], Dr.T.K.V. Kesava Rao[1], Mrs. Buddha Sravana Sree[1]

[1]*Department of Pharmaceutical Analysis, Pydah College of Pharmacy Patavala, Andhra University, Kakinada, Andhra Pradesh, India*

*Author for Correspondence: Gubbala Haritha
Email: harithagubbala19@gmail.com

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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.

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]

Every country has legislation on bulk drugs and their pharmaceutical formulations that sets standards and obligatory quality indices for them. These regulations are presented in separate articles relating to individual drugs and are published in the form of book called "Pharmacopoeia" (e.g. IP, USP, and BP). Quantitative chemical analysis is an important tool to assure that the raw material used and the intermediate products meet the required specifications. Every year number of drugs is introduced into the market. Also quality is important in every product or service, but it is vital in medicines as it involves life.

There is a time lag from the date of introduction of a drug into the market to the date of its inclusion in pharmacopoeias. This happens because of the possible uncertainties in the continuous and wider usage of these drugs, report of new toxicities and development of patient resistance and introduction of better drugs by the competitors. Under these conditions standard and analytical procedures for these drugs may not be available in Pharmacopoeias. In instrumental analysis, a physical property of the substance is measured to determine its chemical composition. Pharmaceutical analysis comprises those procedures necessary to determine the identity, strength, quality and purity of substances of therapeutic importance.[2]

Pharmaceutical analysis deals not only with medicaments (drugs and their formulations) but also with their precursors i.e. with the raw material on which degree of purity and quality of medicament depends. The quality of the drug is determined after establishing its authenticity by testing its purity and the quality of pure substance in the drug and its formulations.

Quality control is a concept which strives to produce a perfect product by series of measures designed to prevent and eliminate errors at different stages of production. The decision to release or reject a product is based on one or more type of control action. With the growth of pharmaceutical industry during last several years, there has been rapid progress in the field of pharmaceutical analysis involving complex instrumentation. Providing simple analytical procedure for complex formulation is a matter of most importance. So, it becomes necessary to develop new analytical methods for such drugs. In brief the reasons for the development of newer methods of drugs analysis are:

1. The drug or drug combination may not be official in any pharmacopoeias.
2. A proper analytical procedure for the drug may not be available in the literature due to Patent regulations.
3. Analytical methods for a drug in combination with other drugs may not be available.
4. Analytical methods for the quantitation of the drug in biological fluids may not be available.
5. The existing analytical procedures may require expensive reagents and solvents. It may also involve cumbersome extraction and separation procedures and these may not be reliable.[1, 2]

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–MassSpectrometry), 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).

MATERIALS AND METHODS

Levodopa-Sura labs, Benserazide-Sura labs, Water and Methanol for HPLC-LICHROSOLV (MERCK), Acetonitrile for HPLC-Merck.

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]

EXPERIMENTAL METHODS

Sulbactam -Provided by Sura labs, Durlobactam (Pure)-Provided by Sura labs, Water and Methanol for HPLC-LICHROSOLV (MERCK), Acetonitrile for HPLC- Merck, Placeda tablets 0.5/10mg (Marketed product)-Mankind Pharma

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

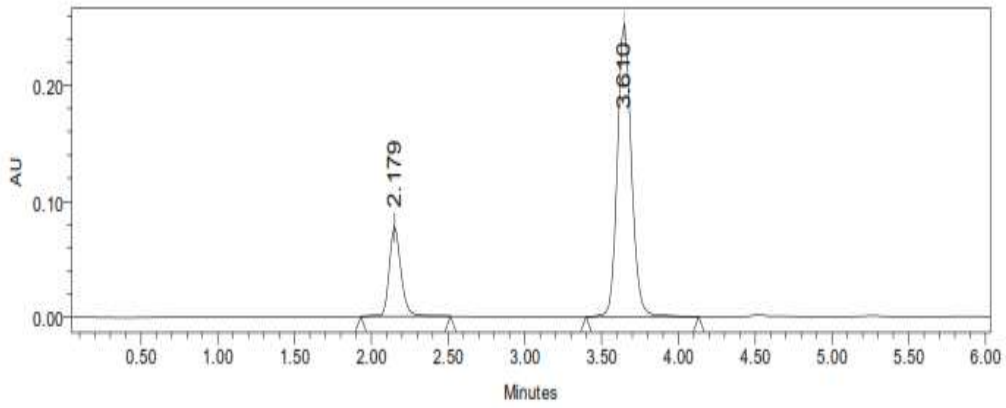

Fig 1: Optimized Chromatogram (Standard)

Table 1: 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

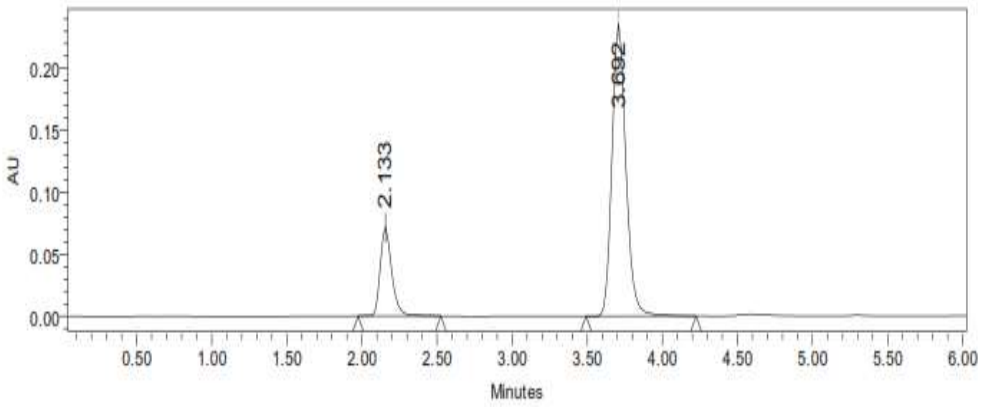

Fig 2: Optimized Chromatogram (Sample)

Table 2: 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

- *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.*

System Suitability

Table 3: Results of system suitability for Sulbactam

S.No	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Sulbactam	2.152	513652	78542	4698	1.2
2	Sulbactam	2.157	513524	78654	4785	1.2
3	Sulbactam	2.141	513425	78541	4682	1.2
4	Sulbactam	2.133	513647	78454	4854	1.2
5	Sulbactam	2.166	514824	78655	4872	1.2
Mean			513814.4			
Std. Dev.			572.2004			
% RSD			0.111363			

- *%RSD of five different sample solutions should not more than 2.*
- *The %RSD obtained is within the limit, hence the method is suitable.*

Table 4: Results of system suitability for Durlobactam

S.No	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing	Resolution
1	Durlobactam	3.674	1635285	265421	7985	1.1	10.1
2	Durlobactam	3.631	1635241	265484	7898	1.1	10.1
3	Durlobactam	3.625	1652547	253498	7954	1.1	10.1
4	Durlobactam	3.692	1658458	265241	7965	1.1	10.1
5	Durlobactam	3.629	1652894	265348	7985	1.1	10.1
Mean			1646885				
Std. Dev.			10865.58				
% RSD			0.659766				

- *%RSD of five different sample solutions should not more than 2.*
- *The %RSD obtained is within the limit, hence the method is suitable.*

Assay (Standard)

Table 5: 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 6: 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 7: 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 8: 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

Table 9: Showing Assay Results

S.No.	Name of Compound	Label Claim	Amount Taken (from Combination Tablet)	% Purity
1	Sulbactam	0.5mg	0.4	99.57%
2	Durlobactam	10 mg	9.8	99.57%

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

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

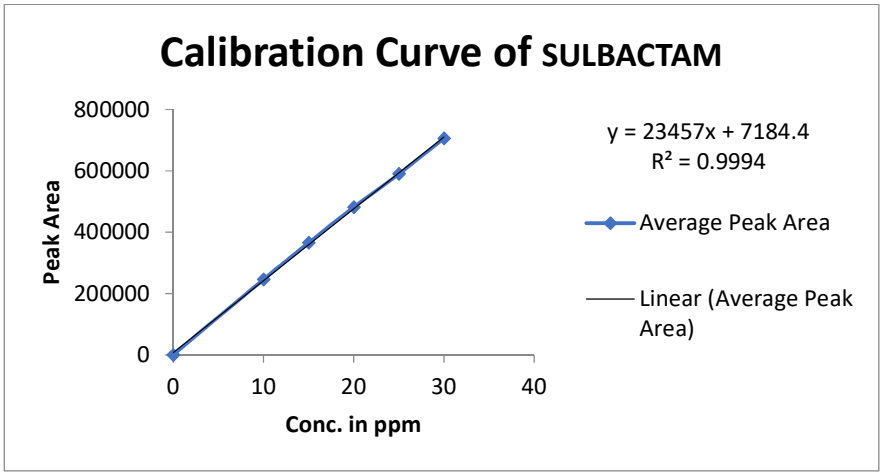

Fig 3: Calibration Graph of Sulbactam

CHROMATOGRAPHIC DATA FOR LINEARITY STUDY OF DURLOBACTAM

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

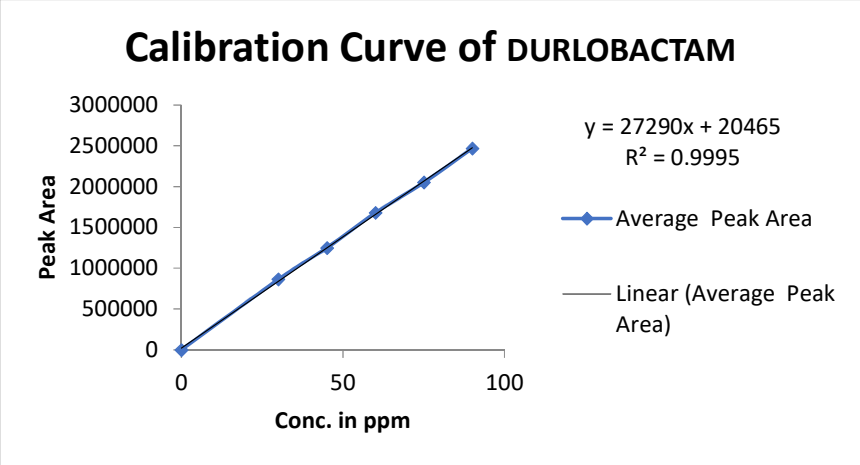

Fig 4: Calibration Curve of Durlobactam

REPEATABILITY

Table 10: 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			

- *%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 11: 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 12: 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			

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

Table 13: 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				

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

Table 14: 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			

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

Table 15: 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				

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

ACCURACY

Table 16: 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%	

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

Table 17: 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.

Table 18: 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

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

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.

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