International Journal of Pharmacy and Industrial Research (IJPIR)

IJPIR |Vol.14 | Issue 4 | Oct - Dec -2024

www.ijpir.com

DOI : https://doi.org/10.61096/ijpir.v14.iss4.2024.523-532

ISSN: 2231-3656

Print: 2231-3648

Research

Validated rp-hplc method development for the simutaneous estimation of decitabine and cedazuridine in its combined dosage forms

Merla Sudha*[1], Buddha Sravana Sree[1], Dr. T. K. V. Kesava Rao[1],

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

*Author for Correspondence: Merla Sudha
Email: sudhamerla2000@gmail.com

Published on: 26 Nov 2024

Published by:
DrSriram Publications

Abstract

A new, simple, precise, accurate and reproducible RP-HPLC method for Simultaneous estimation of Decitabine and Cedazuridine in bulk and pharmaceutical formulations. Separation of Decitabine and Cedazuridine was successfully achieved on a Phenomenex Luna C18 (4.6×250mm, 5µm) particle size or equivalent in an isocratic mode utilizing Acetonitrile: Phosphate Buffer (pH-4.6) (45:55 v/v) at a flow rate of 1.0mL/min and elutes was monitored at 245nm, with a retention time of 2.102 and 3.537 minutes for Decitabine and Cedazuridine respectively. The method was validated and the response was found to be linear in the drug concentration range of 6µg/mL to 14µg/mL for Decitabine and 18µg/mL to 42µg/mL for Cedazuridine. The values of the slope and the correlation coefficient were found to be 77824 and 0.999 for Decitabine and 10515 and 0.999 for Cedazuridine respectively. The LOD and LOQ for Cedazuridine were found to be 0.8 µg/mL and 2.4µg/mL respectively. This method was found to be good percentage recovery for Decitabine and Cedazuridine were found to be 100.351 and 100.93 respectively indicates that the proposed method is highly accurate. The specificity of the method shows good correlation between retention times of standard with the sample so, the method specifically determines the analytes in the sample without interference from excipients of tablet dosage forms. The method was extensively validated according to ICH guidelines for Linearity, Range, Accuracy, Precision, Specificity and Robustness.

Keywords: Decitabine and Cedazuridine, RP-HPLC, Accuracy, Precision, ICH Guidelines.

INTRODUCTION

Pharmaceutical analysis comprises those procedures necessary to determine “identity, strength, quality and purity of the drug substances and drug products. Pharmaceutical analyst plays a major role in all quality controlling divisions of industry. Analytical chemistry involves separating, identifying, and determining the

relative amounts of components in a sample matrix. The number of new drugs is constantly growing. This requires new methods for controlling the quality. Modern pharmaceutical analysis must need the following requirements[1.]

1. The analysis should take a minimal time.
2. The accuracy of the analysis should meet the demands of the Pharmacopoeia.
3. The analysis should be performed with a minimal cost.
4. Precision and selectivity of the selected method should be good.

Typical Instrumental Techniques

The methods of estimation of drugs are divided into physical, chemical, physicochemical and biological ones of them, physical and physicochemical methods are used mostly. Physical methods of analysis involve the studying of the physical properties of a substance. They include determination of the solubility, transparency or degree of turbidity, colour density or specific gravity (for liquids), moisture content, melting, freezing and boiling points. Physicochemical methods are used to study the physical phenomenon that occurs as a result of chemical reactions. Among the physicochemical methods are optical refractometry, polarimetry, emission and fluorescent methods of analysis, photometry including photocolorimetry, spectrophotometry, nephelometry and turbidometry, electrochemical (potentiometry, amperometry, coulometer, polarography) and chromatography (column, paper, thin layer, gas, high performance liquid) methods are generally preferable.

Methods involving nuclear reactions such as nuclear magnetic resonance (NMR) and paramagnetic resonance (PMR) are becoming more popular. The combination of mass spectroscopy with gas chromatography is one of the most powerful tools available. The chemical methods include the gravimetric and volumetric procedures, which are based on complex formation, acid-base and precipitation and redox reactions. Titrations in non-aqueous media and complexometry have been widely used in pharmaceutical analysis whenever the existing amounts are in milligram level and the interference is negligible. The methods (LC-MS,[4] HPLC, GLC, NMR and Mass Spectroscopy) of choice for assay involve sophisticated equipment that are very costly and pose problems of maintenance. Hence, they are not in the reach of most laboratories and small-scale industries, which produce bulk drugs and pharmaceutical formulations.

The visible Spectrophotometric methods which fall in the wavelength region 400-800 nm and fluorimetric methods (may fall in UV & Visible regions) are very simple, cheap and easy to carry out estimations of drugs in bulk form and their formulations. The limitations of many colorimetric or fluorimetric methods of analysis lie in the chemical reactions upon which the procedures are based rather than the instruments available. Many of the reactions involve colour or fluorescence of a drug are quite selective or can be rendered selective through the introduction of masking agents, control of PH, use of solvent extraction technique, adjustment of oxidation states or by prior removal of interfering ingredients with the aid of chromatographic separation.

1. This is preferably followed by general methodology for UV-Visible and HPLC method developments.
2. Followed by literature of drugs used in Analysis

HPLC

Russian botanist Tswett invented chromatography as a separation technique. He describes in detail the separation of pigments, the colour substances by filtration through column, followed by developments with pure solvents. High-performance liquid chromatography (HPLC) [5] is the fastest growing analytical technique for analysis of drugs. Its simplicity, high specificity and wide range of sensitivity make it ideal for the analysis of many drugs in both dosage forms and biological fluids.

According to IUPAC, chromatography [6] is a physical method of separation in which components will be separated or distributed between stationary and mobile phases. The importance of chromatography is increasing rapidly in pharmaceutical analysis for the exact differentiation, selective identification and quantitative determination of structurally closely related compounds. Another important field of application of chromatographic methods is the purity testing of final products and the intermediates. The reasons for the popularity of the method is its sensitivity, its ready adaptability to accurate quantitative determinations, its suitability for separating non-volatile species or thermally fragile ones and its wide spread applicability to substances that are of prime interest to the industry. Sensitive detectors have transformed liquid column chromatography into high speed, efficient, accurate and highly resolved method of separation.

MATERIALS AND METHOD

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

HPLC method development

Trails

Preparation of standard solution: Accurately weigh and transfer 10 mg of Decitabine and Cedazuridine working standard into a 10ml of clean dry volumetric flasks add about 7ml of Methanol and sonicate to dissolve and removal of air completely and make volume up to the mark with the same Methanol. Further pipette 0.1ml of the above Decitabineand 0.3ml of the Cedazuridine 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 Water: Acetonitrile and Methanol: Phosphate Buffer: ACN with varying proportions. Finally, the mobile phase was optimized to Acetonitrile: Phosphate Buffer in proportion 45:55 v/v respectively.

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

Optimized chromatographic conditions

Instrument used	:	Waters HPLC with auto sampler and PDA Detector 996 model.
Temperature	:	35ºC
Column	:	Phenomenex Luna C18 (4.6×250mm, 5µm) particle size
Buffer	:	Dissolve 6.8043 of potassium dihydrogen phosphate in 1000 ml HPLC water and adjust the pH 4.6 with diluted orthophosphoric acid. Filter and sonicate the solution by vacuum filtration and ultra sonication.
pH	:	4.6
Mobile phase	:	Acetonitrile: Phosphate Buffer (45:55 v/v)
Flow rate	:	1ml/min
Wavelength	:	245 nm
Injection volume	:	10 µl
Run time	:	7 min

Validation

Preparation of buffer and mobile phase:

Preparation of Potassium dihydrogen Phosphate (KH2PO4) buffer (pH-4.6): Dissolve 6.8043 of potassium dihydrogen phosphate in 1000 ml HPLC water and adjust the pH 4.6 with diluted orthophosphoric acid. Filter and sonicate the solution by vacuum filtration and ultra sonication.

Preparation of mobile phase: Accurately measured 450 ml (45%) of Methanol, 550 ml of Phosphate buffer (55%) were mixed and degassed in digital ultrasonicater for 15 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	:	Acetonitrile: Phosphate Buffer (pH-4.6) (45:55 v/v)
Column	:	Phenomenex Luna C18 (4.6×250mm, 5µm) particle size
Flow rate	:	1 ml/min
Wavelength	:	245 nm
Column temp	:	35ºC
Injection Volume	:	10 µl
Run time	:	7 minutes

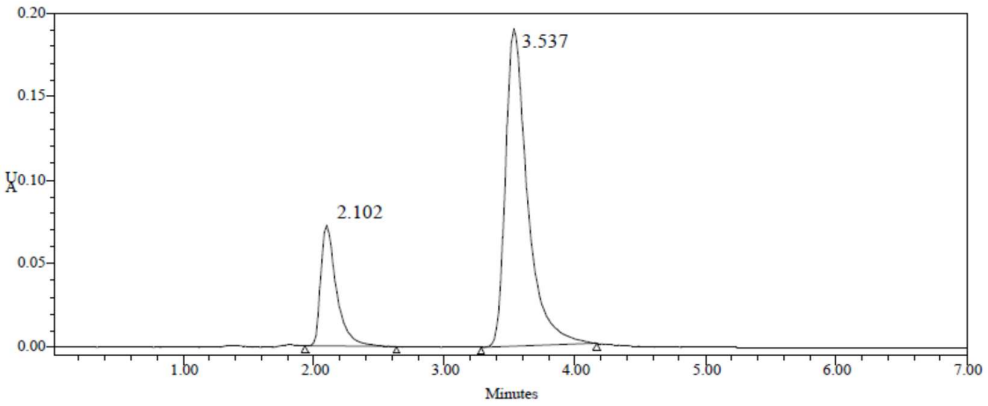

Fig 1: Optimized Chromatogram

Table 1: Peak results for Optimized Chromatogram

S. No	Peak name	R_t	Area	Height	USP Resolution	USP Tailing	USP plate count
1	Decitabine	2.102	765788	69583		0.98	5588.0
2	Cedazuridine	3.537	2532157	190048	2.98	1.27	5399.0

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

Optimized Chromatogram (Sample)

Mobile phase : Acetonitrile: Phosphate Buffer (pH-4.6) (45:55 v/v)
Column : Phenomenex Luna C18 (4.6×250mm, 5µm) particle size
Flow rate : 1 ml/min
Wavelength : 245 nm
Column temp : 35ºC
Injection Volume : 10 µl
Run time : 7 minutes

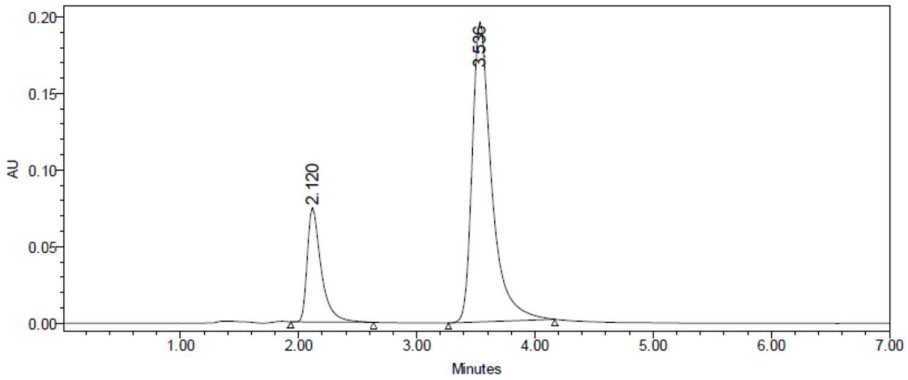

Fig 2: Optimized Chromatogram (Sample)

Table 2: Optimized Chromatogram (Sample)

S. No	Peak name	R_t	Area	Height	USP Resolution	USP Tailing	USP plate count
1	Decitabine	2.120	775683	13123		0.98	6364.0
2	Cedazuridine	3.536	2658479	937406	5.07	1.24	7459.0

- *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 Decitabine

S.No	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Decitabine	2.117	765842	69588	5588	1.9
2	Decitabine	2.118	766595	69853	5577	1.6
3	Decitabine	2.116	765486	70212	5659	1.6
4	Decitabine	2.109	765929	69214	5643	1.7
5	Decitabine	2.102	765427	69559	5686	1.6
Mean			765855.8			
Std. Dev			467.0618			
% RSD			0.060986			

- *%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 Cedazuridine

Sno	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Cedazuridine	3.547	2534659	190059	5366	1.2	2.06
2	Cedazuridine	3.539	2536853	190053	5349	1.4	2.04
3	Cedazuridine	3.547	2535878	190079	5388	1.5	2.0
4	Cedazuridine	3.565	2533565	190036	5346	1.6	2.02
5	Cedazuridine	3.537	2534213	190084	5365	1.6	2.03
Mean			2535033.6				
Std. Dev			1322.282421				
% RSD			0.052160351				

- *%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.*

Assay (Standard)

Table 5: Peak results for assay standard

Sno	Name	Rt	Area	Height	USP Resolution	USP Tailing	USP plate count	Injection
1	Decitabine	2.102	759869	71256		1.7	5688	1
2	Cedazuridine	3.537	2458753	215653	2.03	1.6	5363	1
3	Decitabine	2.105	759459	72542		1.7	5747	2
4	Cedazuridine	3.552	2465886	226566	2.01	1.6	5451	2
5	Decitabine	2.112	759244	72583		1.7	5585	3
6	Cedazuridine	3.560	2489577	221541	2.05	1.6	5457	3

Assay (sample)

Table 6: Peak results for Assay sample

Sno	Name	Rt	Area	Height	USP Resolution	USP Tailing	USP plate count	Injection
1	Decitabine	2.120	756986	68959		0.97	7254	1
2	Cedazuridine	3.536	2569857	198563	2.06	1.24	8837	1
3	Decitabine	2.120	758744	69858		1.06	6531	2
4	Cedazuridine	3.537	2598653	195681	2.05	0.98	7272	2
5	Decitabine	2.102	756849	69587		1.8	7587	3
6	Cedazuridine	3.537	2587457	192542	2.04	1.5	8372	3

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

The % purity of Decitabine and Cedazuridine in pharmaceutical dosage form was found to be 99.8%.

Linearity
Chromatographic data for linearity study
Decitabine

Concentration µg/ml	Average Peak Area
6	467848
8	619853
10	768785
12	928978
14	1095699

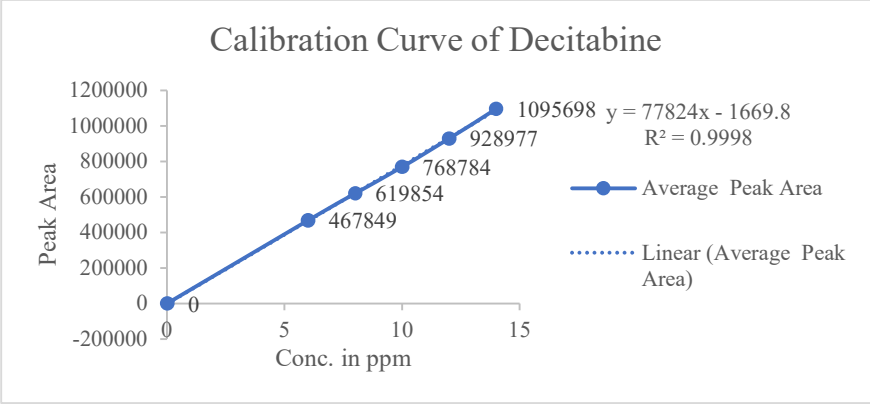

Fig 3: Calibration Graph for Decitabine

Cedazuridine

Concentration µg/ml	Average Peak Area
18	1789547
24	2456988
30	3085986
36	3759863
42	4406588

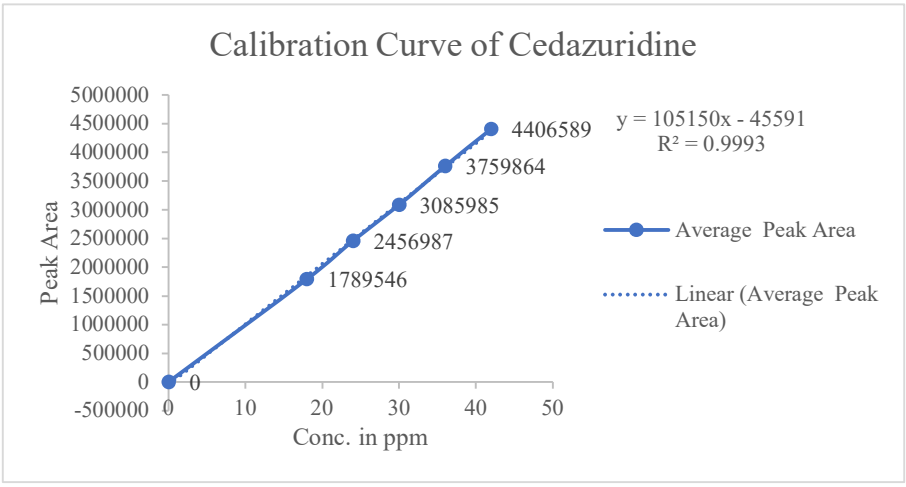

Fig 4: Calibration Graph for Cedazuridine

Repeatability

Table 7: Results of Repeatability for Decitabine:

Sno	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Decitabine	2.108	766853	702563	5686	1.6
2	Decitabine	2.105	765885	698788	5583	1.4
3	Decitabine	2.113	765843	701236	5522	1.6
4	Decitabine	2.109	768986	700125	5526	1.9
5	Decitabine	2.109	765844	698987	5579	1.7
Mean			766682.2			
Std. Dev			1358.219			
% RSD			0.177155			

- *%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 8: Results of method precision for Cedazuridine

Sno	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Cedazuridine	3.552	2569866	2231112	5366	1.6
2	Cedazuridine	3.550	2578473	2674211	5424	1.6
3	Cedazuridine	3.564	2568986	2231262	5369	1.5
4	Cedazuridine	3.564	2586844	2421303	5358	1.5
5	Cedazuridine	3.565	2545899	2324714	5497	1.6
Mean			2570014			
Std. Dev			15308.62			
% RSD			0.595663			

- *%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.*

Intermediate precision

Day 1

Table 9: Results of Intermediate precision for Decitabine

Sno	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Decitabine	2.108	758956	68987	5786	1.6
2	Decitabine	2.105	759868	68958	5699	1.4
3	Decitabine	2.113	758984	68546	5688	1.6
4	Decitabine	2.109	756893	68953	5782	1.9
5	Decitabine	2.109	759855	68596	5786	1.7
6	Decitabine	2.102	756986	68953	5694	1.6
Mean			758590.3			
Std. Dev			1339.793			
% RSD			0.176616			

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

Table 10: Results of Intermediate precision for Cedazuridine

S.No.	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Cedazuridine	3.552	2659853	190026	5486	1.5	2.04
2	Cedazuridine	3.550	2648572	190049	5422	1.6	2.03
3	Cedazuridine	3.564	2659866	190053	5469	1.6	2.01
4	Cedazuridine	3.564	2658548	190079	5488	1.6	2.05
5	Cedazuridine	3.565	2648982	190017	5493	1.6	2.02
6	Cedazuridine	3.537	2654653	190058	5464	1.6	2.03
Mean			2655079				

Std. Dev	5242.086
% RSD	0.197436

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

Day 2

Table 11: Results of Intermediate precision Day 2 for Decitabine

Sno	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Decitabine	2.102	766896	69859	5587	1.5
2	Decitabine	2.105	765989	69853	5635	1.6
3	Decitabine	2.112	766533	69825	5433	1.6
4	Decitabine	2.113	766215	69876	5469	1.6
5	Decitabine	2.109	765898	69855	5547	1.9
6	Decitabine	2.109	765246	69849	5508	1.7
Mean			766128.5			
Std. Dev			567.7234			
% RSD			0.074103			

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

Table 12: Results of Intermediate precision for Cedazuridine

Sno	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Cedazuridine	3.537	2653253	190111	5429	1.6	7.99
2	Cedazuridine	3.552	2648986	190059	5453	1.6	6.5
3	Cedazuridine	3.560	2658212	190143	5497	1.6	8.8
4	Cedazuridine	3.564	2653651	190033	5443	1.5	8.2
5	Cedazuridine	3.564	2648979	190059	5488	1.5	7.6
6	Cedazuridine	3.565	2658986	190048	5462	1.6	5.4
Mean			2653678				
Std. Dev			4313.355				
% RSD			0.162543				

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

Accuracy

Table 13: The accuracy results for Decitabine

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	392892.7	5	5.028	100.541%	100.352%
100%	781997	10	10.027	100.262%	
150%	1171989	15	15.039	100.254%	

Table 14: The accuracy results for Cedazuridine

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	204963	15	15.157	101.041%	100.94%
100%	365019	30	30.379	101.261%	
150%	521063.3	45	45.217	100.485%	

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

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

Robustness

Table 15: Results for Robustness

Decitabine

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 mL/min	765788	2.102	5588	1.7
Less Flow rate of 0.9 mL/min	758699	2.330	5459	1.7
More Flow rate of 1.1 mL/min	7689585	1.950	5697	1.7
Less organic phase	758413	2.290	5585	1.4
More organic phase	769851	1.998	5354	1.5

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

Cedazuridine

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 mL/min	2532159	3.537	5399	1.6
Less Flow rate of 0.9 mL/min	2458693	3.885	5328	1.7
More Flow rate of 1.1 mL/min	2658641	3.263	5257	1.7
Less organic phase	2452149	4.435	5213	1.2
More organic phase	2653895	3.009	5525	1.0

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

CONCLUSION

A new method was established for simultaneous estimation of Decitabine and Cedazuridine by RP-HPLC method. The chromatographic conditions were successfully developed for the separation of Decitabine and Cedazuridine by using Phenomenex Luna C18 (4.6×250mm, 5µm) particle size, flow rate was 1ml/min, mobile phase ratio was (45:55 v/v) Acetonitrile: Phosphate Buffer (pH-4.6 was adjusted with orthophosphoric acid), detection wave length was 245nm. The instrument used was WATERS HPLC Auto Sampler, Separation module 2695, photo diode array detector 996, Empower-software version-2. The retention times were found to be 2.102mins and 3.537mins. The % purity of Decitabine and Cedazuridine was found to be 99.8%. The system suitability parameters for Decitabine and Cedazuridine such as theoretical plates and tailing factor were found to be within limits. The analytical method was validated according to ICH guidelines (ICH, Q2 (R1)). The linearity study n Decitabine and Cedazuridine was found in concentration range of 6µg-14µg and 18µg-42µg and correlation coefficient (r2) was found to be 0.999 and 0.999, % recovery was found to be 100.351% and 100.93%, %RSD for repeatability was 0.177 and 0.595. The precision study was precise, robust, and repeatable. LOD value was 0.6 and 0.8, and LOQ value was 1.8 and 2.4 respectively. Hence the suggested RP-HPLC method can be used for routine analysis of Decitabine and Cedazuridine in API and Pharmaceutical dosage form.

ACKNOWLEDGEMENT

The Authors are thankful to the Management and Principal, Department of Pharmacy, Pydah College of Pharmacy, Kakinada, Andhra Pradesh for extending support to carry out the research work. Finally, the authors express their gratitude to the Sura Labs, Dilsukhnagar, Hyderabad, for providing research equipment and facilities.

REFERENCES

1. Kealey and P.J Haines, Analytical Chemistry, 1st edition, Bios Publisher, (2002), PP 1-7.
2. A.BraithWait and F.J.Smith, Chromatographic Methods, 5th edition, Kluwer Academic Publisher, (1996), PP 1-2.
3. Andrea Weston and Phyllisr. Brown, HPLC Principle and Practice, 1st edition, Academic press, (1997), PP 24-37.
4. Yuri Kazakevich and Rosario Lobrutto, HPLC for Pharmaceutical Scientists, 1st edition, Wiley Interscience A JohnWiley & Sons, Inc., Publication, (2007), PP 15-23.
5. Chromatography, (online). URL:http://en.wikipedia.org/wiki/Chromatography.
6. Meyer V.R. Practical High-Performance Liquid Chromatography, 4th Ed. England, John Wiley & Sons Ltd, (2004), PP 7-8.
7. Sahajwalla CG a new drug development, vol 141, Marcel Dekker Inc., New York, (2004), PP 421–426.

8. D.H. Shewiy, E. Kaale, P. G. Risha, B. Dejaegher, J. S. Verbeke, Y. V. Heyden, Journal Pharmaceut. Biomed. Anal, 66, 2012, 11-23.
9. M.D. Rockville, General Tests, Chapter 621 – Chromatography System Suitability, United States Pharmacopeial Convention (USP), USP 31, 2009.
10. FDA Guidance for Industry-Analytical Procedures and Method Validation, Chemistry, Manufacturing, and Controls Documentation, Centre for Drug Evaluation and Research (CDER) and Centre for Biologics Evaluation and Research (CBER), 2000.
11. Korany MA, Mahgoub H, Ossama TF, Hadir MM. Application of artificial neural networks for response surface modelling in HPLC method development. J Adv Res, 3, 2012, 53-63.
12. Swartz ME, Jone MD, Fowler P, Andrew MA. Automated HPLC method development and transfer. Lc Gc N. Am, 75, 2002, 49-50.
13. Snyder LR, Kirkland JJ, Glajach JL. X. In Practical HPLC Methods Development. John Wiley, New York, 295, 1997, 643-712.
14. Swartz M, Murphy MB. New Fronties in Chromatography. Am Lab, 37, 2005, 22-27.
15. Dolan JW. Peak tailing and resolution. Lc Gc N. Am, 20, 2002, 430-436.