Publisher Name: ZenToks Books
Editorial Book: Zentoks Series in Pharmacy Science
ISBN: 978-81-958975-5-1

CHAPTER 1

RP-HPLC Method Development and Validation for the estimation of Decitabine in Drug Dosage form

Kumar Raja Jayavarapu[1*], Praveen Kumar Dasari[1] and G. Manasa[1]

[1] *Department of Pharmaceutical Analysis, Mother Teresa Pharmacy College, Sathupally, Telangana – 507303, India.*
[*] *Corresponding Author.*

Abstract

A new, precise, rapid, accurate RP-HPLC method was developed for the estimation of Decitabine in pharmaceutical dosage form. After optimization the good chromatographic separation was achieved by Isocratic mode with a mixture of Ammonium Acetate buffer of pH 4.5: Acetonitrile (985:15) v/v as the mobile phase with Develosil RP Aqueous-AR-5 (150 x 4.6 mm, 5 μm), column as stationary phase at flow rate of 1.5 mL/min and detection wavelength of 244 nm. The Retention time of decitabine was found to be 3.786 min. The linearity of this method was found in the concentration range of 50-150 μg/mL. The correlation coefficient R2 value is found to be 0.998. The LOD for this method was found to be 0.0003 μg/mL. The LOQ for this method was found to be 0.0009 μg/mL. This method was found to be good percentage recovery about 99.77 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 analyte in the sample without interference from excipients of formulation. The method was extensively validated according to ICH guidelines for Linearity, Range, Accuracy, Precision, specificity and Robustness.

Keywords: UV Spectrophotometer, Decitabine, High Performance Liquid Chromatography.

1. Introduction

Decitabine 1 is chemically 4-amino-1-(2-deoxy-β -D-erythro-pentofuranosyl)-1, 3, 5-triazin-2 (1H) -one and the structural formula is shown in Fig. 1. The molecular formula is C8 H12N4O4 and molecular weight is 228.21g/mol. It is stable at room temperature, sparingly soluble in ethanol soluble in water and freely soluble in DMSO. Decitabine is believed to exert its antineoplastic 2 effects after phosphorylation and direct in corporation into DNA and inhibition of DNA methyl transferase, causing hypomethylation of DNA and cellular differentiation or apoptosis. It useful in treatment of Myelodys plastic syndrome (MDS) and antineoplastic agent. From the literature survey, it was found that Decitabine was estimated by analytical methods such as few UV-Visible methods and high-performance liquid chromatographic 3 (HPLC) method. The present developed method was simple, precise, specific and accurate.

How To Cite: K.R. Jayavarapu, P.K. Dassari, G. Manasa *RP-HPLC Method Development and Validation for the estimation of Decitabine in Drug Dosage form. In A. Rajput (Eds). Zentoks Series in Pharmacy Science. Pages 1–6, 2025. ZenToks Books, India. ISBN: 978-81-958975-5-1*

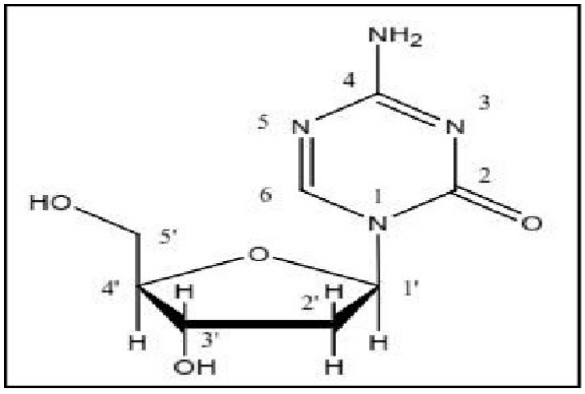

Figure 1: Structural Formula 0f Decitabine

2. Materials and Methods

For HPLC, Waters 2690 series model equipped with Auto sample, PDA (2998) detector 4, 5 with Empower 2 software was employed for the investigation. All the chemicals used in the investigation were of HPLC grade. The chromatographic analysis was performed on a Develosil RP Aqueous-AR-5 column 6 (150 x 4.6 mm, 5μm). The mobile phase consists of Ammonium acetate buffer pH 7, 8, 9 of 4.5 and acetonitrile in the ratio of 985:15v/v. The optimized chromatographic conditions are summarized in Table 1, 2.

Table 1: Optimized chromatographic conditions for the proposed method for Decitabine.

S. No	Parameter	Optimized condition
1	Linear range (μg/ml)	50-150
2	Detection wavelength (nm)	244nm
3	Temperature (°C)	20-25°C
4	Retention time (Min)	3.401min
5	Limit of detection (μg/ml)	0.0003
6	Limit of quantification (μg/ml)	0.0009
7	Flow Rate	1.5 mL/min

Table 2: System Suitability test Parameters for the Proposed Method for Decitabine.

S. No.	Parameter	Optimized condition
1	Retention time (Min)	3.653min
2	Theoretical plates	6599

2.1. Preparation of Ammonium Acetate buffer pH 4.5

About 3.85 gm of Ammonium Acetate was weighedand dissolved in 1000 mL of water. Adjust the pHto 4.5$\pm$0.02 using ortho phosphoric acid. The buffer was filtered through 0.45μm membrane filter paper to remove all fine particles.

2.2. Preparation of Standard solution

About 100 mg of decitabine was weighed into a 100 mL volumetric flask, to this 25mL of mobile phase was added, sonicated and the volume was made up with the mobile phase.

2.3. Preparation of Sample solution

Sample name : Decogen
Manufacture name : Pfizer
Weigh a quantity of powder equivalent to 100 mg of decitabine in 100 mL volumetric flask and make up mark with mobile phase. From above solution Pipette 1 mL of the clear solution in to 10 mL volumetric flask and make up volume with mobile phase. The resulting solution is used to record the chromatogram (Fig. 2).

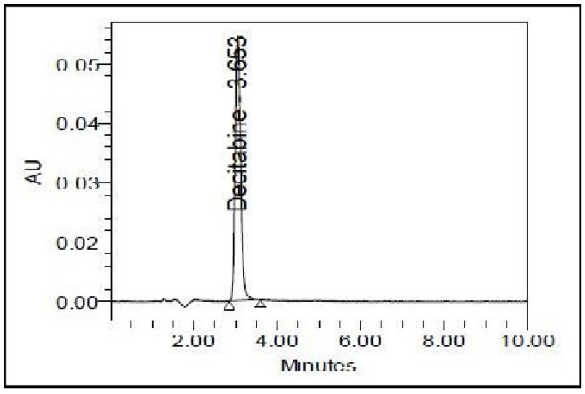

Figure 2: Chromatogram of Decitabine

Table 3: Results for Decitabine.

S.NO	Name	RT	Area	TP	TF
1	decitabine	3.653	345505	6599	1.18

Table 4: Results of assay.

Drug	Label claim(mg)	Amount found(mg)	% Assay
Decitabine	50	49.95	99.9

Observation
So the % assay found to be within the limits. The percentage purity of Decitabine was found to be within the limits that is 98-102 %.

3. Hplc Method Validation

3.1. System Suitability

To verify that the analytical system is working properly and can giveaccurate and precise results were evaluated by 100 μg/mL of Decitabine was injected six times and the chromatograms were recorded for the same.

Table 5: Results for system suitability of decitabine.

Injection	**RT**	**Peakarea**	**Theoretical plates (TP)**	**Tailing factor (TF)**
1	3.793	345849	6679	1.22
2	3.799	345177	6441	1.23
3	3.72	344045	6552	1.22
4	3.726	345849	6551	1.24
5	3.797	347590	6283	1.21
6	3.799	345784	6329	1.23
Mean	3.772333	345715.7	-	-
SD	0.038323	1151.924	-	-
%RSD	1.015901	0.3332		–

3.2. Acceptance criteria

• The % RSD for the retention times of decitabine Peaks from 6 replicate injections of each Standard solution should be not more than 2.0
• The % RSD for the peak area responses of decitabine peaks from 6 replicate injections of each standard solution should be not more than 2.0%.
• The number of theoretical plates (N) for the decitabine peaks is not less than 2000.
• The Tailing factor (TP) for the decitabine peak is not more than 2.0.

3.3. Result

The plate count and tailing factor results were found to be satisfactory and are found to be within the limit. The % RSD was found to be 0.33.

3.4. System precision

The system precision was determined by analysing standard preparation of decitabine for six times. The chromatograms were recorded and the results were summarized in Table 6.

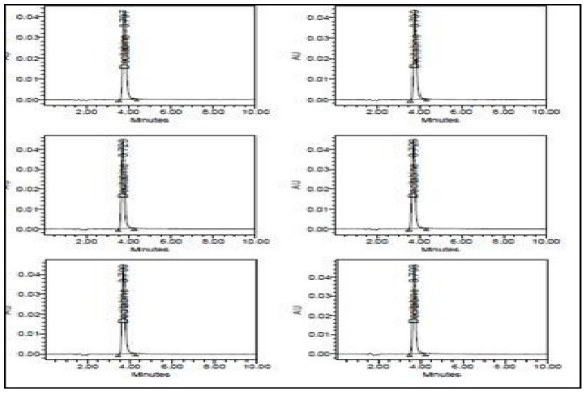

Figure 3: List of Chromatograms for system precision

Table 6: Results for system precision.

Injection	Decitabine			
	Retentiontimes	**Area**	**TP**	**Tf**
1	3.793	345849	6679	1.22
2	3.799	345177	6441	1.23
3	3.72	344045	6552	1.22
4	3.726	345849	6551	1.24
5	3.797	347590	6283	1.21
6	3.799	345784	6329	1.23
Average	3.772333	345715.7	6472.5	1.225
SD	0.038323	1151.924	-	-
%RSD	1.015901	0.3332	-	-

3.5. Result

% RSD of 6 determinations of decitabine for System precision found to be within the acceptance criteria of less than 2.0%.

3.6. Method precision

Method precision was determined by injecting sample solutions of concentration decitabine (100μg/mL) for six times are prepared separately. The chromatograms were recorded and the results were summarized in Table 7.

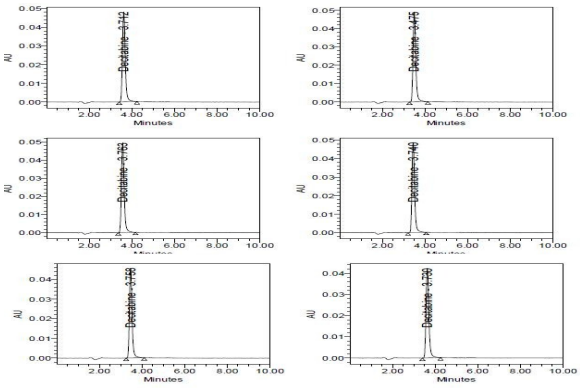

Figure 4: List of Chromatograms for method precision

3.7. Result

The % RSD of 6 determinations of decitabine for System precision found to be within the acceptance criteria of less than 2.0%.

Table 7: Results for method precision.

Injection	Decitabine			
	Retentiontimes	**Area**	**TP**	**Tf**
1	3.74	345746	6883	1.25
2	3.758	344016	6140	1.23
3	3.795	347992	6829	1.22
4	3.763	346110	6874	1.22
5	3.712	345853	6822	1.21
6	3.73	345690	6932	1.22
Average	3.749667	345901.2	6746.667	1.225
SD	0.028987	1268.072	-	-
%RSD	0.773065	0.3666	-	-

3.8. Linearity and range

Preparation of standard stock solution

Standard stock solutions of decitabine (mg/mL) were prepared by dissolving 100 mg of decitabine in 100 mL of mobile phase. After that filtered the solution using 0.45-micron syringe filter and Sonicated for 5 min further dilutions were given in the Table 8.

Table 8: Linearity data of Decitabine.

S. No.	Concentration (μg/mL)	Area
1	50	174482
2	70	232730
3	100	345818
4	120	423221
5	150	518543

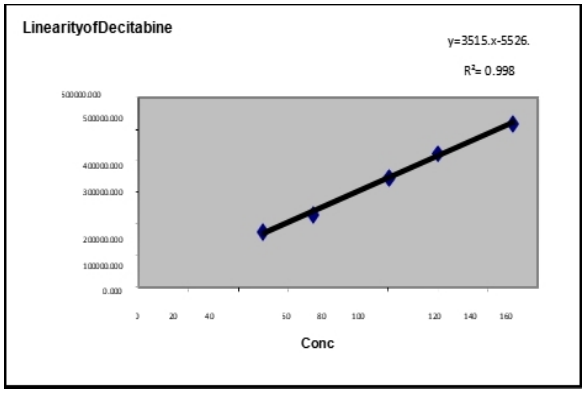

Figure 5: Graph for Linearity data of Decitabine

Table 9: Observation for linearity.

S. No.	Parameter	Decitabine
1	Correlation coefficient	0.998
2	Slope	3515
3	Intercept	5526

3.9. Acceptance criteria

The relationship between the concentration (in %) and area of decitabine should be linear in the specified range and the correlation should not be less than 0.99.

3.10. Result

The correlation coefficient for linear curve obtained between concentration vs. Area for standard preparations of decitabine was found to be 0.998 respectively.

3.11. Robustness

The Robustness of the method was determined. The results obtained by deliberate variation in method parameters are summarized below in Table 10.

Table 10: Results for Robustness of Decitabine.

Chromatographic Changes		Retentiontime(min)	Tailing factor
Wavelength (nm)	242	3.783	1.07
	244	3.768	1.24
	246	3.770	1.11
Mobile phase composition (v/v)	983:18	3.751	1.06
	985:15	3.768	1.24
	987:13	3.728	1.05

3.12. Result

The tailing factor was found to be within the limits on small variation of flow rate and wavelength.

4. Conclusion

A new precise, accurate, rapid method has been developed for the simultaneous estimation of Decitabine in pharmaceutical dosage form by RP- HPLC. The optimum wavelength for the determination of Decitabine was selected at 244 nm. Various trials were performed with different mobile phases in different ratios, but Ammonium Acetate buffer pH 4.5: ACN (985:15) was selected as good peak symmetry. The Retention time of decitabine was found to be 3.786 min.The different analytical performance parameters such as linearity, precision, accuracy, and specificity, LOD, LOQ were determined according to International Conference on Harmonization ICH Q2B guidelines. The calibration curves were obtained by plotting peak area versus the concentration over the range of 50-150 μg/mL. From linearity the correlation coefficient R^2 value was found to be 0.998. The proposed HPLC method was also validated for system suitability, system precision and method precision. The % RSD in the peak area of drug was found to be less than 2%. The number of theoretical plates was found to be more than 2000, which indicates efficient performance of the column. The LOD for this method was found to be 0.0003 μg/mL. The LOQ for this method was found to be 0.0009 μg/mL, indicates the sensitivity of the method. The percentage of recovery of was found to be 99.77 shows that the proposed method is highly accurate.

References

[1] The Drugs and Cosmetics Act and Rules, 1940.

[2] Methods of Analysis-http://www.pharmatutor.org/pharma-analysis

[3] Douglas,A.;Skoog,F.;James,H.;Stanley,R.C.LiquidChromatography. In Instrumental Analysis, 9th ed.; Cengage Learning India Pvt.Ltd.:NewDelhi,2007;893-934.

[4] Skoog; Holler; Crouch; Liquid Chromatography. In Instrumental Analysis, Cengage Learning India.:NewDelhi. 2011;893.

[5] Chatwal, R. G.; Anand, K. S. High Performance Liquid Chromatography. In Instrumental Methods Of Chemical Analysis, 5thed.; Himalaya Publishers.:Mumbai,2010;2.570- 2.629.

[6] Sharma, B. K. High Performance Liquid Chromatography. In Instrumental Methods Of Chemical Analysis, 24th ed.; Goel Publishers.: Meerut, 2005;295- 300.

[7] Alfonso, R. G.; Ara, H. D. M.; Glen, R. H.; Thomas, M.; Nicholas, G. P.;Roger, L.S.; Steve, H. W. Chromatography. In Remington: The Science and Practice of Pharmacy, 20thed.; Lippincott Williams & Wilkins:Philadelphia, 2000;587

[8] Typesofelution-http://chemwiki.ucdavis.edu/@api/deki/pages/402/pdf

[9] Braithwaite, A.; Smith, F. J. Liquid Phase Chromatography on Columns. In Chromatographic methods,5thed.;Kluwer Academic Publishers:Netherlands, 1999;129.

[10] Prakash Chander, Tushar N. Mehta. Fast Analysis of Decitabine Using a Solid Core HILIC Column themoscientific.2013,1-3.

[11] Gloryhepsiba,B.B.Teja,K. Ashok kumar,Y.Ravindra reddy. Stability indicating RP-HPLC Method Devlopment and Validation of Decitabine Drug in Formulation International Journal of Pharm Tech Research. 2011,3910,237-243.