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Analytical Method Development and Validation of Gemcitabine in Tablets by HPLC by Different Analytical Techniques

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Abstract: *An isocratic reverse phase liquid chromatography (RP-HPLC) method has been developed and subsequently validate for the determination of Gemcitabine in pharmaceutical formulation. In this method, Agilent TC C18 (250*4.6mm ;) particle size 5µm column with mobile phase consisting of Acetonitrile and water in ratio of 50:50 v/v was used. The detection wavelength is 270nm and the flow rate 1.0mL/ min. The linearity was found in the range of 80µg/ml and shows a correlation coefficient of 0.9992. The method precision for the determination of assay was below 2.0% RSD. The developed method was validating by determining specificity, accuracy, precision and linearity. The developed method is simple , fast, accurate and precise hence can be applied for the routine quality control analysis of Gemcitabine in pure and pharmaceutical formulation.*

Key words: *HPLC, Gemcitabine, Agilent TC C18, correlation coefficient and Acetonitrile.*

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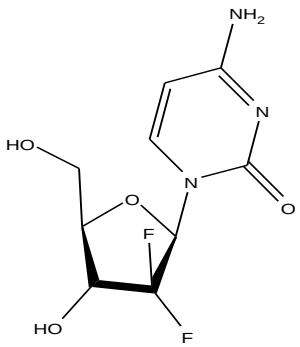
I. Introduction:

Gemcitabine is deoxy cytidine analogue having (antineoplastic or cytotoxic) activity. It is broad spectrum Antimetabolite used in treatment of various forms of cancer such as pancreatic adenocarcinoma, ovarian, cancer small cell lung. Cancer, bladder cancer. It acts by inhibition of thymidilate kinase and DNA synthesis. It is a prodrug and converted to its active metabolites. Following influx through the cell membrane via nucleoside transporters, gemcitabine undergoes complex intra cellular conversion to the nucleotides gemcitabine diphosphate (dfdCDP) and triphosphate (dfdCTP) responsible for its cytotoxic actions. The cytotoxic activity of gemcitabine may be the result of several actions on DNA synthesis. dfdCTP completes with deoxycytidine triphosphate (dCTP) is an inhibition of DNA polymerase. dfdCDP is a potent inhibition of ribonucleoside reductase resulting in depletion of deoxyribonucleoside pools necessary for DNA synthesis and by potentiating the effects of dfdCTP. DfdCTP is incorporated into DNA and the incorporation of one or more nucleotide leads to DNA stand termination. This extra nucleotide may be important in hiding the dfdCTP from DNA repair enzymes, as incorporation if dfdCTP into DNA appears to be resistant to the normal mechanisms of DNA repair. Gemcitabine HCl is soluble in water, slightly soluble in methanol, and practically insoluble in ethanol and polar organic solvents.

GEMCITABINE

Structure:



Chemical name: *4-amino-1-(2-deoxy-2, 2- difluoro-β-D –erythro –pentofuranosyl) pyrimidin-2(1H)-one .*
Molecular weight: 269.198g/mol.

II. Method development of gemcitabine by HPLC:

Selection of detector wave length:

An UV spectrum of 100µg/ ml Gemcitabine in diluents (water) was recorded by scanning in the range of 200nm to 400nm. From the UV spectrum wavelength of 270nm was selected. At this wave length Gemcitabine standard showed good absorbance.

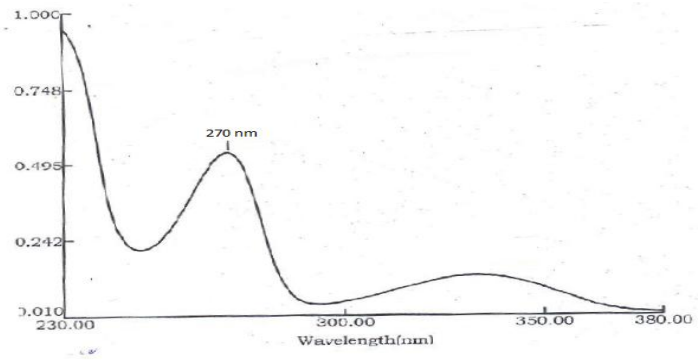


Fig. No. 1: Spectra of Gemcitabine showing λ_{max} at 270nm

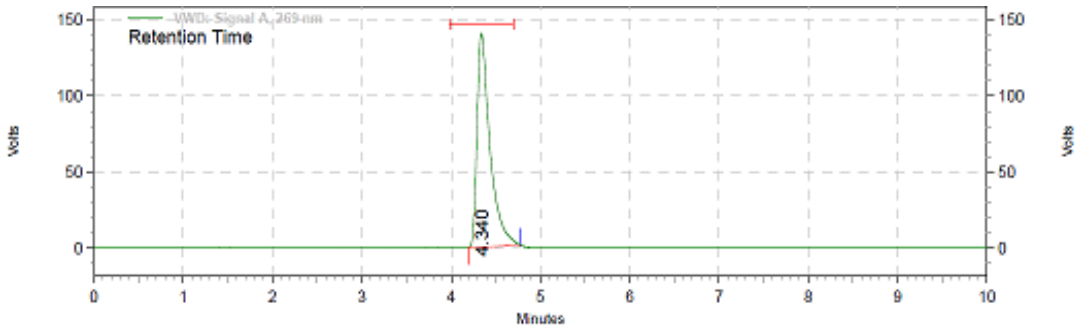


Fig. No. 2: Chromatogram of gemcitabine

Peak name	RT	Area	% Area	Theoretical plates	Peak purity index
Gemcitabine	4.340	26988929	9168	1,00000	1.51

Table No.1: Typical chromatogram of Gemcitabine

Method Validation

System Suitability:

The system suitability studies were done with the 50mg of standard drug. The % of RSD values are below are2% , theoretical plate count is above 2000 and tailing factor is less than 2, indicating that the method is suitable . The chromatogram is recorded.

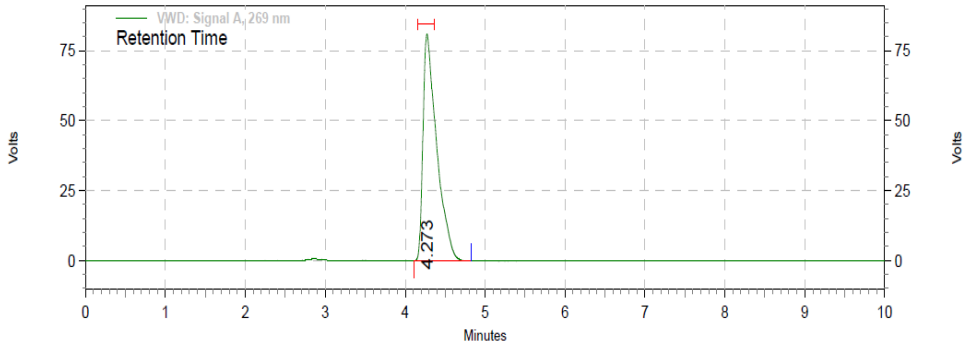


Fig. No. 3: Standard Chromatogram showing System suitability

S.No.	Peak Name	Rt(min)	Area	USP Tailing	Plate count
1	Gemcitabine	4.273	21894553	1.11	5413
2	Gemcitabine	4.271	21854321	1.12	5377
3	Gemcitabine	4.270	21857295	1.12	5396
4	Gemcitabine	4.273	21894553	1.12	5385
5	Gemcitabine	4.272	21895496	1.11	5369
6	Gemcitabine	4.275	21895489	1.11	5364
Mean			21895534	1.12	5384
SD			2218.7		
%RSD			0.14		

Table No.2: Results from system suitability study

Linearity:

The linearity study was performed for the concentration of 80µg/ml to 120 µg/ml level. Each level was injected into chromatographic system. The area of each level was used for calculated of correlation coefficient.

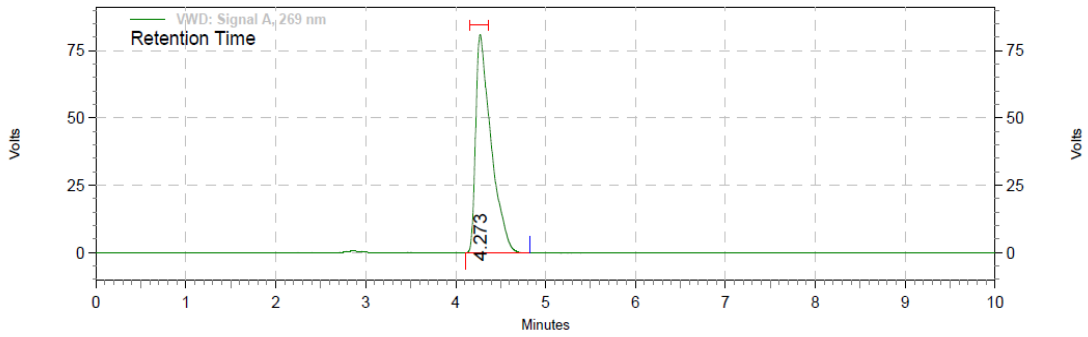


Fig. No. 4: Chromatogram showing linearity level-1

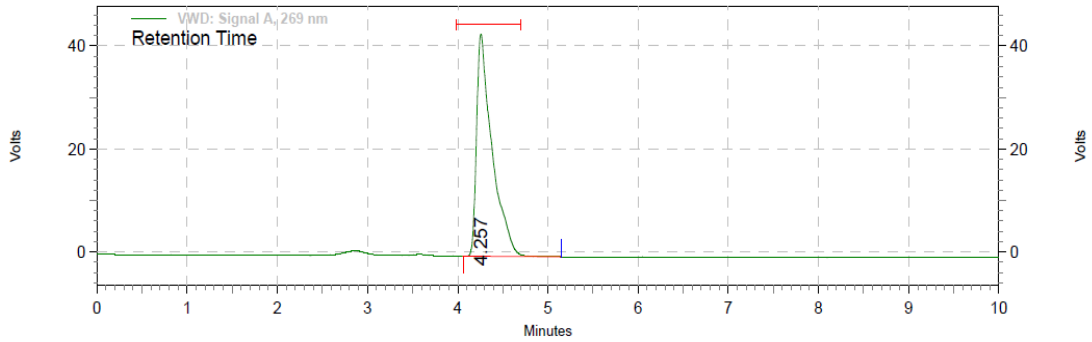


Fig. No. 5: Chromatogram showing linearity level -2

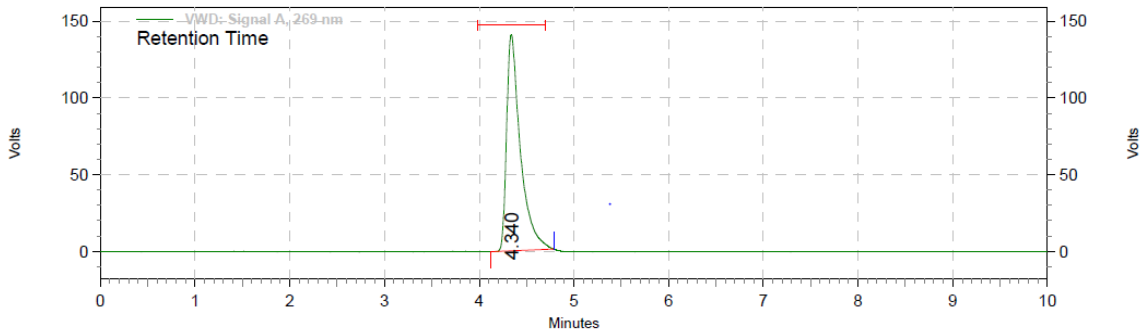


Fig. No. 6: Chromatogram showing linearity level -3

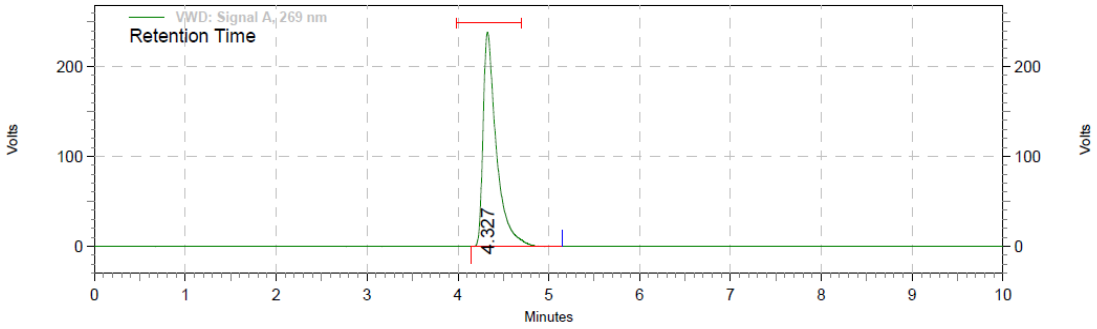


Fig. No. 7: Chromatogram showing linearity level -4

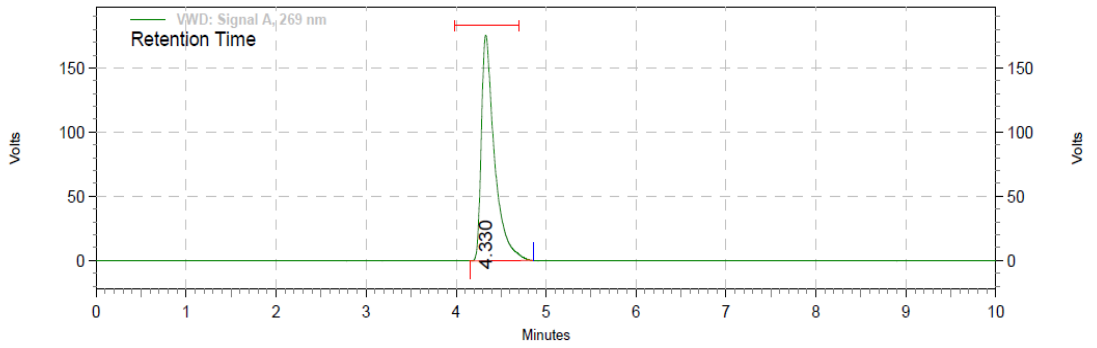


Fig. No. 8: Chromatogram showing linearity level- 5

S.No.	Linearity level	Concentration	Peak area
1	80µg/ ml	80	21894553
2	90µg/ml	90	24557980
3	100µg/ml	100	26988929
4	110µg/ l)	110	29423432
5	120µg/ml	120	31776601
Correlation coefficient			0.9992

Table No.3: Linearity levels

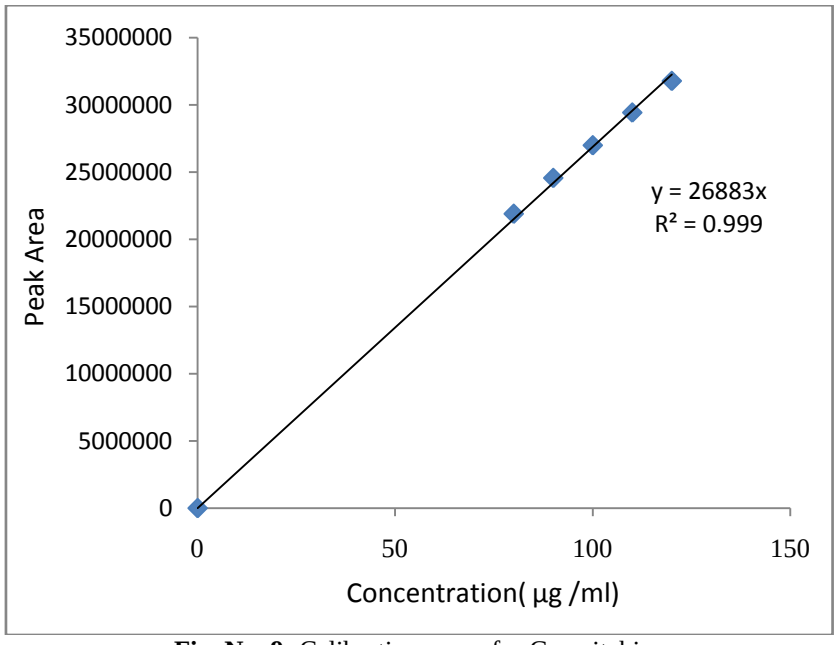


Fig. No. 9: Calibration curve for Gemcitabine

The linearity study was performed the correlation coefficient of Gemcitabine was found to be 0.9992 respectively (NLT 0.99).

Specificity:

The specificity test was performed for Gemcitabine. It was found that there was no interference of impurities in retention time of analytical peak. The method showed excellent specificity with Gemcitabine eluting at retention of 4.340 minutes. No interference was observed with mobile phase.

Accuracy:

The accuracy study was performed for 50µg/ml for Gemcitabine. Each level was injected in triplicate into chromatographic system. The area of each level was used for calculation of % recovery.

Level recovery	Amount of drug spiked (µg/ml)	Drug recovered	%Recovery	Mean	SD	% RSD
50	9.6	9.62	100.2	100.4	0.346	0.34
		9.62	100.2			
		9.68	100.8			
100	12	12.23	101.9	101.4	0.974	0.95
		12.08	100.6			
		12.21	102.5			
		14.26	99.02			
150	12.4	14.21	99.8	99.50	0.6451	0.64
		14.45	100.3			

Table No.4: Results from accuracy study

Precision:

Conc µg/ml	Peak area	Statistical parameters
50	21579032	Mean: 21576038 S.D: 3123.5 % R.S.D.
	21574827	
	21579953	
	21578064	
100	26988929	Mean: 26988561 S.D:1410.15 % 0.10
	26980451	
	26989058	
	26988935	
	26988847	
	26988956	
150	31776749	Mean: 31777632 S.D: 1227.72 % R.S.D: 0.07
	31778120	
	31776549	
	31776436	
	31776659	
	31776439	

Table No.5: Results from precision study- Intra day

III. Results and Discussion:

Validation parameter	Acceptance Criteria	Results
System suitability	The RSD should be NMT 2% for each peak	%RSD IS 0.14
Specificity	The interference of the diluents/ placebo is considered insignificant , if the chromatogram of the placebo shows no peak, at the retention time of analyte peak	No peaks are eluted at the retention time of Gemcitabine
System precision	The % RSD of 6 replicate injections should be NMT 2.0%	% RSD is 0.13
Method precision	The % RSD calculated on 6 determinations of assay value should be NMT 2 % `	% RSD is 0.12
Linearity	The correlation coefficient should be NLT 0.99	0.9992
Accuracy	The method is considered accurate if average recovery is NLT 98 % and NMT 102 %	Accuracy for the average of triplicate in each concentration samples are within the limit.

Table No.6: Results and discussion

IV. Conclusion:

The developed method was validated for specificity, accuracy, precision, recovery linearity, robustness ruggedness and system suitability. The percentage of recovery of Gemcitabine was found to be 99.5% to 101.4% level. The low standard deviation values and good recoveries indicate the reproducibility and accuracy of the developed method. As well the % RSD values for the precision study also were within acceptable limit

Hence the developed chromatographic (HPLC) method for Gemcitabine is said to be rapid, simple, precise, accurate, and cost effective that can be effectively applied for the routine analysis in research institution, quality control department in industries, approved testing laboratories, biopharmaceutical studies, and clinical pharmacokinetic studies.

From the overall results obtained it was concluded that the developed method was more accurate, precise, specific and robust with $\pm 2^0$ C in temperature,± 0.2 mL min in flow rate, ± 10% variation in organic phase.

Bibliography:

[1]. M. R. Jayapal, A Santosh Kumar Sreevatsav, K. Shyam Sunder , "RP-HPLC method for the estimation of Gemcitabine HCl 1 gm injection in parenteral dosage form", International Journal of Pharmaceutical Chemistry, Vol 3,No. 3, 2013.

[2]. N. A. Parshina, T. V. Pleteneva, V. N. Baikova, M. N. Narimanov, S. A. Tyulyandin, "Quantitative Estimation of Gemcitabine by HPLC in Plasma", Pharmaceuticals Chemistry Journal, May 2008, Volume 42, Issue 5, Pages 288-290.

[3]. Prakash.S.Sarsambi, Kalyan.K, Chaitanya.S, "Stability indicating RP-HPLC method for the determination of Gemcitabine in bulk drug and pharmaceutical dosage forms", RGUHS Journal of Pharmaceutical Sciences, 1(3), 2011, Pages: 243-247.

[4]. R. Murali Krishna, M. Ramesh, M. Buela, T. Siva Kumar "Method Development and Validation for the estimation of Gemcitabine HCl in Pharmaceutical Dosage forms by RP-HPLC", Indo American Journal of Pharmaceutical Research, Vol: 1(8), 2011, Pages: 189-195.

[5]. Shaik Mastanamma, G. Ramkumar, D. Anantha Kumar, and J. V. L. N. Seshagiri Rao, "A Stability indicating RP-HPLC method for the estimation of Gemcitabine HCl in injectable dosage forms", Volume 7, 2010, S1, Pages: S239-244.

[6]. K. K. Kumar, K. E. V. Nagoji, and R. V. Nadh, "A validated RP-HPLC method for the estimation of Lapatinib in tablet dosage form using Gemcitabine HCl as an internal standard", Indian Journal Of Pharmaceutical Sciences, 74(6), 2012 Nov-Dec, Pages: 580–583.

[7]. R. Losa , M.I. Sierra, C. Guardado, A. Fernández, M.O. Gión, D. Blanco, J.M. Buesa , "Development and Validation of an ion-pair HPLC method for Gemcitabine and 2',2'-diflouro-2'-deoxyuridine determination", Elsevier Analytica Chimica Acta, Volume 528, Issue 2, 10 January 2005, Pages: 255-260.

[8]. Bruce Keith, Yan Xu, Jean L. Grem, "Measurement of the anti-cancer agent Gemcitabine in human plasma by high performance liquid chromatography", Elsevier Journal of Chromatography B, Volume 785, Issue 1, 25 February 2003, Pages 65-72.

[9]. Qinmei Zhou, Liucheng Liu, Dengshan Zhang, and XingFeng Fan , "Analysis of Gemcitabine liposome injection by HPLC with evaporative light scattering detection", December 2012, Vol. 22, No. 4, Pages 263-269.

[10]. Rajesh, V.; Anupama, B.; Jagathi, V.; Sai Praveen, P, "Simultaneous Estimation of Gemcitabine HCl and Capecitabine HCl in combined tablet dosage form by RP-HPLC method", E-Journal of Chemistry, 2011, Vol. 8, Issue 3, Pages 1212.

[11]. H. Elkhaïli, S. Niedergang, D. Pompei, L. Linger, D. Leveque, F. Jehl, "High Performance Liquid Chromatographic Assay for Meropenem in Serum", Elsevier Journal of Chromatography B: Biomedical Sciences and Applications, Volume 686, Issue 1, 8 November 1996, Pages 19-26.

[12]. W. V. Caufield, J. T. Stewart, "HPLC separations of Meropenem and selected pharmaceuticals using a polar endcapped octadecylsilane narrow bore column", Chromatographia, March 2000, Volume 51, Issue5-6, pages: 308-314.

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