International Journal of Pharmaceutical Chemistry
ISSN: 2249-734X (Online)
Journal DOI:10.7439/ijpc
CODEN:IJPCI4 (American Chemical Society)

Research Article

RP-HPLC method for estimation of Gemcitabine HCl 1gm injection in parenteral dosage form

A Santosh Kumar Sreevatsav[*1], K. Shyam Sunder[2] and Jayapal Maleraju[3]

[1]*Department of Pharmaceutical Analysis, MRR College of Pharmacy, Nadergul, A.P.*
[2]*Department of Pharmaceutical Chemistry, MLR Institute of Pharmacy, Dundigal (V), Hyd-43.*
[3]*Department of Chemistry S. V. University Tiruapti -517502*

Abstract

A Simple, precise, rapid and stability indicating reverse phase liquid chromatography method has been developed and validated in order to determine Gemcitabine HCl in parenteral dosage form. A L-7 column made up ot stainless steel having C-18 as stationary phase with an internal diameter of 250 X 4.6mm is used. Phosphate buffer : methanol in the ration of 97:3 is used as the mobile phase using 0.2µl membrane filter. A UV detector is used where 266nm is the wavelength set for detection with a flow rate of 1.0ml/min and the runtime was for 10 min. The method is then validated for linearity, selectivity, precision (method, intermediate, system), robustness. These parameters generated results which are under the limits.

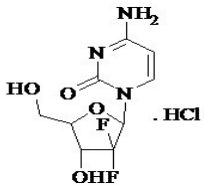
Key words: Gemcitabine HCl, Robustness, Liquid Chromatography

1. Introduction

Gemcitabine[1] (2'-2'difluorodeoxycytidine) is a cytotoxic agent with wide spectrum of Anti tumor activity, used in cancer of the pancreas, lungs, ovaries, breast and urinary bladder. In blood and liver, Gemcitabine is metabolized to 2'-2'-difluorodeoxyuridine by deamination[2]. Gemcitabine is a pyrimidine antimetabolite, structurally related to cytosine arabinoside and is effective in treating a wide range of solid tumors. Gemcitabine is associated with specific inhibition of DNA synthesis (S-Phase) and this activity depends upon its intracellular phosphorylation by nucleoside kinase to the active diphosphate and triphosphate nucleosides[3]. A literature survey reveals the report of a few analytical methods for determination of gemcitabine in pharmaceutical dosage forms and in biological fluids by HPLC and LC-MS/MS. But, no stability indicating method is being developed[4]. The present work describes the development of a validated RP-HPLCmethod in pharmaceutical dosage form. The present study was validated following the ICH guidelines[5-10].

Gemlive 1000 Injection is Clear, Colourless liquid.

Structure:



Gemcitabine HCl

* Correspondence Info
A Santosh Kumar Sreevatsav
Department of Pharmaceutical Analysis,
MRR College of Pharmacy, Nadergul, A.P. India
Email:sreevatsav_a@yahoo.com

2. Materials and Methods

2.1 Instruments / Equipments Used

H.P.L.C- Waters - Alliance 510 with UV- 484 Data Ace software (Instrument I.D: AL-011)
HPLC - Agilent 1100 Series with Chromeleon software (Instrument I.D: AL-013)
HPLC Analytical column L-7, 25cm x 4.6mm x 5µm
Analytical weighing balance - Mettler Toledo B204S
Millipore Nylon 0.2µm
Laboratory accessories

2.2 Chemicals Used

Gemcitabine HCl working standard (WRS No-WS-005; Purity – 99.95% on dry basis).
Gemlive 1000 Injection
Monobasic Sodium phosphate –AR
phosphoric acid
Methanol –AR
Water – HPLC Grade.

2.3 Chromatographic Conditions:

Column	:L-7, 25cm x 4.6mm x 5µm
Mobile Phase	:Weigh accurately and transfer 13.8 g of Monobasic Sodium phosphate and 2.5ml phosphoric acid to a 1000 ml volumetric flask. Add about 980 ml of water, dissolve and dilute to volume with water. Adjust pH to 6.5 with OPA. Mix buffer and Methanol in the ratio of 97:3. Filter through 0.2 µm Nylon membrane filter paper and degas prior to use.
Wavelength	: 266 nm
Flow Rate	: 1.0 ml / minute
Injection volume	: 20 µl
Run time	: 10 minutes
Blank solution	: Use Mobile phase as blank
Diluent	: Use Mobile phase as diluents

a) Preparation of Gemcitabine HCl standard Solution: Weigh accurately about 40 mg of Gemcitabine HCl working standard and transfer to a 100 ml volumetric flask. Add 70 ml of diluent and sonicate to dissolve. Dilute to volume with diluent and mix. Transfer 1.0 ml of solution into a 10 ml of volumetric flask and dilute to volume with the diluent and mix. Filter the solution through 0.2µm nylon membrane filter. (Dilution scheme: 40mg ® 100.0 ml ® 1 ml /10.0 ml)

b) Preparation of Test Solution: Pippete out 1ml of sample solution into a 100 ml volumetric flask. Add about 70 ml of diluent and shake for 5 minutes by mechanical means or manually and further sonicate for 5 minutes. Transfer 1.0 ml of solution into a 10 ml of volumetric flask and dilute to volume with the diluent and mix. Filter the solution through 0.2µm nylon membrane filter.

(Dilution scheme: 1 ml ®100.0 ml ® 1 ml /10.0 ml)

2.4 Assay Method: Separately inject equal volumes of blank, five replicate injections of system suitability solution (Gemcitabine HCl standard working solution). Then inject two injections of test solution and record the chromatograms. Disregard any peak due to blank in the test solution. Calculate % RSD of five replicate injections of system suitability solution (Gemcitabine HCl standard working solution). Check tailing factor and theoretical plates of the peak in the chromatogram obtained with 5^{th} injection of system suitability solution (Gemcitabine HCl standard working solution).

3. Results and Discussions

3.1 Validation Results: The system suitability parameters were monitored throughout the validation study and are recorded in the validation report. The validation data is summarized below:

3.1.1 Specificity / Selectivity: Selectivity was performed by injecting the diluent blank solution, system suitability solution, test solution.

3.1.2 Acceptance criteria: The Gemcitabine HCl peak should be well resolved from any other peak and from each other. The diluent blank solution should not show any peak at the retention time of the Gemcitabine Hcl. The system suitability criteria were found to meet the pre-established acceptance criteria as per the analytical method.

Table - 2: System suitability – Selectivity

Sr. No.	Area of Gemcitabine HCl
1	2654.869
2	2701.88
3	2689.57
4	2681.13
5	2613.68
Mean	2671.57
Standard Deviation (±)	39.52
(%) Relative Standard Deviation	1.48

All the injections were processed at the wavelength provided in the method. There was no interference observed from diluent blank solution with Gemcitabine HCl peak.

3.1.3 Remarks: The method is selective.

3.2 Linearity

3.2.1 Linearity and Range for standard: For the linearity study five standard solutions of Gemcitabine HCl were prepared from the range starting from 50% to 150% of the theoretical concentration of assay preparation.

The system suitability solution and the linearity solutions were injected as per the protocol. The linearity graph of concentration against peak response was plotted and the correlation coefficient was determined.

3.2.2 Acceptance criteria: Correlation coefficient should be greater than or equal to 0.999.

The system suitability criteria were found to meet the pre-established acceptance criteria as per the analytical method. (Refer to Table-3 for system suitability results).

Table 3: System suitability - Linearity of standard

Sr. No.	Area of Gemcitabine HCl
1	2659.33
2	2699.99
3	2638.13
4	2630.47
5	2696.10
Mean	2664.80
Standard Deviation (±)	32.16
(%) Relative Standard Deviation	1.21

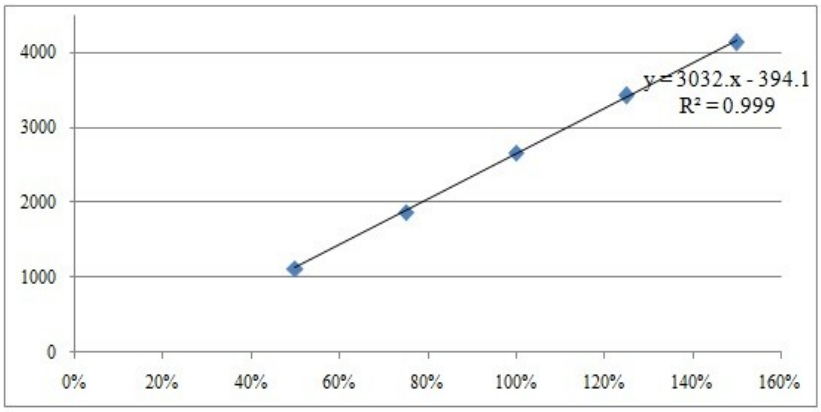
The average peak area of Gemcitabine HCl peak at each concentration level was determined and the linearity graph was plotted against the sample concentration in percentage. The results of linearity study are as given in Table 4.

Table 4: Results of linearity of standard

Linearity Level	Sample Concentration (in %)	Sample Concentration (in ppm)	Peak Area	Correlation Coefficient
Level – 1	50	20	1120.38	0.999
Level – 2	75	30	1863.22	
Level – 3	100	40	2654.37	
Level – 4	125	50	3420.21	
Level – 5	150	60	4132.10	

The linearity plot of peak area of **Gemcitabine HCl** Vs. standard concentration in percentage is presented in figure-1.

Figure 1: Linearity graph of Gemcitabine HCl standard



3.2.3 Remark :

a. A linearity graph of the average area at each level against the concentration (%) is plotted and is found to be a straight line graph.

b. The correlation coefficient is found to be more than 0.999.

c. Hence it is concluded that, the method is found to be linear in the range of 50% to 150% of the working concentration.

d. The range for the analytical method is 20 ppm to 60 ppm.

3.3. Precision:

3.3.1 System Precision:

3.3.1.1 Procedure: The system precision was performed by injecting 10 replicate injections of system suitability solution and the chromatograms are reviewed for the system suitability criteria.

3.3.1.2 Acceptance criteria: % RSD of peak areas of ten replicate injections of system suitability solution should not be more than 2.0% and system suitability criteria should pass as per analytical method.

3.3.1.3 Results: The system suitability criteria were found to meet the pre-established acceptance criteria as per the analytical method.

Table 5: System precision

Sr. No.	Area of Gemcitabine HCl
1	2705.59
2	2719.07
3	2756.41
4	2770.52
5	2693.21
6	2696.10
7	2643.40
8	2774.58
9	2701.88
10	2724.24
Mean	2718.50
Standard Deviation (±)	40.20
(%) Relative Standard Deviation	1.48

3.3.1.4 Remark: From the above data it is concluded that the system precision is established.

3.3.2 Method Precision:

3.3.2.1 Procedure: Six test solutions of Gemcitabine HCl in Gemlive 1000 Injectionand were prepared as per the analytical method. The % RSD of % assay of six test solutions was calculated.

3.3.2.2 Acceptance criteria: % RSD of the results of six test solutions should not be more than 2.0%.

3.3.2.3 Results: The system suitability criterion was found to meet the pre-established acceptance criteria as per the analytical method. The results of assay obtained from six test solutions preparations are presented in Table – 7.

Table - 6: System suitability - Method precision

Analyst – 1
HPLC No.: EH/R&D/HPLC-024

Sr. No.	Area of Gemcitabine HCl
1	2754.12
2	2736.60
3	2704.44
4	2758.37
5	2788.41
Mean	2748.39
Standard Deviation (±)	30.84
(%) Relative Standard Deviation	1.12

Table - 7: Results of method precision

Test Solution	% Assay of Gemcitabine HCl
1	98.45
2	101.24
3	100.9
4	101.54
5	101.24
6	101.96
Mean	100.89
Standard Deviation (±)	1.25
(%) Relative Standard Deviation	1.24

3.3.2.4 Remark : The % RSD of the six assay results is found less than 2.0% and meets the pre-established acceptance criteria. Hence, it is concluded that the method is precise.

3.3.3 Intermediate Precision:

3.3.3.1 Procedure: Six test solutions of Gemlive 1000 Injection were prepared as per the analytical method on different day. These test solutions were analyzed by a different analyst using different HPLC column of same make but having different serial number and different HPLC system. The % RSD of % assay results of twelve test solutions (six samples from method precision and six samples from intermediate precision) was calculated.

3.3.3.2 Acceptance criteria: % RSD of the results of twelve test solutions (six of method precision and six of intermediate precision) should not be more than 2.0%.

3.3.3.3 Results: The system suitability criteria were found to meet the pre-established acceptance criteria as per the analytical method. (Refer to Table -8 for system suitability results). The results of assay obtained from six test solutions are presented in Table - 9. % RSD of assay results from method precision and intermediate precision (12 results) are presented in Table - 10.

Table - 8: System suitability - Intermediate precision

Analyst – 2; HPLC No.: EH/R&D/HPLC-023

Sr. No.	Area of Gemcitabine HCl
1	2701.88
2	2689.57
3	2681.13
4	2724.24
5	2616.93
Mean	2682.75
Standard Deviation (±)	40.21
(%) Relative Standard Deviation	1.50

Table - 9: Results of intermediate precision

Test Solution	% Assay of Gemcitabine HCl
1	101.64
2	101.71
3	101.24
4	101.17
5	101.25
6	100.59
Mean	101.27
Standard Deviation (±)	0.40
(%) Relative Standard Deviation	0.40

Table - 10: Results of twelve test solutions of Gemcitabine HCl in Gemlive 1000 Injection

(six of method precision & six of intermediate precision)

Analysis performed during method precision study By Analyst 1 on system 1 and on column 1 on day 1	
Same column	**% Assay of Gemcitabine HCl**
1	98.45
2	101.24
3	100.9
4	101.54
5	101.24
6	101.96
Analysis performed during intermediate precision study By Analyst 2 on system 2 and on column 2 on day 2	
Column sr. no.	015337030136 01
Test Solution	**% Assay of Gemcitabine HCl**
7	101.64
8	101.71
9	101.24
10	101.17
11	101.25
12	100.59
Mean of twelve samples	101.08
Standard Deviation (±)	0.90
(%) Relative Standard Deviation	0.89

3.3.3.4 Remark: The analysis was carried out on six test solutions of the same lot of the drug product by two different analysts using two different equipments within the same laboratory using two different columns of the same make but having different serial numbers on two different days. The % RSD of the twelve assay results (six of method precision and six from intermediate precision) is found to be less than 2.0%.

Thus, the method is found to be rugged and precise.

3.4 Robustness:

3.4.1 Experiment: Prepare two test solutions of the same lot (as used in 7.0.a and 7.0.b) of Gemcitabine HCl in Gemlive 1000 Injectionas per analytical method. Inject this solution along with diluent blank solution and system suitability solution along different chromatographic conditions as shown below:

Change in column lot (same make, different serial no.)

Change in flow rate (± 0.2 ml/minute)

Change in wavelength (± 2 nm)

Change in pH of mobile phase

Change in Column Lot:

Normal Experimental Condition: L-7, 25cm x 4.6mm x 5µm

The system suitability criteria were found to meet the pre-established acceptance criteria as per the analytical method. (Refer to Table - 11 for system suitability results).

Table - 11: System suitability - Robustness with Change in Column L

Flow rate ®	Same column	Diff column
Sample	% Assay	
Test solution	101.24	99.08
Average assay result from method precision	100.89	100.89
Mean	101.07	99.99
Standard Deviation (±)	0.25	1.28
(%) Relative Standard Deviation	0.24	1.28

The assay results obtained with different flow rate conditions are as given in Table - 14.

Table - 14: Results for Change in Column Lot

Sr. No.	Area of Gemcitabine HCl	
	Same column	Diff column
1	2754.12	3161.99
2	2736.60	3155.72
Mean	2745.36	3158.85
Standard Deviation (±)	12.39	4.44
(%) Relative Standard Deviation	0.45	0.14

Change in Flow Rate (± 0.2 mL/minute):

(Normal Experimental Condition: 1.0ml/minute)

The system suitability criteria were found to meet the pre-established acceptance criteria as per the analytical method. (Refer to Table - 15 for system suitability results).

Table - 12: System suitability - Robustness with change in flow rate

Sr. No.	Area of Gemcitabine HCl	
	0.8mL/minute	1.2 mL/minute
1	3160.42	2638.01
2	3161.46	2632.69
Mean	3160.94	2635.35
Standard Deviation (±)	0.73	3.76
(%) Relative Standard Deviation	0.02	0.14

The assay results obtained with different flow rate conditions are as given in Table13.

Table - 13: Results for change in flow rate

Flow rate ®	0.8mL/minute	1.2 mL/minute
Sample	% Assay	
Test solution	100.98	102.02
Average assay result from method precision	100.89	100.89
Mean	100.94	101.46
Standard Deviation (±)	0.06	0.80
(%) Relative Standard Deviation	0.06	0.79

Change in Wavelength (± 2 nm):

(Normal Experimental Condition: 266nm)

The system suitability criteria were found to meet the pre-established acceptance criteria as per the analytical method. (Refer to Table - 17 for system suitability results).

Table - 14: System suitability - Robustness with change in wavelength

Sr. No.	Area of Gemcitabine HCl	
	264nm	268 nm
1	2643.40	2681.98
2	2616.93	2638.15
Mean	2630.17	2660.06
Standard Deviation (±)	18.72	30.99
(%) Relative Standard Deviation	0.71	1.17

The assay results obtained with different wavelength conditions are as given in Table –15.

Table -15: Results for change in wavelength

Wavelength ®	264nm	268 nm
Sample	% Assay	
Test solution	101.81	99.45
Average assay result from method precision	100.89	100.89
Mean	101.35	100.17
Standard Deviation (±)	0.65	1.02
(%) Relative Standard Deviation	0.64	1.02

Change in pH of mobile phase (± 0.2):

(Normal Experimental Condition: 6.5 pH)

The system suitability criteria were found to meet the pre-established acceptance criteria as per the analytical method. (Refer to Table - 16 for system suitability results).

Table - 16: System suitability - Robustness with change in pH of mobile phase

Sr. No.	Area of Gemcitabine HCl	
	6.3 pH	6.7 pH
1	2638.01	2696.10
2	2666.13	2633.79
Mean	2652.07	2664.95
Standard Deviation (±)	19.88	44.06
(%) Relative Standard Deviation	0.75	1.65

The assay results obtained with different wavelength conditions are as given in Table –17.

Table -17: Results for change in in pH of mobile phase

Wavelength ®	6.3 pH	6.7 pH
Sample	% Assay	
Test solution	99.78	101.53
Average assay result from method precision	100.89	100.89
Mean	100.34	101.21
Standard Deviation (±)	0.78	0.45
(%) Relative Standard Deviation	0.78	0.45

3.4.2 Remarks: The analysis of the same lot of Gemlive 1000 Injectionwas carried out at different conditions of column lot, flow rate, wavelength. The system suitability was found to meet the pre-established criteria at all the conditions and the % RSD between results obtained with changed condition and average result of method precision is not more than 2.0%.

The analytical method meets the pre-established acceptance criteria for robustness study as per protocol. Thus, the method is robust.

3.5 Stability of Analytical Solution:

3.5.1 Procedure: System suitability solution and test solution of Gemlive 1000 Injection were prepared on 0^{th},12^{th}, 24^{th}, 36^{th} and 48^{th} hour of experiment and stored these solutions at room temperature for every time interval up to 48 hrs and analyzed these solutions on 48 hrs with freshly prepared test solution. The system suitability solution was prepared freshly at the time of analysis. The assay of Gemlive 1000 Injection in the sample was calculated.

3.5.2 Acceptance criteria: The analyte is considered stable if there is no significant change in % assay.

3.5.3 Results: The system suitability criteria were found to meet the pre-established acceptance criteria as per the analytical method (Refer to Table - 17 for system suitability results)

Table – 18: System suitability – Solution stability

Time	Std Area	Avg std area	Spl area	Avg Spl area
0th hr	2659.32	2682.45	2719.07	2678.6
	2705.59		2638.13	
12th hr	2756.41	2763.46	2809.88	2752.99
	2770.52		2696.1	
24 hr	2603.78	2648.49	2694.01	2668.7
	2693.2		2643.4	
36 hr	2598.09	2607.51	2724.23	2702.68
	2616.93		2681.13	
48 hr	2701.87	2738.22	2654.86	2635.89
	2774.57		2616.93	
Mean	2688.03	2688.03	2687.77	2687.77
Standard Deviation (±)	67.13	63.81	55.68	43.63
(%) Relative Standard Deviation	2.50	2.37	2.07	1.62

Table – 19: Results for solution stability

% Assay results calculated against the freshly prepared system suitability standard	
Sample	% Assay of Gemcitabine HCl
0th hr	100.55
12th hr	100.54
24 hr	101.47
36 hr	104.38
48 hr	96.94
Mean	100.78
Standard Deviation (±)	2.66
(%) Relative Standard Deviation	2.64

3.5.4 Remark: The system suitability was found to meet the pre-established criteria and the % RSD between assay results obtained for freshly prepared test solution and the stored test solutions is less than 3.0%. There is no significant change in assay level observed up to 48Hrs for test solution at room temperature. Thus, it can be concluded that the solution is stable up to 48hrs at room temperature.

References

1. The Merck Index, XIII edition, Merck research laboratories, 755(2001).
2. N.A.Parshina, T.V.Pleteneva, V.N.Baikova, H.N.Narimanov and S.A.Tyulyandin, Quantitative estimation of Gemcitabine by HPLC in Plasma, *pharmaceutical chemistry journal*, vol.42, no.5, 2008.
3. L.Cattel, M.Airoldi, L.Delprino, R.Passera, P.Milla and F.Pedani, Pharmacokinetic evaluation of Gemcitabine and 2',2'-difluorodeoxyvytidine-5'-triphosphate after prolonged infusion in patients affected by different solid tumors, *annals of Oncoogy* 17(supplement 05): V142-V147, 2006.
4. Marangon E, Federicas, Orazio.C, Gallifioni.E, Maurizio D, and Massimo Z, *Journal of Mass spectrum*, 2008, 43(2), 216-223.
5. Validation of Analytical procedures, Methodology, ICH harmonized tripartite guidelines, 1996,1-8.
6. C.Sean Sweetman, Martindale The Complete Drug Reference, 34:558(2005).
7. Losa R, Sierra MI, Guardado, C.Fernandez, A.Gion, M.O.Blanco, D.Buesa, J.M. *Analytica chimica Acta,* 528(2), 255-260 (English) (2005).
8. Cristina S, Massimo Z, Marco S, Maurizio B, and Clandio M, Rapid Commun Mass spectrum, 2004, 18(10), 1017-1023.
9. Shaik Mastanamma, G.Ram Kumar, D.Anantha kumar, and JVLN. Seshagiri Rao, A stability indicating RPHPLC method for the estimation of Gemcitabine HCl in injectable Dosage forms, *E-journal of Chemistry*, 2010, 7(s1), s239-s244.
10. Shilpa P, YR Anitha, Vasanth PM, Ramesh M, Ramesh T, Development and validation of RP-HPLC method for the determination of Gemcitabine HCl in bulk and parenteral dosage form, *international journal of Bio-Pharma Research* 2012, 01(02), 16-19.