ISSN No: 2287-6898
Research Article

IJBPR

Development and Validation of RP-HPLC Method for the Determination of Gemcitabne Hydrochloride in Bulk and Parentral Dosage Form

Shilpa P[1], YR Anitha[1], Vasanth PM[1], Ramesh M[2], Ramesh T*[2]

[1]Department of Pharmaceutical Analysis, UCEV-JNTUK, Vizianagaram Campus, A.P., INDIA

[2]Department of Biotechnology, UCEV-JNTUK, Vizianagaram Campus, A.P., INDIA

Received for publication: July 10th 2012; **Accepted**: October 29th 2012.

Corresponding Author: Dr. Ramesh Malothu, Head and Assistant Professor, Department of Pharmacy and Biotechnology, UCEV-JNTUK, Vizianagaram-535002, A.P, India

ABSTRACT

A simple, rapid, precise, accurate and sensitive reverse phase liquid chromatographic method has been developed for the determination of Gemcitabine hydrochloride in bulk and parentral dosage form. The chromatographic method was standardized using ODS column with PDA detection at 285 nm and mobile phase consisting of ammonium acetate (0.025M): methanol (40:60 v/v) at a flow rate of 1.0 mL/min. The proposed method was successfully applied to the determination of Gemcitabine hydrochloride in bulk and parentral dosage form. The method was linear over the range of 5 - 40µg/mL. The recovery was in the range of 98.17 to 100. 40% and limit of quantification was found to be 0.16µg/mL. Different analytical performance parameters such as precision, accuracy, limit of detection, limit of quantification and robustness were determined according to International Conference on Harmonization (ICH) Q2Bguide.

Keywords: Gemcitabine hydrochloride, RP-HPLC, Bulk and Parentral dosage form.

INTRODUCTION

Gemcitabine HCl is a difluoro analog of deoxycitidine used as anticancer drug. Only limited methods have been described in the literature for the determination of gemcitabine HCl which includes the use of HPLC with variable walength detector detection [(4,5)] and LC-MS [(6)]. The aim of this study is development of a simple, precise, rapid and accurate reverse phase HPLC method for the estimation of Gemcitabine HCl in parenteral dosage form.The present work describes the development of a validated. RP-HPLC method in pharmaceutical dosage form. The present RP-HPLC method was validated following the ICH guidelines[(8)].

MATRIALS AND METHODS

Reagents and chemicals:

The HPLC solvents were used of Merck (India) Ltd. Ammonium acetate (HPLC grade) HPLC grade water was used to prepare all the solutions required for the method.

Apparatus and chromatographic conditions:

Quantitative HPLC was performed on an isocratic high pressure liquid chromatography (Shimadzu SPDM20A) equipped with a series LC-20 AT pump, photo diode array detector. Analytical ODS column (4.6mm x 250mm, 5µ) was used and the system connected with the help of LC-prominence software in a computer system for data integration and processing.

Chromatographic conditions:

The mobile phase mixture, ammonium acetate (0.025 M) and methanol (40:60 v/v) was filtered through 0.45µm membrane filter, sonicate for 10 minutes to degas the mixture. The flow rate of the mobile phase was maintained at 1.0mL/min. The column temperature was set at 25 ± 10C and the detection was carried out at 285 nm. The run time was set at 10 minutes and the volume of injection loop was 20µL.

Preparation of standard solutions:

About 50mg of Gemicitabine hydrochloride RS was taken in 50mL volumetric flask and made up to volume with methanol to get a concentration of 1000µg/mL. The solution was filtered and 5mL of filtrate was transferred to 50mL volumetric flask and volume adjusted up to mark with methanol to give a solution containing 100µg/mL Gemicitabine hydrochloride.

Preparation of sample solutions:

The lyophilized powder equivalent to 50mg was transferred to a 50mL standard flask. The powder was vortexes with methanol and made to volume with the same to get a concentration of 1000µg/mL. The solution was filtered through 0.45µm membrane filter and 5mL of filtrate

was diluted to 50mL with methanol solution to get a concentration of 100µg/mL.

Assay method:

With the optimized chromatographic conditions, a steady baseline was recorded, the mixed standard solution was injected and the chromatogram was recorded. The retention time of Gemcitabine hydrochloride was found to be 6.7min respectively. This procedure was repeated for the sample solution obtained from the formulation. The response factor (peak area ratio of standard peak area and internal standard peak area) of the standard solution and Sample solution was calculated. The concentration of the drugs was calculated using following formula:

$$\text{Concentration of drugs} = \frac{\text{Response factor of the sample}}{\text{Response factor of the standard}} \times \text{Concentration of standard}$$

RESULTS

Estimation of Gemcitabine hydrochloride in dosage forms:

The HPLC procedure was optimized with a view to develop precise and stable assay method. The pure drug Gemcitabine hydrochloride run with a ODS column (4.6mm x 250mm i.d., 5u) with a mobile phase of a mixture of ammonium acetate and methanol (adjusted to at a flow rate of 1.0 ml/min with detection at 285 nm gave sharp and symmetrical peaks with retention time 6.7min for Gemcitabine hydrochloride respectively. The typical chromatogram of sample solution is shown in Figure.1. Detection was done at 285 nm. The peak area ratio of standard and sample solutions was calculated. The assay procedures were repeated for six times and mean peak area and mean weight of standard drug was calculated. The percentage of individual drug found in formulations, mean, standard deviation in formulations were calculated and presented in Table 1. The results of analysis shows that the amounts of drug were in good agreement with the label claim of the formulation.

Method validation: [8-15]

Accuracy and precision: The accuracy of the method was determined by recovery experiments. The recovery studies were carried out three times and the percentage recovery and standard deviation of the percentage recovery were calculated and presented in Table.1. From the data obtained, added recoveries of standard drugs were found to be accurate.

The method was found to be accurate with the percentage recovery 98.17 to 100.40%. Precision of the method was demonstrated by inter day and intraday variation studies. In the intraday studies, six repeated injections of standard and sample solutions were made and the response factor of drug peaks are calculated and presented in Table.2. In the inter day variation studies, six repeated injections of standard and sample solutions were made for three consecutive days and response factor of drug peaks are calculated and presented in Table 2. From the data obtained, the developed RP-HPLC method was found to be precise.

Table.1. Results of analysis of formulation and recovery Studies

Drug	Intial amount (mg)	Fortified amount (mg)	Recovery (%)	RSD (%)
Brand I	250	25	99.43	1.32
		50	98.17	1.28
		100	98.57	1.70
Brand II	250	25	100.40	1.81
		50	98.30	1.10
		100	99.71	1.53

Table.2: Intraday and interday precision studies of Gemcitabine hydrochloride

Drug	Concentration (ug/ml)	Intra-day precision Mean RRF ± SD	RSD (%)	Inter-day precision Mean RRF ± SD	RSD (%)
Brand I	10	3.58 ± 0.04	1.11	3.47 ± 0.06	1.72
	15	5.49 ± 0.07	1.27	5.12 ± 0.11	2.14
	20	7.17 ± 0.13	1.81	7.25 ± 0.12	1.65
Brand II	10	4.17 ± 0.09	2.15	4.23 ± 0.03	0.70
	15	6.35 ± 0.12	1.88	6.13 ± 0.08	1.30
	20	8.15 ± 0.10	1.17	8.21 ± 0.15	1.82

The method was found to be with the %RSD 1.11 TO 2.15 intraday 0.70 to 2.14 for inter day

Linearity and Range:

Linearity was studied by preparing standard solution at different concentration levels. The linearity range was found to be 5-40µg/mL. The LOD and LOQ for Gemcitabine hydrochloride were found. The results are tabulated in Table.3.

Table 3: Analytical performance parameters of Gemcitabine hydrochloride

Statistical parameters	Gemcitabine hydrochloride
Linearity range, ug/ml	5-25
Correlation coefficient (R)	0.9995
Regression coefficient (R^2)	0.9989
Limit of detection, ug/ml	0.05
Limit of quantification, ug/ml	0.16

Robustness:

The study was done by small deliberate changes in the chromatographic conditions at different levels and relative retention factor (RRF) is noted for Gemcitabine hydrochloride. Small variations in the mobile phase ratio

and flow rate were done and the results were presented in table 4.

Table.4: Robustness study for Gemcitabine hydrochloride

Condition	RRF	RSD(%)
Mobile phase ratio (w/v)		
36:64	5.51	0.65
38:62	5.45	1.76
40:60	5.44	1.01
Flow rate (ml/min)		
0.9	5.39	1.30
1.0	5.41	1.23
1.1	5.44	0.98

The method exhibited good robustness because the changes made in chromatographic conditions did not influence the analytical results.

Solution stability:

In order to demonstrate the stability of both standard and sample solutions during analysis, both solutions were analyzed over a period of 5 h at room temperature. The results show that for both solutions, the retention time and peak area of Gemcitabine hydrochloride remained almost unchanged and no significant degradation within the indicated period, thus indicated that both solutions were stable for at least 5 h, which was sufficient to complete the whole analytical process.

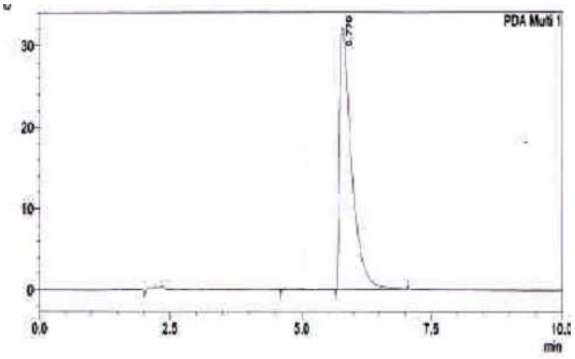

Figure.1: Typical chromatogram of Gemcitabine

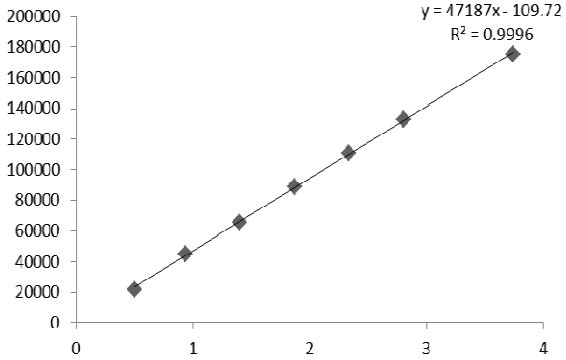

Figure.2: Calibration curve of Gemcitabine hydrochloride

CONCLUSION

An analytical liquid chromatographic method was developed and validated for the determination of Gemcitabine hydrochloride in parentral dosage form. The developed method was found to be specific, accurate, precise, robust, economic and rapid for its intended use.

REFERENCES

1. TV Pleteneva, NA Parshina, VN Baikova, MNNarimanov and SA Tyulyandin, Quantitative estimation of gemcitabine by HPLC in plasma, Pharmaceutical chemistry journal, vol-42, no.5, 288-290.

2. Kuna Mangamma, VenkataRao, Varasala Sandhya Mohan, Adireddy Prasanna, Method Development and Validation of Gemcitabine and Irinotecan by RP-HPLC in Pharmaceutical Formulation, International Journal of Chemical and Analytical Science, August, 2012, 3(8), 1500-1502.

3. Wang, Ling-Zhi, Goh, Boon-Cher, Lee, How-Sung Noordhuis, Pau, Peters, Godefridus, An Expedient Assay for Determination of Gemcitabine and Its Metabolite in Human Plasma Using Isocratic Ion-Pair Reversed-Phase High-Performance Liquid Chromatography, Therapeutic Drug Monitoring: October 2012.

4. Sanjay LBorisagar, Harsha U Patel, Chhaganbhai N Patel, A Validated Stability-Indicating HPTLC Method For The Estimation Of Gemcitabine Hcl In Its Dosage Form, JPC-Journal of Planar Chromatography February.

5. Prakash S Sarsambi, Kalyan Chakravarthy, K Purushotham Rao, Spectrophotometric Estimation of Gemcitabine in bulk drug and its Formulation, IJPI's Journal of Analytical Chemistry.

6. GRamkumar, Shaik Mastanamma, D Anantha Kumar And JVLN Seshagiri Rao, A Stability Indicating RP-HPLC method For The Estimation Of Gemcitabinehcl In Injectable Dosage Forms E-Journal Of Chemistry 2010.

7. Mark N Kirstein, Edward L Tobinick, Kai Chen and Xiaoyuan Chen, Sensitive And Specific HPLC-UV Method For Gemcitabine And DFDU.(2010)

8. Validation of Analytical Procedures: Methodology, ICH Harmonized tripartite Guideline, 1996, 1-8.

9. Lloyd R Snyder, Joseph J Kirkl and Joseph L Glajesh., Practical HPLC Method Development, 2nd edition, 1997, 1-14.

10. Craig S Young and Raymond J Weig, An efficient approach to column selection in HPLC method development, Altech Associates.

11. Lloyd RSnyder, Joseph JKirkland., Joseph LGlajesh, "Practical HPLC method development for pharmaceuticals, vol 8, 2007, 175-20

12. Fox Z, Dragsted UB, Gerstoft J, Phillips AN, Kjaer J, Mathiesen L, Youle M, Katlama C, Hill A, Bruun JN, Clumeck N, Dellamonica P, Lundgren JD: A randomized trial to evaluate the process related impurities regimen: the COLATE trialAntivir TherYear.2006; 11(6), pp:761-

13. Sharma BK, Instrumental Methods of Chemical Analysis., Goel Publishing House, Meerut, 24th edition, 2005, C-210-215.

14. Chatwal GR and Shan K Anand, Instrumental methods of Chemical analysis., 9th edition, 2004,2.624-2.634

15. Dav Jr RA, Underwood AL, Quantitative Analysis Prentice-Hall of India publishers, New Delhi, 6th edition, 2006, 556-560.

16. Kaur H, 2006, High Performance Liquid Chromatography, Instrumental methods of chemical analysis, 3rd edition.., Pragathi prakashan, Meerut, Pg No.798 -809.

17. J Mark Green, Analytical Chemistry, 1996, May; 68, 305- 309.

18. Sethi PD, HPLC: Quantitative Analysis of Pharmaceutical formulations, CBS Publishers, New Delhi, 2001, 3-137

Source of support: Nil

Conflict of interest: None Declared