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RP-HPLC Method for the Estimation Gemcitabine in API and Parenteral Dosage form

Sarala Kudikala, Sreenivas Reddy Malladi, Sridhar Thota, Venisetty Raj Kumar*
St. Peters Institute of Pharmaceutical Sciences, Vidyanagar, Hanamkonda, Warangal, Andhra Pradesh-506002, India.

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ABSTRACT

A stability-indicating RP-HPLC method was developed and validated for the determination gemcitabine HCl in active pharmaceutical ingredient and parenteral dosage form. The chromatographic conditions comprised of a reversed phase Enable C_{18} G column (250×4.6, 5µm particle size), with a mobile phase composed of acetonitrile and methanol in the ratio of 55:45v/v respectively. Flow rate was adjusted to 1.0 ml/min. Detection was carried out at 285 nm. The retention time of gemcitabine HCl was found to be 2.79 min. The linear regression analysis data for the calibration plots showed good linear relationship within the concentration range 1-45 µg/ml. The value of correlation coefficient was found to be 0.999. The recovery of gemcitabine HCl was about 100.7 to 101.6 %. Gemcitabine HCl was subjected to stress conditions including acidic, alkaline, oxidation, photolysis and thermal degradation. Gemcitabine HCl is more sensitive to wards acidic degradation. The method was validated as per ICH guidelines. Developed method was successfully applied for estimation of gemcitabine HCl in parenteral dosage form.

Keywords: *Gemcitabine HCl, RP-HPLC, Stability Studies.*

INTRODUCTION

Gemcitabine is a new anticancer nucleoside that is an analog of deoxycytidine. It is used as chemotherapeutic agent in the treatment of lung cancer, pancreatic cancer, bladder cancer and breast cancer. It is being investigated for use in esophageal cancer, and is used experimentally in lymphomas and various other tumors [1]. Chemically it is 2´-deoxy-2´,2´-difluorocytidine monohydro-chloride (**Fig 1**). It is a pro-drug and once transported into the cell, must be phosphorylated by deoxycytidine kinase to an active form and inhibit processes required for DNA synthesis. Incorporation of gemcitabine diphosphate into DNA is most likely the major mechanism by which gemcitabine causes cell death [1, 2]. Extensive literature survey reveals that only few methods were reported for the determination of gemcitabine HCl which includes spectrophotometric [3] and HPLC [4-7]. So far no stability indicating RP-HPLC method has been reported using simple acetonitrile and methanol as mobile phase. In view of these points an attempt was made to develop a simple, accurate and validated stability indicating RP-HPLC method for the estimation of gemcitabine HCl in bulk drug and parenteral dosage forms.

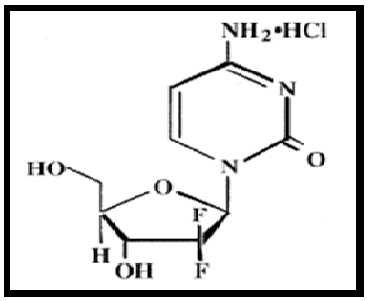

Fig.1. Chemical structure of gemcitabine HCl

***Corresponding author:**
Venisetty Raj Kumar
St. Peters institute of pharmaceutical sciences,
Hanamkonda, Warangal, Andhra Pradesh -506001, India.
Tel.: +91 9848207485.
**E.Mail: vrk10@hotmail.com*

MATERIALS AND METHODS

Gemcitabine HCl (API) was obtained as a gift sample from Dr. Reddy's pvt. Ltd, Hyderabad Methanol and acetomitrile of HPLC grade were obtained from Sigma Aldrich Chemicals limited. Maharastra. Marketed injection formulation (Gemcite ®) manufactured by Eli lilly pharma Ltd. was purchased.

Chromatographic Conditions:

Chromatographic separation was performed with isocratic elution. The following optimised parameters were used as a final method for the estimation of gemcitabine HCl.

Stationary phase	: Enable C_{18}G (250×4.6mm, 5µm)
Mobile phase	: Acetonitrile: methanol (55:45)
Flow-rate	: 1.0 ml/min
Injection volume	: 20 µL
Detection wavelength	: 285 nm
Temperature	: ambient temperature
Run-time	: 10 min

Preparation of a standard solution: Stock solution (1 mg/mL) of gemcitabine HCl was prepared by dissolving 100 mg of drug in 100 mL volumetric flasks containing 50 mL of methanol and sonicated for about 15 min and made up to volume with methanol. From this secondary stock was prepared by diluting 10 mL to 100 mL with methanol. Working standard of gemcitabine HCl was prepared by suitably diluting the stock solution with mobile phase to get the concentration range of 1-45µg/ mL.

Preparation of the sample solution: The powder of the sample vial claimed to contain 200 mg of gemcitabine HCl. The accurate quantity equivalent to 100 mg of an active ingredient was extracted with methanol and filtered through 0.45µm membrane filter, followed by adding methanol up to 100 ml to get the stock solution of 1mg/mL. Stock solution was further diluted step wise with mobile phase as under the preparation of standard solution to get the required concentration.

Method Validation:

The proposed chromatographic method was validated as per ICH guidelines. Peak calibration curve was constructed by plotting peak area Vs concentration. Accuracy was determined by recovery studies with known concentration of drugs and the percentage recoveries of the added drugs were determined. Precision was evaluated in terms of intra-day and inter-day

precision. The precision was investigated using six replicates of same concentrations of standard solutions. LOD and LOQ values were calculated from the calibration curve. Robustness of the method was determined by deliberately varying certain parameters like flow-rate, analytical wavelength and column temperature [8].

Forced Degradation studies:

The study was intended to ensure the effective separation of gemcitabine HCl and its degradation peaks of formulation ingredients at the retention time of gemcitabine HCl. Forced degradation studies were performed to evaluate the stability indicating properties and specificity of the method.[9] Gemcitabine HCl standard solution of concentration 1000 µg/ml was prepared with mobile phase and treated with 5 ml of 1N HCl. The resultant solution was analysed for every 24 h after prior dilution. For alkaline degradation study gemcitabine HCl standard solution of concentration 1000 µg/ml was prepared with mobile phase and treated with 5 ml of 1N NaOH. The resultant solution was analysed for every 24 h after prior dilution. Gemcitabine HCl powder was exposed to dry heat at 60^0 C and powder was removed for every 24 h and diluted as mentioned above and analysed for thermal degradation study.

RESULTS AND DISCUSSION

Method development:

Initially wavelength was selected for the method development and different compositions, pH and flow rate of the mobile phase were tried during method development. The 285 nm was selected for the current method since at this wavelength gemcitabine HCl can be selected with high sensitivity. In the course of optimizing the composition of mobile phase, methanol in combination with acetonitrile in different ratios were tried. After a series of preliminary experiments it was concluded that acetonitrile: methanol (55:45) gave sharp and symmetrical peaks with retention time 2.7 min for gemcitabine HCl.

Method validation:

Linearity: The calibration curve was constructed between peak area and respective concentrations. The calibration curve was linear over the range of 1-45 µg/ml. Correlation coefficient was found to be 0.999. The regression equation for calibration curve was found to be y=19922x-953.2. Results of linearity are shown in **Fig 2** and **Table 1.**

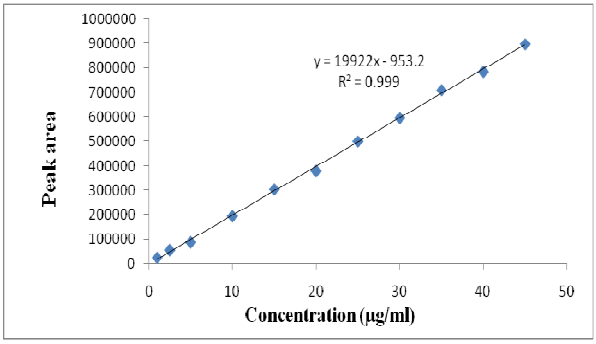

Fig. 2: Standard calibration curve for gemcitabine HCl (1-45µg/mL)

Table No. 1: Calibration curve parameters of gemcitabine HCl

Statistical parameters	Gemcitabine HCl
Linearity range (µg/ml)	1- 45
Slope(b)	19922
Intercept (a)	953.2

Accuracy: The accuracy of the method was determined by recovery experiments. The recovery studies were carried out six times and the percentage recovery and standard deviation of the percentage recovery were calculated. 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 100.7-101.6%. The results were shown in **Table 2**

Table.2. Accuracy results for Gemcitabine HCl

Method	Concentration (in µg)	Amount found (in µg)	%Assay
Standard addition (n=3)	20	20.1µg	100.7%
	25	25.2µg	101.0%
Percentage (n=3)	100	101.6	101.6%

Precision: 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 is calculated. In the inter day variation studies, six repeated injections of standard and sample solutions were made for six consecutive days and response factor of drug peaks are calculated. The % RSD obtained for intraday and inte-rday precision was less than 2% as shown in **Table 3**

Table No. 3: Results for *Intra-day* and *Inter-day* precision of gemcitabine HCl

S. No.	Concentration (µg/ml)	%RSD	
		Intra-day	Inter-day
1	10	0.15	0.91
2	20	0.32	1.23
3	30	0.46	1.06

LOD and LOQ: LOD and LOQ values decide about the sensitivity of the method. LOD is the lowest detectable concentration of the analyte, while LOQ is the lowest quantifiable concentration. LOD was found to be 1.69µg/ml and LOQ was found to be 5.13 µg/ml. The results for LOD & LOQ values shows that the method is quite sensitive for gemcitabine HCl compared with previously reported methods.

Robustness: Robustness of the method was determined by deliberately changing parameters like flow, wavelength and column temperature. Samples were analysed in triplicates and %RSD was calculated from peak areas. Results of robustness are summarized in **Table 4**.

Table No. 4: Robustness results for gemcitabine HCl

Factor		%RSD
Flow rate (ml/min)	0.8	0.6
	1	1.2
	1.2	1.8
Wavelength (nm)	283	0.9
	285	0.3
	287	0.7
Column temperature (^{0} C)	28	1.3
	30	1.1
	32	1.4

Forced degradation studies: All the stressed samples in acid, alkaline degradation studies were decomposed to 50% and 35% respectively. No decomposition was seen on exposure of solid drug to dry heat. The forced degradation studies data are summarized in **Table 5**

Table No. 5: Data of forced degradation studies

S. NO.	Stress condition	Time	Degradation (%)
1	Acid hydrolysis (1N HCl)	24 h	50
2	Alkaline hydrolysis (1N NaOH)	48 h	35
4	Thermal degradation (60^0C)	7 days	Stable

Assay: The validated method was applied to the determination of gemcitabine HCl in commercially available Gemcite ® injection. The percentage assay was found to be 99.98%. The results of assay indicate that the developed method is selective. The retention time of gemcitabine HCl in bulk drug was found to be 2.79, and in its injection was found to be 2.78. The percentage assay was found to be 99.98%. The results of assay indicate that the developed method is selective. Assay results and chromatogram of marketed formulation were shown in **Table 6** & **Fig 3** respectively.

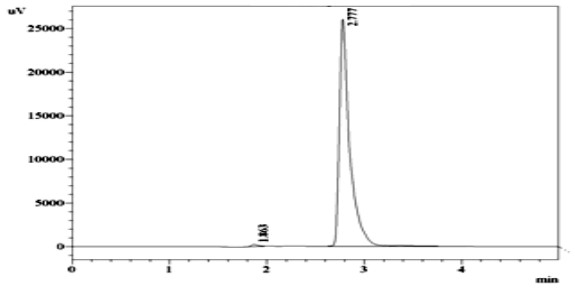

Fig. 3: Chromatogram for marketed formulation

Table No. 6: Assay of gemcitabine HCl marketed parenteral formulation (GEMCITE®)

Peak areas		Label claim (µg)	Amount found (µg)	Assay (%)
Standard	Drug			
205414	205376	10	9.99	99.98

CONCLUSION

The chromatographic method developed for gemcitabine HCl was new, rapid, fast, accurate, and reliable and precise. %RSD is less than 2% showed high degree of precision of the proposed method. The developed method was also specific as it was capable of determining gemcitabine HCl in presence of its degradation products. The forced degradation study of gemcitabine HCl shows that the drug was degraded 50% at acidic condition and 35% at basic condition after 90 min. With the above fact the developed method can be accepted as a novel stability indicating RP-HPLC method which uses acetonitrile: methanol as the mobile phase. This method has shown satisfactory results with a new combination of mobile phase and with low retention time it would be very useful for reference. This method can be used for the routine determination of gemcitabine HCl in bulk drug and its parenteral dosage form.

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Conflict of interest: The authors have declared that no conflict of interest exists.

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