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Research Article **Analysis**

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RP-HPLC METHOD FOR THE QUANTIFICATION OF GEMCITABINEIN FORMULATIONS

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ABSTRACT

A simple, precise and accurate RP-HPLC method was developed and validated for rapid assay of Gemcitabine in tablet dosage form. Isocratic elution at a flow rate of 1ml/min was employed on a symmetry Chromosil C18 (250x4.6mm, 5µm in particle size) at ambient temperature. The mobile phase consisted of Methanol: Acetonitrile: Water 80:15:5v/v. The UV detector wavelength was fixed at 282nm. In chromatogram the retention of Gemcitabine was 5.02min. The percentage RSD for precision and accuracy of the method was found to be less than 2%. The method was validated as per the ICH guidelines. The method was successfully applied for regular analysis of Gemcitabine in tablet dosage form.

KEYWORDS: Gemcitabine, RP-HPLC, UV detection, recovery, precise, 282nm

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INTRODUCTION

Gemcitabine is antineoplastic agent, also called as an anticancer drug. Gemcitabine is used in the treatment of different cancers such as in palliative treatment of non-small cell lung cancer to slow or stop the growth of abnormal cells. Gemcitabine is used in combination with different anticancer drugs to obtain best therapeutic effects and to reduce toxicity or side effects. Gemcitabine was first synthesized in Larry Hertel's lab at Eli Lilly during the early 1980s.[6] It was intended as an antiviral drug, but preclinical testing showed that it killed leukemia cells *in vitro*.[6]

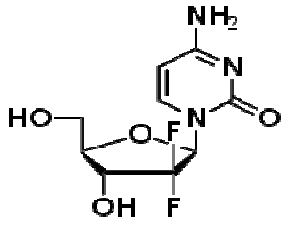

Figure 1
Stricture of Gemcitabine

Gemcitabine is used in various carcinomas such as non-small cell 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 tumor types. Gemcitabine represents an advance in pancreatic cancer care. It is also not as debilitating as some other forms of chemotherapy. Another target of gemcitabine is the enzyme ribonucleotide reductase (RNR). The diphosphate analogue binds to RNR active site and inactivates the enzyme irreversibly. Once RNR is inhibited, the cell cannot produce the deoxyribonucleotides required for DNA replication and repair, and cell apoptosis is induced.[1] A few of the most common side effects of gemcitabine include increased liver enzymes, leukopenia, and nausea and vomiting. Some side effects occur less frequently such as difficulty breathing, blood in the stool, or anys allergic reaction.

EPERIMENTAL

Materials

Working standard of Gemcitabine was obtained from well reputed research laboratories. HPLC grade water, Methanol was purchased from E. Merck (Mumbai, India).

Apparatus

In this study PEAK LC7000 isocratic HPLC system was used for development and validation of a new method. The analytical column Chromosil C18 (250×4.6mm) used for separation., Electronic balance-DENVER (SI234), The manual injector (Rhoedyne) with a 20µl loop was used for the injection of sample. UV 2301 Spectrophotometer was used in the determination of the suitable wavelength.

Determination of wavelength of maximum absorbance

The standard solutions of Gemcitabine were scanned in the range of 200 -400 nm against mobile phase as a blank. Gemcitabine showed maximum absorbance at 282nm. So the wavelength selected for the determination of Gemcitabine was 282nm.(7)

Chromatographic equipment and conditions

To develop a High Pressure Liquid Chromatographic method for quantitative estimation of GEMCITABINE an isocratic PEAKHPLC instrument with Zodiac C18 column (250 mm x 4.6 mm, 5µ) was used. The instrument is equipped with a LC 20AT pump for solvent delivery and variable wavelength programmable LC – 7000 UV-

detector. A 20µL Rheodyne inject port was used for injecting the samples. Data was analyzed by using PEAK software. The mobile phase consisted of Methanol:Acetonitrile:Water 85:15:5 v/v,(P^H 4.7)Injections were carried out using a 20µl loop at room temperature (20 + 2 °C) and the flow rate was 1 ml/min. Detection was performed at 282nm with 10min runtime.

Standard and sample solutions

A 10 mg amount of Gemcitabine reference substance was accurately weighed and dissolved in 10 ml mobile phase in a 10 ml volumetric flask to obtain 1000µg/ml concentrated solution. Required concentrations were prepared by serial dilution of this solution. Sample solution was prepared by dissolving 200mg Gemzar injection powder containing Gemcitabine.10 mg of Gemcitabine was accurately weighed and quantitatively transferred into a 100 ml volumetric flask. Approximately 25 ml mobile phase were added and the solution was sonicated for 15 min. The flask was filled to volume with mobile phase, and mixed. After filtration, an amount of the solution was diluted with mobile phase to a concentration of 60µg/ml.

Method validation

Method validation was performed following ICH specifications for specificity, range of linearity, accuracy, precision and robustness.

RESULTS AND DISCUSSION

System Suitability

By the optimization of HPLC conditions like mobile phase, solvent ratio, wavelength, floe, columns method was developed aand achived a sharp peak with maximum theoretical plates. Based on Tailing factor, Capacity factor, system suitability condtions are fixed for analysis of Gemicitabine. System suitability parameters were shown in Table.1. Standard chromatogram was given in Figure.2

Table 1
System suitability parameters of Gemcitabine

API Concentration	60µg/ml
Mobile Phase	Methanol:Acetonitrile:Water80:15:5
Wavelength	282nm
Column	C_{18} Column
P^H	4.7
Retention Time	5.02
Run Time	10min
Area	285700.5
Th. Plates	6766
Tailing Factor	1.84
Pump Pressure	10.5 MPa
Flow Rate	1ml/min

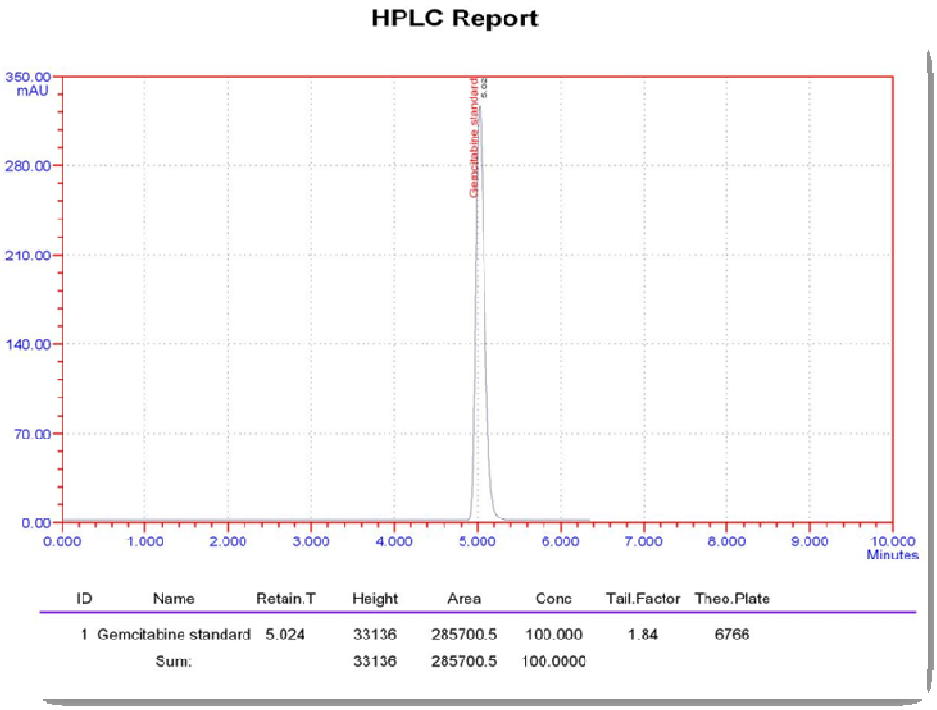

Figure 2
Standard chromatogram of Gemcitabine

Range of linearity

Standard curves were constructed daily, for three consecutive days, using seven standard concentrations in a range of 30, 40, 50, 60, 70, 80, 90, and 100µg/ml for Gemcitabine. The linearity of peak area responses versus concentrations was demonstrated by linear least square regression analysis. The linear regression equation was y = -22278.58 + 4681.633x (r= 0.999). Linearity values can shown in Table: 2

Table.2
Linearity results of Gemcitabine

S.No	Concentration (µg/ml)	Area
1	30	133403.6
2	40	180168.5
3	50	233682.5
4	60	285700.5
5	70	322996
6	80	373398.7
7	90	417181
8	100	464186
	Slope	4681.633
	Intercept	-2188.35
	CC	0.99967

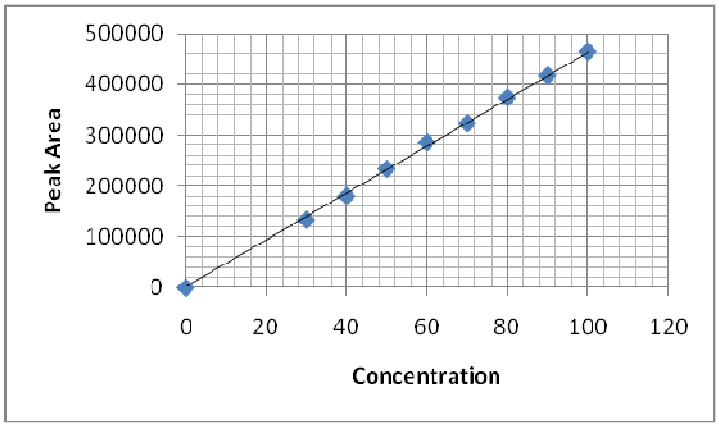

Figure 3
Calibration curve of Gemcitabine

Precision

To study precision, six replicate standard solutions of Gemcitabine(120µg/ml) were prepared and analyzed using the proposed method. The percent relative standard deviation (% RSD) for peak responses was calculated and it was found to be which is well within the acceptance criteria of not more than 2.0%. Results of system precision studies are shown in Table.3 and Table.4.

Table 3
Intraday Precision Results for Gemcitabine

Sample (µg/ml)	Area
1	286132.9
2	282005.2
3	289364.1
4	281692.3
5	281137.2
6	288816.3
RSD	1.30979

Table 4
Interday Precision results of Gemcitabine

Sample (µg/ml)	Area
1	285700.5
2	280403.4
3	284920.8
4	280248.6
5	281020.9
6	286157.7
RSD	0.988

Limit of Detection and Limit of Quantification: µg/ml

To determine the Limit of Detection (LOD) sample was dissolved by using Mobile phase and injected until peak was disappeared. After 1.5µg/ml dilution Peak was not clearly observed, based on which 1.5µg/ml is considered as Limit of Detection and Limit of Quantification is 5µg/ml.

Table 5
LOD and LOQ results of Gemcitabine

Parameter	Measured Value
Limit of Quantification	5µg/ml
Limit of Detection	1.5µg/ml

Robustness

Typical variations in liquid chromatography conditions were used to evaluate the robustness of the assay method. The robustness study was performed by slight modification in flow rate of the mobile phase, composition of the mobile phase and wavelength of the detector. Gemcitabine at standard concentration was analyzed under these changed experimental conditions. It was observed that there were no marked changes in chromatograms, which demonstrated that the developed method was robust in nature. The robustness acceptance criteria set in the validation were the same established on system suitability test describe above. Results were shown in table 6.

Table 6
Robustness results of Gemcitabine

S.NO	Parameter	Change	Area	% of Change
1	Standard	……	285700.5	……
2	MP	Meoh :ACN:H_2O		
		85:12.5:2.5	283774.2	0.67
		75:17.5:7.5	280403.4	1.86
3	PH	4.8	280248.6	0.96
		4.6	281840.0	1.35
4	WL	287nm	283859.7	1.9
		277nm	280675.9	1.75

Ruggedness

Ruggedness was performed by using six replicate injections of standard and sample solutions of concentrations which were prepared and analyzed by different analyst on three different. Ruggedness also expressed in terms of percentage relative standard deviation.

Sample (µg/ml)	Area
1	278786.1
2	277587.1
3	274405.1
4	270732.8
5	274791.4
6	279822.9
RSD	1.21938

Recovery

The accuracy of the method was determined by standard addition method. A known amount of standard drug was added to the fixed amount of pre-analyzed tablet solution. Percent recovery was calculated by comparing the area before and after the addition of the standard drug. Recovery test was performed at 3 different concentrations i.e.60µg/ml, 80µg/ml, 100µg/ml. The percent recovery was calculated and results are presented in Table. Satisfactory recoveries ranging from 98.07 to 101.14 were obtained by the proposed method. This indicates that the proposed method was accurate. Results are given in table.8.

Table 8
Recovery results of Gemcitabine

% Recovery	Target Conc., (µg/ml)	Spiked conc, (µg/ml)	Final Conc, (µg/ml)	Conc., Obtained	% of Recovery
50%	40	20	60	59.711	99.52
	40	20	60	59.836	99.72
	40	20	60	59.431	99.05
100%	40	40	80	101.3	101.3
	40	40	80	101.65	101.65
	40	40	80	100.88	100.88
150%	40	60	100	79.047	98.809
	40	60	100	80.040	100.05
	40	60	100	81.207	101.50

Table 9
Formulation Analysis

Formulation assay was carried out by dissolving 200mg Gemzar injection powder with mobile phase and diluted to prepare 60µg/ml concentrated Gemcitabine sample solution. The results were given in the table 9 and it is has god agreement with the label claim.

Table 9
formulation analysis of Gemcitabine

Formulation	Dosage	Concentration	Amount found	% Assay
Gemzar	200mg	60µg/ml	59.77	99.61

CONCLUSION

The proposed method for the assay of Gemcitabine is simple and rapid method.Due to absence of solid buffer column life span will prolong than normal methods. The isocratic method is simple than gradient method.The developed method is applicable for analysis of Gemcitabine in formulation and bulk drugs.

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