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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF GEMCITABINE IN PHARMACEUTICAL FORMULATION USING RP-HPLC

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ABSTRACT

The scope and objective of the present work is to optimize condition to develop estimation of Gemcitabine by HPLC method. For HPLC method, Acetonotirle and Water (50:50) was used as mobile phase which gives good resolution and good peak shapes for Gemcitabine. The flow rate was set at 1.0 mL/min, and the detection was carried out with UV detector at 270 nm. The correlation coefficient of Gemcitabine was found to be 0.9992. 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 precision study also were within acceptable limit**.**

Key words: Gemcitabine, HPLC method.

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INTRODUCTION

The drug analysis plays an important role in the development, manufacture and therapeutic use of drugs. Most of the pharmaceutical industries do the quantitative chemical analysis to ensure that the raw material used and the final product thus obtained meets certain specification and to determine how much of each component is present in the final product. Standard analytical procedure for newer drugs or formulation may not be available in Pharmacopoeias; hence it is essential to develop newer analytical

method and validation of drug. For the Method Development and Validation of new drug present in dosage forms UV- Spectrophotometer, HPLC and HPTLC methods are considered to be most suitable. Since these are powerful and rugged methods and also extremely precise, accurate, sensitive, specific, linear and rapid. Literature survey has revealed that various methods were reported for estimation of Gemcitabine those are Colorimetry, UV Spectrophotometry, HPLC, UPLC, LC/MS and HPTLC. The objective of the proposed method is to develop simple and accurate method for the estimation of Gemcitabine pharmaceutical dosage forms by HPLC (1-10). Hence, on the basis of literature survey it was thought to develop a precise, accurate, simple and reliable, less time consuming and less cost effective method for the estimation of Gemcitabine.

MATERIALS AND METHODS

Standard Preparation

10mg of Gemcitabine working Standard was accurately weighed and transferred into a 10ml clean dry volumetric flask, and about 10 ml of diluent was added, and sonicated to dissolve. The solution was cooled to room temperature and diluted to volume with diluent and mixed.

Sample preparation

The sample equivalent to 10 mg of Gemcitabine was accurately weighed and transferred into a 10ml volumetric flask. About 10 ml of diluent was added and shaken for 10 minutes on orbital shaker and sonicated for 10 minutes with occasional shaking. The solution was cooled to room temperature and diluted to volume with diluent. The solution was filtered.

Procedure

About 10 µl of blank, standard and sample preparations was separately injected in to the chromatograph and the chromatograms were recorded and the peak area responses were measured for the analyte peaks. The % content of Gemcitabine in the portion of Gemcitabine tablets was calculated using the formula.

$$\% \text{ Assay} = \frac{Gu}{Gs} \times \frac{Ws}{100} \times \frac{250}{Wr} \times \frac{P}{100} \times \frac{\text{Avg Wt.}}{LA} \times 100$$

The developed method is validated for parameters such as Accuracy, Precision, Specificity, Linearity, System suitability, Limit of detection, Limit of quantification, Robustness and Ruggedness (11, 12).

RESULTS AND DISCUSSION

The system suitability studies were done with the 50 mg of standard drug. The % of RSD values are below 2%, theoretical plate count is above 2000 and tailing factor is less than 2, indicating that the method is suitable. The chromatogram is recorded and are shown in fig-1

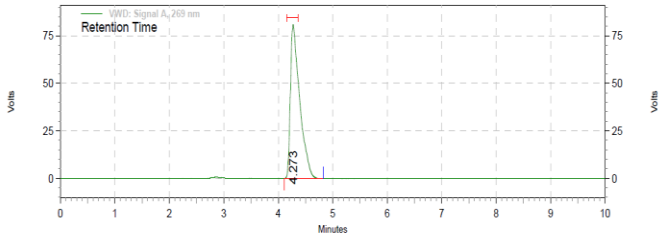

Fig-1 Standard Chromatogram showing system suitability

The system suitability studies were done with accurately weighing equivalent to 10mg of Gemcitabine dosage form. The % of RSD values are below 2%, theoretical plate count is above 2000 and tailing factor is less than 2, indicating that the method is suitable. The chromatograms are recorded and are shown in fig-2.

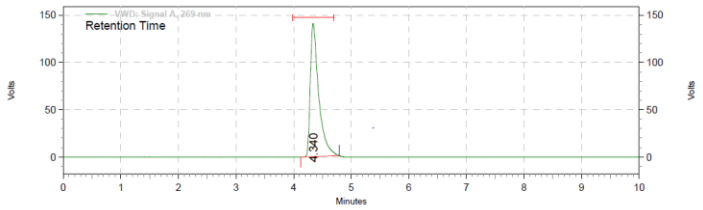

Fig-2 Sample Chromatogram showing system suitability

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 calculation of correlation coefficient. The linearity study was performed the correlation coefficient of Gemcitabine was found to be 0.9992. Calibration Curve for Gemcitabine is shown in Fig-3 and results are tabulated in table-1.

Table-1 Showing results from linearity study

S.No	Linearit Leavel	Concentration (µg/ml)	Peak area
1	I	80	21894553
2	II	90	24557980
3	III	100	26988929
4	IV	110	29423432
5	V	120	31776601
Correlation Coefficient			**0.9992**

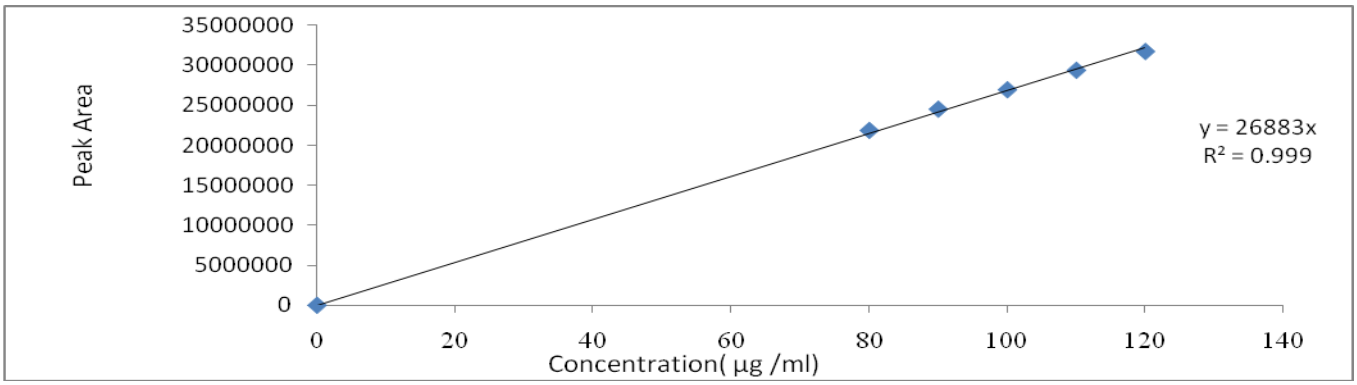

Fig-3 Calibration curve of Gemcitabine

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.

The accuracy study was performed for 50 µg/ml, 100 µg/ml and 150 µg/ml for Gemcitabine. Each level was injected in triplicate into chromatographic system. The area of each level was used for calculation of % recovery. Results are tabulated in Table-2. The accuracy study was performed for % recovery. The % recovery was found to be 101.4 to 99.50%.

Table-2 Showing result from accuracy study

Level of 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			
		12.31	102.5			
150	14.4	14.26	99.02	99.50	0.6451	0.64
		14.21	99.8			
		14.45	100.3			

The

The precision study was performed for six injections of Gemcitabine. Each standard injection was injected into chromatographic system. The area of each standard injection was used for calculation of %RSD. Results are tabulated in Table-3 and 4.

Table-3 Showing results from method precision study-repeatability (100 µg/ml)

S.No	Peak Name	Peak area
1	Gemcitabine	26988929
2	Gemcitabine	26987590
3	Gemcitabine	26988501
4	Gemcitabine	26981593
5	Gemcitabine	26989476
6	Gemcitabine	26988927
Mean		26988534
SD		1523.76
%RSD		0.12

Table-4 Showing results from System precision study-repeatability (100µg/ml)

S.No	Peak Name	Peak area
1	Gemcitabine	26988929
2	Gemcitabine	26987068
3	Gemcitabine	26989476
4	Gemcitabine	26988927
5	Gemcitabine	26987590
6	Gemcitabine	26991201
Mean		26988572
SD		1525.33
%RSD		0.13

The precision of method was determined by replicate injection of sample solution. The %RSD of area of intraday precision is 0.4%, 0.10% and 0.07%. %RSD of interday precision was found to be 0.3%, 0.09% and 0.07%. Precision results are within the limits.

CONCLUSION

RP-HPLC method was developed and validated. The developed method has adavantages such as- No tedious extraction procedures were involved; The run time required for recording chromatogram was 10 min all the peaks are eluting finely with developed method; and Suitable for the analysis of raw materials and formulations.

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 ±2º C in temperature, ±0.2 mL/min in flow rate, ±10% variation in organic phase.

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