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The estimation of gemcitabine hydrochloride in parenteral dosage forms by gradient RP-HPLC

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ABSTRACT

A simple, precise, rapid and accurate reverse phase HPLC method developed for the estimation of Gemcitabine hydrochloride in parenteral dosage form. A Hypersil BDS C18, 150 x 4.6 mm, 5 µm particle size, with mobile phase consisting of ammonium acetate in water (0.03M) and methanol was used in gradient mode. The flow rate was 1 ml/min and the effluents were monitored at 268 nm. The retention time was 7.24 min. The detector response was linear in the concentration of 10-120 mcg/ml. The respective linear regression equation being Y=3322.25x+29349.70. The limit of detection and limit of quantification was 0.5 and 1.5 mcg/ ml respectively. The percentage assay of Gemcitabine hydrochloride was 99.22 %. The method was validated by determining its accuracy, precision and system suitability. Conditions were investigated to provide the best peak resolution concerning symmetry & tailing; reduce runtime as proposed advantage& present low cost and simple modus operandi. The results of the study showed that the proposed Gradient RP-HPLC method is simple, rapid, precise and accurate, which is useful for the routine determination of Gemcitabine hydrochloride in bulk drug and in its pharmaceutical dosage forms.

Key words: Gemcitabine hydrochloride, Gradient RP-HPLC, Estimation, and Parenterals.

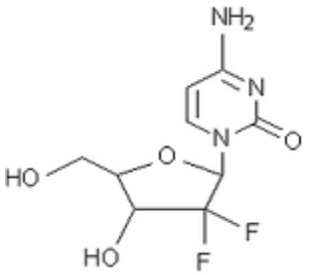
INTRODUCTION

Gemcitabine hydrochloride[1] is a antineoplastic agent[1]; with a chemical name 2'-deoxy-2,2'-difluoro cytidine with molecular formula $C_9H_{11}F_2N_3O_4$ HCl and a molecular weight of 299.77. It is used as an anticancer agent in pancreatic carcinoma[2,3]. Literature survey reveals many Chromatographic methods[2-8] for the determination of Gemcitabine hydrochloride in combination with other anticancer agents, in biological fluids and in cancer cells. So far, only one assay procedure has been reported for the estimation of Gemcitabine HCl from pharmaceutical dosage forms by RP-HPLC in isocratic mode. The availability of an HPLC method with high sensitivity and selectivity will be very useful for the determination of Gemcitabine hydrochloride in pharmaceutical formulations. The aim of the study was to develop a simple, precise and accurate reversed-phase HPLC method in gradient mode for the estimation of Gemcitabine hydrochloride in bulk drug samples and in pharmaceutical dosage forms.

EXPERIMENTAL

Materials and Methods

Gemcitabine hydrochloride was obtained as a gift sample from M/s Shilpa Medicare, Raichur.



Structure of Gemcitabine

Ammonium acetate was of analytical grade, and supplied by M/s S.D.Fine Chem Limited, Mumbai. Methanol and water used were of HPLC grade (Qualigens). Commercially available Gemcitabine HCl powder for injection (Gemcite 200mg, Elililly) were procured from local market.

Instrument

Quantitative HPLC was performed on liquid Chromatograph, Waters separation 2996, PDA detector module equipped with automatic injector with injection volume 20 µl, and 2693 pump. A RP C-18 Hypersil BDS (150×4.6 mm; particle size 5 µm) was used. The HPLC system was equipped with Empower Software.

HPLC conditions

The contents of the mobile phase is mixture of 0.03 M ammonium acteate in water (solvent A) and methanol (solvent B) was set in the proportion of 15:85 over 12 minutes, was found to be most suitable for gradient mode. They were filtered before use through a 0.45 µm membrane filter, and pumped from the respective solvent reservoirs to the column at a flow rate of 1.0 ml/min. The run time was set at 20.0 min and the column temperature was ambient. Prior to the injection of the drug solution, the column was equilibrated for at least 30 min with the mobile phase flowing through the system. The eluents were monitored at 268 nm.

Preparation of standard stock solution

A standard stock solution of the drug was prepared by dissolving 100 mg of Gemcitabine HCl in 100 ml volumetric flask containing 30 ml of diluent (50:50 v/v acetonitrile: water), sonicated for about 15 min and then made up to 100 ml with diluent to get a 1mg/ml standard stock solution.

Working standard solution

5 ml of the above stock solution was taken in 50 ml volumetric flask and made up to 50 ml with diluent to get a concentration 100 µg/ml.

Preparation of sample solution

Transfer 106.3 mg of sample of the powder for injection, which is equivalent to 100 mg of the active ingredient, was mixed with 50 ml of diluent. The mixture was allowed to stand for 1 hr with intermittent sonication to ensure complete solubility of the drug, and then filtered through a 0.45 µm membrane filter, followed by adding diluent to obtain a stock solution of 1.0 mg/ml. An aliquot of this solution was transferred to a 10 ml volumetric flask and made up to sufficient volume with mobile phase to give the concentration of 100mcg/ml.

Time programming of the gradient elution used to determine Gemcitbine HCl

Time(min)	Flow rate (ml/min)	%Solvent A	% Solvent B
0.01-2	1.0	100.0	0.0
2.0-12	1.0	15	85.0
12-15	1.0	100	0.0
15-20	1.0	100	0.0

Solvent A: 0.03 M Ammonium acetate in water
Solvent B: Methanol.

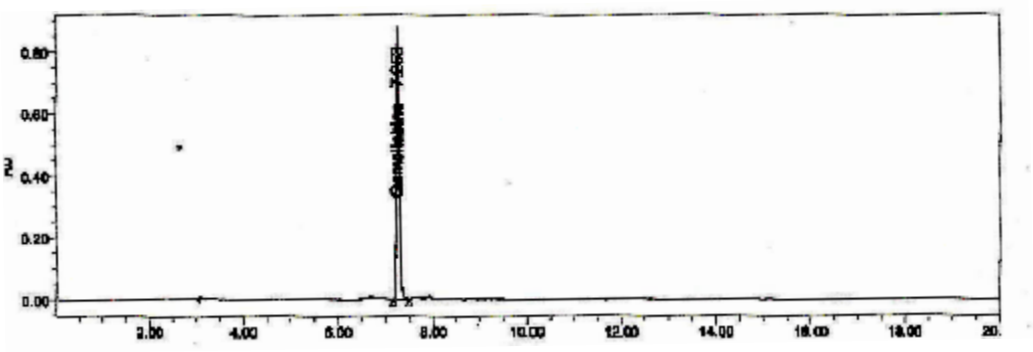


Fig. 1: Typical Chromatogram of Gemcitabine HCl by Gradient- HPLC

Linearity

Aliquots of standard Gemcitabine HCl stock solution were taken in different 10 ml volumetric flasks and diluted up to the mark with the diluent such that the final concentrations of Gemcitabine HCl are in the range of 10-120 mcg/ml. Each of these drug solutions (20 µL) was injected three times into the column, and the peak areas and retention times were recorded. Evaluation was performed with PDA detector at 268 nm and a Calibration graph was obtained by plotting peak area versus concentration of Gemcitabine HCl (Fig 2). The plot of peak area of each sample against respective concentration of Gemcitabine HCl was found to be linear in the range of 10–120 µg/ml with correlation coefficient of 0.9999. Linear regression least square fit data obtained from the measurements are given in table I. The respective linear regression equation being Y=3322.25 ×+29349.70. The regression characteristics, such as slope, intercept, and %RSD were calculated for this method and given in Table 1.

Assay

20 µl of sample solution was injected into the injector of liquid chromatograph. The retention time was found to be 7.248 minutes. The amount of drug present per parenteral was calculated by comparing the peak area of the sample solution with that of the standard solution. The data are presented in Table 2.

Table 1: Linear regression data for calibration curves

Drug	Gemcitabine HCl
Concentration range (mcg/ml)	10-120
Slope (m)	33225.25
Intercept (b)	29349.7096
Correlation coefficient	0.9999
% RSD	0.08
% Range of error (Confidence limits)	
0.05 level	0.993
0.01 level	0.987

Table 2: Results of HPLC assay and Recovery studies

Sample	Amount claim (mg/powder for injection)	% found by the proposed method (mg/ powder for injection.)	% Recovery* by the Proposed method
1.	200	99.85	103.37
2.	200	100.52	102.00
3.	200	99.26	98.37

*Average of three different concentration levels.

Recovery studies

Accuracy was determined by recovery studies of Gemcitabine HCl, known amount of standard was added to the preanalysed sample and subjected to the proposed HPLC analysis by gradient mode. Results of recovery study are shown in Table 2. The study was done at three different concentration levels.

RESULTS AND DISCUSSION

The system suitability tests were carried out on freshly prepared standard stock solution of Gemcitabine HCl. Parameters that were studied

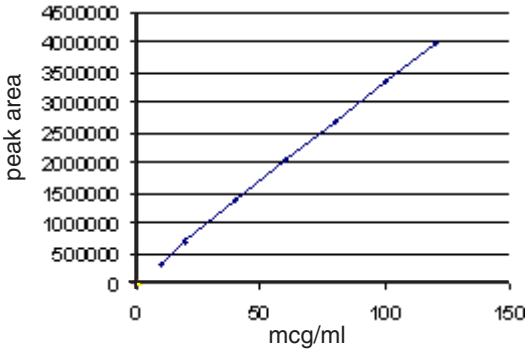


Fig. 2: Calibration curve of Gemcitabine HCl by Gradient-HPLC

to evaluate the suitability of the system are given in Table 3.

Limit of Detection (LOD) and Limit of Quantification (LOQ)

The limit of detection (LOD) and limit of quantification (LOQ) for Gemcitabine HCl were found to be 0.5 and 0.15 µg/ml respectively. The signal to noise ratio is 3 for LOD and 10 for LOQ. From the typical chromatogram of Gemcitabine HCl as shown in fig 1, it was found that the retention time was 7.24 min. A mixture of 0.03 M ammonium acteate in water(solvent A)and methanol(solvent B) was set in the proportion of 15:85 over 12 minutes, found to be most suitable to obtain a peak symmetrical and free from tailing. The time programming of gradient used in the determination of Gemcitabine HCl is described in the tabular form. In the present developed HPLC method, the standard and sample preparation required less time and no tedious extraction were involved. A good linear relationship (r=0.9999) was observed between the concentration range of 10-120 mcg/ml. Low values of standard deviation are indicative of the high precision of the method. The assay of Gemcitabine HCl parenterals was found to be 99.22%. From the recovery studies it was found that about 101.24 % of Gemcitabine HCl was recovered which indicates high accuracy of the method. The absence of additional peaks in the chromatogram indicates non-interference of the common excipients used in the parenterals. This demonstrates that the developed HPLC method is simple, linear, accurate, sensitive and reproducible. Thus, the developed method can be easily used for the routine quality control of bulk and parenteral dosage form of Gemcitabine HCl within a short analysis time

Table 3: Validation Summary

Validation Parameter	Results
System Suitability	
Theoretical Plates (N)	7259
Tailing factor	1.05
Retention time in minutes	7.24
LOD (mcg/ml)	0.5
LOQ (mcg/ml)	0.15

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