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Analytical Method Development and Validation of RP-HPLC Method for the Determination of Gemcitabine in Bulk and Pharmaceutical Dosage Forms

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ABSTRACT

A rapid and sensitive high performance liquid chromatographic method was developed for the estimation of Gemcitabine in bulk and pharmaceutical dosage forms. Gemcitabine was chromatographed on a Zorbax R_XC_8, (250mm× 4.6mm, 5µ) using a mobile phase consisting of phosphate buffer (pH 3.0) and methanol in the ratio of 85:15 v/v. The flow rate was maintained at 1.2 ml/min and eluents were detected at 275 nm. The retention time of Gemcitabine was found to be 11.78 min. The proposed method was validated by determining accuracy, precision, specificity and system suitability parameters. Linearity was observed in the range of 25-150 µg/ml. The mean recovery of 100.0±0.32 of the drug was indicating high level of accuracy of the method. Due to its simplicity, accuracy and high precision the proposed HPLC method was found to be appropriate for the estimation of Gemcitabine in bulk and pharmaceutical dosage forms.

Key words: Estimation of Gemcitabine, HPLC and Pharmaceutical dosage forms.

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INTRODUCTION

Gemcitabine hydrochloride[1-5], 2-deoxy-2'2'diflurocytidine monohydrochloride has antitumour activity. The cytotoxic effect of Gemcitabine is attributed to combination of two actions of the diphosphate and the triphosphate nucleosides, which leads to inhibition of DNA synthesis. After the Gemcitabine nucleotide is incorporated into DNA, only one additional nucleotide is added to the growing DNA strands. After this addition, there is inhibition of further DNA synthesis. DNA polymerase epsilon is unable to remove the Gemcitabine nucleotide and repair the growing DNA strands (masked chain termination). In lymphoblastoid cells, Gemcitabine induces internucleosomal DNA fragmentation, one of the characteristics of programmed cell death.

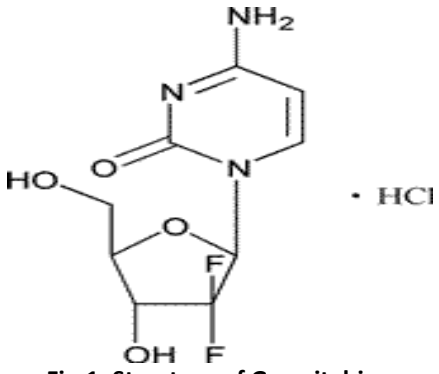

Fig-1: Structure of Gemcitabine

It is useful in combination with paclitaxel as first-line therapy for metastatic breast cancer. It is useful for initial treatment in patients with inoperable, locally advanced (stage IIIA or IIIB) or metastatic (stage IV) non-small cell lung cancer in combination with cisplatin. It is useful as first-line drug for the treatment of adrenocarcinoma of pancreas, also used as second-line therapy in patients previously treated with fluorouracil. It is used alone or in combination with cisplatin for the treatment of advanced or metastatic bladder cancer. It is currently being investigated for use in the treatment of advanced epithelial cell cancer.

Literature review indicated that there are LC-MS [6-8], HPLC [9-15] methods for the determination of Gemcitabine individually and in combination with other drugs in dosage forms and biological fluids. The present investigation by the authors describes a rapid, accurate and precise RP-HPLC method for the determination of Gemcitabine in pharmaceutical dosage forms.

MATERIALS AND METHODS

Alliance-Waters 2695 separation module with Waters 2996 photo diode array detector equipped with empower software. Monobasic sodium phosphate and phosphoric acid used were of AR grade and methanol of HPLC grade was used. Zorbax R_XC_8 (250mm×4.6mm, 5µ) column was used. Hamitton injection syringe was used for sample injection

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HPLC parameters

The mobile phase consisting of monobasic phosphate buffer (pH 3.0) and methanol in the ratio of 85:15 v/v was pumped at a flow rate of 1.2 ml/min. The volume of each injection was 20 µL. The column was equilibrated for 40 min with the mobile phase. The eluents were monitored at 275 nm. Diluent used was water.

Selection of the mobile phase and column

The separation was tried with various columns such as ODS C18 *column* (250×4.6 mm, 5µ), Alltima C18 *column (*100×2.1 mm, 5µm), Zorbax R_XC_8, (250mm×4.6mm, 5µ) and various mobile phases like methanol, phosphate buffer (pH adjusted with ortho phosphoric acid), acetonitrile. Finally Zorbax R_XC_8, (250mm×4.6mm, 5µ) column and mobile phase comprising of phosphate buffer of pH 3 and methanol in the ratio of 85:15 v/v were selected for the method.

Preparation of mobile phase

13.8g of monobasic sodium phosphate was accurately weighed and transferred in to 1000 ml volumetric flask, 2.5ml of phosphoric acid was added and 300 ml water was added. The solute was made to dissolve. Then the volume was made up to 1000 ml with water. pH was adjusted to 3.0. The solution was filtered through a 0.45 µm membrane filter.

Phosphate buffer (pH 3.0) and acetonitrile was mixed in the ratio of 85:15, v/v. Then the solution was degassed with a helium spurge for 20 min.

Procedure

Stock solution of Gemcitabine was prepared by transferring an accurately weighed quantity of 50mg of drug in to a 50 ml volumetric flask containing 20 ml of diluent, sonicated for 15 min and made up to the volume with diluent. Working standard of 100µg/ml was prepared from the stock solution by suitable dilution.

Calibration plot

Solutions ranging from 25-150 µg/ml were prepared and injected 20 µl of each of these solutions into the chromatographic system for five times. Chromatograms were recorded and the mean peak area was calculated for each determination. A calibration plot was constructed between concentration and peak area. The regression of Gemcitabine concentration over its peak area was found to be y=40557x-9432. The regression coefficient was found to be 0.9999

Assay of Gemcitabine in tablets

Twenty tablets were weighed and average weight of a tablet was calculated. A quantity of tablet powder equivalent to 20 mg was weighed and transferred in to 200ml a volumetric

flask containing 50 ml of diluent. The solution was sonicated for about 20 min and filtered through a 0.45 µm membrane filter. Volume was up to 200 ml with diluent. The solution was then injected for five times and the mean peak area was calculated and the drug content in the tablets was quantified using the regression equation obtained from the calibration curve.

RESULTS AND DISCUSSION

Method development

In the present study we have developed a rapid and sensitive high performance liquid chromatographic method for the estimation of Gemcitabine in bulk and pharmaceutical dosage forms. A Zorbax R_XC_8, (250mm× 4.6mm, 5µ) column and mobile phase consisting of phosphate buffer (pH 3) and methanol in the ratio 85:15, v/v were used. The mobile phase was pumped at a flow rate of 1.2 ml/min. The wavelength selected for detection was 275 nm. The developed method was found to be appropriate for the determination of Gemcitabine in bulk and pharmaceutical dosage forms. A chromatogram of standard solution was shown in fig-1.

Validation
Linearity

A good linearity was observed between the concentration of Gemcitabine and respective mean of the peak area in the concentration range of 25-150 µg/ml. The regression coefficient was found to be 0.999. Calibration plot was shown in fig-2. Linearity values were shown in table-1.

Precision

Intra-day and inter-day precision was studied and the results of precision studies were shown in table-2. The %RSD was found to be satisfactory.

Accuracy

Accuracy was checked by recovery studies. Spiking was made and the prepared solutions were injected. chromatograms were recorded the % recovery was calculated. The results of recovery studies were shown in table 3.

Specificity

Specificity was determined by forced degradation studies. No interference was observed from the excipients and degradation products. Hence the method was found to be specific.

Robustness

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Robustness of the method was checked by changing flow rate, organic phase proportion of the mobile phase and column temperature. The results of robustness were shown in table-4.

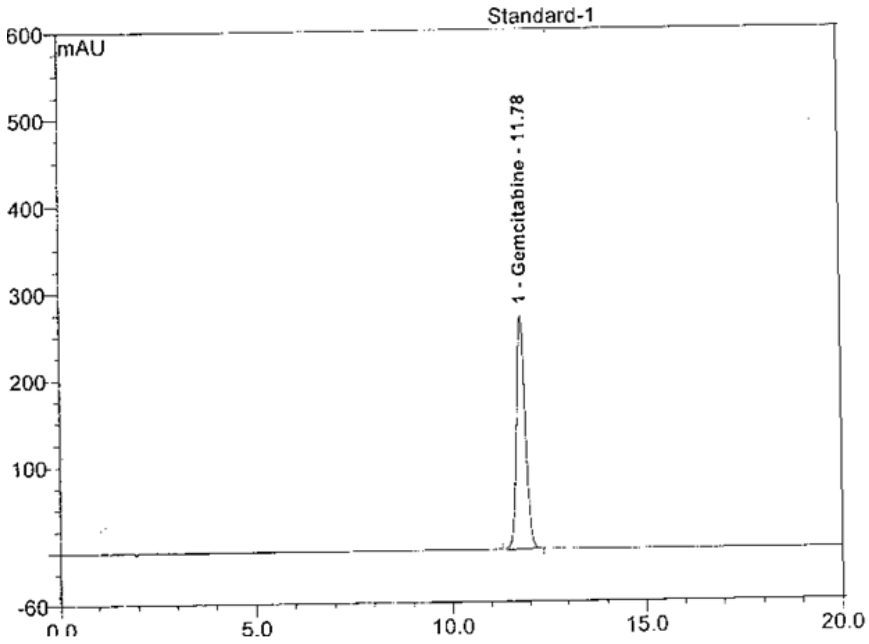

Fig-2: A typical chromatogram of Gemcitabine standard

Table-1: Results of linearity

Concentration (µg/ml)	Average Area of Gemcitabine
25	1005795
50	2025637
75	3030196
100	4047034
125	5029518
150	6097537
correlation coefficient	0.9999

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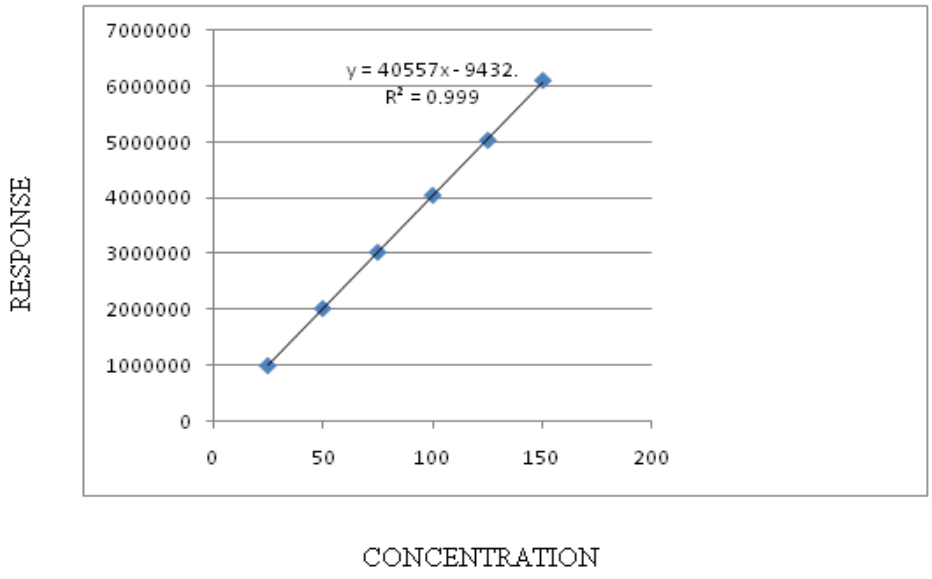

Fig-3: Calibration plot of the proposed method

Table-2: Results of intra-day and inter-day precision

Concentration of Gemcitabine (µg/ml)	Intra-day precision			Inter-day precision		
	Mean amount found	Percent amount found	Percent RSD	Mean amount found	Percent amount found	Percent RSD
50	49.66	99.32	0.36	49.99	99.98	0.68
75	74.64	99.52	0.72	75.15	100.2	0.47
100	100.15	100.15	0.77	100.23	100.23	0.14

Table-3: Results of recovery studies

Amount taken (µg)	Amount found (µg)	Percent recovery	% Mean recovery	% RSD
25+50	74.63	99.50	99.86	0.51
25+50	75.21	100.28		
25+50	74.90	99.80		
25+75	100.21	100.21	100.14	0.32
25+75	100.42	100.42		
25+75	99.80	99.80		
25+100	126.43	101.14	100.32	0.67
25+100	124.72	99.77		
25+100	125.09	100.07		

Table-4: Results of robustness studies

Result	Flow rate (ml/min)			Organic proportion of the mobile phase		
	1.0	1.2	1.4	80	85	90
Percent assay	99.36	99.82	99.55	100.61	99.82	99.66

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