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RP-HPLC Analysis of Gemcitabine in Pure Form and in Pharmaceutical Dosage Forms

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A rapid and precise reverse phase high performance liquid chromatographic method has been developed for the estimation of gemcitabine in pure form as well as in its tablet dosage forms. The quantification was carried out using an ODS column (250 × 4.6 mm ID), 5 µ particle size with a mobile phase comprising of water and acetonitrile in the ratio 30:70 (v/v) in isocratic mode at a flow rate of 1.0 mL/min. The eluent was monitored at 234 nm. The retention time was 2.20 min. Racecadotril was used as an internal standard. The method was duly validated by evaluation of the required parameters. The calibration curve was linear in the concentration range of 1-300 µg/mL and the percentage recovery ranged from 99.98-100.30.

Key Words: Gemcitabine, Estimation, Tablets, RP-HPLC.

INTRODUCTION

Gemcitabine[1] is 2'-deoxy-2',2'-difluorocytidine monohydrochloride (β-isomer). Gemcitabine is a nucleoside analogue that exhibits antitumour activity by killing cells undergoing DNA synthesis. A few HPLC[2-5] methods have been reported in the literature for the estimation of gemcitabine. The authors now proposed a more sensitive, highly accurate and precise HPLC method for the estimation of gemcetabine in pure as well as in tablet dosage forms.

EXPERIMENTAL

A Shimadzu LC-10AT high pressure liquid chromatographic instrument provided with an ODS reversed phase column (250 × 4.6 mm id), 25 µL Hamilton syringe and supported by Windows software was employed in the study.

Gemcitabine was a gift sample from M/s Cipla laboratories, India. HPLC grade acetonitrile (E. Merck India) and milli-Q water were used for preparing the mobile phase.

Chromatographic conditions: The mobile phase used was water and acetonitrile in the ratio 30:70 (v/v). Water and acetonitrile were filtered through 0.45 µ membrane filter and sonicated before use. The mobile phase was pumped from the solvent reservoir in the ratio of 30:70 to the column at a flow rate of 1 mL/min. The run time was set at 10 min. The column was maintained at 30°C and the volume of each injection was 20 µL. Prior to injecting solutions, the column was equilibrated for at least 0.5 h with the mobile phase flowing through the system. The detector sensitivity was set at 0.0001 AUFS and eluent monitored at 234 nm.

Procedure: About 100 mg of pure sample of gemcitabine was weighed accurately and transferred to a 100 mL volumetric flask and dissolved in 70 mL of acetonitrile. The solution was sonicated for 20 min and then the volume made up with a further quantity of acetonitrile to get 1 mg/mL solution. Subsequent dilutions of this solution ranging from 1-300 µg/mL were made in 10 mL volumetric flasks. The solutions prepared as above were filtered through 0.45 µ membrane filter and then 20 µ of filtrate was injected each time in to the column at a flow rate of 1 mL/min. Each concentration was injected 6 times into the column and corresponding chromatograms were obtained. Detection of the drug was done at 234 nm. From the chromatograms, the retention time and mean peak area ratios were recorded for all the concentrations. A calibration curve of peak area ratio *vs.* the respective concentration was plotted. The regression of drug concentration over the peak area ratio was computed using least squares method of analysis. This regression equation was used to estimate the amount of gemcitabine in pharmaceutical formulations.

Estimation of gemcitabine in tablet dosage forms: Two commercial brands of tablets were chosen for testing the suitability of the proposed method to estimate gemcitabine in tablet dosage forms. For this, 20 tablets were weighed and powdered. Accurately weighed portion of the tablet powder equivalent to 100 mg was taken in 100 mL volumetric flask and 50 mL acetonitrile was added, shaken well and allowed to stand for 15 min with intermittent sonication to ensure complete solubility of the drug. The mixture was then thoroughly mixed and made upto the mark with acetonitrile and filtered through a 0.45 µ membrane filter. From the filtrate, different aliquots were taken in separate 10 mL volumetric flasks. The contents of the flask were made up to the volume with the acetonitrile and mixed well. Each of these solutions was then injected into the column. All the determinations were conducted 3 times from the peak area ratios. The drug content in the tablets was quantified using the regression equation obtained from the pure sample.

RESULTS AND DISCUSSION

The aim of this study was to develop a simple, rapid, accurate and precise HPLC method for the analysis of gemcitabine in bulk and tablet dosage forms using most commonly employed RP C-18 column with UV detection.

The run time of the method was set at 10 min and gemcitabine appeared on chromatogram at 2.20 min. This indicates that the present HPLC method is rapid, which in turn shows that the method consumes less volume of HPLC solvents. When the same drug solution was injected 6 times, the retention time of the drug was found to be the same. The retention time for the internal standard (racecadotril) was observed to be 5.93 min.

The peak area ratios from such different concentrations set up as above were calculated and are shown in Table-1. A good linear relationship was observed between the concentration of gemcetabine and the respective peak area ratio. The regression curve was constructed by least squares method and its mathematical expression was $y = 0.1783 x + 0.0311$ (where y is the peak area ratio and x is the concentration of gemcetabine). This regression equation was used to estimate the amount of gemcitabine in tablet dosage forms.

TABLE-1
CALIBRATION OF THE PROPOSED METHOD

Concentration (μg/mL)	Peak area ratios	Concentration (μg/mL)	Peak area ratios
1	0.178	100	18.203
2	0.356	150	26.752
5	0.894	200	35.653
10	1.780	250	44.601
25	4.463	300	53.499
50	8.901	–	–

Regression equation from 1-300 (μg/mL): $y = 0.1783 x + 0.0311$ ($r = 0.999$)

To ensure reliability and accuracy of the method, a known quantity of the drug was mixed with preanalyzed sample. Recovery studies were carried out by the proposed method. The values obtained are shown in Table-2. About 100.10 % of gemcitabine could be recovered from the preanalyzed samples indicating high accuracy of the proposed HPLC method.

The HPLC method, developed in the present study, has also been used to quantify gemcitabine in tablet dosage forms. Gemcitabine tablets (containing 200 mg) were quantified using the proposed analytical method and the results are given in Table-3. No interfering peaks were found in the chromatogram indicating that the tablet excepients did not interfere with

the estimation of drug by the proposed HPLC method. The tablets were found to contain 100.05-101.0 % of the drug. A known amount of drug solution was added to the sample of tablet dosage form and subjected to estimation of drug by proposed method. There was a high recovery of gemcitabine (99.98-100.3 %) indicating that the proposed procedure for determination of gemcitabine in tablet dosage form is highly accurate.

TABLE-2
RESULTS OF RECOVERY STUDY

Amount of gemcitabine	Recovery from drug solution		Recovery from formulation	
	Mean amount	Mean (%) recovery	Mean amount	Mean (%) recovery
100	100.2	100.2	100.30	100.30
200	199.6	99.8	199.99	99.98
300	300.3	100.1	300.10	100.03

TABLE-3
ASSAY OF GEMCITABINE IN TABLET DOSAGE FORMS

Labeled amount (mg)	Mean ± SD amount (mg) recovered (n=3)	Mean ± SD % of recovery (n=3)
200	199.98 ± 0.02	100.06 ± 0.04
200	199.99 ± 0.01	100.05 ± 0.05

Hence, the proposed HPLC method is simple, precise, accurate and rapid for the determination of gemcitabine in dosage forms. It can be easily and conveniently adopted for routine quality control analysis of the drug.

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