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RESEARCH ARTICLE

Development and Validation of pH-Independent Spectroscopic Method for Estimation of Gemcitabine HCl in Pharmaceutical Formulation

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ABSTRACT

This method has been developed by measuring the absorbance of Gemcitabine HCL at 254 nm (pH 3.0 – 10.9). The method is simple, accurate and precise, giving linearity in the range of 14 – 34 µg/mL for Gemcitabine HCL with R2= 0.999 (n= 6). The method was employed in testing the concentration of Gemcitabine HCL in marketed formulations, and the results were found in agreement with the labeled amount.

KEYWORDS

UV spectrophotometry, Isosbestic point, pH independent, Gemcitabine HCL

INTRODUCTION

Gemcitabine is a synthetic pyrimidine, which is used as an anti – cancer prodrug. The drug replaces cytidine during DNA replication and thereby inhibits cell – division, which leads to apoptosis. It is used in non-small cell lung cancer, pancreatic cancer, bladder cancer and breast cancer. It is colorless, odorless, soluble in water, slightly soluble in methanol and sparingly soluble in acetone.

Chemically it is 4-amino-1-[(2R,4R,5R)-3,3-difluoro-4-hydroxy-5- (hydroxymethyl)oxolan-2-yl]-1,2-dihydropyrimidin-2-one[Figure 1][1]. It is a white powder, odorless powder and practically insoluble in acetone, slightly soluble in methanol, soluble in water. It is official in British Pharmacopoeia[2], European Pharmacopoeia[3], United State Pharmacopoeia[4]. Literature survey reveals HPLC[5,6,7,8,9], HPTLC[10] and colorimetry [11] methods for estimation of Gemcitabine HCL in single dosage form.

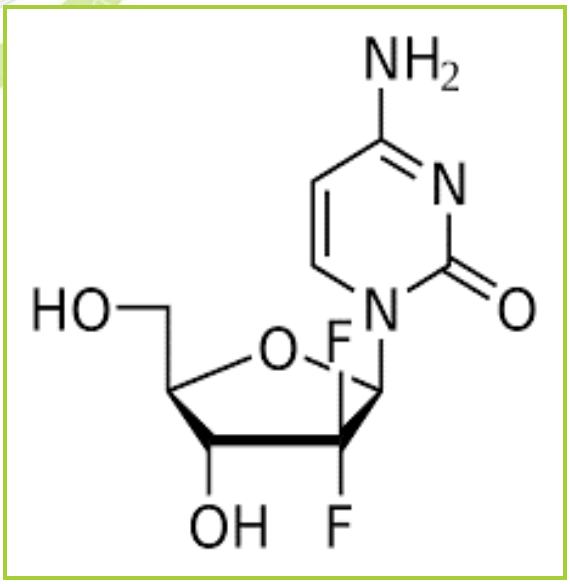

Figure 1: Chemical structure of Gemcitabine

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MATERIALS & METHODS

A Shimadzu model 1700 (Japan) double beam UV/Visible spectrophotometer with a spectral width of 2 nm, wavelength accuracy of 0.5 nm and a pair of 10 mm matched quartz cell was used to measure the absorbance of all the solutions. Spectra were automatically obtained by UV-Probe system software. A Sartorius CP224S analytical balance (Gottingen, Germany), an ultrasonic bath (Frontline FS 4, Mumbai, India), Digital pH meter (LI 712 pH analyzer, Elico Ltd., Ahmedabad) was used in the study. GEM bulk powder was kindly gifted by was kindly supplied as a gift sample from Intas Pharma Pvt. Ltd., Ahmedabad. Tablet of Gemcitabine HCL was purchased from a local pharmacy.

Method

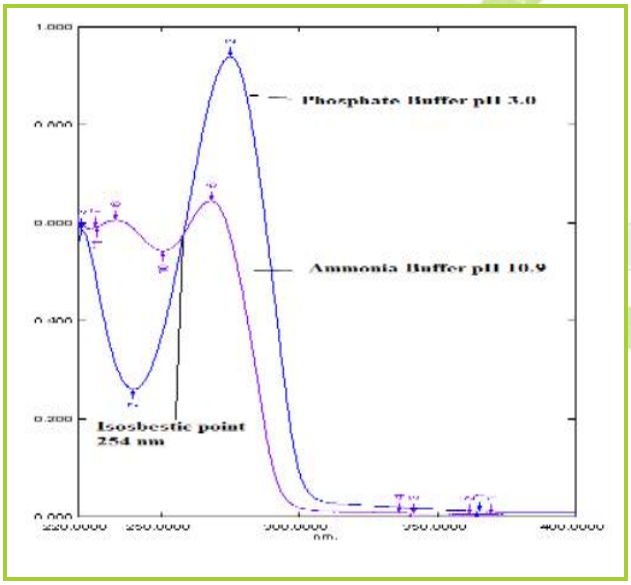

Figure 2: Overlain absorption spectra of Gemcitabine HCl at pH 3.0&pH 10.9

In pH independent spectrophotometric method the isosbestic point of drug solutions in different pH was measured. (Figure 2). For this measurement, an equimolar solution of Gemcitabine was prepared separately in Phosphate Buffer pH 3.0 as well as in Ammonia Buffer pH 10.9 at a concentration of 20 µg /ml. They were scanned in the wavelength range of 200-400 nm. The isosbestic points were recorded at 254 nm. There was no change in isosbestic points, which reveals that there was no interference by additives.

A standard stock solution of Gemcitabine HCL (100 µg/ml) was prepared as follows:-

Phosphate buffer pH 3 and Ammonia buffer pH 10.9 were taken in two separate 100ml volumetric flasks, and 10 mg of pure drug was dissolved therein. Then it was diluted with the respective solvents, so as to obtain a final concentration of 14 -34 µg/ml. The solution was scanned in UV photo spectrometer, using water as a blank. Absorbance was measured at 254 nm, and a calibration curve was prepared.

METHOD VALIDATION

The proposed method was validated according to the International Conference on Harmonization (ICH) guidelines [12]

Linearity (Calibration curve):

Standard working solutions of GEM (1.4,1.8,2.2,2.6,3.0and3.4ml) were transferred to two sets of 10 ml volumetric flasks, one containing Phosphate buffer pH 3 and the other containing Ammonia buffer pH 10.9. Absorbance was measured at 254 nm against water as a blank. A calibration curve was prepared by plotting absorbance v/s concentration of GEM and regression equations were calculated.

Accuracy (% Recovery)

Recoveries of GEM by standard addition method were calculated to check the accuracy of the proposed method.

Known amounts of standard solutions of GEM were added at 50%, 100% and 150% levels to quantified solutions of GEM (20 µg/ml).

Repeatability

Repeated scanning and measuring of absorbance of solutions (n = 6) of GEM (22 µg/ml) was conducted, without changing the parameters of the proposed method; so as to

check the precision of the instrument. The results were expressed as percentage relative standard deviation. (%RSD).

Intermediate precision

The intraday and interday accuracy of the proposed method was determined by analyzing the corresponding responses three times on the same day and three different days over a period of 1 week for three different concentrations of standard solutions of GEM(18, 22 and 26 µg/ml). The results are reported regarding percentage relative standard deviation (%RSD).

Limit of detection (LOD) and limit of quantification (LOQ)

The limit of detection (LOD) and limit of quantification (LOQ) of the proposed method were calculated by using the

following equations as per ICH guideline.

$$LOD = 3.3 \times \sigma / S$$

$$LOQ = 10 \times \sigma / S$$

Where, σ = the standard deviation of the response, S = slope of the calibration curve

Estimation of Gemcitabine in its dosage form

For analysis of Gemcitabine in tablet dosage form, five tablets were accurately weighed and powdered. A quantity of carefully weighed the tablet powder equivalent to 10 mg of Gemcitabine was transferred to 2 sets of 100 ml volumetric flask containing 60 ml Phosphate buffer pH 3 and Ammonia buffer pH 10.9, sonicated for 10 min. Finally, volume was made up to the mark with buffer solutions and further shaken for 15 min for complete extraction of from its matrix. Above solution filtered through Whatman filter paper No.42 and diluted up to mark with methanol. An aliquot of the above-prepared sample solution was suitably diluted with buffer solutions to obtain a solution of Gemcitabine (20 µg/ml) and analyzed by the pH-independent spectrophotometric method.

RESULTS & DISCUSSION

Parameters	pH independent spectrophotometric method GEM
Wavelength	254nm
Concentration range (µg/ml)	14-34µg/ml
Regression equation (y=mx+ c)	0.020x+0.025
Correlation coefficient (r^2)	0.999
Slope (m)	0.020
Intercept (c)	0.025
Limit of detection (µg/ml)	0.978
Limit of quantitation (µg/ml)	2.964
Repeatability(n =6)(% RSD)	0.344
Interday precision (n=3)(% RSD)	0.659 – 1.710
Intraday precision (n=3)(% RSD)	0.276 – 0.729
Accuracy± SD (n=3)	100.345 ±0.294
%Assay± SD (n=5)	99.266 ± 0.503

Table 1: Regression analysis data and summary of validation parameters for the proposed method

The proposed method was found to be simple, sensitive, rapid, accurate, precise and economical for the routine simultaneous estimation of two drugs. The linearity ranges for both drugs were found to be 14-34 µg/ml. Characteristic parameters for regression equation and correlation are given in Table 1. The method was successfully used to determine the amounts of GEM present in dosage forms. The results obtained are in good agreement with

the corresponding labeled amount. By observing the validation parameters, the method was found to be sensitive, accurate and precise and hence it can be employed for the routine analysis GEM in pharmaceutical dosage form.

CONCLUSION

The proposed dual wavelength method was found to be linear between the range of 14-34 µg/ml for GEM. The mean percentage recovery was found 100.345% for Gemcitabine at three different levels of standard additions. The precision (repeatability, Intra-day, and inter-day) of methods were found within limits (RSD <2%). It could be concluded from the results obtained in the present investigation that the proposed method for the estimation of Gemcitabine from its pharmaceutical dosage form is simple, rapid, accurate, precise and economic and can be used, successfully in the quality control of pharmaceutical formulations and other routine laboratory analysis.

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