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Quantitative analysis of gemcitibine in commercial dosage forms by UV-visible spectrophotometry

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ABSTRACT

Two simple sensitive selective accurate and economical spectrophotometric methods A and B for the determination of Gemcitibine in bulk drug and pharmaceutical formulations (tablets) have been described in the present work. These methods are based on the formation of purple color and red-violet colored chromogens obtained when the drug was diazotized with nitrous acid followed by coupling with phloroglucinol and resorcinol, exhibiting λ_{max} at 525 and 610 nm. These methods obey Beer's law in the concentration ranges of 2-10 and 4-20 µg/mL.

Key words: Gemcitibine, chromogen, spectrophotometry.

INTRODUCTION

Gemcitibine (2'-deoxy-2'2'di fluro cytidine) is a novel deoxycytidine analogue that has a broader therapeutic index against several solid tumors, of which non-small-cell lung cancer, breast cancer, and pancreas cancer[1-3]. Gemcitibine also demonstrates good efficacy both administering alone and in combination with other anticancer drugs. After intravenous administration, gemcitibine is rapidly metabolized in the liver, kidney, and other tissues to a noncytotoxic metabolite (2'-deoxy-2'2'di fluro cytidine)[4,5]. Several methods, including enzyme linked immuno-sorbance assay (ELISA)[5], F-NMR[6], high-performance liquid chromatography tandem-mass spectrometry (HPLC/NS)[7], derivative-spectrophotometric[8], and HPLC, have been reported for determining the concentrations of gemcitibine in plasma, cerebrospinal fluid, urine, and human carcinoma cells[9-15]. The present paper describes two simple, sensitive and selective visible spectrophotometric methods for the assay of gemcitibine in drug formulations through diazo coupling reactions. The results of analysis for the two methods have been validated statistically.

EXPERIMENTAL

Apparatus

All spectral measurements were made on ELICO SL 159 double beam, UV-visible spectrophotometer with 1 cm quartz cells.

Reagents and Chemicals

- All chemicals used were of analytical grade.
- Aqueous solutions of sodium nitrite (0.1% w/v),
- Aqueous solutions of sodium hydroxide (4% w/v)
- Dilute hydrochloric acid (0.25M) were freshly prepared.

Standard solutions

Gemcitibine (pure form)(100mg) was accurately weighed and dissolved in 20ml of distilled water and transferred to a standard 100ml volumetric flask. The final volume was made up to the mark with distilled water. The final concentration was brought to 100µg/mL with distilled water.

Procedure for the assay of gemcitibine in pharmaceutical formulations

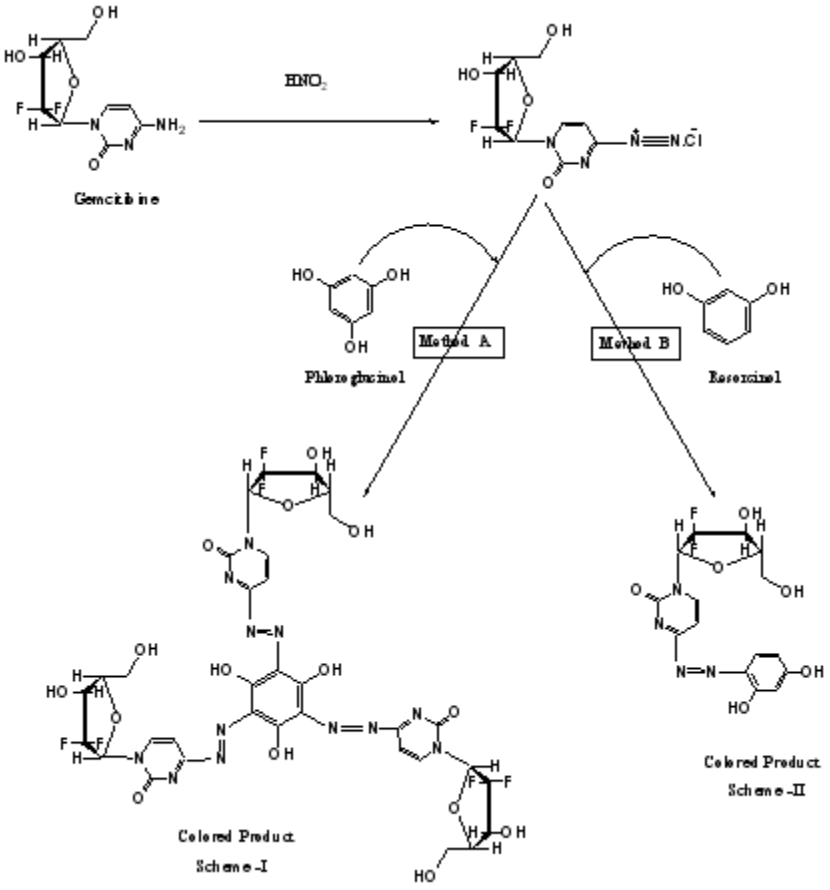
Twenty tablets containing Gemcitibine were weighed and finely powdered. An accurately weighed portion of the powder equivalent to 25mg of GEM was dissolved in a 25ml of methanol and mixed for about 5 minutes and then filtered. The methanol was evaporated to dryness. The remaining portion of solution was diluted in a 25ml volumetric flask to the volume with distilled water. The general procedure was then followed in the concentration ranges mentioned above.

Procedures for the determination of gemcitibine
Method A

Aliquots of gemcitibine (0.5-2.5ml, 2-10µg/mL) were transferred into a series of 25ml volumetric flasks. To each of the above aliquots, hydrochloric acid (dilute) (1.0ml) and 1.0ml cold aqueous solution of sodium nitrite (0.1% w/v) were added and set aside for 10 min at 0-5°C temperature. Later 1.0ml of phloroglucinol (0.1% w/v) and 1.5ml of aqueous sodium hydroxide (4% w/v) were added successively, and then the volume in each tube was made up to 25ml with distilled water. The absorbance was measured at 525nm against reagent blank. The amount of Gemcitibine was computed from calibration curve. The color was found to be stable for more than 2 hours at room temperature. The concentration of Gemcitibine was calculated either from calibration curve or from regression equation.

Method B

Aliquots of drug solution (0.5-2.5ml, 4-20µg/mL) were transferred into a series of 25ml volumetric flasks. To each of the above aliquots, hydrochloric acid (dilute) (1.0ml) and 1.0ml cold aqueous solution of sodium nitrite (0.1% w/v) were added and set aside for 10 min at 0-5° C temperature. Later 1.0ml of resorcinol (0.1% w/v) and 1.5ml of aqueous sodium hydroxide (4% w/v) were added successively, and then the volume in each tube was made up to 25ml with distilled water.



The absorbance was measured at 610nm against reagent blank. The amount of Gemcitibine was computed from calibration curve. The color was found to be stable for more than 4 hours at room temperature. A calibration graph was drawn and the corresponding regression equation was computed to obtain the concentration of Gemcitibine.

RESULTS AND DISCUSSION

The presence of amino group in Gemcitibine enabled the use of diazotization of the drug with nitrous acid and coupling the resulting diazonium salt with phloroglucinol, to form purple colored chromogen in method A (Scheme-I) exhibiting λ_{max} at 525nm. In method B (Scheme-II) same diazotization reaction was followed by coupling with resorcinol in presence of sodium hydroxide solution resulting in the formation of red-violet chromongens exhibiting λ_{max} at 610nm. The Beer's law was obeyed by these two methods in the concentration ranges 2-10 and 4-20 µg/mL respectively. The optical characteristics such as Beer's law limits, absorption maxima, molar absorptivity, Sandell's sensitivity, percent relative standard deviation (%RSD) calculated from six measurements containing ¾ th of the amount of the upper Beer's law limits of Gemcitibine. The percent range of errors (0.05 level and 0.01 confidence limits) were calculated for the two methods are reported in Table 1.

The optimum conditions for the color development in method A and method B were established by varying the parameters one at a time and keeping the other parameters fixed while observing the effects on the absorbance of colored species. The optimum concentration for the estimation of Gemcitibine was established by varying the drug concentration, keeping reagent concentration fixed. After establishing the optimum concentration for the drug, the reagent concentration was varied. The above ranges of drug and reagent concentrations were chosen because the colored species formed gave better absorbance and obeyed Beer's law. The values obtained for the determination of Gemcitibine in different brands of Tablet samples 1, 2 and 3 by the proposed and U.V methods are compared in Table 2. To evaluate the validity and reproducibility of the methods, known amounts of pure drug were added to the previously analyzed pharmaceutical preparation and the mixtures were analyzed by the proposed methods. The percent recoveries are given in Table 2.

The methods reported here are found to be simple, sensitive, accurate and precise. Further, spectrophotometric methods involve simple

Table 1: Optical characteristics and statistical data for the regression equation of the proposed methods

Optical characteristics	Method A	Method B
λmax (run)	525	610
Beer's law limits(µg/mL)(C)	2 - 10	4 - 20
Molar absorptivity (lit.mol^{-1}cm^{-1})	9.470×10^3	8.378×10^3
Sandell's sensitivity (µg/cm^2)-0.001ab units	0.0241	0.0584
Regression equation(Y = a + bc)*		
Slope (b)	0.03946	0.03480
Intercept (a)	0.00924	0.00416
Correlation coefficient (r)	0.9992	0.9999
% RSD		
Range of errors**	0.3609	0.6363
Confidence limits with 0.05 level	0.3788	0.6680
Confidence limits with 0.01 level	0.59421	1.0476

* Y is the absorbance and C is the concentration µg/mL

** For six measurements

Table 2: Estimation of gemcitibine in pharmaceutical formulations

Sample	Labelled amount (mg)	Amount obtained (mg) proposed methods*		UV method	% Recovery of proposed methods**	
		Method A	Method B		Method A	Method B
Tablet - 1(Gemzar)	200	198.6	199.5	199.7	99.44	99.87

*Average of six determinations

** Mean and standard deviation of six determinations

instrumentation which is cost effective compared with other instrumental techniques, which ordinary laboratories cannot afford to have. The present methods involve the formation of highly stable colored species which makes it easier for the determination of Gemcitibine from pharmaceutical dosage forms in a routine manner. Common execipients usually present in the tablet form, did not interfere at their regularly added levels.

CONCLUSIONS

The proposed spectrophotometric methods for the determination of Gemcitibine are simple accurate, precise and cheap. The statistical analysis shows that the data from the proposed methods are in good agreement with those of the reported method. The color reaction does not require stringent conditions nor any specific reagent or buffer. The color is stable up to 45 min, which is sufficient for the analyst to perform the analysis.

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