CHAPTER 3
DRUG PROFILES
&
ANALYTICAL METHODS

3.1 DRUG PROFILE

3.1.1 Gemcitabine Hydrochloride

I. Description (USP 2007)

a. Drug Category: Radiation-Sensitizing Agents; Immunosuppressive Agents; Antimetabolites; Antineoplastic agents; Antiviral agents

b. Drug Type: Small Molecule; Approved

c. Systematic (IUPAC) name: 2'-Deoxy-2',2'-difluorocytidine hydrochloride

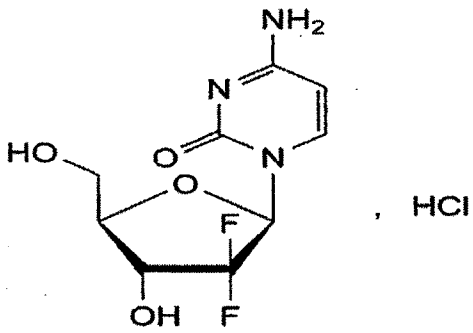
d. Marketed preparations available: Gemzar

e. Empirical formula: $C_9H_{11}F_2N_3O_4,HCl$

f. Molecular weight: 299.7

g. Melting Range: 286-292 degree C

h. Structure:



i. **Physical properties**

1) **Appearance:** White or almost white powder.

2) **Solubility:** Soluble in water, slightly soluble in methanol, practically insoluble in insoluble in ethanol and polar organic solvents.

j. **Application of Gemcitabine Hydrochloride**

Gemcitabine Hydrochloride (Gemcitabine HCl) is a chemotherapeutic drug used in the treatment of various types of cancer. Gemcitabine HCl is most commonly used to treat non small cell lung cancer, pancreatic, bladder cancer and breast cancer.

k. **Protein Binding:** Plasma protein binding is negligible (<10%)

l. **Mechanism of action:** The prodrug gemcitabine is converted intracellularly via deoxycytidine kinase to difluorodeoxycytidine monophosphate, which is

further converted to two active metabolites, dFdCDP and dFdCTP, di- and triphosphate respectively. Firstly, dFdCDP inhibits the catalysing enzyme ribonucleotide reductase resulting in a reduced amount of deoxynucleotide, deoxycytidine triphosphate (dCTP), available for DNA synthesis. Secondly, dFdCTP competes with dCTP for incorporation into DNA. Incorporating dFdCTP results in chain termination after the further addition of one more nucleotide and thus to apoptosis. Thus dFdC affects the synthesis phase of cell metabolism in two different ways and exhibits a selfpotentiating effect (Noble and Goa, 2007; Gemzer, 2006).

m. **Pharmacokinetics**

1) **Oral Absorption:** no information found

2) **Distribution:** Gemcitabine pharmacokinetics is linear. The volume of distribution is affected by the duration of infusion, age and gender. Crossing of blood brain barrier were unknown and PPB was negligible.

3) **Metabolism:** Metabolized via nucleoside kinases to two active metabolites, gemcitabine diphosphate and gemcitabine triphosphate. GEM is rapidly deaminated in the blood, liver, kidneys and other tissues. GEM undergoes deamination via cytidine deaminase to an inactive uracil metabolite (dFdU). The active metabolite was dFdCTP and inactive metabolite is dFdU (difluorouridine form).

4) **Excretion:** Gemcitabine clearance is lower in women and in the elderly. The inactive metabolite is excreted almost entirely in urine and its elimination is dependent on renal excretion. Urine was 92-98% (< 10% unchanged), $t_{1/2}$ 32 to 94 min

5) **Indications and Status**

- Locally advanced (unresectable) or metastatic adenocarcinoma of the pancreas.
- Locally advanced or metastatic non-small cell lung cancer (NSCLC) as a single agent or in combination with cisplatin.

- In combination with cisplatin for locally advanced or metastatic transitional cell carcinoma (TCC) of the bladder.
- In combination with paclitaxel for unresectable, locally recurrent or metastatic breast cancer, who have good performance status and have relapsed following adjuvant anthracycline- based chemotherapy.
- In combination with docetaxel in metastatic leiomyosarcoma of the uterus or metastatic breast cancer

6) **Adverse effects**
   - Cardiovascular- Myocardial Infarction, Edema, heart failure, Arrhythmia, Hypertension
   - Dermatological- Rash, Alopecia, Toxic epidermal necrosis
   - Gastrointestinal- Constipation, Diarrhea (12%), Nausea and vomiting(64%) Stomatitis, Anorexia
   - Hematological- Infection, Neutropenia, Thrombocytopenia.
   - Hepatic- Hepatotoxicity, Liverfailure(rare)
   - Renal / Metabolic- proteinuria, Hematuria (36%)
   - Others- Fatigue, Myalgia, Anaphylaxis, Fever (37%), Flu-like syndrome (19%), Pain(16%)
   - The major dose-limiting toxicity with gemcitabine is myelosuppression which is non-cumulative.

7) **Toxicity:** The cytotoxic activity of gemcitabine *in vivo* is dose and dosage regimen dependent. This means the activity and the toxicity are related to the dose given and the dosage interval of the treatment. The problem with Gemcitabine is its short plasma t½ and its quick metabolism into dFdU followed by elimination from the body. Therefore high doses of dFdC are required in order to achieve sufficient cytotoxic concentrations of dFdCTP. Due to the narrow therapeutic window, high administered doses increase the possibility of toxicities and concentration dependent side effects for patients. According to clinical studies, the primary dose limiting toxic effect is myelosuppression, neutropenia, leucopenia, anaemia and thrombocytopenia. In addition, together with other side effects, such as hepatic abnormalities,

nausea and vomiting, 10 % of patients ceased treatment. (Brandl and Massing, 2003; Gemzer, 2006; Moog et al., 2002; Noble and Goa, 2007).

8) Dosage

Pancreatic Cancer	Cycle 1: 1000mg/m^2 weekly for 7 weeks with 1 week rest Cycle 2 on: 1000mg/m^2 for 3 weeks with 1 week rest
NSCLC	1000mg/m^2 for 3 weeks ± Cisplatin 100mg/m^2 after infusion Day 1 only 1250mg/m^2 for 3 weeks ± Cisplatin 100mg/m^2 after infusion Day 1 only
Breast Cancer	1250mg/m^2 weekly for 2 weeks with paclitaxel 175mg/m^2 on day 1 only

Reference

1. Brandl M, Massing U. Vesicular phospholipid gels. In: Torchilin VP, Weissig V, editors. Liposomes. 2nd ed: Oxford University Press; 2003;353-372.
2. British Pharmacopoeia 200;1:686.
3. Gemzar (gemcitabine HCl) for injection. [Online]. 2006 [cited 2006 May 6]; Available from: URL:http://pi.lilly.com/us/gemzar.pdf
4. Moog R, Burger AM, Brandl M, Schüler J, Schubert R, Unger C, et al. Change in pharmacokinetic and pharmacodynamic behaviour of gemcitabine in human tumor xenografts upon entrapment in vesicular phospholipid gels. Cancer Chemother Pharmacol 2002;49:356-366.
5. Noble S, Goa KL. Gemcitabine. A review of its pharmacology and clinical potential in non-small cell lung cancer and pancreatic cancer. Drugs 1997;54(3):447-472.
6. United States Pharmacopoeia 30, NF25, United States pharamcopoeial convention, Rockeville, Page 2215.

3.2 ANALYTICAL METHOD

3.2.1 Introduction

Analytical methods are important tools to estimate the drug content in the formulations and to assess the stability of the drugs in the formulations over the period of time. The analytical methods are of volumetric methods and instrumental methods. Instrumental methods have advantages over volumetric methods because of their sensitivity, low sample requirement and accuracy. UV spectrophotometric method is the simplest instrumentation method capable of drug estimation in micrograms.

3.3 Materials and Instruments

3.3.1 Instrument and software for UV spectrophotometric measurement

Spectrophotometric measurements were carried out on a Shimadzu 1700 double beam UV Visible spectrophotometer with a fix slit width of 1nm coupled with HP7540 computer loaded with UV PC software of version 2.10. The spectral bandwidth was 1 nm and the wavelength scanning speed was 2800 nm/min. Matched quartz cuvettes (1cm) were used for all the spectral measurements.

3.4 Methods

3.4.1 UV spectroscopic method

3.4.1.1 Methodology

Preparation of stock solution of drugs

Stock solution containing 1mg/mL was prepared by dissolving drug in the solvent.

Preparation of standard solution of drugs

Standard solutions were prepared by pipetting out required volume of stock solution in 10 mL volumetric flasks and making the volume up to the mark with solvent to obtain known final concentrations in µg/mL. The spectras of the standard solutions were recorded using UV Visible spectrophotometer for 200nm to 400nm range against solvent as blank. The observations were recorded in triplicate.

Estimation of drugs in formulations

A definite volume of the sample to be estimated was taken in a 10mL volumetric flask and diluted up to the mark with solvent. The resultant solution was then keep it 2 min at ambient temperature and the spectra of the standard solutions was recorded using

UV Visible spectrophotometer in 200nm to 400nm range against solvent as blank. The observations were recorded in triplicate.

3.4.1.2 Method validation

1. Linearity and Range

Linearity of an analytical method is the ability to elicit the test results that are directly or by well defined transformation proportional to the concentration of the analyte in the samples within the given range.

2. Accuracy:

The accuracy of an analytical method is the closeness of test results obtained by that method to the true value. The accuracy of the method was determined by calculating the recoveries of the analyte by the method of standard additions. Known amounts of standard drug (80%, 100% and120%) were added to the preanalysed samples and the absorbances were measured.

3. Precision/ Repeatability/stability:

The precision of an analytical method is the degree of agreement among the individual test results when the procedure is applied repeatedly to multiple sampling of homogeneous sample. The precision of an analytical method is usually expressed as the SD (Standard Deviation) or RSD (% Relative Standard Deviation)

4. Limit of detection (LOD) and Limit of quantification (LOQ):

The limit of detection is a quantitative parameter and can be defined as the lowest concentration of the analyte in a sample that can be detected with acceptable precision and accuracy under stated experimental conditions, but not necessarily quantities as an exact value (USP, 2007). It is expressed as the concentration of analyte in the sample. Anything that changes the sensitivity of a method, including instrument and sample preparation will change the detection limit.

Limit of quantification is the lowest concentration of an analyte in a sample that may be measured in a sample matrix such as impurities in bulk drug substances and degradation products in finished products. The LOQ is almost 10 times higher than that of the blank.

LOD (or) LOQ = k. S_B/ S

Where k - Constant (3 for LOD, 10 for LOQ)

S_B - Standard deviation of the analytical blank

S - Slope of the concentration/response graph.

3.5 Experimental Conditions

3.5.1 UV spectroscopic method

The Spectrophotometric method described in USP was used for the estimation of gemcitabine in formulation.

Materials

Gemcitabine HCl, water, Methanol.

Table 3.1 Experimental conditions for Gemcitabine HCl by UV method

1.	Solvent	Water, Water and Methanol
2.	Stock solution conc.	1mg/mL
3.	Serial Conc. Range	5 – 30 µg/mL
4.	Spectrum range	200nm to 400nm
5.	Spectrum Blank	Water, Water and Methanol
6.	Peak	at 268nm

3.6 Results

3.6.1 UV spectroscopic method

Table 3.2 Absorbance of Gemcitabine HCl at 268nm in Water and Methanol

Sl. No.	Conc. (µg/mL)	Absorbance at 268 nm	SD	%RSD	Variance	Std. Error
1	5	0.143	0.002121	1.478272	4.5E-06	0.001226
2	10	0.307	0.003536	1.149767	1.25E-05	0.002044
3	15	0.509	0.004950	0.971491	2.45E-05	0.002861
4	20	0.656	0.007778	1.184794	6.05E-05	0.004496
5	25	0.852	0.004243	0.499134	0.000018	0.002452
6	30	0.971	0.008485	0.874771	7.2E-05	0.004905

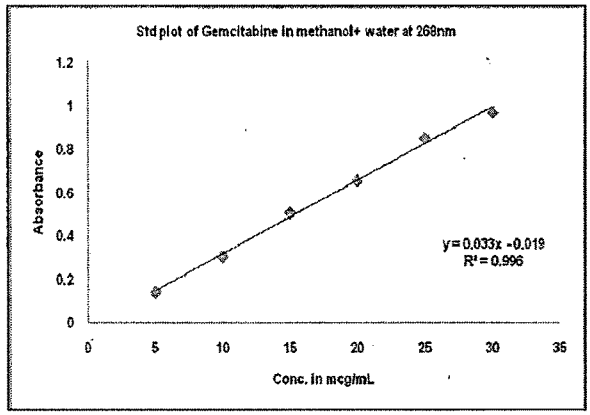


Figure 3.1 Standard plot of Gemcitabine in methanol and water at 268nm

Table 3.3 Absorbance of Gemcitabine HCl at 268nm in Water

Sl. No.	Conc. (µg/mL)	Absorbance at 268 nm	SD	%RSD	Variance	Std. Error
1	5	0.153	0.00495	3.224591	2.45E-05	0.002861
2	10	0.314	0.004243	1.351159	0.000018	0.002452
3	15	0.448	0.007778	1.734264	6.05E-05	0.004496
4	20	0.619	0.008485	1.370805	7.2E-05	0.004905
5	25	0.770	0.017678	2.294311	0.000313	0.010218
6	30	0.957	0.016971	1.773309	0.000288	0.00981

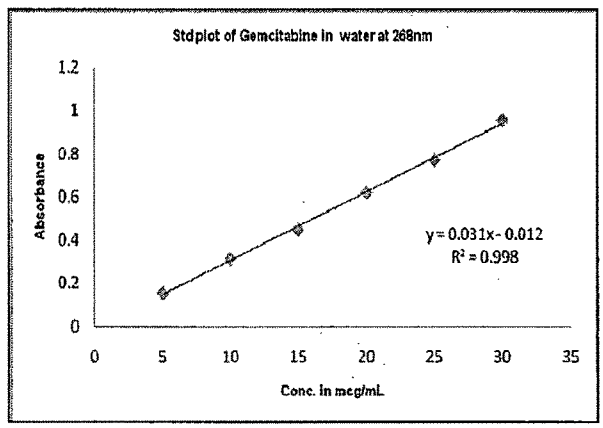


Figure 3.2 Standard plot of Gemcitabine in water at 268nm

3.7 Discussion

Gemcitabine HCl

The UV spectroscopic method described in the USP was used for the gemcitabine estimation. On screening of 5-30µg /mL solution, absorption maxima were obtained at 268nm (Pasut et al., 2008). The method was validated for linearity, accuracy and precision. The validation parameters were found to meet the "readily pass criteria" specified in the USP and % RSD were found to be less than 5% (Table 3.2 and 3.3). The absorbance was found to be linear in the range of 5-30µg /mL with r^2 value of 0.996 in water and methanol and 0.998 in water (figure 3.1 and 3.2). There was no lipids were found to be interfering while drug estimation in the solubility study.

Reference

1. Pasut G, Canal F, Dalla Via L, et al. Antitumoral activity of PEG–gemcitabine prodrugs targeted by folic acid. Journal of Controlled Release 2008; 127(3):239-248.
2. The United States Pharmacopoeia 30, NF25, United States pharamcopoeial convention, Rockeville. 2007;2215.
3. British Pharmacopoeia 200;1:686.