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IN VITRO PLANT REGENERATION FROM SHOOT TIP AND NODAL EXPLANTS OF RHINACANTHUS NASUTUS (L.) KURZ.

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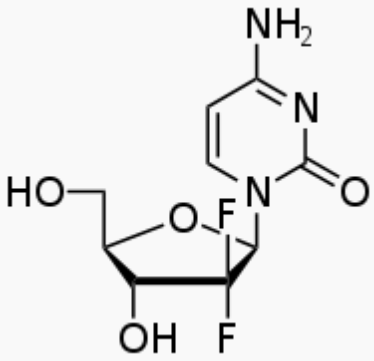
ABSTRACT

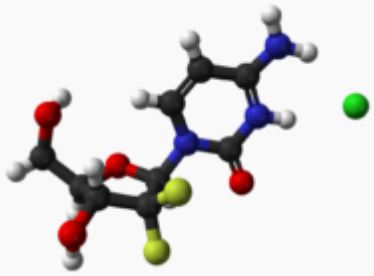
A simple, precise and accurate RP-HPLC method was developed and validated for rapid assay of Gemcitabinein tablet dosage form. Isocratic elution at a flow rate of 1ml/min was employed on a symmetry Chromosil C18 (250x4.6mm, 5µm in particle size) at ambient temperature. The mobile phase consisted of Acetonitril: Methanol 82: 18 v/v, (P^H 5.9 with 0.1M Phosphate buffer). The UV detection wavelength was 252nm and 20µl sample was injected. The retention time for Gemcitabinewas 6.89min. The percentage RSD for precision and accuracy of the method was found to be less than 2%. The method was validated as per the ICH guidelines. The method was successfully applied for routine analysis of Gemcitabinein tablet dosage form and bulk drug.

Key Words: Gemcitabine, RP-HPLC, UV detection, recovery, precise, 282nm

INTRODUCTION: Gemcitabine is antineoplastic agent, also called as anticancer drug. Gemcitabine is used in the treatment of different cancers such as in palliative treatment of non-small cell lung cancer to slow or stop the growth of abnormal cells. Gemcitabine is used in combination with different anticancer drugs to obtain best therapeutic effects and to reduce toxicity or side effects.

Gemcitabine was first synthesized in Larry Hertel's lab at Eli Lilly during the early 1980s.[6] It was intended as an antiviral drug, but preclinical testing showed that it killed leukemia cells in vitro.[6]





Systematic (IUPAC) name

4-amino-1-(2-deoxy-2,2-difluoro-β-D-erythro-pentofuranosyl)pyrimidin-2(1H)-on

Figure 1: Stricture of Gemcitabine

Gemcitabine is used in various carcinomas: non-small cell lung cancer, pancreatic cancer, bladder cancer and breast cancer. It is being investigated for use in esophageal cancer, and is used experimentally in lymphomas and various other tumor types. Gemcitabine represents an advance in pancreatic cancer care[citation needed]. It is also not as debilitating as some other forms of chemotherapy. Another target of gemcitabine is the enzyme ribonucleotide reductase (RNR). The diphosphate analogue binds to RNR active site and inactivates the enzyme irreversibly. Once RNR is inhibited, the cell cannot produce the deoxyribonucleotides required for DNA replication and repair, and cell apoptosis is induced.[1]

Side effects: A few of the most common side effects of gemcitabine include increased liver enzymes, leukopenia, and nausea and vomiting. Some side effects occur less frequently, but are much more serious and should be reported to a healthcare provider immediately. Seek medical attention right away if you develop serious gemcitabine side effects such as difficulty breathing, blood in the stool, or any signs of an allergic reaction.

EXPERIMENTAL

Materials

Working standard of Gemcitabine was obtained from well reputed research laboratories. HPLC grade water, Methanol was purchased from E. Merck (Mumbai, India).

Apparatus

A Series HPLC [6-11] system PEAK LC 7000 isocratic HPLC with PEAK 7000 delivery system. Rheodyne manual sample injector with switch (77251), Analytical column Chromosil C18. 250×4.6mm, Electronic balance-DENVER (SI234), manual Rheodyne injector with a 20 µl loop was used for the injection of sample. PEAK LC software was used. UV 2301 Spectrophotometer was used to determine the wavelength of maximum absorbance

Determination of wavelength of maximum absorbance

The standard solutions of Gemcitabine were scanned in the range of 200 -400 nm against mobile phase as a blank. Gemcitabine showed maximum absorbance at 282nm. So the wavelength selected for the determination of Gemcitabine was 282nm.

Chromatographic equipment and conditions

To develop a High Pressure Liquid Chromatographic method for quantitative estimation of GEMCITABINE an isocratic PEAK HPLC instrument with Zodiac C18 column (250 mm x 4.6 mm, 5µ) was used. The instrument is equipped with a LC 20AT pump for solvent delivery and variable wavelength programmable LC – 7000 UV-detector. A 20µL Rheodyne inject port was used for injecting the samples. Data was analyzed by using PEAK software.

The mobile phase consisted of Methanol:Acetonitrile:Water 85:15:5 v/v,(P^H 4.7)Injections were carried out using a 20 µl loop at room temperature (20 + 2 °C) and the flow rate was 1 ml/min. Detection was performed at 252nm with 10min runtime.

Standard and sample solutions

A 10 mg amount of Gemcitabine reference substance was accurately weighed and dissolved in 10 ml mobile phase in a 10 ml volumetric flask to obtain 1000 ppm concentrated solution. Required concentrations were prepared by serial dilution of this solution.

A composite of 20 (INTELENCE) tablets was prepared by grinding them to a fine, uniform size powder. 10 mg of Gemcitabine was accurately weighed and quantitatively transferred into a 100 ml volumetric flask. Approximately 25 ml mobile phase were added and the solution was sonicated for 15 min. The flask was filled to volume with mobile phase, and mixed. After filtration, an amount of the solution was diluted with mobile phase to a concentration of 120ppm.

Method validation

Method validation was performed following ICH specifications for specificity, range of linearity, accuracy, precision and robustness.

RESULTS AND DISCUSSION

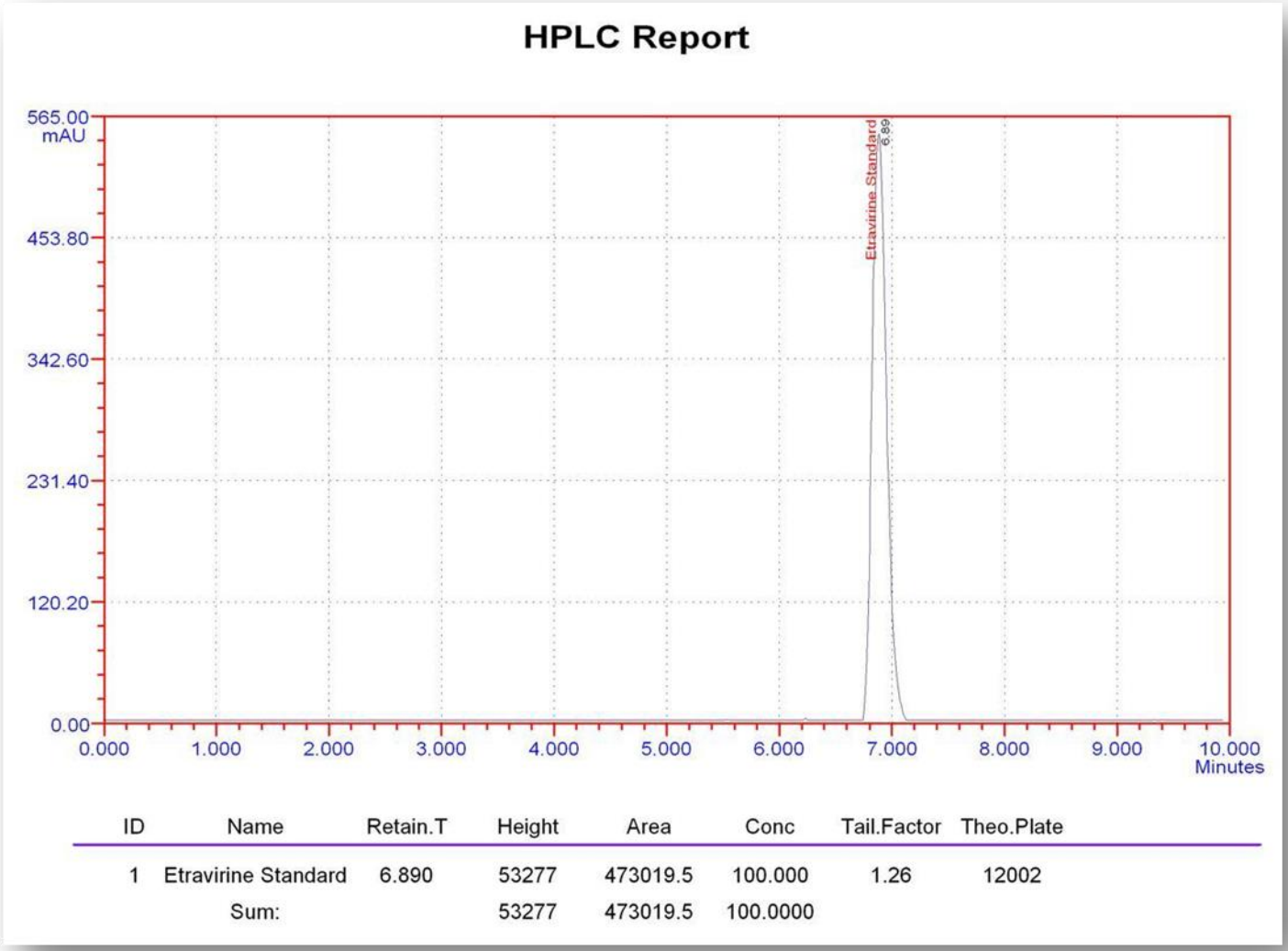
System Suitability

Having optimized the efficiency of a chromatographic separation, the quality of the chromatograph was monitored by applying the following system suitability tests: capacity factor, tailing factor and theoretical plates. The system suitability method acceptance criteria set in each validation run were: capacity factor >2.0, tailing factor ≤2.0 and theoretical plates >2500. In allcases, the relative standard deviation (R.S.D) for the analytic peak area for two consecutive injections was < 2.0%. A chromatogram obtained from reference substance solution is presented. System suitability parameters were shown in Table.1. Standard chromatogram was given in Figure.2

Table.1 System suitability parameters of Gemcitabine

Api Concentration	60 ppm

Mobile Phase	
Wavelength	282nm
Column	C_{18} Column
P^H	
Retention Time	
Run Time	10min
Area	285700.5
Th. Plates	6766
Tailing Factor	1.84
Pump Pressure	10.5 MPa
Flow Rate	1ml/min



ID	Name	Retain.T	Height	Area	Conc	Tail.Factor	Theo.Plate
1	Etravirine Standard	6.890	53277	473019.5	100.000	1.26	12002
	Sum:		53277	473019.5	100.0000		

Figure.2: Standard chromatogram of Gemcitabine

Range of linearity

Standard curves were constructed daily, for three consecutive days, using seven standard concentrations in a range of 30,40,50,60,70,80,90,and 100ppm for Gemcitabine. The linearity of peak area responses versus concentrations was demonstrated by linear least square regression analysis. The linear regression equation was y = 3862 + 3123x (r= 0.999). Linearity values can shown in Table: 2

Table.2: Linearity results of Gemcitabine

S.No	Concentration (µg/ml)	Area
1	30	133403.6

2	40	180168.5
3	50	233682.5
4	60	285700.5
5	70	327033.6
6	80	373398.7
7	90	417181
8	100	464186
	Slope Intercept CC	4681.633 -2188.35 0.99967

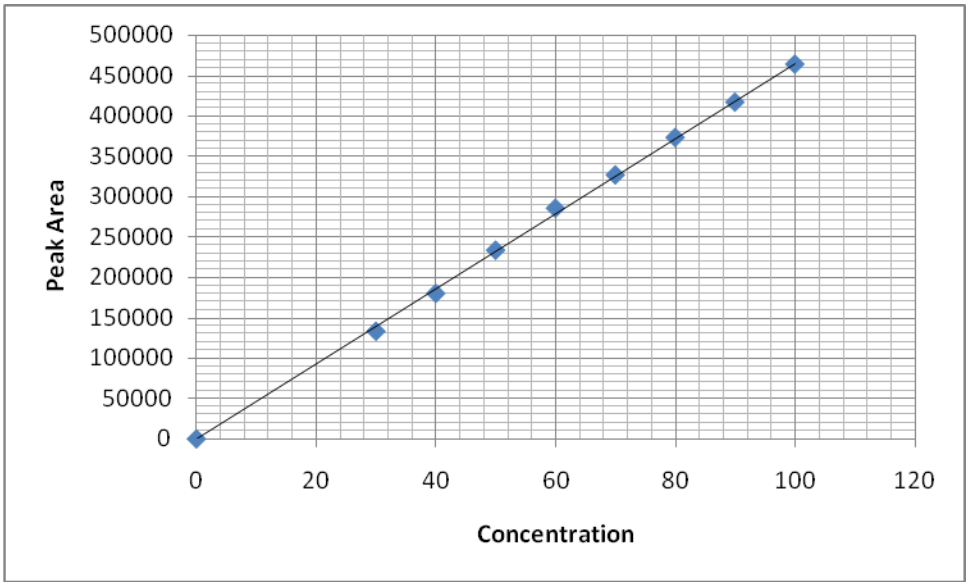


Figure 3: Calibration curve of Gemcitabine

Precision

To study precision, six replicate standard solutions of Gemcitabine (120ppm) were prepared and analyzed using the proposed method. The percent relative standard deviation (% RSD) for peak responses was calculated

and it was found to be which is well within the acceptance criteria of not more than 2.0%. Results of system precision studies are shown in Table.3 and Table.4.

Table 3: Intraday Precision Results for Gemcitabine:

Sample (µg/ml)	Area
1	286132.9
2	282005.2
3	289364.1
4	281692.3
5	281137.2
6	288816.3
RSD	1.30979

Table 4: Inter day Precision results of Gemcitabine

Sample (µg/ml)	Area
1	285700.5
2	280403.4
3	284920.8
4	280248.6
5	281020.9
6	286157.7
RSD	0.988

Limit of Detection and Limit of Quantification:

To determine the Limit of Detection (LOD) sample was dissolved by using Mobile phase and injected until peak was disappeared. After 0.5ppm dilution Peak was not clearly observed, based on which 0.5ppm is considered as Limit of Detection and Limit of Quantification is 0.15ppm.

Table.5: LOD and LOQ results of Gemcitabine

Parameter	**Measured Value**
Limit of Quantification	5ppm
Limit of Detection	1.5ppm

Robustness

Typical variations in liquid chromatography conditions were used to evaluate the robustness of the assay method. The robustness study was performed by slight modification in flow rate of the mobile phase, composition of the mobile phase and wavelength of the detector. Gemcitabine at standard concentration was analyzed under these changed experimental conditions. It was observed that there were no marked changes in chromatograms, which demonstrated that the developed method was robust in nature. The robustness acceptance criteria set in the validation were the same established on system suitability test describe above. Results were shown in table 6.

Table.6: Robustness results of Gemcitabine

S.NO	Parameter	Change	Area	% of Change
1	Standard	……	285700.5	……
2	MP	Meoh :ACN:H_2O 85:12.5:2.5 75:17.5:7.5	 283774.2 280403.4	 0.67 1.86
3	PH	4.8 4.6	280248.6 281840.0	0.96 1.35
4	WL	287nm 277nm	283859.7 280675.9	1.9 1.75

Ruggedness:

Ruggedness was performed by using six replicate injections of standard and sample solutions of concentrations which were prepared and analyzed by different analyst on three different. Ruggedness also expressed in terms of percentage relative standard deviation.

Sample (µg/ml)	Area
1	278786.1
2	277587.1
3	274405.1
4	270732.8
5	274791.4
6	279822.9
RSD	1.21938

Recovery

The accuracy of the method was determined by standard addition method. A known amount of standard drug was added to the fixed amount of pre-analyzed tablet solution. Percent recovery was calculated by comparing the area before and after the addition of the standard drug. Recovery test was performed at 3 different concentrations i.e. 60ppm, 80ppm, 100ppm. The percent recovery was calculated and results are presented in Table. Satisfactory recoveries ranging from 98.07 to 101.14 were obtained by the proposed method. This indicates that the proposed method was accurate. Results are given in table.8

Table.8: Recovery results of Gemcitabine

% Recovery	Target Conc., (ppm)	Spiked conc, (ppm)	Final Conc, (ppm)	Conc., Obtained	% of Recovery
50%	40	20	60	59.711	99.52
	40	20	60	59.836	99.72
	40	20	60	59.431	99.05
100%	40	40	80	101.3	101.3
	40	40	80	101.65	101.65
	40	40	80	100.88	100.88
150%	40	60	100	79.047	98.809
	40	60	100	80.040	100.05
	40	60	100	81.207	101.50

Table.9: Formulation Analysis

Formulation	Dosage	Concentration	Amount found	% Assay
INTELENCE	100mg	120ppm	119.80	99.83

CONCLUSION

The proposed method for the assay of Gemcitabine in tablets or capsules is very simple and rapid. It should be emphasized it is isocratic and the mobile phase do not contain any buffer. The method was validated for specificity, linearity, precision, accuracy and robustness. Although the method could effectively separate the drug from its products, further studies should be performed in order to use it to evaluate the stability of pharmaceutical formulations.

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