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STABILITY INDICATING RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF GEMCITABINE AND CLARITHROMYCIN IN BULK AND PHARMACEUTICAL DOSAGE FORM

Y. Anil Kumar*, Y. Sirisha, A. L. Harini and A. Prathyusha

Department of Pharmacology, Hindu College of Pharmacy, Amaravathi road, Department of Pharmaceutical Analysis, Sims College of Pharmacy, Guntur-522 002, A. P, India.

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***Corresponding Author**
Y. Anil Kumar
Department of Pharmacology, Hindu College of Pharmacy, Amaravathi road, Department of Pharmaceutical Analysis, Sims College of Pharmacy, Guntur-522 002, A. P, India.

ABSTRACT

A simple, accurate and precise Stability indicating RP-HPLC method for the estimation of in pure and pharmaceutical dosage form has been reported. Quantitative estimation of Gemcitabine and Clarithromycin was done by using WATERS HPLC 2965 SYSTEM with Auto Injector and PDA Detector on a Chromosil C_{18} Column (150mm x 4.6mm) 5µg.). A 10µL syringe was used for injecting the samples. Data was analyzed by using Empower 2 software. UV-VIS spectrophotometer shimadzu with special bandwidth of 2mm and 10mm and matched quartz was used for measuring absorbance for Gemcitabine and Clarithromycin solutions. The mobile phase consists of a water: phosphate Buffer: Methanol (35:65v/v) And at a flow rate of 1 milliliter/minute. Gemcitabine and Clarithromycin were eluted at approximately 7 minutes. The wavelength was found to be 254nm. A linear response was observed in the concentration ranges of 20-60µg/ml with a regression coefficient of 0.999. Forced degradation studies were performed on pure sample of Gemcitabine and Clarithromycin using acid (0.1 Normal (N) hydrochloric acid), base (0.1 N sodium hydroxide), peroxide (30% H_2O_2) and thermal (105°C) conditions. The developed method was validated with respect to specificity, precision (% RSD about 0.4%), linearity (linearity of range about 20-60 µg/mL), robustness, LOD and LOQ values were found to be 0.001µg/ml, 0.005µg/ml and 0.004µg/ml 0.015µg/ml respectively.

KEYWORDS: High performance liquid chromatography, Gemcitabine and Clarithromycin.

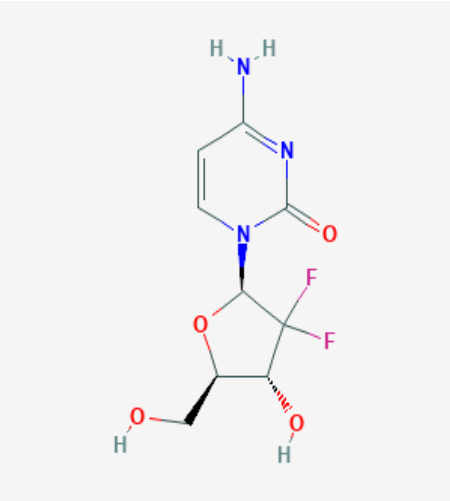
INTRODUCTION

Gemcitabine[1]

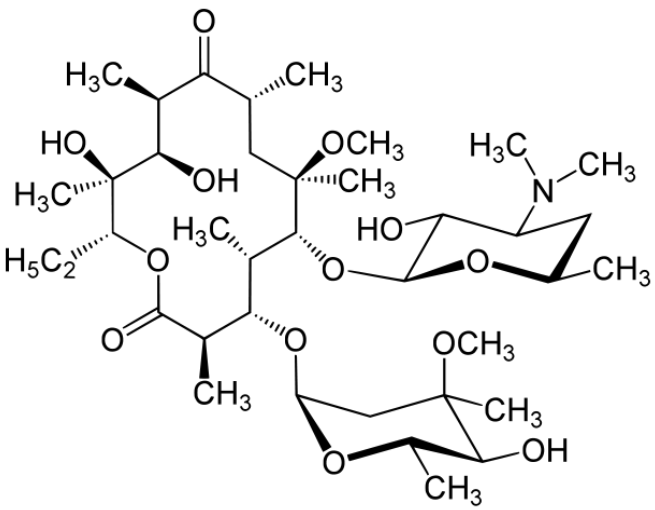
Gemcitabine, sold under the brand name **Gemzar**, among others, is a chemotherapy medication used to treat a number of types of cancer. These cancers include breast cancer, ovarian cancer, non-small cell lung cancer, pancreatic cancer, and bladder cancer. It is given by slow injection into a vein. Common side effects include bone marrow suppression, liver and kidney problems, nausea, fever, rash, shortness of breath, and hair loss. Use during pregnancy will likely result in harm to the baby. Gemcitabine is in the nucleoside analog family of medication. It works by blocking the creation of new DNA, which results in cell death.

Clarithromycin[2]

Clarithromycin, sold under the brand name **Biaxin** .among others, is an antibiotic used to treat various bacterial infections. This includes strep throat, pneumonia, skin infections, H. pylori infection, and Lyme disease, among others.[1] Clarithromycin can be taken by mouth as a pill or liquid. .Common side effects include nausea, vomiting, headaches, and diarrhea. Severe allergic reactions are rare. Liver problems have been reported. It may cause harm if taken during pregnancy. It is in the macrolide class and works by decreasing protein production of some bacteria.



Gemcitabine



Clarithromycin

MATERIALS AND METHODS

Gemcitabine and Clarithromycin Gemcitabine and Clarithromycin tablets, HPLC grade water, acetonitrile, phosphate buffer, ammonium acetate buffer, glacial acetic acid, methanol, potassium dihydrogen phosphate buffer, tetra hydro furan, tri ethyl amine, ortho-phosphoric acid, 2N Hcl,2N NaoH,20% H_2O_2 etc.

INSTRUMENTATION[3-6]

Quantitative estimation of was done by using WATERS HPLC 2965 SYSTEM with Auto Injector and PDA Detector on a BDS C18 column (250 mm x 4.6 mm, 5µ). A 10µL syringe was used for injecting the samples. Data was analyzed by using Empower 2 software. UV-VIS spectrophotometer shimadzu with special bandwidth of 2mm and 10mm and matched quartz was used for measuring absorbance for Gemcitabine and Clarithromycin solutions. Degassing of the mobile phase was done by using a shimadzu ultrasonic bath sonicator. A Shimadzu balance was used for weighing the materials.

CHROMATOGRAPHIC CONDITIONS[7]

Chromatographic separation achieved using an analytical column; Chromosil C_{18} Column (150mm x 4.6mm) 5µg. Mobile phase was consisted of water: Acetonitrile P^H 2.5 (30:70 v/v). The elution was achieved isocratically at a flow rate of 1 mL/min with injection volume of 10 µL. Column temperature was maintained at 30°C and chromatograph was recorded at wavelength 254nm.

Preparation of Sample

Standard preparation

Weigh accurately 10mg Gemcitabine Working Reference Standard and 15mg of Clarithromycin Working Reference Standard is taken in to 100ml volumetric flask and then it was dissolved and diluted to volume with mobile phase up to the mark. After that 50ml of the above solution was taken into 100ml standard flask and made up with mobile phase. Further pipette 0.5ml of the above stock solution in to a 10ml volumetric flask and dilute up to the mark with diluent.

Sample preparation

Amount of 694.2mg of the tablet powder was taken in to 100ml standard flask. A volume of 70ml of mobile phase was added and sonicate for 30min. Then the solution was cooled and diluted to volume with mobile phase and filtered through 0.45µm membrane filter. Further

pipette 0.25ml of Gemcitabine and Clarithromycin of the above stock solution in to a 10ml volumetric flask and dilute up to the mark with diluent.

RESULTS AND DISCUSSION

Method development and validation

Some important parameters like pH of the mobile phase, concentration of the acid or buffer solution, etc., were tested for a good chromatographic separation. Trials showed that mobile phase with reverse phase C_{18} column gives symmetric and sharp peaks. After the optimization of chromatographic conditions, estimation is carried out by the developed RP-HPLC method. Standard solution of drug was injected separately and chromatogram of Gemcitabine and Clarithromycin recorded in Fig.1 now the sample solution was injected separately and chromatogram was recorded until the reproducibility of the peak areas were satisfactory.

Validation[8]

HPLC method was validated according to the International Conference on Harmonization Guidelines (ICH Q2B, validation of analytical procedures, methodology). The method was validated for parameters such as system suitability, linearity, precision, accuracy, and robustness.

Linearity

Linear range of Gemcitabine and Clarithromycin was found to be 20-60µg/ml. And regression co-efficient was 0.999.

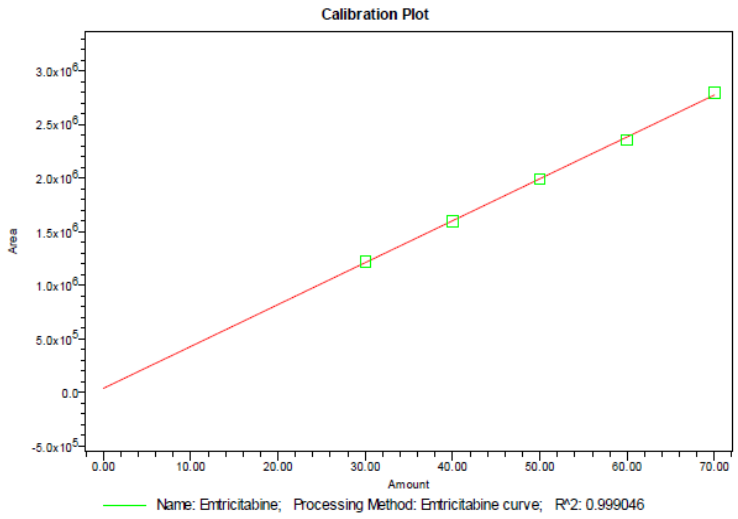


Fig 1: Showing calibration graph for Gemcitabine.

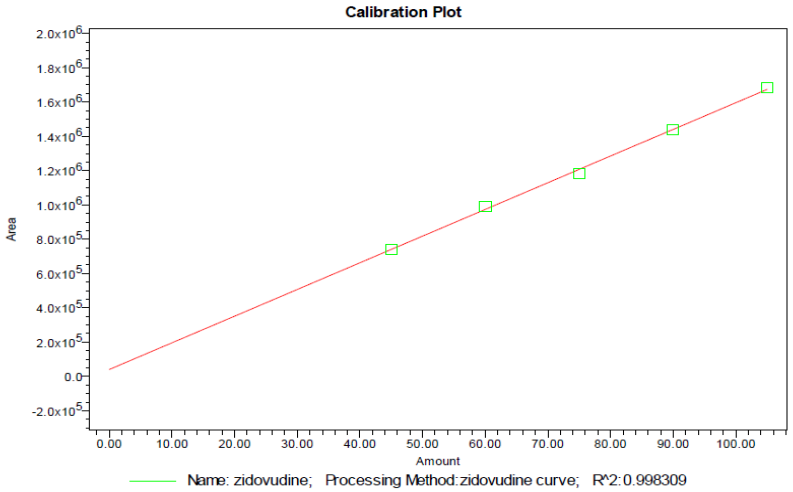


Fig 2: Showing calibration graph for Clarithromycin.

Tab. 1: Linearity of Gemcitabine and Clarithromycin.

Sample ID	Gemcitabine		Clarithromycin	
	Concentration (mcg/ml)	Area	Concentration (mcg/ml)	Area
20% of operating concentration	20	1224140	10	740046
40% of operating concentration	30	1595681	15	990204
60% of operating concentration	**40***	**1992966**	**20***	**1183023**
80% of operating concentration	50	2356546	25	1439886
100% of operating concentration	60	2797214	30	1682302
Correlation Coefficient			0.999	

Accuracy

The percentage recovery of Gemcitabine was found to be 101.39%, 100.01% and100.39% & percentage recovery of Clarithromycin was found to be 101.91%, 99. 66% and 102.09% for accuracy 50%, 100% and 150% samples respectively. The %RSD was found to be less than 2. in **Tab.2.**

Tab 2: Accuracy for Gemcitabine.

Recovery level	Accuracy of Gemcitabine					Average % Recovery
	Amount taken (mcg/ml)	Area	Average area	Amount recovered (mcg/ml)	Percentage Recovery	
50%	5.05	1011326	1017498.5	101.3927	101.3927	100.599%
	5.05	1015029				
	5.05	1026141				
100%	10	1986534	1987384.8	100.0106	100.0106	
	10	1987425				
	10	1988195				
150%	15	2989367	2992493.4	100.3936	100.3936	
	15	2991556				
	15	2996557				

Tab 3: Accuracy for Clarithromycin.

Recovery level	Accuracy of Clarithromycin					Average % Recovery
	Amount taken (mcg/ml)	Area	Average area	Amount recovered (mcg/ml)	% Recovery	
50%	8.1	646754	648293.3	101.91	101.91	101.22%
	8.1	648998				
	8.1	649128				
100%	15	1172743	1174011.1	99.66	99.66	
	15	1174031				
	15	1175259				
150%	23.3	1866742	1868236.3	102.09	102.09	
	23.3	1867956				
	23.3	1870011				

Precision

The % Relative standard deviations of Gemcitabine and Clarithromycin were found to be 0.5 and 0.2 respectively. Hence the %RSD values indicate a good degree of precision within the specified range.

Tab 4: Method precision Gemcitabine,

S.No	Injection	Peak Name	R_t	Area	Height
1	Injection-1	Gemcitabine	2.586	2010800	346322
2	Injection-2	Gemcitabine	2.588	2002956	340800
3	Injection-3	Gemcitabine	2.590	2012800	346911
4	Injection-4	Gemcitabine	2.590	2005243	344089
5	Injection-5	Gemcitabine	2.591	2011092	345720
Average				2008578.1	
Standard Deviation				4237	
%RSD				0.2	

Clarithromycin

Tab 5: Method precision Clarithromycin.

S.No	Injection	Peak Name	R_t	Area	Height
1	Injection-1	Clarithromycin	3.713	1184689	162348
2	Injection-2	Clarithromycin	3.714	1188199	163120
3	Injection-3	Clarithromycin	3.734	1195842	163500
4	Injection-4	Clarithromycin	3.737	1184210	160362
5	Injection-5	Clarithromycin	3.741	1198327	162484
Average				1190253.2	
Standard Deviation				6483.1	
%RSD				0.5	

Limit of Detection

The LOD for this method was found to be 0.001µg/ml for Gemcitabine and 0.005µg/ml for Clarithromycin.

Limit of Quantification

The LOQ for this method was found to be 0.004µg/ml a Gemcitabine nd 0.015µg/ml for Clarithromycin.

Robustness: The robustness was tested by changing the, mobile phase composition and P^H. The %RSD Values by changing the above parameters were found to be within the acceptance criteria i.e. less than 2.

Tab 6: Flow rate results for Gemcitabine.

S.No	Flow Rate (ml/min)	System Suitability Results	
		USP Plate Count	USP Tailing
1	0.8	5752	1.4
2	1.0	5026.5	1.3
3	1.2	4476	

Tab7: Flow rate results for Clarithromycin.

S.No	Flow Rate (ml/min)	System Suitability Results	
		USP Plate Count	USP Tailing
1	0.8	7187	1.2
2	1.0	6381.5	1.2
3	1.2	6471	5.0

Specificity

Specificity is the ability of the analytical method to measure the analyte free from interference due to other components. Specificity was determined by comparing test results

obtained from analyses of sample solution containing ingredients with that of test results those obtained from standard drug. Chromatograms for standard & samples were recorded and they represent no interference.

System Suitability

All the system suitability parameters such as % RSD, Tailing factor, Theoretical plate count are within the acceptance criteria.

CONCLUSION

The RP-LC method developed for the analysis of Gemcitabine and Clarithromycin in their pharmaceutical preparations is simple, precise, and accurate. The method is useful for routine analysis due to short run time.

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