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A Validated RP-HPLC method for Simultaneous Estimation of Gemcitabine and Clarithromycin in Bulk and Pharmaceutical Dosage form

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A B S T R A C T

A reverse phase high performance liquid chromatographic method was developed for the determination of Gemcitabine and Clarithromycin in bulk and pharmaceutical dosage form. The separation was carried out on a [Column: Agilent C18 (4.6 x 250mm, 5µ m, Make: Waters)] using a mobile phase mixture of Methanol, Sodium acetate buffer in a isocratic elution at a flow rate of1ml/min. The detection was made at 274 nm. The retention time of Gemcitabine and Clarithromycin was found to be 2.369 and 5.992 min respectively, Calibration curve was linear over the concentration range of 10µg-50µg and 120µg-300µg of Gemcitabine and Clarithromycin. The propose method was validated as per the ICH guidelines. The method was accurate, precise, specific and rapid found to be suitable for t he quantitative estimation of related substances in drug an d pharmaceutical dosage form.

Keywords: Gemcitabine and Clarithromycin, HPL C, Validation studies

A R T I C L E I N F O

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1. Introduction

Gemcitabine and Clarithromycin is an Anti-Bacterial Agents, Protein Synthesis Inhibitors, Macrolides. Gemcitabine inhibits thymidylate synthetase, leading to inhibition of DNA synthesis and cell death. Gemcitabine is a prodrug so activity occurs as a result of intracellular conversion to two active metabolites, gemcitabine

diphosphate and gemcitabine triphosphate by deoxycitidine kinase. Gemcitabine diphosphate also inhibits ribonucleotide reductase, the enzyme responsible for catalyzing synthesis of deoxynucleoside triphosphates required for DNA synthesis. Finally, Gemcitabine triphosphate(difluorodeoxycytidine triphosphate) competes with endogenous deoxynucleoside triphosphates for incorporation into DNA. Clarithromycin is first metabolized to 14-OH clarithromycin, which is active and works synergistically with its parent compound. Like other macrolides, it then penetrates bacteria cell wall and reversibly binds to domain V of the 23S ribosomal RNA of the 50S subunit of the bacterial ribosome, blocking translocation of aminoacyl transfer-RNA and polypeptide synthesis. Clarithromycin also inhibits the hepatic microsomal CYP3A4 is enzyme and P-glycoprotein, an energy-dependent drug efflux pump.

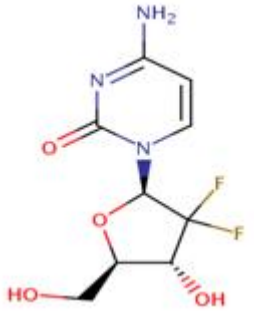


Figure 1: Gemcitabine

Figure 2: Clarithromycin

2. Materials and Methods

Preparation of mobile phase:

Take 24 gm of Sodium acetate into 1000ml volumetric flask dissolved in HPLC grated water and adjust Ph up to 3 with ortho phosphoric acid. From the above prepared buffer take 300 ml (30%) and 700ml Methanol (70%) HPLC were mixed and degassed in ultrasonic water bath for 5 minutes and was filtered through 0.45 µ filter under vacuum filtration.

Preparation of standard solution (Clarithromycin and Gemcitabine):

10 mg of Gemcitabine and 10mg of Clarithromycin were accurately weighed and transferred into a 10 ml clean dry volumetric flask, about 7 ml of diluent was added and sonicated to dissolve it completely. The volume was made up to the mark with the same solvent to give the concentration of 1000 µg/ml. (Stock solution) .Further 0.3 ml and 1.8 ml was pipette out from the above stock solutions into a 10ml volumetric flask and diluted up to the mark with diluent to give the concentration of 30 µg/ml and 180 µg/ml respectively.

Method development & Optimization:

Using Mobile phase consisting of different buffers and methanol at different concentrations and different mobile phase's pH values are attempted .The peak was observed that the shape and retention time of voriconazole was found to be broad compared to the mixed phosphate buffer and acetonitrile composition of mobile phase. After selecting the best conditions based on peak performance , mixed Methanol and Sodium acetate buffer in the ratio 70:30 and HPLC using column is Agilent C18 (150*4.6 *5µ) , the run times of the proposed method was 10 mins with isocratic solution. Column temperature is 25°C, flow rate is 1ml/min, PDA Detector is mainly used this purpose, after inject the standard solution volume was found to be 10mL.

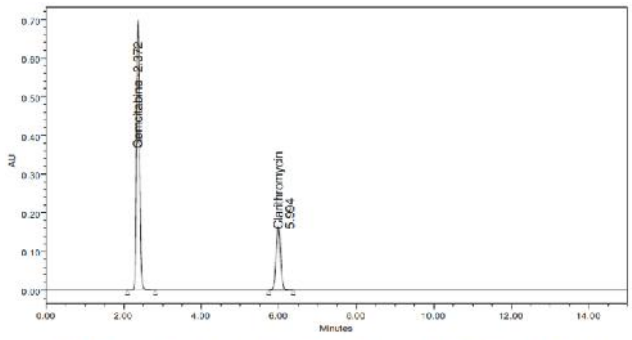
Method validation:

Gemcitabine and Clarithromycin standards taken to the10 mg was accurately weighed and transferred into a 25ml of volumetric flask containing HPLC grade Methanol s diluents. It was sonicated, dissolves completely and made volume up to the mark with the same solvent. The method was validated in accordance with ICH guidelines. The parameters assessed were precision, accuracy, linearity, specificity, robustness.

3. Results and Discussion

A new method was established for simultaneous estimation of Gemcitabine and Clarithromycin by RP-HPLC method. The chromatographic conditions were successfully developed for the separation of Gemcitabine and Clarithromycin by using Agilent C18 5µm (4.6*250mm) column, flow rate was 1ml/min, mobile phase ratio was Methanol: sodium acetate buffer (70:30%v/v), detection wave length was 274nm. The instrument used was WATERS HPLC Auto Sampler, Separation module 2695, PDA Detector 996, Empower-software version-2.The retention times were found to be 2.369 mins and 5.992 mins. The % purity of Gemcitabine and Clarithromycin was found to be 99.82% and 98.89% respectively. The system suitability parameters for Gemcitabine and Clarithromycin such as theoretical plates and tailing factor were found to be 1.2, 3857 and 1.1, 13186 the resolution was found to be 20.25.The analytical method was validated according to ICH guidelines (ICH, Q2 (R1)). The linearity study for Gemcitabine and Clarithromycin was found in concentration range of 10µg-50µg and 120-300µg and correlation coefficient (r2) was found to be 0.999 and 0.999, % mean recovery was found to be 99.68% and 99.89%, %RSD for repeatability was 0.2 and 0.1, % RSD for intermediate precision was 0.3 and 0.1 respectively. The precision study was precise, robust, and repeatable. LOD value was 3.01 and 3.08, and LOQ value was 9.79 and 10.3 respectively. Hence the suggested RP-HPLC

method can be used for routine analysis of Gemcitabine and Clarithromycin in API and Pharmaceutical dosage form.



	Peak Name	RT	Area	Height	% Area	USP Resolution	USP Tailing	USP Plate Count
1	Gemcitabine	2.372	3982538	691563	75.436		1.23	3857.03
2	Clarithromycin	5.994	1299568	165058	24.564	20.28	1.12	13128.40

Figure 3

Method Validation:

Precision:

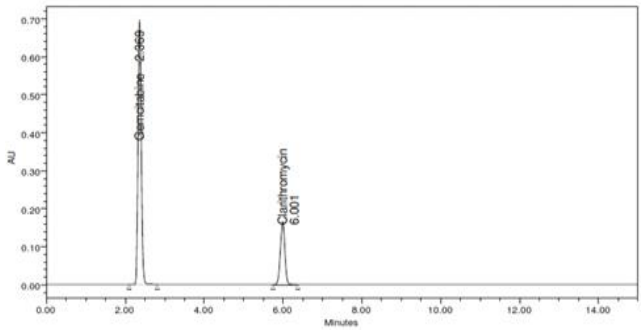


Figure 4

Table 1: Sample Chromatogram values for Repeatability

S.NO	Peak name	RT	Area
1	Gemcitabine	2.369	3982589
2	Gemcitabine	2.368	3975231
3	Gemcitabine	2.370	3991258
4	Gemcitabine	2.367	3962014
5	Gemcitabine	2.371	3982587
6	Gemcitabine	2.372	3971047
Mean			3977454
Std.dev			9377.733
%RSD			0.235772

Table 2

	Peak name	RT	Area
1	Clarithromycin	6.001	1295120
2	Clarithromycin	5.998	1296587
3	Clarithromycin	5.997	1297215
4	Clarithromycin	5.998	1298473
5	Clarithromycin	5.996	1299357
6	Clarithromycin	5.998	1298697
Mean			1297575
Std.dev			1436.058
%RSD			0.110672

Acceptance Criteria: The % RSD for the area and Rt of five standard injections results should not be morethan 2%.

Accuracy: The spiked level was found to be at 50,100,150 and the % recovery was found to be 99.68, 99.89 % respectively.

Acceptance Criteria: The % Recovery for each level should be between 98.0 to 102.0%.

Linearity

Table 3: Linearity results for Gemcitabine

S.No	Linearity Level	Concentration	Area
1	I	10 ppm	939041
2	II	20 ppm	2047315
3	III	30 ppm	2903443
4	IV	40 ppm	3963308
5	V	50 ppm	5086560
Correlation Coefficient			0.999

Table 4: Linearity results for Clarithromycin

S.No	Linearity Level	Concentration	Area
1	I	10 ppm	305153
2	II	20 ppm	664510
3	III	30 ppm	948707
4	IV	40 ppm	1288825
5	V	50 ppm	1656474
Correlation Coefficient			0.999

Acceptance Criteria:

Correlation coefficient should be not less than 0.999

Plotting of calibration graphs:

The resultant areas of linearity peaks are plotted against Concentration

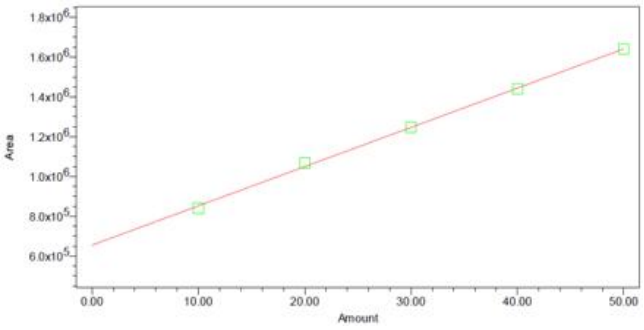


Figure 5: Calibration curve of Gemcitabine

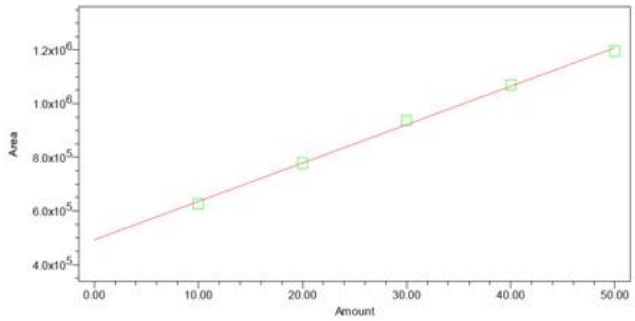
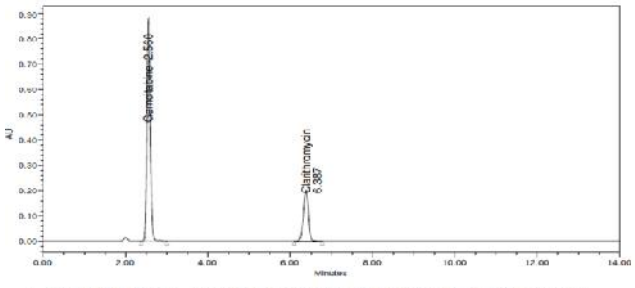


Figure 6: Calibration curve of Clarithromycin

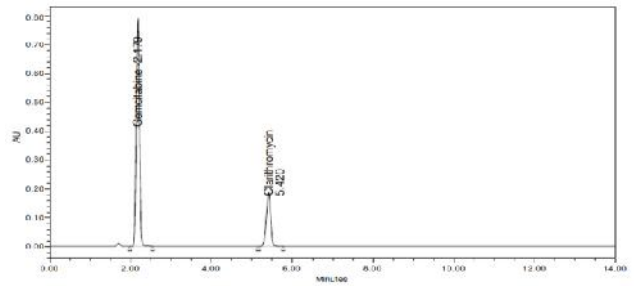
Table 5: Calibration parameters for Gemcitabine and Clarithromycin

Parameter	Results for Gemcitabine	Results for Clarithromycin
Slope	19618	1511
Intercept	65398	48120
Correlation co-efficient	0 .999	0.999

Robustness:

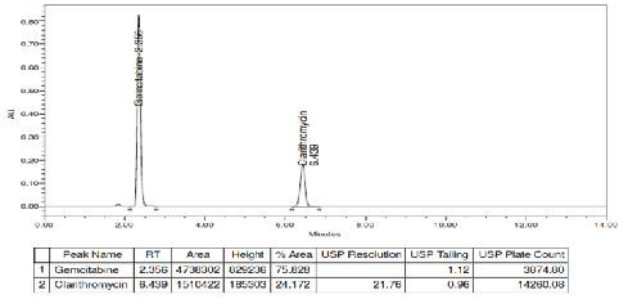


	Peak Name	RT	Area	Height	% Area	USP Resolution	USP Tailing	USP Plate Count
1	Gemcitabine	2.560	5195425	807629	75.646		1.11	4402.81
2	Clarithromycin	6.387	1689446	200805	24.354	20.06	0.93	13416.90

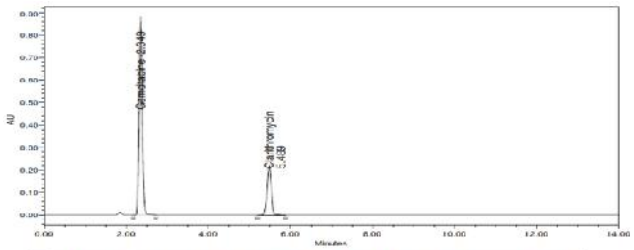


	Peak Name	RT	Area	Height	% Area	USP Resolution	USP Tailing	USP Plate Count
1	Gemcitabine	2.179	4347307	787810	75.572		1.09	3548.56
2	Clarithromycin	5.420	1405286	187898	24.428	18.58	0.95	11867.41

Figure 7: Chromatograms for Robustness Flow rate



	Peak Name	RT	Area	Height	% Area	USP Resolution	USP Tailing	USP Plate Count
1	Gemcitabine	2.356	4738302	629236	75.828		1.12	3874.80
2	Clarithromycin	6.439	1510422	185903	24.172	21.76	0.96	14260.08



	Peak Name	RT	Area	Height	% Area	USP Resolution	USP Tailing	USP Plate Count
1	Gemcitabine	2.349	4869445	873441	75.457		1.10	4010.14
2	Clarithromycin	6.489	1585865	218875	24.543	18.90	0.94	13128.95

Figure 8: Chromatogram for Robustness organic composition

4. Conclusion

The method was found to be precise, accurate and linear over the linear concentration range. The method developed is unique in determining the impurities even at low levels than that of specifications. The analytical method validation of Gemcitabine and Clarithromycin in tablet dosage form by RP-HPLC was found to be satisfactory and could be used for the routine pharmaceutical analysis Method was validated as per ICH guidelines like system suitability, accuracy, precision, linearity, specificity, robustness and solution stability. Therefore, this HPLC method can be used as a routine analysis of these drugs in pharmaceutical formulations.

Table 6: Details of Instrument

S.No	Instrument	Model No.	Software	Manufacturer's name
1	HPLC Alliance	Waters 2695	Empower	Waters
2	PDA Detector	Waters 996	UV Win 5	Lab India
3	UV double beam	UV 3000	-	Satorius
4	spectrophotometer	BSA224SCW	-	Lab India

Table 7: Accuracy results of Gemcitabine

% Concentration (at specification Level)	Area	Amount added (m)	Amount found (m)	% Recovey	Mean Recovery
50%	1251446	5	4.9	96.8%	99.89%
100%	23652005	10	9.98	99.9%	
150%	2339845	15	15.0	990.0%	

Acceptance Criteria: The % Recovery for each level should be between 98.0 to 102.0%.

Table 8: Accuracy results of Clarithromycin

% Concentration (at specification Level)	Area	Amount added (m)	Amount found (m)	% Recovey	Mean Recovery
50%	325148	5	5.0	95.3%	98.69%
100%	2453370	10	9.96	99.6%	
150%	1655857	15	14.9	99.3%	

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