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"VALIDATED RP-HPLC METHOD FOR THE SIMULTANEOUS ESTIMATION OF GEMCITABINE AND CLARITHROMYCIN IN ITS BULK AND DOSAGE FORM"

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ABSTRACT
A rapid and precise reverse phase HPLC method has been developed for the validation of Clarithromycin and Gemcitabine, in its un mixed form as well as in solid dosage form. Chromatography was carried out on a Phenomenex Luna C18 (4.6×150mm, 5µ) column using a mixture of Acetonitrile: Triethylamine Buffer pH 3.8 (75:25v/v) as the mobile phase at a flow rate of 0.9ml/min, the detection was carried out at 210nm. The retention time of the Clarithromycin and Gemcitabine was 1.933, 3.396 ±0.02min respectively. The method produce linear responses in the concentration range of 5-25mg/ml of Clarithromycin and 10-50mg/ml of Gemcitabine. The method precision for the determination of assay was below 2.0%RSD. The method is useful in the quality control of bulk and pharmaceutical formulations.

KEYWORDS: Clarithromycin, Gemcitabine, RP-HPLC, Validation.

INTRODUCTION
Analytical chemistry[1] involves the application of a range of techniques and methodologies to obtain and assess qualitative, quantitative and structural information on the nature of matter. Clarithromycin[2-7], chemically (3*R*, 4*S*, 5*S*, 6*R*, 7*R*, 9*R*, 11*S*, 12*R*, 13*S*, 14*S*)-6-{[(2*S*, 3*R*, 4*S*, 6*R*)-4-(dimethylamino)-3-hydroxy-6-methyloxan-2-yl]oxy}-14-ethyl-12,13-dihydroxy-4 {[(2*R*, 4*S*, 5*S*, 6*S*)-5-hydroxy-4-methoxy-4, 6-dimethyloxan-2-yl]oxy}-7-methoxy-3,5,7,9,11,13-hexamethyl-1-oxacyclotetradecane-2,10-dione, is a Anti-Bacterial Agents, predominantly metabolized by CYP3A4 resulting in numerous drug interactions. Clarithromycin is first used to 14-OH clarithromycin, which is dynamic and works synergistically with its parent compound. at that point infiltrates microscopic organisms cell divider and reversibly ties to area V of the 23S ribosomal RNA of the 50S subunit of the bacterial ribosome, blocking translocation of aminoacyl move RNA and polypeptide bond.

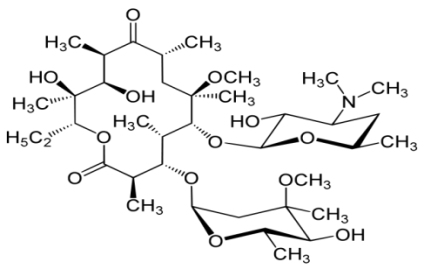

Fig. 1: structure of Clarithromycin.

Gemcitabine[8-9], chemically 4-amino-1-(2-deoxy-2, 2-difluoro-β-D- erythro pentofuranosyl) pyrimidin-2(1H)-one, is an Antiviral Agent hinders thymidylate synthetase, prompting restraint of DNA amalgamation and cell passing. Gemcitabine is a prodrug so movement happens because of intracellular transformation to two dynamic metabolites, gemcitabine diphosphate and gemcitabine triphosphate by deoxycitidine kinase. Gemcitabine diphosphate additionally represses ribonucleotide reductase, the catalyst in charge of catalyzing union of deoxynucleoside triphosphates required for DNA blend. At long last, Gemcitabine triphosphate (diflurorodeoxycytidine triphosphate) rivals endogenous deoxynucleoside triphosphates for joining into DNA.

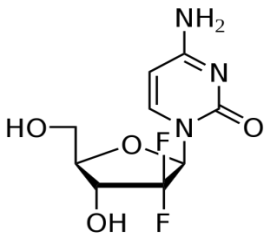

Fig. 2: Structure of Gemcitabine.

Review Structure of Gemcitabine Review of writing for Clarithromycin and Gemcitabine gave data in regards to its physical and substance properties, different expository strategies that were directed alone and in blend with

other Clarithromycin and Gemcitabine. Writing review[10-19] uncovers that specific chromatographic techniques were accounted for synchronous estimation of Clarithromycin and Gemcitabine and single strategy is accessible for such estimation by RP-HPLC. In perspective on the requirement for an appropriate RP-HPLC technique for routine examination of Clarithromycin and Gemcitabine in plans, endeavors were made to create basic, exact and precise investigative strategy for synchronous estimation of Clarithromycin and Gemcitabine and expand it for their assurance in detailing. The utility of the created strategy to decide the substance of Clarithromycin and Gemcitabine in business detailing was likewise illustrated. Approval of the strategy was done as per USP and ICH rule[20] for the measure of dynamic fixing. The technique was approved for parameters like framework reasonableness, linearity, exactness, precision, particularity, roughness, strength, farthest point of recognition and cutoff of measurement. This strategy gives intends to evaluate the segment. This proposed technique was appropriate for the examination of Pharmaceutical dosage forms.

MATERIALS AND METHODS

Materials: All the reagents used in the experiment were HPLC grade solvents, Clarithromycin, Gemcitabine, milli-Q water, acetonitrile, Methanol etc. HPLC instrument was used of WATERS HPLC 2965 SYSTEM with Auto Injector and PDA Detector. Software used is Empower 2.Digital pH meter and Digital ultra sonicator.

METHOD DEVELOPMENT[21-25]

Preparation of standard solution: Accurately weigh and transfer 10 mg of Gemcitabine and Clarithromycin working standard into a 10ml of clean dry volumetric jars include about 7ml of Methanol and sonicate to break up and expulsion of air totally and make volume sufficient with a similar Methanol. Further pipette 0.15ml of Clarithromycin and 0.3ml the above Gemcitabine stock arrangements into a 10ml volumetric carafe and weaken sufficient with Methanol.

Mobile Phase Optimization: Initially the mobile phase tried was Methanol: Water, Acetonitrile: water and Phosphate buffer pH 4.0: Methanol with varying proportions. Finally, the mobile phase was optimized to Acetonitrile: Triethylamine pH 3.8 in proportion 75:25v/v respectively.

Optimization of Column: The method was performed with various columns like C18 and C8 columns, Symmetry and Xterra column. Phenomenex Luna C18 (4.6×150mm, 5µ) was found to be ideal as it gave good peak shape and resolution at 0.9ml/min flow.

Optimized chromatographic conditions:

Instrument used	:	Waters Alliance 2695 HPLC with PDA Detector 996 model.
Temperature	:	35ºc
Column	:	Phenomenex Luna C18 (4.6×150mm, 5µ)
Mobile phase	:	Acetonitrile: Triethylamine pH 3.8 (75:25v/v)
Flow rate	:	0.9ml/min
Wavelength	:	210 nm
Injection volume	:	10 µl
Run time	:	6min

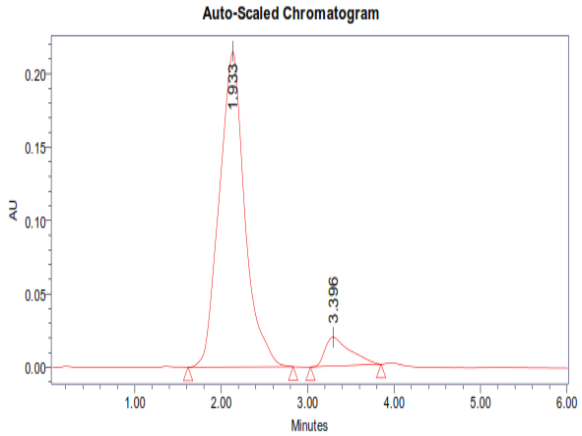

Fig. 3: Optimized Chromatogram (Standard).

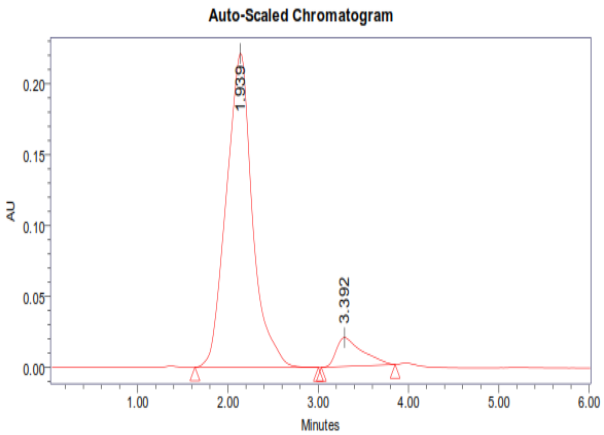

Fig. 4: Optimized Chromatogram (Sample).

METHOD VALIDATION[26-31]

Every one of the arrangements were set up as indicated by the systems given under planning of standard and test arrangements. The created strategy was approved according to ICH rules.

Preparation of Triethylamine buffer (pH-3.8): Take 6.0ml of Triethylamine in to 750ml of HPLC water in a 1000ml volumetric flask and mix well. Make up the volume up to mark with water and adjust the pH 3.8 by using Orthophosphoric acid, filter and sonicate.

Preparation of mobile phase: Accurately measured 750 ml (75%) of Acetonitrile and 250 ml of Triethylamine Buffer pH3.8 (25%) were blended and degassed in advanced ultra sonicater for 10 minutes and afterward sifted through 0.45 µ channel under vacuum filtration. The Mobile phase was used as the Diluent.

Preparation of Standard Solution: Accurately weigh and transfer 10 mg of Gemcitabine and 10mg of Clarithromycin working standard into a 10ml of clean dry volumetric carafes include about 7mL of Diluents and sonicate to disintegrate it totally and make volume sufficient with a similar dissolvable (Stock arrangement). Further pipette 0.15ml of Clarithromycin and 0.3ml the above Gemcitabine stock arrangements into a 10ml volumetric carafe and weaken sufficient with Diluent.

Preparation of Sample Solution: Take total weight of the powder and weight 10 mg equivalent weight of Gemcitabine and Clarithromycin tests into a 10mL clean dry volumetric cup and include about 7mL of Diluent and sonicate to break down it totally and make volume sufficient with a similar dissolvable. Further pipette 0.15ml of Clarithromycin and 0.3ml the above Gemcitabine stock arrangements into a 10ml volumetric cup and weaken sufficient with Diluent.

RESULTS AND DISCUSSION

Results: Specificity: Analytical method was tested for specificity to measure accurately quantitates Gemcitabine and Clarithromycin in drug product.

Assay of Standard

Table 1: Peak results for assay standard of Gemcitabine.

S.No	RT	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	1.939	407105	219674	5249	1.14
2	1.943	407333	218266	5248	1.14
3	1.949	409824	221080	5254	1.13
4	1.949	403182	221866	5255	1.12
5	1.953	407276	221578	5253	1.13
Mean		406944			
Std. Dev.		2384.036			
% RSD		0.585839			

Table 2: Peak results for assay standard of Clarithromycin.

S.No	RT	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing	Resolution
1	3.390	390942	20057	6569	1.23	4.9
2	3.397	392296	20602	6613	1.29	4.9
3	3.395	398056	21296	6672	1.29	4.9
4	3.391	393286	21242	6619	1.29	4.9
5	3.388	392284	21592	6672	1.22	4.9
Mean		393372.8				
Std. Dev.		2747.438				
% RSD		0.698431				

Assay of Sample

Table 3: Peak results for Assay sample of Gemcitabine.

S.No	Rt	Area	Height	USP Tailing	USP Plate Count	Injection
1	1.955	409895	218842	1.16	5218	1
2	1.956	409411	221359	1.14	5216	2
3	1.956	409066	219684	1.14	5427	3

Table 4: Peak results for Assay sample of Clarithromycin.

S.No	Rt	Area	Height	USP Tailing	USP Plate Count	Resolution
1	3.395	387469	21283	1.20	4612	4.9
2	3.388	387471	22171	1.25	4690	4.9
3	3.392	386604	21731	1.20	4640	4.9

The % purity of Gemcitabine and Clarithromycin in pharmaceutical dosage form was found to be 100.7%.

Linearity: Correlation Coefficient (r) is 0.99 and the intercept is 3593. These values meet the validation criteria.

Table 5: Chromatographic data for linearity study of Gemcitabine.

Concentration Level (%)	Concentration (µg/ml)	Average Peak Area
33	10	154449
66	20	280463
100	30	449653
133	40	590193
166	50	755619

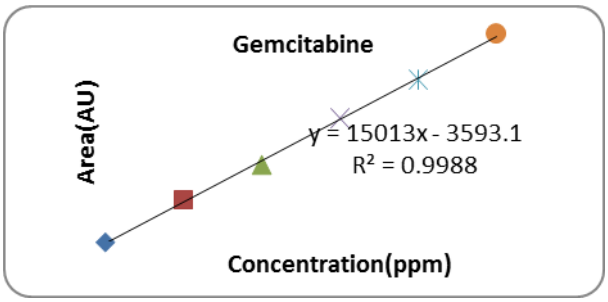

Fig. 5: Linearity Plot of Gemcitabine.

Table 6: Chromatographic data for linearity study of Clarithromycin

Concentration Level (%)	Concentration (µg/ml)	Average Peak Area
33	5	156581
66	10	267461
100	15	394576
133	20	528761
166	25	644180

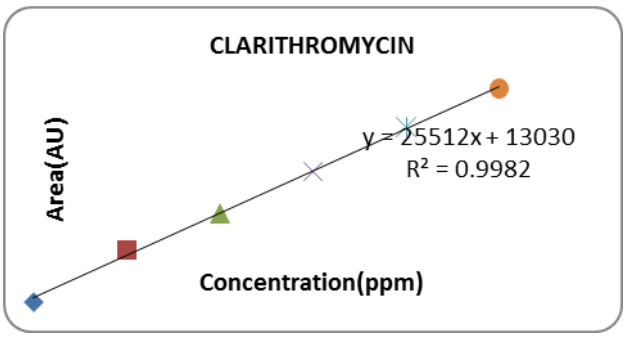

Fig. 6: Linearity Plot of Clarithromycin.

Correlation Coefficient (r) is 0.99, and the intercept is 13030. These values meet the validation criteria.

Precision: The accuracy of a diagnostic methodology communicates the closeness of understanding (level of dissipate) between a progression of estimations got from numerous examining of the equivalent homogeneous example under the endorsed conditions.

Repeatability: Obtained Five (5) imitates of 100% precision arrangement according to test conditions. Recorded the pinnacle territories and determined % RSD.

Table 7: Results of repeatability for Gemcitabine

S. No	Retention time	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	1.961	409349	208879	5200	1.18
2	1.966	409980	214656	5213	1.16
3	1.966	407839	214544	5208	1.17
4	1.968	409731	212354	5202	1.18
5	1.966	408042	218482	5193	1.16

Mean	408988.2			
Std.dev	985.0826			
%RSD	0.240858			

Table 8: Results of repeatability for Clarithromycin:

S. No	Retention time	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	3.389	317876	20821	7639	1.28
2	3.388	320133	21502	6718	1.22
3	3.386	323930	22054	6762	1.21
4	3.387	324517	22022	6748	1.23
5	3.386	323107	21455	6878	1.21
Mean		321912.6			
Std.dev		2816.936			
%RSD		0.875062			

Table 9: Results of Intermediate precision Day-1for Gemcitabine.

S.No	RT	Area (µV*sec)	Height (µV)	USP Plate count	USP Tailing
1	1.968	409600	200415	5192	1.1
2	1.972	409792	204737	5202	1.1
3	1.971	409710	202315	5198	1.1
4	1.978	408131	210538	5213	1.1
5	1.978	409596	208031	5213	1.1
6	1.976	409932	206543	5217	1.1
Mean		409460.2			
Std. Dev.		663.3016			
% RSD		0.161994			

Table 10: Results of Intermediate precision Day-1for Clarithromycin.

S.No	RT	Area (µV*sec)	Height (µV)	USP Plate count	USP Tailing	Resolution
1	3.386	323199	20851	6281	1.2	4.9
2	3.388	324588	21266	6392	1.2	4.9
3	3.386	321726	21070	6293	1.2	4.9
4	3.387	326955	21217	6039	1.2	4.9
5	3.389	323546	21257	6153	1.2	4.9
6	3.385	327755	20978	6293	1.2	4.9
Mean		324628.2				
Std. Dev.		2316.421				
% RSD		0.713561				

Table 11: Results of Intermediate precision Day 2 for Gemcitabine.

S.No	RT	Area (µV*sec)	Height (µV)	USP Plate count	USP Tailing
1	1.980	409042	209754	5237	1.1
2	1.982	409920	210411	5023	1.1
3	1.979	407912	208055	5983	1.1
4	1.979	409213	207720	5294	1.1
5	1.963	406475	206740	5819	1.1
6	1.965	409079	209516	5183	1.1
Mean		408606.8			
Std. Dev.		1227.327			
% RSD		0.300369			

Table 12: Results of Intermediate precision Day 2 for Clarithromycin.

S.No	RT	Area (µV*sec)	Height (µV)	USP Plate count	USP Tailing	Resolution
1	3.379	323744	21401	6173	1.2	4.9
2	3.379	325554	21446	6183	1.2	4.9
3	3.376	323154	21266	6103	1.2	4.9
4	3.377	331213	21312	6482	1.2	4.9
5	3.323	323263	21750	6831	1.2	4.9
6	3.317	328951	21602	6153	1.2	4.9
Mean		325979.8				
Std. Dev.		3369.293				
% RSD		1.033589				

Accuracy: Accuracy at different concentrations (50%, 100%, and 150%) was prepared and the % recovery was calculated.

Table 13: The accuracy results for Gemcitabine.

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	222026.7	15	15.0	100.0	99.8%
100%	443552.3	30	29.3	99.6	
150%	674558	45	45.3	100.3	

Table 14: The accuracy results for Clarithromycin.

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	202430	7.5	7.2	98.6	99.8%
100%	394993.7	15	14.6	99.8	
150%	593559	22.5	22.7	101.1	

Robustness: The strength was performed for the stream rate varieties from 0.9 ml/min to 1.1ml/min and versatile stage proportion variety from increasingly natural stage to less natural stage proportion for Gemcitabine and Clarithromycin. The technique is strong just in less stream condition and the strategy is vigorous even by change in the Mobile stage ±5%. The standard and tests of Gemcitabine and Clarithromycin were infused by changing the states of chromatography. There was no noteworthy change in the parameters like goals, following variable, hilter kilter factor, and plate check.

Table 15: Robustness Results of Gemcitabine.

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 mL/min	409905	1.933	4242	1.1
Less Flow rate of 0.9 mL/min	407262	2.451	5405	1.6
More Flow rate of 1.1 mL/min	409250	1.630	5365	1.5
Less organic phase	407722	2.064	4393	1.6
More Organic phase	406458	1.960	4358	1.5

Table 16: Robustness Results of Clarithromycin.

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 mL/min	392596	3.396	6515	1.7
Less Flow rate of 0.9 mL/min	322247	4.178	4698	1.1
More Flow rate of 1.1 mL/min	321244	2.754	7934	1.7
Less organic phase	317397	3.455	4368	1.4
More Organic phase	318735	3.287	5371	1.3

DISCUSSION

The explanatory technique was created by considering various parameters. As a matter of first importance, greatest absorbance was observed to be at 229nm and the pinnacle immaculateness was incredible. Infusion volume was chosen to be 10µl which gave a decent pinnacle territory. The segment utilized for study was Luna C18 (4.6×150mm, 5µ) in light of the fact that it was giving great pinnacle. 35 ° C temperatures was observed to be appropriate for the idea of medication

arrangement. The stream rate was fixed at 0.9ml/min on account of good pinnacle region and tasteful maintenance time. Portable stage is Acetonitrile: Triethylamine pH 3.8 (75:25v/v) was fixed because of good symmetrical pinnacle. So this versatile stage was utilized for the proposed investigation. Run time was chosen to be 6min in light of the fact that investigate gave top around 1.9, 3.3 and furthermore to decrease the all-out run time. The percent recuperation was observed to be 98.0-102.0 was direct and exact over a similar range. Both framework and strategy exactness was observed to be precise and well inside range. The logical technique was discovered linearity over the range 5-25mg/ml of Clarithromycin and 10-50mg/ml of Gemcitabine of the objective focus. The expository breezed through both strength and roughness tests. On the two cases, relative standard deviation was well acceptable..

CONCLUSION

In the present examination, a basic, delicate, exact and precise RP-HPLC technique was produced for the quantitative estimation of Clarithromycin and Gemcitabine mass medication and pharmaceutical measurement structures. This technique was straightforward, since weakened examples are legitimately utilized with no fundamental substance Derivatization or cleansing advances. Clarithromycin and Gemcitabine was unreservedly dissolvable in ethanol, methanol and sparingly solvent in water. Acetonitrile: Triethylamine Buffer pH 3.8 (75:25v/v) was picked as the portable stage. The dissolvable framework utilized in this technique was practical. The %RSD esteems were inside 2 and the strategy was observed to be precise. The results communicated in Tables for RP-HPLC technique was promising. The RP-HPLC strategy is increasingly delicate, exact and exact contrasted with the Spectrophotometric techniques. This technique can be utilized for the standard assurance of Clarithromycin and Gemcitabine in mass medication and in Pharmaceutical measurements structures.

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