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A NEW RP HPLC METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF GEMCITABINE & CAPECITABINE IN BULK AND PHARMACEUTICAL DOSAGE FORMS

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ABSTRACT
A simple and selective LC method is described for the determination of Gemcitabine and Capecitabine in tablet dosage forms. Chromatographic separation was achieved on a c_{18} column using mobile phase consisting of a mixture of 80 volumes of methanol and 20 volumes of water with detection of 240 nm. Linearity was observed in the range 6-14 µg /ml for Gemcitabine (r^2 =0.998) and 6-14 µg /ml for Capecitabine (r^2 =0.996) for the amount of drugs estimated by the proposed methods was in good agreement with the label claim. The proposed methods were validated. The accuracy of the methods was assessed by recovery studies at three different levels. Recovery experiments indicated the absence of interference from commonly encountered pharmaceutical additives. The method was found to be precise as indicated by the repeatability analysis, showing %RSD less than 2. All statistical data proves validity of the methods and can be used for routine analysis of pharmaceutical dosage form.
Key Words: Gemcitabine, Capecitabine, pharmaceutical dosage form

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INTRODUCTION
Chromatography is a non-destructive procedure for resolving a multi-component mixture of traces, minor or constituents in to individual fractions. It is a method of separating a mixture of components into individual components through porous medium under the influence of solvent. For many years, researchers have looked at “fast LC” as away to speed up analyses. The need for speed, the availability of affordable and easy to use mass spectrometers. Smaller columns and faster flow rates (amongst to their parameters) have been used.

Elevated temperature, having the dual advantages of flowering viscosity, and increasing mass transfer by increasing the diffusivity of the analytes, has also been investigated· However, using conventional particle

Size and pressures, limitations are soon reached and compromises must be made, sacrificing resolution. HPLC technology simply doesn’t have the capability to take full advantages of sub-2µm particles.UPLC can be regarded as new invention for liquid chromatography. UPLC refers to Ultra Performance Liquid Chromatography. UPLC brings dramatic improvements in sensitivity, resolution and speed of analysis can be calculated. It has instrumentation that operates at high pressure than that used in HPLC & in this system uses fine particles (lessthan2.5µm) & mobile phases at high linear velocities decreases the

length of column, reduces solvent consumption & saves time. According to the van Deemter equation, as the particle size decreases to less than 2.5µm, there is a significant gain inefficiency, while the efficiency does not diminish at increased flow rates or linear velocities

Therefore by using smaller particles, speed and peak capacity (number of peaks resolved per unit time ingredient separations) can be extended to new limits, termed Ultra Performance Liquid Chromatography, or UPLC. The technology takes full advantage of chromatographic principles to run separations. Using columns packed with smaller particles (lessthan2.5µm) and / or higher flow rates for increased speed, this gives superior resolution and sensitivity (1-3). Aim is **t**o develop new UPLC method for the simultaneous estimation of Gemcitabine and Capacitabine pharmaceutical dosage form.

MATERIALS AND METHODS

Determination Of Working Wavelength (λmax)

In simultaneous estimation of two drugs isobestic wavelength is used. Isobestic point is the wavelength where the molar absorptivity is the same for two substances that are interconvertible. So this wavelength is used in simultaneous estimation to estimate both drugs accurately.

Preparation of standard stock solution of gemcitabine

10 mg of Gemcitabine was weighed and transferred in to 100ml volumetric flask and dissolved in methanol and then make up to the mark with methanol and prepare 10 µg /ml of solution by diluting 1ml to 10ml with methanol.

Preparation of standard stock solution of capecitabine

10mg of capecitabine was weighed in to 100ml volumetric flask and dissolved in Methanol and then dilute up to the mark with methanol and prepare 10 µg /ml of solution by diluting 1ml to 10ml with methanol.

Preparation of samples for Assay

Preparation of mixed standard solution

weigh accurately 10 mg of gemcitabineand 10 mg of capecitabinein 100 ml of volumetric flask and dissolve in 10ml of mobile phase and make up the volume with mobile phase. From above stock solution 14 µg/ml of gemcitabineand 10 µg/ml of capecitabineis prepared by diluting 2.4ml to 10ml with mobile phase. This solution is used for recording chromatogram (4-6).

Tablet sample

10 tablets (each tablet contains capecitabine- 200mg &gemcitabine- 200mg) were weighed and taken into a mortar and crushed to fine powder and uniformly mixed. Tablet stock solutions of capecitabineand gemcitabinewere prepared by dissolving weight equivalent to 10 mg of capecitabineand 10 mg of gemcitabineand dissolved in sufficient mobile phase. After that filtered the solution using 0.45-micron syringe filter and Sonicated for 5 min and dilute to 50ml with mobile phase. Further dilutions are prepared in 5 replicates of 10ug/ml of capecitabineand 14ug/ml of gemcitabinewas made by adding 2.4 ml of stock solution to 10 ml of mobile phase.The amount of CAPECITABINE and GEMCITABINEpresent in the formulation by using the formula given below,

$$\%\ \text{Assay} = \frac{AT}{AS} \times \frac{WS}{DS} \times \frac{DT}{WT} \times \frac{P}{100} \times \frac{AW}{LC} \times 100$$

RESULTS AND DISCUSSION

The wavelength of maximum absorption (λ_{max}) of the drug, 10 µg/ml solution of the drugs in methanol were scanned using UV-Visible spectrophotometer within the wavelength region of 200–400 nm against methanol as blank.The absorption curve shows characteristic absorption for gemcitabine and capecitabine and 240 nm for the combination.So the both drugs % assay found to be within the limits. The percentage purity of both gemcitabine and capecitabinewere found to be within the limits that is 98-102% (Table-1).

Table-1 Results of assay

Drug	Label claim(mg)	Amount found(mg)	% Assay
Gemcitabine	200	120.1	98.7
Capecitabine	200	115.4	99.3

The % RSD for the retention times and peak area of gemcitabine and capecitabine were found to be less than 2%. The plate count and tailing factor results were found to be satisfactory and are found to be within the limit (Table-2 and 3).

Table-2 Results for system suitability of gemcitabine

Injection	Retention time (min)	Peak area	Theoretical plates (TP)	Tailing factor (TF)
1	1.763	30310742	4017	1.39
2	1.765	30310854	4022	1.31
3	1.767	30311067	4120	1.38
4	1.761	30311084	4157	1.36
5	1.767	30311145	4130	1.37
Mean	1.7646	30310978.4	-	-
SD	0.002608	17.840915	-	-
%RSD	0.147777	0.056693	-	-

Table-3 Results for system suitability of capecitabine

Injection	Retention time (min)	Peak area	Theoretical plates	Tailing factor
1	3.340	48846135	7488	1.39
2	3.345	48845137	7480	1.40
3	3.342	48846347	7487	1.34
4	3.341	48846541	7481	1.31
5	3.343	48846842	7470	1.30
Mean	3.3422	48846200.4	-	-
SD	0.001924	48.892903	-	-
%RSD	0.057553	0.0132844	-	-

The correlation coefficient for linear curve obtained between concentration vs. Area for standard preparations of gemcitabine and capecitabineis 0.998 and 0.996. The relationship between the concentration of gemcitabine and capecitabine and area of gemcitabine and CAPECITABINE is linear in the range examined since all points lie in a straight line and the correlation coefficient is well within limits (Fig-1 and 2).

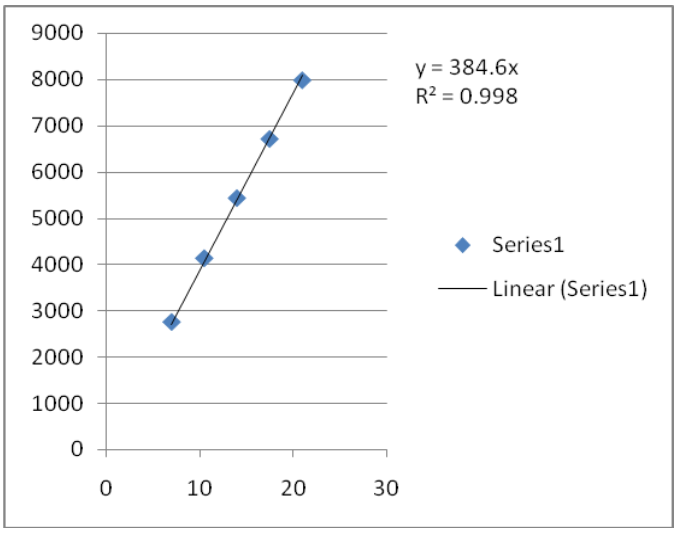

Fig-1 Linearity graph of gemcitabine

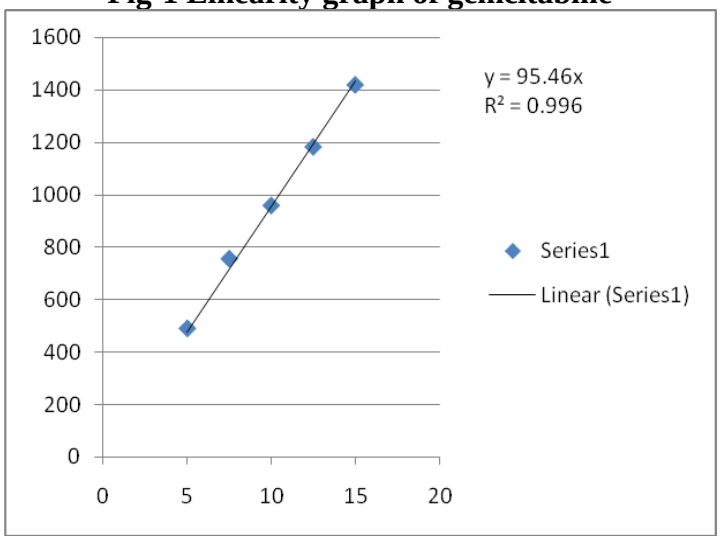

Fig-2 Linearity graph of capecitabine

The % recovery of GEMCITABINE and CAPECITABINE should lie between 98% and 102% (fig-3).

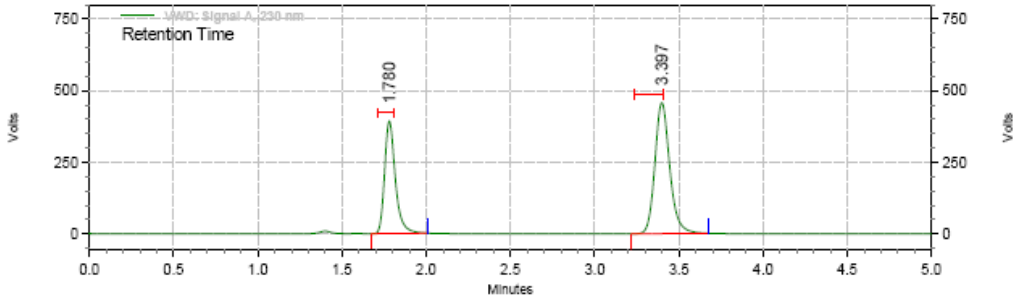

Fig-3 Chromatogram of 100% recovery

From the observation the %RSD between two analysts Assay values not greater than 2.0%, hence the method was rugged (Table-4).

Table-4 Results for Ruggedness

GEMCITABINE	%Assay	CAPECITABINE	%Assay
Analyst 01	100.53	**Analyst 01**	98.65
Anaylst 02	100.40	**Anaylst 02**	100.41
% RSD	0.67	**% RSD**	0.58

CONCLUSION

Experimental results and parameters it was concluded that, this newly developed method for the simultaneous estimation ofGemcitabine and Capacitabinewas found to be simple, precise, accurate and high resolution and shorter retention time makes this method more acceptable and cost effective and it can be effectively applied for routine analysis in research institutions, quality control department in meant in industries, approved testing laboratories studies in near future.

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