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Research Article

ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF CAPECITABINE IN BULK BY RP-HPLC METHOD

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ABSTRACT
A simple, rapid and selective RP-HPLC method has been developed for quantification of Capecitabine from bulk drug using a mobile phase consisting mixture of methanol and buffer (70:30) (v/v) at the flow rate of 1.0 ml/min. A Phenomenex C18 (250×4.6mm, 5µ particle size) column was used as stationary phase. The retention time for Capecitabine was 4.1min. Linearity was observed in the concentration range of 10 to 50 µg/ml, with good linearity response greater than 0.997. The mean % recovery obtained is 99.996%. The proposed method is precise, accurate, selective and rapid for the determination of Capecitabine in bulk.
Key words: Capecitabine, RP-HPLC Method, Validation.

INTRODUCTION

Capecitabine [CPC] is Pentyl [1-(3,4-dihydroxy-5-methyl-tetrahydrofuran-2-yl)- 5-fluoro-2-oxo-1H-pyrimidin- 4-yl]aminomethanoate[1] or 5'-Deoxy-5-fluoro-N4-pentyloxycarbonyl-cytidine . Literature survey reveals a few LC-MS methods reported for the determination of Capecitabine and its metabolites in biological fluids[2-5] and a single HPLC[6] method in tablet formulation.CPC is an orally-administered chemotherapeutic agent used in the treatment of metastatic breast and colorectal cancers. CPC is a prodrug, that is enzymatically converted to 5-fluorouracil in the tumor, where it inhibits DNA synthesis and slows growth of tumor tissue. CPC belongs to a group of drugs called antimetabolites. 5-FU also exhibits significant toxicity[7]. It is a fluoropyrimidine carbamate that mimics continuous-infusion 5-FU after being converted via a 3-step enzymatic cascade to 5-FU. The final conversion involves thymidine phosphorylase (abbreviated TP). Thymidine phosphorylase is also known as platelet-derived endothelial cell growth factor and is found more abundantly in prostate cancer cells than in normal tissue. TP is a tumor-associated angiogenesis factor. 5-flurouracil (5-FU) inhibits DNA synthesis as well as the production of proteins which are necessary for cell division and growth.

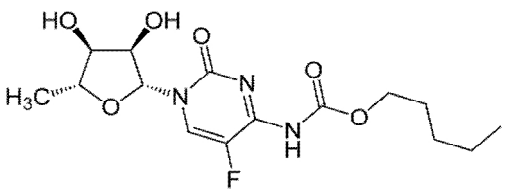

Figure 1: Chemical Structure of Capecitabine

MATERIALS AND METHOD

Instruments

High performance liquid chromatographic system (HPLC) (Shimadzu) equipped with two LC 20AT liquid pumps, Rheodyne injector, pH meter and analytical balance.

Chemicals

Capecitabine has been obtained as a gift sample form Dr Reddy's Laboratories Private Limited (Hyderabad, India), methanol of HPLC grade, Ammonium acetate, glacial acetic acid, Milli-Q water.

Preparation of Standard Stock Solution

10mg of CPC was accurately weighed, transferred into 10ml of clean dry volumetric flask and dissolved in methanol, the volume was made up to the mark with methanol to give 1000ppm.

Optimized Chromatographic Conditions

The analysis was performed by using HPLC, Column of C18, 250×4.6mm, 5µ was used with a flow rate of 1.0ml/min. The mobile phase consists of methanol and ammonium acetate buffer, pH 4, in the ratio of 70:30, the injection volume was 20µL and the detection was at 240 nm using U.V. detector.

Calibration Curve for Capecitabine

Appropriate aliquots of standard stock solution were taken in different 10 ml volumetric flasks and diluted up to mark with mobile phase to obtain final concentration of 10ppm, 20ppm, 30ppm, 40ppm and 50ppm of Capecitabine respectively. The solutions were injected using a 20µg/ml fixed loop system and chromatograms were recorded. Calibration curve was drawn by plotting average peak area versus concentration as shown in figure 2. The linearity table of Capecitabine is shown in Table-1.

METHOD VALIDATION

Linearity

The linearity range was found to be 10-50µg/ml. The regression equation for Capecitabine was found to be y =37070x + 21673 and correlation co-efficient (r^2 = 0.999).

Precision

The precision of the analytical method is determined by assaying sufficient number of sample and relative standard deviation was calculated.

Method

Preparation of Standard Stock Solution

Accurately weigh 10mg of capecitabine and transferred into 10ml volumetric flask and dissolved and volumes were made up with diluents. 1ml of above solution is diluted to 10ml with diluent to obtain the concentration of 100µg/ml of Capecitabine.

Preparation of Working Standard Solution

From the standard stock solution a volume of 1, 2, 3, 4,5ml were transferred it into five different 10ml volumetric flasks.

The volumes were made up with the diluent to obtain the concentration of 10, 20, 30, 40, 50µg/ml of capecitabine.

20µl of various mixed working standard solutions were injected and obtained chromatograms were recorded. The correlation coefficient and % curve fitting slope were calculated. The results are given in Table-2.

Accuracy

Accuracy was found out by recovery study[8] using standard addition method. It was conducted by three replicate measurements at three different concentrations as low, medium, high quality control samples. The data is given in Table-3.

Robustness

The robustness of the analytical method is determined by analysis of aliquots from homogenous Lots by varying different physical parameters, but still within specified parameters of the assay. For example change parameters like flow rate, mobile phase ratio and detection wavelength.

Method

20µl of working standard solution were injected in different chromatographic conditions and chromatograms were recorded. The data when flow rate and mobile phase composition were changed is given in tables-4&5 respectively.

Limit of Detection

It is the lowest amount of analyte in a sample that can be detected but Not necessarily quantified by the analytical method. The detection limit is usually expressed as the concentration of analyte (parts per million).

It is Determined basing on standard deviation (σ) of response and the slope(S). The detection limit may be expressed as

$$DL = 3.3\sigma/S$$

From the formula the limit of detection was found to be 0.40µg.

Limit of Quantification

The quantitation limit of an analyte procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy.

Based on standard deviation (σ) of response and the slope(S). Quantitation limit may be expressed as

$$QL = 10\sigma/S$$

From the formula the limit of quantification was found to be 0.017µg.

RESULTS AND DISCUSSIONS

The results from development activity are that a suitable, easy, less time consuming validated method has been developed for capecitabine. The RP-HPLC procedure was optimized with a view to develop accurate and stable assay method with the pure drug. A C18 phenomenex, 250*4.6mm column in isocratic mode, with mobile phase methanol: buffer (70:30) was used. The flow rate was 1.0ml/min and identical components were measured with U.V.Detector at 240nm. Linearity was assessed by plotting concentration vs. Area which is shown in Fig: 2 with the linearity in the range of 10-50µg/ml for CPC with correlation coefficient of 0.999 with good linearity response. The % recovery was found to be with in limits of the acceptance criteria with mean recovery of 99.996%. Robustness, LOD and LOQ were determined and results are given. The results of the validation suggested that the developed RP-HPLC method could be employed successfully for the estimation of Capecitabine.

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TABLE-1 LINEARITY DATA FOR CAPECITABINE

CONCENTRATION (µg/ml)	PEAK AREA
10	582943
20	962145
30	1326467
40	1710169
50	2062428
Correlation coefficient	0.999
Slope (m)	37070
Intercept(c)	21673

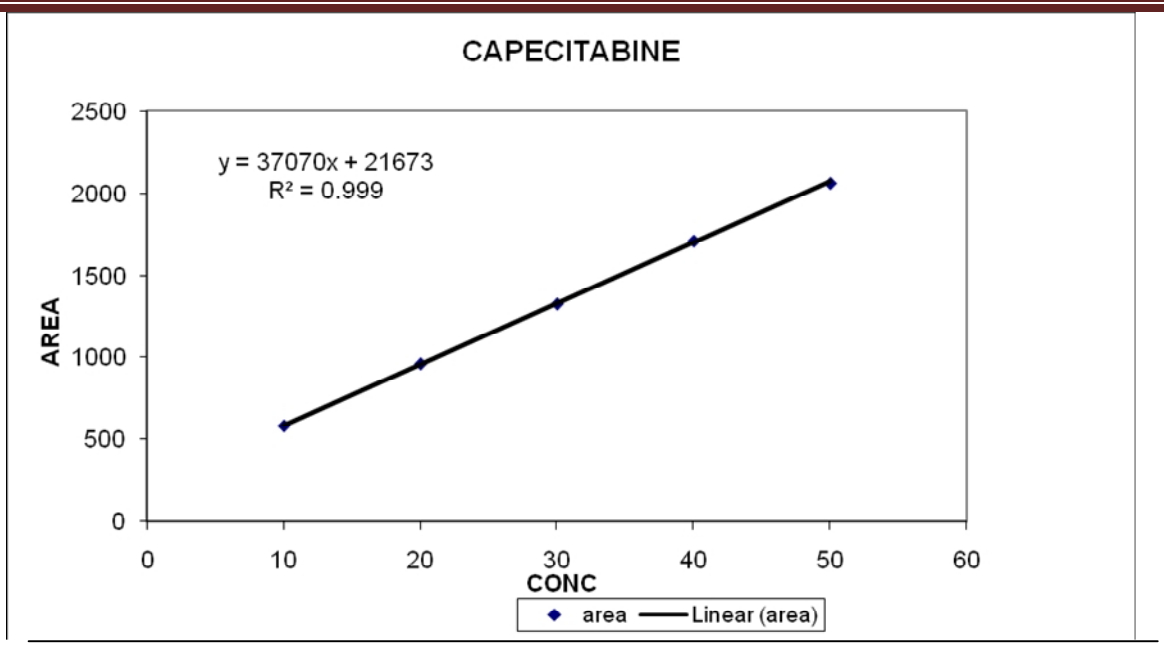

Figure 2: Linearity curve of Capecitabine

TABLE-2 PRECISION OF PROPOSED HPLC METHOD

PREPARATION	RETENTION TIME	AREA OBTAINED
Preparation-1	4.103	2062428
Preparation-2	4.117	2159461
Preparation-3	4.112	2112604
Preparation-4	4.118	2154085
Preparation-5	4.124	2157113
Average	4.114	2129138
Standard Deviation	0.007855	41976.29
Relative Standard Deviation	0.001909	0.0191715
% RSD	0.19	1.9

TABLE-3 ACCURACY STUDIES

Recovery level	% Recovery
50% accuracy	
Preparation -1	98.99
Preparation -2	99.27
Preparation -3	101.73
Average	99.996
100% accuracy	
Preparation -1	98
Preparation -2	102
Preparation -3	99.9
Average	99.96
120% accuracy	
Preparation -1	97.67
Preparation -2	102.27
Preparation -3	100.05
Average	99.996

TABLE-4 ROBUSTNESS (FLOW RATE)

S.NO	DRUG	FLOW RATE 0.8 ML/MINUTE		FLOW RATE 1ML/MINUTE		FLOW RATE 1.2 ML/MINUTE	
		Retention time	Area obtained	Retention time	Area obtained	Retention time	Area obtained
1.	CAPECITABINE	5.084	2614585	4.103	2137542	3.457	1760902
2.		5.073	2607515	4.117	2159461	3.467	1756696
3.		5.072	2609579	4.112	2112604	3.453	1758740
4.		5.089	2611542	4.118	2154085	3.459	1758960
5.		5.088	2619522	4.124	2157113	3.462	1761020
Standard Deviation		0.008167	4726.172	0.007855	19621.32	0.005273	1783.83
RSD		0.001607	0.001809	0.001909	0.009151	0.001524	0.001014
%RSD		0.167	0.1104	0.19	0.9	0.1524	0.10

TABLE-5 ROBUSTNESS (MOBILE PHASE COMPOSITION)

S.NO	DRUG	68:32v/v methanol: buffer		70:30v/v methanol: buffer		72:28v/v methanol: buffer	
		Retention time	Area obtained	Retention time	Area obtained	Retention time	Area obtained
1.	CAPECITABINE	4.272	2614585	4.118	2154085	3.937	2122195
2.		4.264	2607515	4.124	2157113	3.941	2109052
3.		4.281	2609579	4.112	2112604	3.950	2188997
4.		4.278	2611542	4.103	2137542	3.942	2163042
5.		4.289	2619522	4.117	2159461	3.942	2161648
Standard Deviation		0.009418	4726.172	0.007855	19621.32	0.001198	32677.44
RSD		0.0022	0.001809	0.001909	0.009151	0.001198	0.015206
%RSD		0.2	0.1104	0.19	0.9	0.11	1.5

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