A NOVEL RP-HPLC METHOD FOR THE DETERMINATION OF CAPECITABINE IN PHARMACEUTICAL DRUG PRODUCTS

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ABSTRACT

A simple, precise and accurate RP-HPLC method was developed and validated for rapid assay of Capecitabine in tablet dosage form. Isocratic elution at a flow rate of 1ml/min was employed on a symmetry Chromosil C18 (250x4.6mm, 5µm in particle size) at ambient temperature. The mobile phase consisted of Methanol:Water 80: 20 v/v, (P^H 4.9). The UV detection wavelength was 271 nm and 20µl sample was injected. The retention time for Capecitabine was 6.79 min. The percentage RSD for precision and accuracy of the method was found to be less than 2%. The method was validated as per the ICH guidelines. The method was successfully applied for routine analysis of Capecitabine in tablet dosage form and bulk drug.

Key Words: Capecitabine, RP-HPLC, UV detection, recovery, precise, 271nm

INTRODUCTION

Capecitabine is an orally-administered chemotherapeutic agent used in the treatment of metastatic breast and colorectal cancers. It is used in combination with other medications to treat breast cancer that has come back after treatment with other medications. It is also used alone to treat breast cancer that has not improved after treatment with other medications. Capecitabine is also used to treat colon or rectal cancer (cancer that begins in the large intestine) that has gotten worse or spread to other parts of the body. It is also used to prevent colon cancer from spreading in people who have had surgery to remove the tumor. Capecitabine is also sometimes used to treat advanced gastric cancer (cancer of the stomach). Talk to your doctor about the risks of using this medication for your condition.

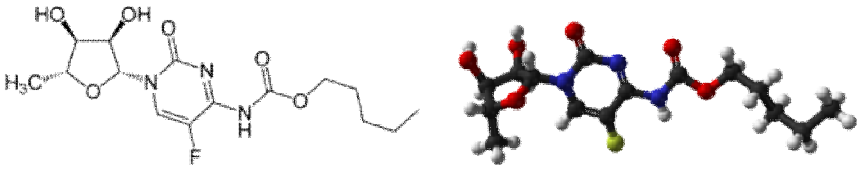

Fig: 1 Structure of Capecitabine

Capecitabine [1-4] works by stopping or slowing the growth of cancer cells. It is a prodrug, that is enzymatically converted to 5-fluorouracil in the tumor, where it inhibits DNA synthesis and slows growth of tumor tissue. The activation of capecitabine follows a pathway with three enzymatic steps and two intermediary metabolites, 5'-deoxy-5-fluorocytidine (5'-DFCR) and 5'-deoxy-5-fluorouridine (5'-DFUR), to form 5-fluorouracil. Capecitabine is in a class of medications called ant metabolites.

Potential major adverse reactions include Cardiovascular- EKG changes, myocardial infarction, angina (these may be more common in patients with pre-existing coronary artery disease), Dermatological- Hand-foot syndrome (numbness, tingling, pain, redness, or blistering of the palms of the hands and soles of the feet). This can lead to the disappearance of fingerprints in some patients. [5-7], gastrointestinal- Diarrhea (sometimes severe), nausea, and stomatitis have occurred. Octreotide has been studied as an anti-diarrheal in cases of refractory diarrhea associated with capecitabine use.[8], Hematological- Neutropenia, anemia, and thrombocytopenia, Hepatic- Hyperbilirubinemia.

EXPERIMENTAL

Materials

Working standard of Capecitabine was obtained from well reputed research laboratories. HPLC grade water, Methanol was purchased from E. Merck (Mumbai, India).

Apparatus

A Series HPLC [6-11] system PEAK LC 7000 isocratic HPLC with PEAK 7000 delivery system. Rheodyne manual sample injector with switch (77251), Analytical column Chromosil C18. 250×4.6mm, Electronic balance-DENVER (SI234), manual Rheodyne injector with a 20 µl loop was used for the injection of sample. PEAK LC software was used. UV 2301 Spectrophotometer was used to determine the wavelength of maximum absorbance

Determination of wavelength of maximum absorbance

The standard solutions of Capecitabine were scanned in the range of 200 -400 nm against mobile phase as a blank. Capecitabine showed maximum absorbance at 271nm. So the wavelength selected for the determination of Capecitabine was 271nm.

Chromatographic equipment and conditions

To develop a High Pressure Liquid Chromatographic method for quantitative estimation of CAPECITABINE an isocratic PEAK HPLC instrument with Zodiac C18 column (250 mm x 4.6 mm, 5µ) was used. The instrument is equipped with a LC 20AT pump for solvent delivery and variable wavelength programmable LC – 7000 UV-detector. A 20µL Rheodyne inject port was used for injecting the samples. Data was analyzed by using PEAK software.

The mobile phase consisted of Methanol: Water 80:20 v/v,(P^H 4.9)Injections were carried out using a 20 µl loop at room temperature (20 + 2 °C) and the flow rate was 1 ml/min. Detection was performed at 271nm with 12min runtime.

Standard and sample solutions

A 10 mg amount of Capecitabine reference substance was accurately weighed and dissolved in 10 ml mobile phase in a 10 ml volumetric flask to obtain 1000 ppm concentrated solution. Required concentrations were prepared by serial dilution of this solution.

A composite of 20 (CAPIIBINE-150mg) tablets was prepared by grinding them to a fine, uniform size powder. 10 mg of Capecitabine was accurately weighed and quantitatively transferred into a 100 ml volumetric flask. Approximately 25 ml mobile phase were added and the solution was sonicated for 15 min.

The flask was filled to volume with mobile phase, and mixed. After filtration, an amount of the solution was diluted with mobile phase to a concentration of 80ppm.

Method validation

Method validation was performed following ICH specifications for specificity, range of linearity, accuracy, precision and robustness.

RESULTS AND DISCUSSION

System Suitability

Having optimized the efficiency of a chromatographic separation, the quality of the chromatograph was monitored by applying the following system suitability tests: capacity factor, tailing factor and theoretical plates. The system suitability method acceptance criteria set in each validation run were: capacity factor >2.0, tailing factor ≤2.0 and theoretical plates >2500. In allcases, the relative standard deviation (R.S.D) for the analytic peak area for two consecutive injections was < 2.0%. A chromatogram obtained from reference substance solution is presented. System suitability parameters were shown in Table.1. Standard chromatogram was given in Figure.2

Api Concentration	80ppm
Mobile Phase	Methanol:Water 80: 20 (v/v)
Wavelength	271nm
Column	C_{18} Column
P^H	4.9
Concentration	80ppm
Retention Time	6.79min
Run Time	12min
Area	533226
Th. Plates	6034
Tailing Factor	0.87
Pump Pressure	11.2 MPa

Table.1 System suitability parameters of Capecitabine:

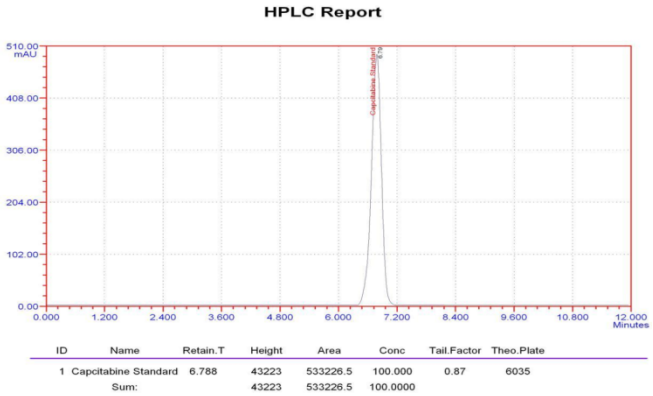

Figure.2: Standard chromatogram of Capecitabine

Range of linearity

Standard curves were constructed daily, for three consecutive days, using seven standard concentrations in a range of 20, 40, 60, 80, 100, 120, 140, and 160 ppm for Capecitabine. The linearity of peak area responses versus concentrations was demonstrated by linear least square regression analysis. The linear regression equation was y = 6425.341 x+ 17157.07 (r= 0.999). Linearity values can shown in Table: 2

S.No	Concentration (ppm)	Area
1	20	164925
2	40	266376
3	60	414130
4	80	533226
5	100	640727
6	120	805412
	140	922937
	160	1032926
	Slope Intercept CC	6425.341 17157.07 0.99914

Table.2: Linearity results of Capecitabine

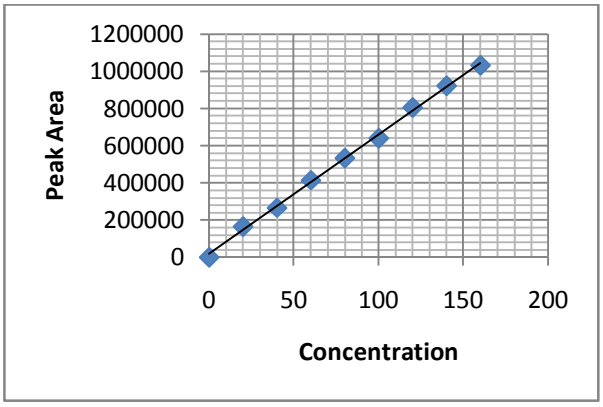

Figure 3: Calibration curve of Capecitabine

Precision

To study precision, six replicate standard solutions of Capecitabine (80ppm) were prepared and analyzed using the proposed method. The percent relative standard deviation (% RSD) for peak responses was calculated and it was found to be which is well within the acceptance criteria of not more than 2.0%. Results of system precision studies are shown in Table.3 and Table.4.

Sample (µg/ml)	Area
1	533226
2	536676
3	534543
4	534581
5	534983
6	538509
RSD	**0.35**

Table 3: Intraday Precision Results for Capecitabine

Sample(µg/ml)	Area
1	522865
2	526764
3	523219
4	523085
5	525543
6	524214
RSD	**0.39**

Table 4: Inter day Precision results of Capecitabine

Limit of Detection and Limit of Quantification:

To determine the Limit of Detection (LOD) sample was dissolved by using Mobile phase and injected until peak was disappeared. After 0.75ppm dilution Peak was not clearly observed, based on which 0.75ppm is considered as Limit of Detection and Limit of Quantification is 2.5 ppm.

Parameter	Measured Value
Limit of Quantification	2.5ppm
Limit of Detection	0.75ppm

Table.5: LOD and LOQ results of Capecitabine

Robustness

Typical variations in liquid chromatography conditions were used to evaluate the robustness of the assay method. The robustness study was performed by slight modification in flow rate of the mobile phase, composition of the mobile phase and wavelength of the detector. Capecitabine at standard concentration was analyzed under these changed experimental conditions. It was observed that there were no marked changes in chromatograms, which demonstrated that the developed method was robust in nature. The robustness acceptance criteria set in the validation were the same established on system suitability test describe above. Results were shown in table 6.

S.NO	Parameter	Change	Area	% of Change
1	Standard	.....	533226	.....
2	MP	Methanol:Water 82:28 88:12	532377 531257	0.15 0.36
3	PH	5.0 4.8	537462 537978	0.79 0.89
4	WL	275nm 264nm	530755 538864	0.46 1.05

Table.6: Robustness results of Capecitabine

Ruggedness:

Ruggedness was performed by using six replicate injections of standard and sample solutions of concentrations which were prepared and analyzed by different analyst on three different. Ruggedness also expressed in terms of percentage relative standard deviation.

Sample (µg/ml)	Area
1	549442
2	540195
3	543052
4	549086
5	547377
6	540112
RSD	**0.79**

Table.7: Ruggedness results of Capecitabine

Recovery

The accuracy of the method was determined by standard addition method. A known amount of standard drug was added to the fixed amount of pre-analyzed tablet solution. Percent recovery was calculated by comparing the area before and after the addition of the standard drug. Recovery test was performed at 3 different concentrations i.e. 60ppm, 80ppm, 100ppm. The percent recovery was calculated and results are presented in Table. Satisfactory recoveries ranging from 98.06 to 100.05 were obtained by the proposed method. This indicates that the proposed method was accurate. Results are given in table.8

% Recovery	Target Conc., (ppm)	Spiked conc, (ppm)	Final Conc, (ppm)	Conc., Obtained	% of Recovery
50%	40	20	60	59.9	99.8
	40	20	60	59.6	99.4
	40	20	60	60.3	100.6
100%	40	40	80	78.4	98.06
	40	40	80	79.02	98.7
	40	40	80	79.6	99.7
150%	40	60	100	100.6	100.6
	40	60	100	100.9	100.9
	40	60	100	100.05	100.05

Table.8: Recovery results of Capecitabine

Formulation	Brand name	Prepared conc	Area	%Assay	Amount found
Capecitabine	CAPIIBINE-150mg	80ppm	524878	98.4	78.95

Table.9: Formulation analysis:

CONCLUSION

The proposed method for the assay of Capecitabine in tablets or capsules is very simple and rapid. It should be emphasized it is isocratic and the mobile phase do not contain any buffer. The method was validated for specificity, linearity, precision, accuracy and robustness. Although the method could effectively separate the drug from its products, further studies should be performed in order to use it to evaluate the stability of pharmaceutical formulations.

REFERENCES

1. NIOSH Alert: Preventing occupational exposures to antineoplastic and other hazardous drugs in healthcare settings. 2004. U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health, DHHS (NIOSH) Publication No. 2004-165.

2. OSHA Technical Manual, TED 1-0.15A, Section VI: Chapter 2. Controlling Occupational Exposure to Hazardous Drugs. OSHA, 1999. http://www.osha.gov/dts/osta/otm/otm_vi/otm_vi_2.html
3. American Society of Health-System Pharmacists. ASHP Guidelines on Handling Hazardous Drugs: Am J Health-Syst Pharm. 2006; 63:1172-1193.
4. Polovich M., White JM, Kelleher LO (eds). Chemotherapy and biotherapy guidelines and recommendations for practice (2nd ed.) 2005. Pittsburgh, PA: Oncology Nursing Society.
5. "Subtopics". Nice.org.uk. Retrieved 2012-08-15.
6. Fingerprints May Vanish With Cancer Drug - US News and World Report
7. Cancer Drug Erases Man's Fingerprints - CNN
8. "Stritch School of Medicine". Stritch.luc.edu. Retrieved 2012-08-15