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RP-HPLC Method for the Estimation of Capecitabine in Tablet Dosage Forms

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A simple, accurate, sensitive and fast reverse phase high performance liquid chromatographic method was developed for the determination of capecitabine in tablet dosage form. A symmetry C_{18} 5 µ, 75 mm × 4.6 mm i.d., in isocratic mode, with a mixture of ammonium acetate with 0.1 % formic acid as buffer and methanol (45:55 v/v) as the mobile phase with flow rate of 1.2 mL/min and analyte was monitored at 305 nm with run time of 5 min. The retention time of the capecitabine was achieved at 3.024 min. The detector response was linear in the concentration of 1.5-22.5 µg/mL, assay is 99.9 % and the limit of detection and limit of quantification was 0.05 and 0.19 µg/mL, respectively. The method was validated by determining its selectivity, sensitivity, precision, linearity, accuracy and LOD and LOQ. The proposed method is simple, fast, sensitive, accurate and precise and hence can be applied for routine quality control of capecitabine in tablet dosage form.

Key Words: Capecitabine, Reverse phase high performance liquid chromatographic, Waters, PDA, Empower software and xeloda tablets.

INTRODUCTION

Capecitabine is an orally-administered chemotherapeutic agent used in the treatment of metastatic breast and colorectal cancers with a chemical name pentyl [1-(3,4-dihydroxy-5-methyl-tetrahydrofuran-2-yl)-5-fluoro-2-oxo-1*H*-pyrimidin- 4-yl] aminomethanoate. Capecitabine 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. It is official in martindale[1] the extra pharmacopoeia. Literature survey reveals few chromatographic methods for the determination of capecitabine in combination with other metabolites in biological fluids[2-8] and one in formulation[9]. Proposed assay method for the estimation of capecitabine in pharmaceutical dosage form is simple, specific and fast. The present paper aims at reporting very sensitive, selective and very short time isocratic RP-HPLC method for the estimation of capecitabine in bulk as well as tablet dosage form.

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EXPERIMENTAL

Waters HPLC with PDA detector was used with empower software. Water (Milli-Q water system), HPLC grade methanol and acetonitrile (Rankem Ltd.), GR grade ammonium acetate and formic acid (85 %, Merck Ltd.) were used. Capecitabine was obtained as a gift sample from NATCO Pharma. Ltd., Hyderabad. The tablet formulation containing capecitabine (Brand name: Xeloda, manufactured by Roche Pharma., Goa.) were procured from local market.

Chromatographic conditions: Chromatographic separation was achieved using a symmetry C_{18} (75 mm × 4.6 mm i.d. 5 µ) analytical column. The mobile phase consisting of 2.35 g of ammonium acetate in 1000 mL with 0.1 % formic acid and methanol (45:55 v/v) was passed through 0.45 µm membrane filter and degassed by ultrasonication. The flow rate was maintained at 1.2 mL/min with injection volume 20 µL and the detection was made at 305 nm. The column and the HPLC system were kept in ambient temperature.

Preparation of standard stock solution: Accurately weighed 30 mg of capecitabine standard was taken in 100 mL volumetric flask added 50 mL of diluent (prepared in 60:40 ratio of methanol and buffer) and sonicated to dissolve and then diluted up to the volume with diluent to get 300 µg/mL standard stock solution.

Working standard solution: 5 mL of the above stock solution was taken into a 100 mL volumetric flask and then diluted to the volume with diluent to get a concentration of 15 µg/mL.

Preparation of sample solution: Twenty tablets were weighed and crushed into fine powder. The powder equivalent to 150 mg was taken in 200 mL volumetric flask added 150 mL of diluent and sonicated for 20 min with intermediate shaking. The sample was allowed to cool at room temperature and the volume was finally made up to volume. The sample solution was centrifuged at 5000 rpm for 5 min to get a clear solution. Then 2 mL of supernatant clear solution was pipette out into 100 mL volumetric flask and made up the volume with diluent to get a concentration of 15µg/mL.

Linearity: Several aliquots of standard stock solutions (1 mL = 300 µg/mL) of capecitabine were taken in different 100 mL volumetric flask and diluted up to the mark with diluent to obtained concentration of 1.5, 3.6, 7.5, 11, 15, 18.6 and 22.5 µg/mL of capecitabine. Evaluation was performed with PDA detector at 305 nm. Peak area was recorded for all the peaks and a calibration graph was obtained by plotting peak area *versus* concentration of capecitabine (Fig. 1).

Assay: Sample solution of 20 µL was injected into HPLC (n = 6) and recorded the chromatograph. The amount of drug present per tablet was calculated by comparing the peak area of the sample solution with that of the standard solution. The data are presented in Table-1.

Recovery studies: Accuracy was determined by adding the known amount of capecitabine pure drug to the pre analyzed samples and subjected to the proposed HPLC method. Results of recovery study are shown in Table-1. The study was done at 50-150 % of test concentration levels.

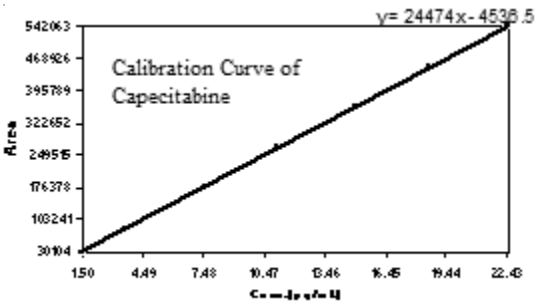

Fig. 1. Calibration curve of capecitabine by HPLC

TABLE-1
RESULTS OF HPLC ASSAY AND RECOVERY STUDIES

Sample	Assay*		Accuracy**			
	Amount claim (mg/tablet)	Amount found (mg/tablet)	Amount added (mg)		Amount found (mg)	
			50 %	150 %	50 %	150 %
Assay	150 mg/tab	149.84	75.10	227.51	74.99	227.80
SD		0.52	0.02	0.29	0.15	0.51
RSD (%)		0.30	0.03	0.13	0.20	0.22

*: Assay average of six determination s (n = 6). **: Accuracy average of three different determinations.

RESULTS AND DISCUSSION

Capecitabine is soluble in methanol and acetonitrile. Different mobile phases were tried to achieve better chromatogram with the short run time. Different mobile phases like methanol:water (50:50), $Na_2H_2PO_4$:methanol (50:50) and ammonium acetate:methanol (55:45) with C_{18} column. Satisfactory separation was achieved with ammonium acetate with 0.1 % formic acid:methanol (45:55) with symmetry C_{18} (75 mm × 4.6 mm i.d. 5µ) analytical column. Capecitabine has λ_{max} at three different wavelengths 214, 240 and 305 nm (Fig. 2). In this proposed method wavelength 305 nm was selected so that there will be no interference from excipients and solvents and maximum absorbance for capecitabine. System suitability tests were carried out on freshly prepared standard stock solution of capecitabine as per the USP-XXVII. Parameters that were studied to evaluate the suitability of the system are given in Table-2.

TABLE-2
VALIDATION AND SYSTEM SUITABILITY PARAMETER

Parameter	Capecitabine
Retention time (min)	3.0240
Asymmetry	1.180
Theoretical plates	1817.000
Calibration range (µg/mL)	1.5-22.5
Limit of detection (µg/mL)	0.050
Limit of quantification (µg/mL)	0.190
Wavelength (nm)	305

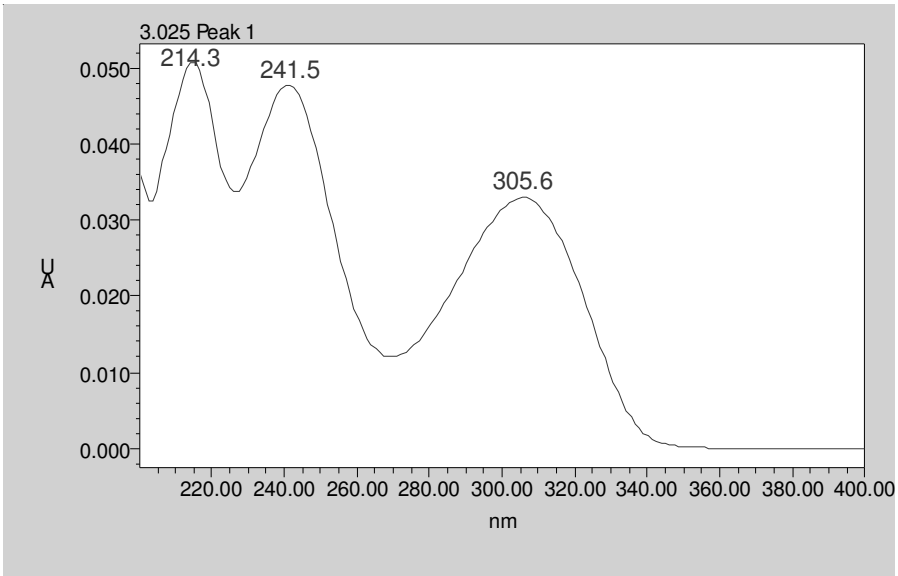

Fig. 2. Spectrum of capecitabine standard by HPLC

Typical chromatogram of capecitabine shown in Fig. 3. It was found that the retention time was 3.024 min. A regression equation obtained for capecitabine was y = 24474x - 4536.5 with concentration range of 1.5-22.5 µg/mL and the correlation coefficient (r = 0.9999) shows that the method is linear. The assay (n = 6) of capecitabine tablets was found to be 99.9 % and recovery studies found that about 100.0 %. The limit of detection (LOD) and limit of quantification (LOQ) for capecitabine were found to be 0.05 and 0.19 µg/mL, respectively. The signal to noise ratio is 3 for LOD and 10 for LOQ. The absence of additional peaks in the chromatogram indicates non-interference of the common excipients used in the tablets. This demonstrates that the developed HPLC method is fast, simple, accurate, sensitive and reproducible and also the standard and sample preparation required less time and no tedious extraction were involved.

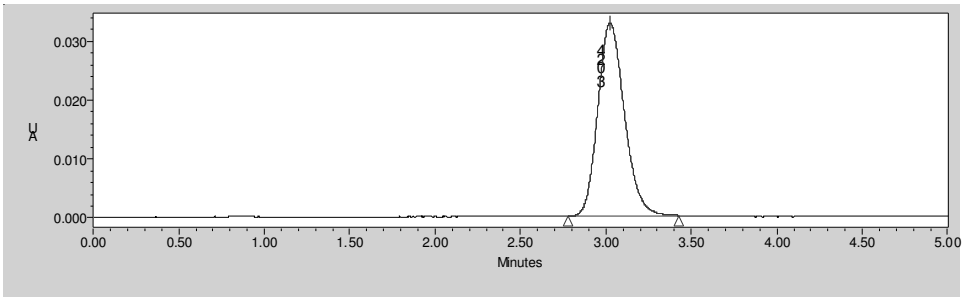

Fig. 3. Typical chromatogram of capecitabine by HPLC

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