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Development and Validation of Stability Indicating HPTLC Method for Estimation of Capecitabine in Tablet Dosage Form

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ABSTRACT

A rapid, accurate and reproducible stability indicating HPTLC method was developed for determination of capecitabine in presence of its degradation products. Separation was done on TLC plate pre-coated with silica gel 60 F254 using toluene: methanol: ethyl acetate: triethylamine (5:3:2:1, v/v/v/v) as mobile phase. This system was found to give compact and dense spots for capecitabine (Rf = 0.68). Quantitative analysis was done through densitometric measurements at wavelength of 240 nm. The developed method was optimized and validated as per ICH guidelines. Method was found linear over the concentration range of 500-2500 ng (nonogram)/spot with correlation coefficient of 0.996. The limits of detection and limit of quantitation were found to be 30.38 ng/spot and 92.068 ng/spot respectively. The percentage recovery of capecitabine was found to be in the range from 98.21 to 100.02%. The drug was subjected to acid, alkali and neutral hydrolysis, oxidation, photolytic, dry heat conditions. The degradation products of capecitabine in acidic, alkali, neutral, oxidative, photolytic condition and dry heat condition were well separated from the pure drugs. The method was applied for estimation of capecitabine in solid oral tablet dosage forms.

KEY WORDS: Capecitabine, High-performance thin layer chromatography, Stability indicating assay, Validation, capiTAB

INTRODUCTION

Capecitabine CAP [N4-pentoxycarbonyl- 5- deoxy-5-fluorocytidine] is an anticancer prodrug of 5- fluorouracil (5-FU) that was designed to undergo preferential conversion to 5-FU within tumors. [1-5]. 5-FU has also been widely used as an anticancer agent in the chemotherapy of solid tumors, but its efficacy is limited by dihydropyrimidine dehydrogenase catalyzed formation of dihydro-5-fluorouracil. [1, 2]

Several methods like HPLC [12], LC-MS [15], HPTLC, UV spectrophotometry have been reported for estimation of capecitabine in bulk, dosage form and in biological fluid. Stability indicating assay method for capecitabine in pharmaceutical drug substance by UPLC has also been reported. Stability indicating HPTLC method for capecitabine was found. [16] In this present work different mobile phase tried and succeed.[11] Most of the reported methods are based on hyphenated techniques, and overall cost of the analysis using these techniques is more as compared to HPTLC. This represented work is a effortless, sensitive, accurate, precise and economic stability-indicating HPTLC procedure was developed [6]. The specific method was optimized and according to ICH guidelines it is validated.

MATERIALS AND METHODS

Materials and Reagents: Pure drug, capecitabine was procured from Intas Pharma, Ahemdabad, Gujarat, India. All reagents and chemicals were of analytical grade; they included Methanol, Ethyl acetate, triethyl amine and Toluene (SD Fine Chemicals Limited, Mumbai, India).

Preparation of Standard Solution: Standard stock solution of API (2000 µg(microgram)/ml(mililitre)) was prepared by dissolving accurately weighed quantity (20 mg) of the drug in 10 ml of methanol.

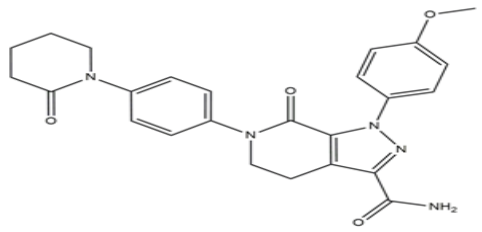

Figure 1 Chemical structure of capecitabine [9]

Chromatographic Conditions:

Selection of Mobile Phase: From the standard stock solution, 100 nl /sec (nanolitre) / (second) of the API was applied to chromatography plates in band form, and these plates were put for a run under different solvent systems. The attempts were made to achieve the preferred Rf value range 0.1-0.8. For efficient separation different solvent combination were tried. From the different mobile phase combinations tested, toluene: methanol: ethyl acetate: triethyl amine (5:3:2:1 v/v/v/v) yields a compact band which showed a symmetrical peak on chromatogram and expected Rf value of 0.68 ± 0.04 for the API.

Choice of Analytical Wavelength for Densitometry Assessment: After proper chromatographic development, scanning of the TLC plate was done over the wavelength range of 200-700 nm. From the spectra Fig. 2, it was observed that capecitabine exhibited strong absorbance at about 240 nm, which was selected as the analytical wavelength for further analysis.

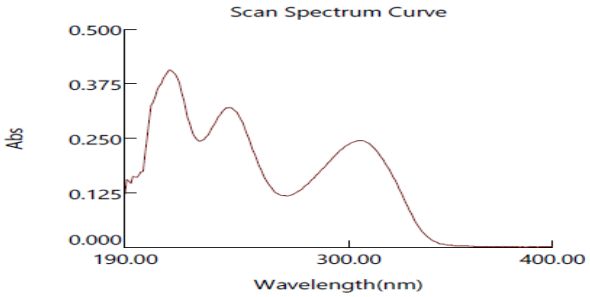

Figure 2 Spectrum of Capecitabine

Instrumentation and Optimized Chromatographic Conditions: The samples were spotted in the form of bands of width 6 mm with Camag microliter syringe on pre-coated silica gel aluminum plate 60 F_{254} (10 cm × 10 cm, 0.2 mm, E. Merck, Germany) by Linomat 5 (Camag, Muttenz, Switzerland) sample applicator was semi-automatic. Before chromatography, methanol was used to prewash the plates which is activated at 60 °C for 15 min. A constant application rate of 100 nl/sec was employed, and space between the two bands was 10.0 mm. linear ascending development was performed in twin trough glass chamber previously saturated with the mobile phase for 30 min. The development distance was 75 mm. After the development, TLC plates were dried in a current of air with the help of an air dryer. Densitometric scanning was performed using Camag TLC scanner 3 in the absorbance mode at 240 nm. The slit dimensions were 4 mm × 0.30 mm. The deuterium lamp is used as radiation source which emitte a continuous UV spectrum covering the range of 200 - 400 nm.

Analysis of Marketed Formulation: Preparation of Sample Solution: Five tablets of capecitabine were weighed and finely powdered. The tablet powder equivalent to 100 mg(milligram) of capecitabine was accurately weighed and transferred to a 10 ml volumetric flask. A 5 ml volume of methanol was added, and the flask was sonicated for 10 min. The volume was made up to 10 ml with methanol mixed well and filtered through Whatman paper no. 41. An aliquot of 1 ml of this solution was transferred to 10 ml volumetric flask and volume was made up to 10 ml with methanol. Further 1 ml of this solution was transferred to 10 ml volumetric flask and volume was made up to mark with methanol. A volume of 15 µl of all the solutions were spotted and analyzed. Amount of drug in the sample was calculated by comparing the mean area of sample band with that of the standard band.

Forced Degradation Studies [5]:- To determine the stability-indicating potentiality of developed HPTLC method ICH guidelines is used, the API powder was stressed under various conditions. In all cases, API (20 mg) was accurately weighed, then subjected to forced degradation conditions such as acid (0.1 N HCL at room temperature for 15 min), base (0.1 N NaOH at room temperature for 15 min), neutral hydrolysis (water at 60 ºC for 30 min), oxidation (3% H_2O_2 at room temperature for 15 min), heat (100 ºC for 10 min), and direct sunlight for 2 hrs (hours). During the stress study, time interval is stipulated, and all stressed solution of API were removed and this stressed samples are diluted and subjected to HPTLC analysis.

Method Validation [7]: This specific method was validated as stated by the ICH guidelines, and various parameters were included like linearity, LOD, LOQ, accuracy, robustness and precision.

Linearity:Linear least-square regression analysis was used to serve the data of peak area versus drug concentration. for regression analysis of API, the slope, intercept, and correlation coefficient (r_2) were calculated.

Limit of Detection (LOD) and Limit of Quantitation (LOQ): From the calibration curve the detection and quantification limits were assess.For the determination of LOD and LOQ following equation were used.

LOD = 3.3 × SD / slope
LOQ = 10 × SD / slope

Accuracy: Known amount of standard capecitabine was added at 80%, 100% and 120% level to pre-analysed sample of capecitabine. The quantity of tablet powder equivalent to 10 mg of capecitabine was transferred to four individual 10 ml volumetric flasks. Known amount of standard capecitabine was added to preanalysed sample of capecitabine according to table. An aliquot of 1 ml of this solution was transferred to 10 ml volumetric flask and volume was made up to 10 ml with methanol. Further 5 ml of this solution was transferred to 10 ml volumetric flask and volume was made up to mark with methanol. A volume of 6 µl of all the solutions were spotted and analyzed.

Precision:

Repeatability of measurement: - Working standard solution of capecitabine (15µl of 100µg/ml) was spotted on a TLC plate and analyzed by the proposed method. The obtained band was scanned seven times without changing plate position and percent RSD for measurement of peak area was calculated.

Repeatability of sample application: - Working standard solution of capecitabine (15µl of 100µg/ml) was spotted on a TLC plate seven times and analyzed by the proposed method. The area of the taken seven spots were measured and % RSD of this peak area were calculated.

Intra-day and inter-day precision: - Within the same day the observed variation in result is called as intra-day precision and in between different days,the variation in results is called as inter day precision. Intra-day precision of the proposed method was evaluated by analyzing the entire calibration range of capecitabine (500-2500 ng/spot), three times on same day. Inter-day precision of the proposed method was evaluated by analyzing the entire calibration range of capecitabine (500-2500 ng/spot) on three different days.

Robustness: The effect of little change tion in the method parameters such as the mobile phase composition, saturation time of chamber, and ratio of volume mobile phase on R_f values and peak area, were observed. The composition of the mobile phase varied up ± 0.1 ml and saturation time of chamber upto ± 10 min(minutes). By calculating the RSD for each parameter, the effect of these variations on R_f values and peak area of the API was studied.

RESULTS AND DISCUSSION

Optimization of method for the Densitometric Measurements: on the TLC plate working standard of the drug was spotted and were developed by solvent systems. By changing the proportions different solvents were tried. Toluene: methanol: ethyl acetate: triethylamine (5:3:2:1v/v/v/v) gave the optimum results. The saturation time for the chamber was 30 mins at room temperature. Dense and compact bands were developed with the R_f value of 0.68 ± 0.04. The representative densitogram is given in Fig. 3.

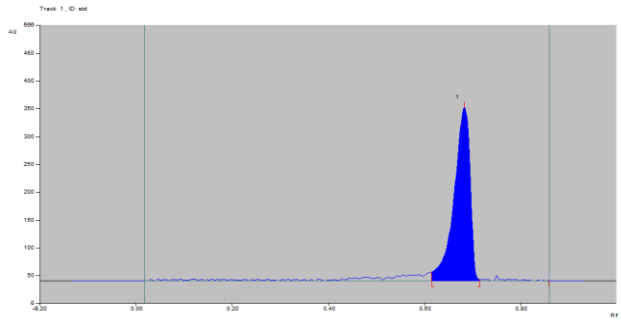

Figure 3 Typical Deensitogram of Capecitabine

Analysis of Marketed Formulation: - The commercial tablet, capitab (500 mg) was quantitatively determined using the developed HPTLC method. The comparison of the mean peak area of the standard band with that of the sample peak area gives result of analysis of tablet formulation. The result has summarized in Table 1.

Table 1 Results of analysis of marketed formulation

Brand name	Label claim (mg)	Amount estimated* (mg)	Percent label SD
CapiTab	500	499.9	99.74 ± 1.03

Validation of Method[6-7]:

Linearity: A linear relationship established between peak area and concentration was found to be in the range of 500-2500 ng/spot. The correlation coefficient of the calibration curve was found to be 0.996.

Calibration curve of capecitbine:-

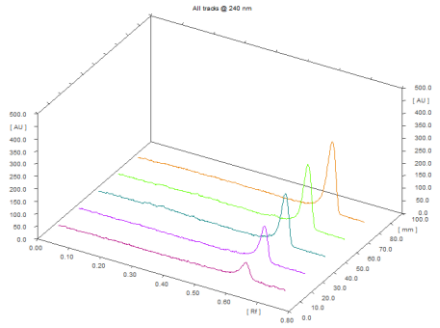

Figure 4 The overlain 3D chromatogram shown in linearity of capecitabine (500-2500 ng/spot).

Table 2 Results of linearity for capecitabine

Parameters	Results
Linearity range	500 - 2500 ng/spot
Regression line equation	y = 3.0815x + 594.03
Mean slope ± S.D.(n=5)	3.07354±0.015051
Mean Y- intercept ± S.D.(n=5)	602.078±28.29757
Correlation coefficient (R2)	0.9965

Limit of Detection and Limit of Quantitation: -
The sensitivity of the developed method was determined in terms of the limit of detection and quantification. The limit of detection and limit of quantification were found to be 30.38 and 92.068 ng/spot, respectively.

Accuracy: - To check the accuracy of the method, recovery studies were carried out by the standard addition method and results of recovery studies obtained within the range of 99.88-100.24% indicates the accuracy of the proposed method. The data obtained from the recovery study are summarized in Table 3.

Table 3 Results of Accuracy

Amount of drug from pre-analysed sample (ng/spot)	Amount of drug spiked (ng/spot)	Total amount spotted (ng/spot)	Total area (Mean)	Amount of drug recovered (ng/spot) ±SD (n=3)	Mean % recovery ±SD (n=3)
1000	-	1000	3345.53	-	-
1000	800	1800	6142.3	800.22	100.02
1000	1000	2000	6713.23	992.89	99.28
1000	1200	2200	7252.36	1178.58	98.21

Precision:- Repeatability or reproducibility of the proposed method was determined by intra-day and inter-day precision study. The drug was assayed three times on the same day (intra-day) and three consecutive days (inter-day). The results of the precision study were expressed in terms of standard deviation (SD) Table 3. The % RSD for both intra-day and the inter-day precision study was found to be less than 2 indicating the repeatability and reproducibility of the method.

Table 4 Results of precision studies

Concentration (ng/spot)	Intraday precision		Interday precision	
	Area (Mean ± SD) (n=3)	%RSD	Area (Mean ± SD) (n=3)	%RSD
500	1965.10±8.834	0.44	1957.10±21.85	1.10
1000	3877.80±21.70	0.55	3859.56±29.45	0.76
1500	5230.26±34.217	0.65	5225.66±27.98	0.53
2000	6852.96±24.53	0.35	6862.86±26.93	0.39
2500	8192.93±75.47	0.92	8177.23±79.83	0.97

Table 5 Results of forced degradation studies.

Force degradation Condition	Condition	R_f value	Capecitabine remaining (%)	Degradation (%)
Acidic Hydrolysis (1N)	(0.1N HCl / Room temperature / 15 min)	0.45 0.65	69.54	30.46
Alkaline hydrolysis (1N)	(0.1N NaOH / Room temperature / 15 min)	0.47 0.65	70.41	29.59
Degradation in neutral condition	(H_2O / 60˚C / 30 min)	0.48 0.65	81.27	18.73
Oxidative degradation	(3% v/v H_2O_2 / Room temperature)	0.47 0.65	82.17	17.83
Photo degradation	2 hours in sunlight	0.48 0.66	85.36	14.64
Thermal Degradation	Dry heat at 100^0C For 10 min	0.44 0.58	93.49	6.6

Degradation Behavior of API: - The degradation behavior of the API was investigated using HPTLC under employed hydrolytic (acid, alkali, neutral), oxidative, thermal, and photolytic stress conditions. The drug showed extensive degradation upon acid, base hydrolytic, neutral hydrolytic, photolytic, and thermal and oxidative stress condition. Various densitogram obtained for API under different stress conditions are shown in Fig. 5, 6, 7, 8, 9 and 10. The obtained results of forced degradation studies are indicated in Table 5. The developed HPTLC method could effectively separate the API from their degradation products, which indicate the potential of stability indicating method.

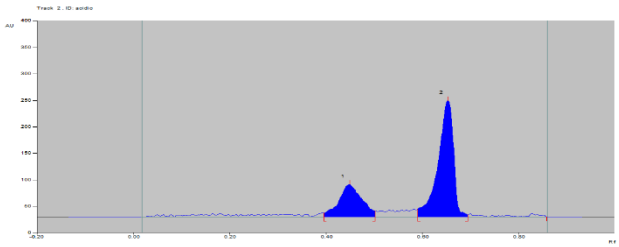

Figure 5 Chromatogram of capecitabine in acidic condition: (0.1N HCl/room temperature /15 min); capecitabine (Rf = 0.65); degradation product (Rf = 0.45).

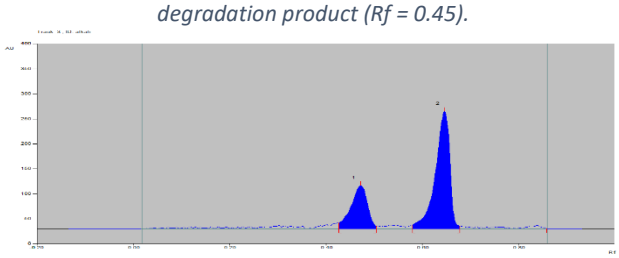

Figure 6 Chromatogram of capecitabine in alkaline condition (0.1N NaOH /room temperature for 15 min); capecitabine (Rf = 0.65); degradation product (Rf = 0.47).

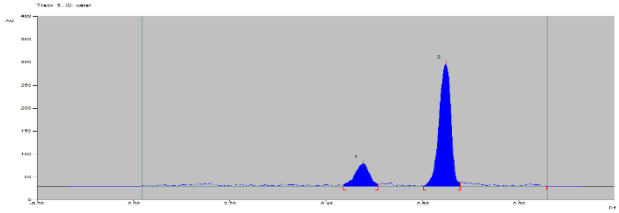

Figure 7 Chromatogram of capecitabine in distilled water (60˚C /30 min); capecitabine (Rf = 0.65)

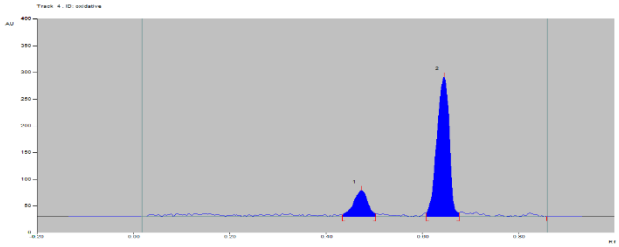

Figure 8 Chromatogram of capecitabine in oxidative condition: (3% H2O2 /room temperature for 15 min); capecitabine (Rf = 0.65); degradation product (Rf = 0.47)

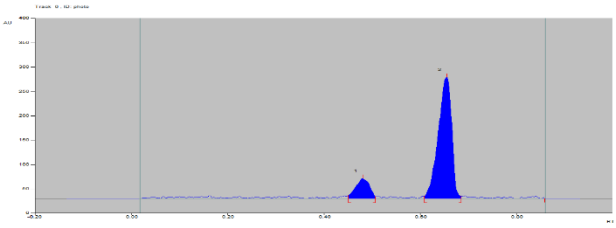

Figure 9 Chromatogram of capecitabine in photolytic degradation study; (drug solution is directly exposed to sunlight/2 hours); capecitabine (Rf = 0.66), degradation product (Rf = 0.48).

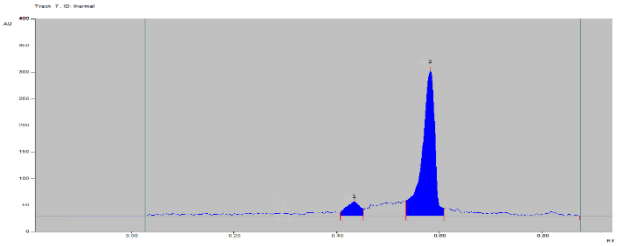

Figure 10 Chromatogram of capecitabine in dry heat degradation study; (drug solution is exposed to 100˚C in oven for 10 min); capecitabine (Rf = 0.58), degradation product (Rf = 0.44).

CONCLUSION

A simple, specific, accurate and precise stability indicating HPTLC method has been developed and validated for estimation of capecitabine in tablets. The method was able to estimate capecitabine accurately in presence of its degradation products. The method was validated as per ICH (Q2 R1) guidelines. The method was applied successfully for estimation of capecitabine in tablets. It can also be used for stability studies of capecitabine tablets.

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