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Original Article

DEVELOPMENT AND VALIDATION OF UV SPECTROPHOTOMETRIC METHOD AND RP-HPLC METHOD FOR ESTIMATION OF CAPECITABINE IN BULK AND TABLET DOSAGE FORMS

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ABSTRACT

Objective: The objective of the present work is to develop and validate a novel, specific, precise and reliable method for estimation of Capecitabine in bulk and pharmaceutical dosage form using UV-visible spectroscopy and RP-HPLC method.

Methods: UV-visible spectrophotometric determination was performed with Elico double beam SL 210 UV-visible spectrophotometer having deuterium lamp at λmax 304 nm using water as a medium. Linearity was noted over a concentration range of 2-20 µg/ml with a correlation coefficient of 0.99. HPLC analysis was performed using Eclipse XDB C18 column with 5 µm particle size having dimensions 4.6 X 250 mm column, Agilent 1260 infinity DAD detector, 1260 infinity quaternary pump using Ezchrome software at a flow rate of 1 ml/min and a run time pressure of 2140 psi. Methanol: acetonitrile: water in the ratio 30: 30: 40 was used as mobile phase and the effluents were analyzed at 304 nm. Both the proposed methods were validated for various parameters like linearity, precision, accuracy, robustness, ruggedness, selectivity, detection, quantification limits, formulation analysis as per International Conference on Harmonization (ICH) guidelines.

Results: Linearity for UV and HPLC method was noted over a concentration range of 2-100 µg/ml with a correlation coefficient of 0.99. The retention time was considered to be 4.60 min. The % RSD for interday and intraday precision studies and recovery analysis of both UV and HPLC methods was found to be less than 1% which is less than the official RSD limit (2%). Recovery analysis performed using marketed formulation capeguard was considered to be greater than 99% for both the methods.

Conclusion: Both the methods developed were validated according to the ICH guidelines. Hence it was evident that the developed methods were novel, sensitive, precise and reliable for estimation of Capecitabine in bulk and were successfully applied for estimation of pharmaceutical dosage forms.

Keywords: Capecitabine, UV-visible spectroscopic method, HPLC method, Validation

INTRODUCTION

Capecitabine (CPTB) is a new, orally administered, enzyme-activated fluoropyrimidine carbamate belonging to the class antimetabolites. The drug is official in the Indian Pharmacopoeia (IP)[1, 2]. The chemical name of Capecitabine is 5-deoxy-5-fluoro-N-(pentyloxy) carbonyl]–cytidine with a molecular formula $C_{15}H_{22}FN_3O_6$ and molecular weight 359.35[3, 4]. The chemical structure is intended to generate high levels of fluorouracil (5-FU) in tumor cells located in the colorectal and breast region. Capecitabine is a prodrug of 5-deoxy-5-fluorouridine (5-DFU), which undergoes enzymatic conversion to be 5-fluorouracil [5-FU] in tumor cells [5, 6]. The 5-FU inhibits the synthesis of thymidine monophosphate, which is an active form of thymidine required for *de novo* synthesis of Deoxy Ribo Nucleic acid [7-10]. The empirical formula of the functional moiety is $C_{15}H_{22}FN_3O_6$. The active moiety acts by mimicking as a normal cell nutrient which is indispensable to the growth of cancer cells. Literature survey disclosed that there are few analytical methods for spectrophotometric [11] and chromatographic estimation of capecitabine [12-16].

A detailed review of the literature regarding the existing methods revealed that there is a need for the development of the spectrophotometric method and chromatographic method, which is simple with less time tedious mobile phase or dilution medium. In the present study modest, novel, sensitive, reliable and accurate methods have been established for the estimation of capecitabine in bulk and in tablet dosage forms. Both the methods have a simple medium like water or feasible mobile phase mixture, which can be used for analysis of capecitabine with better separation of analytes. An attempt was made in the present methods to achieve an accurate, reliable and reproducible result with minimum Relative Standard Deviation (RSD) values than all other existing methods, which was successfully accomplished.

MATERIALS AND METHODS

Chemicals and reagents

The reference sample capecitabine (CPTB) was secured from Torrent Pharmaceuticals Limited. Ahmedabad. All the reagents used stood at analytical grade. Acetonitrile (HPLC grade) and triethylamine (HPLC grade) were acquired from Merck pharmaceutical's private ltd., Mumbai, India. Methanol and water used were of HPLC grade and purchased from Merck specialty's private ltd., Mumbai, India. Commercial tablet CAPEGUARD was procured from the local market.

Instrument specifications

The UV analysis was performed using Elico double beam SL 210 UV-visible spectrophotometer having deuterium lamp associated with spectra treats software. The HPLC analysis was performed using agilent 1260 infinity system (Ezchrome elite software) consisting of DAD VL detector adjusted to a wavelength of 304 nm. The instrument also consisted of an eclipse XDB model C18 column (5 µm particle size, 4.6 x 250 mm) and a 1260 infinity VL quaternary pump.

Spectrophotometric and chromatographic conditions

Spectrophotometric analysis was performed using triple distilled water as mobile phase. The detection was carried out at an absorption maximum (λ_{max}) of 304 nm.

Chromatographic separation was achieved using mobile phase methanol: acetonitrile: water at a ratio 30: 30: 40. A flow rate of 1 ml/min was maintained throughout the separation process. Run time pressure of 2140 psi was maintained. All the contents of the mobile phase were filtered through a 0.45 mm membrane filter and degassing was performed using ROHS sonicator to remove dissolved gases if any. For each trial, 20 µl samples were injected manually, and a total run time of 6 min was maintained. The eluent was detected at 304 nm. Various systems suitability parameters were assessed as mentioned in table 1.

Table 1: System suitability parameters for HPLC

S. No.	Parameter	Results
1	Standard concentration	1 mg/ml
2	Mobile phase	30:30:40 Methanol: acetonitrile: water
3	Elution	Isocratic
4	Wavelength	304 nm
5	Column	Eclipse XDB C18
6	Detector	DAD PDI detector
7	Flow rate	1 ml/min
8	Column Volume	1322.5 mm^3
9	Run time pressure	2140 psi
10	Retention time	4.6 min
11	Runtime	6 min
12	Peak area	4438704.660
13	Tailing factor	0.941
14	Peak Asymmetry Factor	0.882
15	Column dead time	1322.5 min
16	Purging valve pressure	140 psi

Preparation of stock solutions and sample solutions

For the spectrophotometric analysis stock solutions of CPTB was prepared by dissolving 10 mg of the drug in 10 ml distilled water to obtain a final concentration of 1 mg/ml. Serial dilutions were made to prepare diverse sample solutions of concentrations ranging from 2–20 µl/ml. The solutions were analyzed at an absorption maximum of 304 nm against the blank.

For chromatographic analysis of CPTB, the stock solution was prepared by dissolving 10 mg of the drug in 10 ml of the mobile phase to obtain a final concentration of 1 mg/ml. Serial dilutions were made to obtain sample solutions of concentrations ranging from 2–100 µl/ml. All the sample solutions were filtered through 0.45 mm membrane filters and were subjected to degassing. The sample solutions were analyzed at an absorption maximum of 304 nm.

Validation of developed methods [17-19]

Linearity and range

Linearity is defined as the ability to obtain test results, which were directly proportional with the concentration of an analyte in the sample within a given range.

Linearity data for the spectrophotometric method was obtained at an absorption maxima of 304 nm as shown in fig. 1 by using ten concentrations in the range of 2–20 µg/ml. Calibration curve was obtained by plotting absorbance against concentration by considering six observations as shown in fig. 2.

Linearity data for the chromatographic method was obtained by using ten concentrations within the range of 2–100 µg/ml. Calibration curve was obtained plotting peak area against concentration by considering five observations as shown in fig. 3.

Both the methods were studied using six replicates of each sample concentrations.

Precision

The degree of closeness of agreement between a series of measurements obtained from multiple samplings of the same homogeneous sample under the prescribed condition was determined. The intra-day precision was performed by analyzing six replicate standard solutions on the same day, and inter-day precision was performed by analyzing a series of standard solutions for 3 consecutive days using the proposed UV and HPLC methods. The data obtained was presented in table 5**.**

Robustness

Robustness is defined as the measure of its capacity to remain unaffected by small but deliberate variation in method parameters, and it provides an indication to its reliability during normal range. Robustness of both the methods was studied using six replicates of the sample at a concentration level of 60 µg/ml (for HPLC) and 10 µg/ml (for UV).

Ruggedness

Ruggedness was calculated by considering the same sample at different labs by different analysts.

Detection and quantification limits

Limit of detection (LOD) represents the lowest amount of analyte in the sample which can be detected.

Limit of quantification (LOQ) represents the lowest amount of analyte, which can be quantitatively determined.

The above parameters are calculated based on the standard deviation of the response and the slope. The standard deviation was calculated based upon the calibration curve.

$$LOD = 3.3\sigma/SLOQ = 10\sigma/S$$

Selectivity and specificity

The ability to measure accurately and specifically the analyte of interest in the presence of other components like excipients in the tablet formulation was analyzed. The blank, standard, placebo, placebo along with analyte and test preparations were analyzed as per the method to identify interference of blank and placebo with CPTB peaks.

Estimation of an active ingredient in bulk and in tablet dosage form (Formulation analysis)

Twenty tablets (capeguard 500 mg) were weighed accurately and crushed into powder form. Accurately weighed the quantity of powder taken and a standard solution of 1000 µg/ml was prepared using the mobile phase and the diluting fluid. Serial dilutions were taken to ensure the standard solution prepared, and the solutions were analyzed spectrophotometrically and chromatographically using the proposed methods.

RESULTS AND DISCUSSION

Linearity and range

The linearity of CPTB employing UV method was constructed by considering concentration (µg/ml) on X–axis and Absorbance on Y–axis. The regression coefficient was considered to be 0.999 over a concentration range of 2–20 µg/ml. The representative linearity equation was found to be y = 0.0256x+0.0002 as showed in fig. **2** and data were shown in table 3**.**

The linearity of proposed CPTB employing HPLC method was constructed by considering concentration (µg/ml) on X–axis and peak area on Y-axis. The regression coefficient was considered to be 0.999 over a concentration range of 2–100 µg/ml. The representative linearity equation was found to be y = 57534x+17091 as showed in fig. 3 and the corresponding data were shown in table 3.

For both the methods the % RSD was found to be within the acceptable theoretical limits of ≤ 2%.

Table 2: Summary of validation parameters obtained for proposed UV and HPLC methods

Validation parameters	UV	HPLC
Beer's law limit	2-20	2-100
Correlation coefficient (r^2)	0.999	0.999
Regression equation	Y = 0.025x+0.000	y = 57283x+37336
Slope	0.025	57283
Intercept	0.000	37336
LOD	0.234	0.097
LOQ	0.710	0.294

Table 3: Linearity data table for proposed HPLC and UV methods (where n=6)

HPLC linearity data		UV linearity data	
Concentration (mcg/ml)	**Peak area±RSD**	**Concentration (mcg/ml)**	**Absorbance**
2	158716±639.22	2	0.049±0.005
4	262784±12119.81	4	0.106±0.011
6	425330±2064.75	6	0.158±0.006
8	528899±66248.83	8	0.207±0.002
10	638175±1820.09	10	0.258±0.006
20	1167819±60367.12	12	0.303±0.002
40	2266267±100287.5	14	0.355±0.006
60	3468807±374512.7	16	0.415±0.008
80	4496095±3019.35	18	0.463±0.013
100	5885228±139667	20	0.514±0.011
Correlation coefficient	0.999	Correlation coefficient	0.999
Slope	57300	Slope	0.025
Intercept	35375	Intercept	0.000

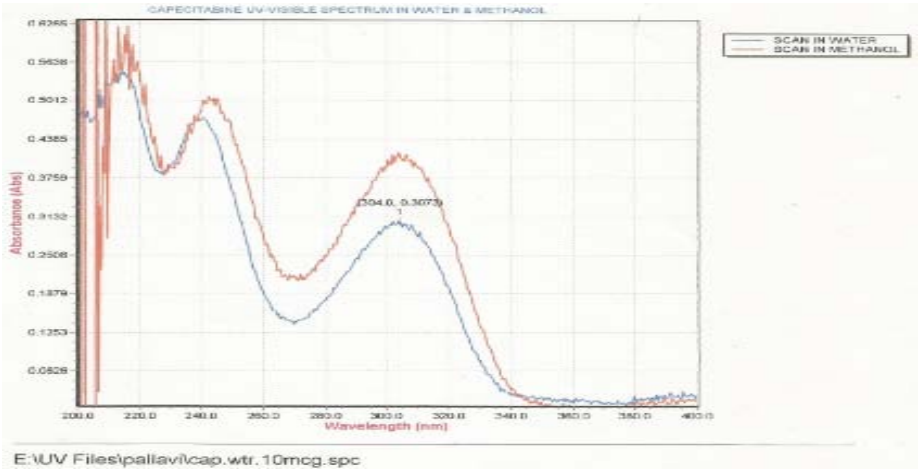

Fig. 1: UV visible spectrum scan of CPTB in water and methanol

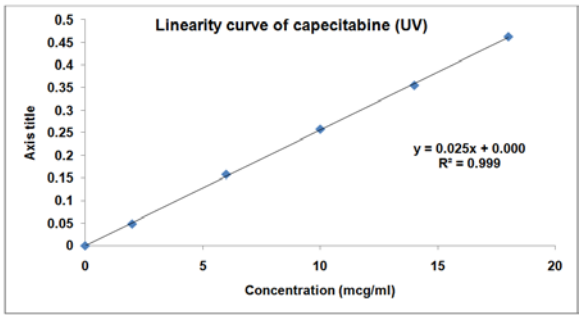

Fig. 2: Linearity curve of CPTB obtained from UV method

Precision

The % RSD for intra-day precision (six independent series in the same day) and inter-day precision (3 consecutive days) analysis performed for six different individual samples of drug solution using the proposed UV and HPLC methods was found to be 0.021%, 0.72% and 0.39%, 0.91% respectively. Since the values obtained as shown in table 4 were within the proposed theoretical limits<2% RSD according to IP, the method was demonstrated to be precise.

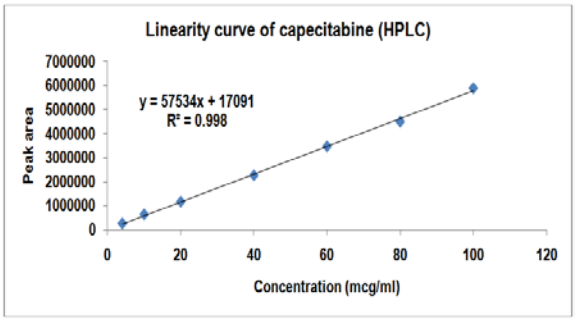

Fig. 3: Linearity curve of CPTB obtained from HPLC method

Table 4: Precision analysis data of CPTB for UV and HPLC

Parameter	UV	HPLC
Interday (% RSD)	0.021	0.39
Intraday (% RSD)	0.72	0.91

Recovery studies

The accuracy of the proposed UV-visible spectroscopic method and HPLC method was established by recovery experiments. The recovery analysis studies were carried out at three different concentration ranges (50, 100 and 150%). All studies were carried in triplicate, and the results obtained were presented in table 5**.** The analyzed samples yielded high recovery values from the proposed methods. % RSD values were found to be less than 0.2% for both UV and HPLC analysis, indicating that the proposed methods were accurate. All the RSD values obtained were less than the theoretical limit of<2% RSD according to IP. F-test results for both the UV and HPLC methods revealed that the F_{cal} value is less than the $F_{tabulated}$ value as shown in table 6 & 7, proving that null hypothesis is accepted. Hence it was proved that there is no significant difference between the actual amount added, and the amount recovered.

Table 5: Recovery analysis for CPTB by the proposed UV and HPLC methods

Method	Concentration level	Amount added (µg/ml)	Total amount (µg/ml)	Amount found (µg/ml)	Amount recovered (µg/ml)	% recovery	% RSD
	50	5	15	14.97	4.97	99.4	0.054
UV	100	10	20	19.73	9.81	98.1	0.063
	150	15	25	24.82	14.78	98.53	0.059
	50	5	15	14.94	4.94	98.8	0.108
HPLC	100	10	20	19.73	9.73	97.3	0.038
	150	15	25	24.82	14.82	98.8	0.092

Table 6: Single factor ANOVA for recovery studies performed using UV method

Source of variation	*SS*	*df*	*MS*	*F cal*	*P-value*	*F tab*
Between Groups	0.032266667	1	0.032267	0.001315	0.972806	7.708647
Within Groups	98.12086667	4	24.53022			

Table 7: Single factor ANOVA for recovery studies performed using HPLC method

Source of variation	*SS*	*Df*	*MS*	*F cal*	*P-value*	*F tab*
Between Groups	0.04335	1	0.04335	0.001755	0.968595	7.708647
Within Groups	98.8222	4	24.70555			

Robustness

The robustness of the proposed HPLC method was checked in terms of variation in mobile phase, flow rate change and wavelength change. Experimental findings proved that the change of mobile phase is the most influential factor on repeatability of the proposed HPLC method. Suitable measures have been adopted to maintain similarity in various instrumental aspects like injection and capillary conditioning. Since % RSD values for all the parameters were found to be less than 0.1% (less than the acceptable theoretical limit of<2% RSD) the proposed HPLC method was found to be robust. The results obtained were presented in table 8**.**

The robustness of the proposed UV method was checked in terms of variation in mobile phase and change in wavelength. Experimental findings proved that change in the mobile phase has a higher influence on repeatability of the proposed UV method compared to change in wavelength. % RSD values for all the parameters were found to be less than 0.02% (less than the acceptable theoretical limit of<2% RSD) which proved that the proposed UV method was found to be robust. The results obtained were presented in table 9**.**

Table 8: Results obtained for robustness study of HPLC method (n=6)

S. No.	Parameter	Condition	Area±RSD	% of change
1	Standard solution (60 mcg)	Standard condition	3468807	----
2	Mobile phase change	Methanol: acetonitrile: water 28: 32: 80	3223986±1023.47	0.071
		Methanol: acetonitrile: water 30: 28: 78	3733627±1945.68	0.076
3	Flow change	0.9 ml/min	3592787±2012.63	0.036
		1.1 ml/min	3686436±1897.13	0.063
4	Wavelength change	302 nm	3492661±1895.85	0.007
		306 nm	3447989±2147.58	0.006

Table 9: Results obtained for robustness study of UV-visible spectrophotometric method (n = 6)

S. No.	Parameter	Condition	Absorbance (nm)	% of change
1	Standard solution (10 mcg)	Standard condition	0.258±0.017	----
2	Mobile phase change	Distilled water: methanol (98: 2)	0.261±0.047	0.0114
		Distilled water: methanol (97: 3)	0.262±0.053	0.0153
3	Wavelength change	302 nm	0.256±0.068	0.007
		306 nm	0.259±0.038	0.004

Ruggedness

Standard solutions of CPTB were analyzed using both the proposed methods for ruggedness.

The results showed that there is not any significant statistical difference between labs, analysts or between instruments. Thus both the methods are proven to have ruggedness.

Detection and quantification limits

The LOD and LOQ for CPTB utilizing the proposed UV method were determined to be 0.234 µg/ml and 0.710 µg/ml respectively.

The LOD and LOQ for CPTB using the proposed HPLC method were found to be 0.0969µg/ml and 0.2936µg/ml respectively. The results obtained were presented in table 2.

Both the methods indicate the accuracy and precision to detect a very low quantity of analyte which is a favorable sign for extending the method to plasma drug analysis.

Specificity

The selectivity and specificity of the proposed methods were tested by studying the effect of various excipients and other additives usually present in the formulations of cCPTB. The chromatograms didn't yield any peaks for mobile phase and placebo when analyzed with the proposed HPLC method. No absorbance was found for blank/dilution fluid when analyzed spectrophotometrically using the proposed UV method. The results obtained were presented in table 10. The well-shaped peaks and the linearity of the results indicate that the proposed methods are selective and specific. A model chromatogram was illustrated in fig. 4.

Table 10: Selectivity and specificity of CPTB samples using proposed UV and HPLC methods

	Mobile phase/Dilution liquid	Placebo	CPTB sample Peak area/Absorbance
UV method	No absorbance	No absorbance	0.258
HPLC method	No peak	No peak	638125

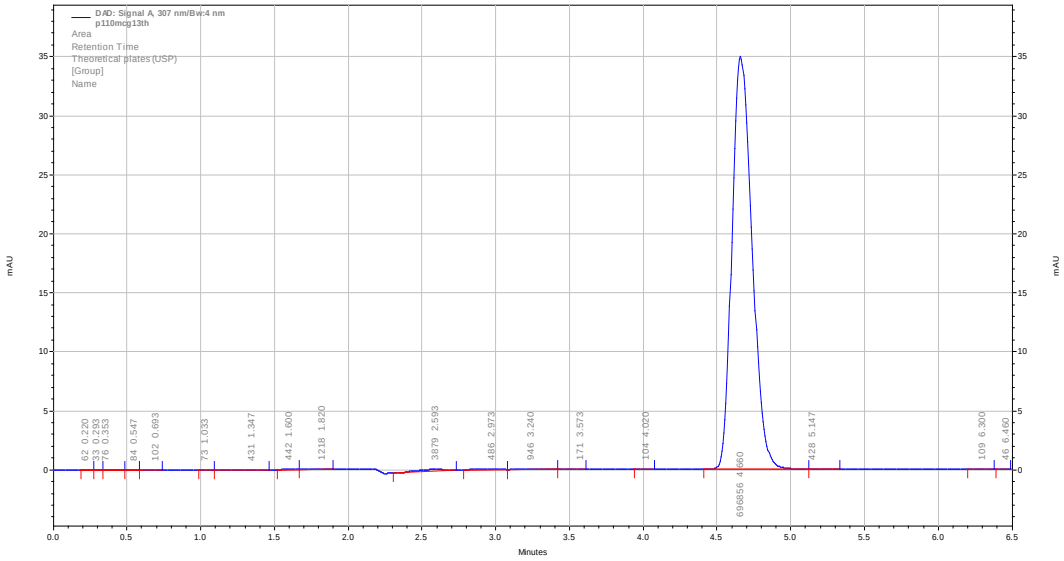

Fig. 4: Typical chromatogram of CPTB

Table 11: Formulation analysis results

S. No.	Tablet name	Dose	Sample concentration	Sample estimated	% of drug estimated in tablet
1	CAPEGUARD (UV)	500 mg	1 mg/ml	0.99±0.0002 mg/ml	99.0 %
2	CAPEGUARD (HPLC)	500 mg	5 mg/ml	4.98±0.0001 mg/ml	99.7%

Determination of an active ingredient in bulk and in tablet dosage form (Formulation analysis)

Twenty solutions of CPTB were prepared using bulk drug and tablet dosage form (capegaurd). The samples were analyzed with both the proposed methods using the same experimental conditions and the drug content was found to be within the limits specified by I. P. The results obtained were presented in table 11.

F-test results for UV and HPLC method revealed that the F_{cal} value<F_{tab} value proving that null hypothesis is accepted. Hence it was proved that in both the methods, there is no significant difference between sample concentration and the sample estimated. The results also assured that both the proposed methods are selective for estimation of formulations.

CONCLUSION

A novel, precise, economical, accessible, reliable and reproducible method for estimation of CPTB in bulk and tablet dosage form using UV and HPLC methods were developed and were validated according to ICH guidelines. The wide range of linearity and use of readily available and economical mobile phase and dilution fluids establishes a further scope of promoting the proposed methods for estimation of capecitabine. The RSD values for all the validation parameters were found to be less than 1, indicating that the proposed UV and HPLC methods were trusts worthy. Both the methods have ample scope and application in industry for estimation of CPTB.

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CONFLICT OF INTERESTS

The authors express no conflict of interests

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