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Original Article

DEVELOPMENT AND VALIDATION OF RP-HPLC AND UV SPECTROPHOTOMETRIC METHODS FOR THE QUANTIFICATION OF CAPECITABINE

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ABSTRACT

Objective: The objective of the present study was to develop and validate a new liquid chromatographic technique and four new spectrophotometric methods for the quantitative estimation of Capecitabine.

Methods: In the first method, the chromatographic technique was carried out in isocratic technique on Shimadzu Model CBM-20A/20 Alite HPLC system, equipped with SPD M20A prominence PDA detector with Zorbax C18 (150 mm × 4.6 mm i. d, 5 µm particle size) column. The method was optimized with a mobile phase consisting of 0.1 % Acetic acid and Acetonitrile (35:65, v/v) with flow rate 0.5 ml/min. In second, third, fourth and fifth methods, spectrophotometric techniques were applied. The absorption maximum (λ_{max}) was observed at 305 nm, 305 nm, 303 nm and 297 nm for method B (developed in 0.1 N hydrochloric acid), C (developed in sodium acetate buffer pH 4.0), D (developed in phosphate buffer pH 7.0) and E (developed in borate buffer pH 9.0) respectively. Different validation parameters such as linearity, precision, accuracy, limit of detection (LOD) and limit of quantification (LOQ), robustness were also determined.

Results: The linearity of the calibration curves for the analyte in the desired concentration range is good for both the HPLC (R^2 = 0.9994) and UV methods. The LOD and LOQ were found to be 0.02354 µg/ml and 0.07162 µg/ml respectively. The % RSD values for the validation parameters (precision and accuracy) were less than 2.0%.

Conclusion: The proposed chromatographic and spectrophotometric methods were validated and can be applied for the determination of Capecitabine in pharmaceutical formulations.

Keywords: Capecitabine, UV spectrophotometric, Forced degradation, RP-HPLC, Method validation

INTRODUCTION

Capecitabine (CTB) (fig. 1) is a fluoropyrimidine carbamate with antineoplastic activity, and it belongs to a class of drugs known as antimetabolites. It is a chemotherapeutic agent administered orally which is used in the treatment of metastatic breast and colorectal cancers. CTB is a prodrug of 5′-deoxy-5-fluorouridine (5'-DFUR), which is enzymatically converted to 5-fluorouracil in the tumor, where it inhibits DNA synthesis and slows the growth of tumor tissue. The activation of CTB 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. Chemically it is 5'-deoxy-5-fluoro-N-[(pentyloxy) carbonyl]-cytidine with empirical formula of C15H22FN3O6 and the molecular weight of 359.35 g/moL [1-5].

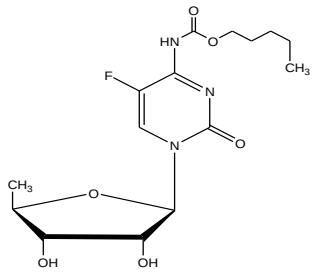

Fig. 1: Chemical structure of capecitabine

Literature available from all possible scientific sources revealed that very few analytical methods have been evoked for the estimation of CTB by spectrophotometric [6-13], HPLC [14-22], visible spectroscopy [23] and Mass spectroscopy [24] methods. Thus, the present study deals with the development of a sensitive, accurate and reliable method for the estimation of CTB in bulk and pharmaceutical dosage forms using a new liquid chromatographic technique and spectrophotometric methods in four different buffer solutions.

MATERIALS AND METHODS

Drugs and chemicals

Analytical grade methanol (Merck), disodium phosphate (Na_2HPO_4) (Merck), monopotassium phosphate (KH_2PO_4) (Merck), boric acid, sodium hydroxide, and glacial acetic acid were used for the analysis. CTB was obtained as a gift sample from Mylan Laboratories limited. All the other reagents used in the analysis were of the high purity analytical grade. All weighing was performed on a calibrated analytical balance. Calibrated glassware's were used throughout the experiments.

Preparation of reagents and solutions

Preparation of 0.1 N HCl

For the preparation of 0.1 N HCl, 8.5 ml of HCl was diluted with 1000 ml of distilled water.

Preparation of sodium acetate buffer (pH 4.0)

For the preparation of sodium acetate buffer (pH 4.0), 2.86 ml of glacial acetic acid and 1.0 ml of 50% w/v solution of sodium hydroxide was added in a 100 ml volumetric flask and made up the volume with HPLC grade water.

Preparation of phosphate buffer (pH 7.0)

For the preparation of phosphate buffer (pH 7.0), 0.5 gms of Na_2HPO_4 and 0.301 gms of KH_2PO_4 was added in a 1000 ml volumetric flask and the volume was made up with distilled water.

Preparation of borate buffer (pH 9.0)

For the preparation of borate buffer (pH 9.0), 6.2 gm of boric acid was dissolved in 500 ml of water and the pH was adjusted to 9.0 with 1 M sodium hydroxide (about 41.5 ml) and diluted with water in a 1000 ml volumetric flask.

Preparation of stock and sample solutions

The standard solution of CTB was prepared by dissolving accurately about 25 mg of the CTB with methanol in a 25 ml volumetric flask. The stock solution was further diluted with 0.1 N HCl, sodium acetate buffer (pH 4.0), phosphate buffer (pH 7.0), and borate buffer (pH 9.0) for method B (1-80 µg/ml), method C (1-60 µg/ml), method D (5-80 µg/ml), and method E (1-60 µg/ml) respectively as per the requirement.

CTB stock standard solution (1 mg/ml)

CTB stock solution (1000 µg/ml) was prepared by dissolving 25 mg of CTB in mobile phase in a 25 ml volumetric flask. Working solutions were prepared from the stock solution with mobile phase, and all the solutions were filtered through 0.45 µm membrane.

Instrumentation and method development

RP-HPLC Instrumentation and chromatographic conditions

Chromatographic separation was achieved by using a Shimadzu Model CBM-20A/20 Alite HPLC system, equipped with SPD M20A prominence PDA detector with Zorbax C18 (150 mm × 4.6 mm i. d, 5 µm particle size) column. A mixture of 0.1% Acetic Acid and acetonitrile (35:65, v/v) was used as the mobile phase. The flow rate was 0.5 ml/min and 20 µl of each sample was injected into the HPLC system.

UV spectrophotometer instrumentation

A double beam UV-VIS spectrophotometer (UV-1800, Shimadzu) loaded with spectra manager software UV Probe was employed with a spectral bandwidth of 1 nm and wavelength accuracy of ±0.3 nm with a pair of 10 mm matched quartz cells. The wavelength range of 200 nm to 400 nm was selected for scanning with medium scanning speed.

Procedure for preparation of solution for spectrophotometric determination

A series of drug solutions 1-80 µg/ml, 1-60 µg/ml, 5-80 µg/ml and 1-60 µg/ml for method B, C, D and E respectively were scanned (200-400 nm) against their reagent blank i.e. 0.1 N HCl for method B, sodium acetate buffer (pH 4.0) for method C, phosphate buffer (pH 7.0) for method D and borate buffer (pH 9.0) for method E and the absorption spectra were recorded. The absorption maximum (λ_{max}) was observed at 305 nm, 305 nm, 303 nm and 297 nm for methods B, C, D and E respectively and the absorbance was recorded against each concentration.

A graph was drawn by taking the concentration of the drug solutions on the x-axis and the corresponding absorbance values on the y-axis for methods B, C, D and E.

Forced degradation studies

Stress studies were performed to evaluate the specificity of the method. All the samples (1 mg/ml) were exposed to stress degradation conditions and diluted with mobile phase (100 µg/ml) and filtered prior to injection [25].

Acid and base induced degradation products

Acidic and alkaline degradations were performed by treating the drug solution (1 mg/ml) with 0.1 N hydrochloric acid and 0.1 N sodium hydroxide respectively. The solutions were refluxed for 1 h at 80 °C, cooled, neutralized and diluted with mobile phase as per the requirement.

Oxidation-induced degradation products

Oxidation degradation was performed by treating the drug solution (1 mg/ml) with 30% hydrogen peroxide. The solution was refluxed for 1 hour at 80 °C, cooled and diluted with mobile phase as per the requirement.

Thermal induced degradation products

For thermal stress testing, 1 mg/ml drug solution was heated in a thermostat at 80 °C for 1 hour, cooled, filtered and diluted as per the requirement before use.

Method validation

Linearity

A series of CTB solutions 0.5–150 µg/ml were prepared from the stock, diluted with mobile phase and 20 µl was injected into the HPLC system. The peak area of each chromatogram was noted. A calibration curve was plotted by taking a concentration of the CTB solutions on the x-axis and the corresponding peak area on the y-axis [25].

Precision and accuracy

The intra-day and inter-day precision of the method were calculated at three different concentration levels (10, 50 and 100 µg/ml) and on three different days respectively and the % RSD was calculated. The accuracy of the assay method was calculated at three different levels (80, 100 and 120%), and the percentage recoveries were calculated [25].

Limit of quantification (LOQ) and limit of detection (LOD)

The limit of quantification (LOQ) and limit of detection (LOD) was based on the standard deviation of the response and the slope of the constructed calibration curve (n=3), as described in International Conference on Harmonization guidelines Q2 (R1) [17, 25].

Method robustness

The robustness of the assay method was established by introducing small changes in the HPLC conditions which included wavelength (238 and 242 nm), the percentage of acetonitrile in the mobile phase (63 and 67%) and flow rate (0.4 and 0.6 ml/min). Robustness of the method was studied using three replicates at a concentration level 100 µg/ml CTB [25].

Assay of marketed formulations of CTB (tablet)

CTB is available as tablets with brand names CACIT 500 (500 mg; BIOCHEM limited, India.), CAPEGARD (500 mg; CIPLA Ltd, India) CAPGET (500 mg; GLS PHARMA Ltd, India) and procured from the local pharmacy store. The contents of each brand of CTB equivalent to 10 mg was extracted with methanol, sonicated and made up to volume with methanol in 10 ml volumetric flasks (1 mg/ml) and filtered. The dilutions were made from this stock as per the requirement for method B, C, D and E the percentage recovery was calculated.

RESULTS AND DISCUSSION

A detailed comparative study of the previously published methods with the present method is discussed in table 1. The satisfactory resolution was achieved with the use of a mixture of 0.1% acetic acid and acetonitrile (35:65, v/v) and C18 column was adopted for the analysis as it has provided a better separation of the analyzes. UV detection was carried out at 240 nm (PDA detector).

The present stability-indicating method for the determination of CTB in pharmaceutical formulations is specific because the drug peak was well separated even in the presence of degradation products. Overall, the data demonstrated that the excipients and the degradation products did not interfere with the CTB peak, indicating the selectivity of the method.

The complete separation of the analytics was accomplished in less than 10 min and the method can be successfully applied to perform long-term and accelerated stability studies of CTB formulations.

Table 1: Comparative table of CTB

Method	Mobile phase	Linearity	Detection	Reference
UV	Ethanol	5-25	307	
HPLC	ACN: phosphate 3.8	30-80	306	[6]
UV	water	5-25	240	[7]
UV	Water	5-40	304	
	Methanol	2-24	303	[8]
	Ethanol	5-25	306	
	Phosphate buffer 7.4	2-38	303	
	Acetonitrile	2-20	306	
Visible	Iodine+HCl+PMAP+Sac	25-150	520	[23]
	Tannic acid+metal volume	10-60	560	
UV	Methanol	2.5-15	243.60	[9]
RP-HPLC	0.05M phosphate buffer (pH 3.0±0.05) buffer and acetonitrile (50:50 % w/v)	70-120	240	[14]
RP-HPLC	70% Ammonium acetate with pH 5.0 and 30% acetonitrile	623.69-8400.59 mg/ml		[15]
RP-HPLC	Phosphate buffer: Acetonitrile (80:20) v/v	50-150	240	[16]
UV	Methanol	5-25	295	[10]
RP-HPLC	Methanol: buffer (70:30)	10-50		[17]
UV	Methanol	3-15	263,281,293	[11]
RP-HPLC	Methanol: buffer (45:55)	10-60		
HPTLC	Chloroform: glacial acetic acid: methanol	100-600		
RP-HPLC	methanol: Acetonitrile: water (80:18:2 V/V)	20-120	230	[18]
RP-HPLC	Methanol: Acetonitrile: Water (50:30:20,v/v, pH adjusted to 4.6 using Triethylamine)	2-10	245	[19]
UV	Phosphate 3	10-20	256.3	[13]
UV	methanol	2-10	245	[12]
RP-HPLC-PDA	Ammonium acetate: methanol (35:65 v/v)	2-10	240	[20]
RP-HPLC	0.005 M dipotassium hydrogen rthophosphate (pH 6.8) and acetonitrile 70:30 v/v	25-300 mg/ml	240	[21]
RP-HPLC	Methanol: water	20-160	271	[22]

Table 2: Spectral characteristics of CTB

Parameters	Method B	Method C	Method D	Method E
Beer-Lambert's limits (µg/ml)	1-80	1-60	5-80	1-60
λ_{max}/Amplitude range (nm)	305	305	303	297
Molar extinction coefficient (Liter/mol. cm)	88.4001 x 10^3	96.3058 x 10^3	98.4619 x 10^3	143.0213 x 10^3
Sandell's sensitivity (µg/cm^2/0.001 absorbance unit)	0.04065	0.03731	0.03649	0.0251
Slope	0.0238	0.0262	0.0265	0.408
Intercept	0.0008	0.0065	0.012	0.0092
Correlation coefficient	0.9992	0.9991	0.9999	0.9993

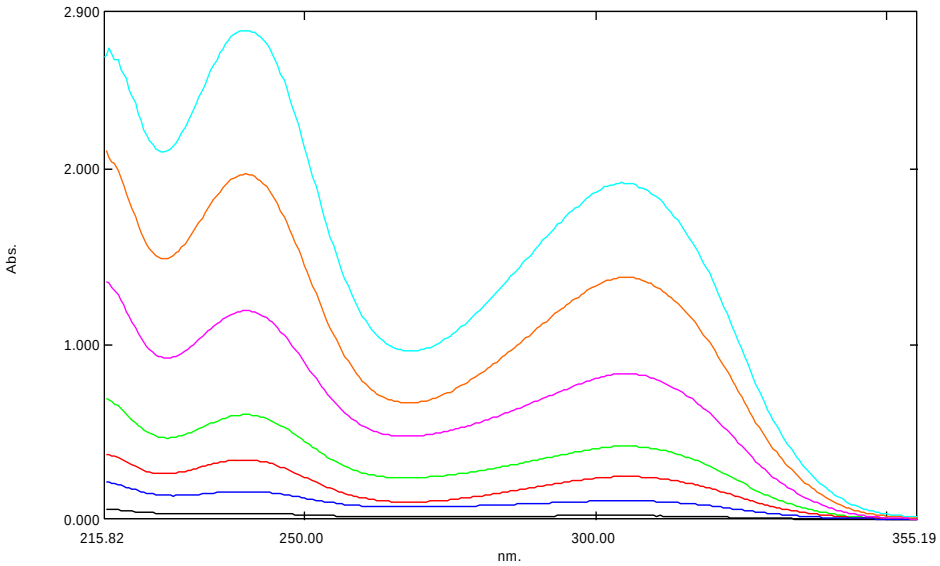

Fig. 2A: Absorption spectrum of CTB in 0.1 N HCl

Spectroscopic method

UV spectrophotometric methods were developed in HCl, sodium acetate buffer (pH 4.0), phosphate buffer (pH 7.0), and borate buffer (pH 9.0) and were recorded. Beer's law was obeyed over the concentration range 1-80 µg/ml, 1-60 µg/ml, 5-80 µg/ml and 1-60 µg/ml for the methods with regression equations 0.0238x+0.0008, 0.262x+0.0065, 0.0265x+0.012 and 0.408x–0.0092 for methods B, C, D and E respectively (table 2), The resulting overlay spectra were shown in fig. 2A, 2B, 2C and 2D and the calibration curves obtained were shown in fig. 3A, 3B, 3C and 3D. The % RSD values in precision studies were found to be 0.31, 0.24, 0.43 and 0.22 for method B, C, D and E respectively (RSD<2%) indicating that the method is more precise. The % RSD values in accuracy studies were found to be 0.457, 0.624, 0.384 and 0.642 for method B, C, D and E respectively (RSD<2%) indicating that the method is more accurate.

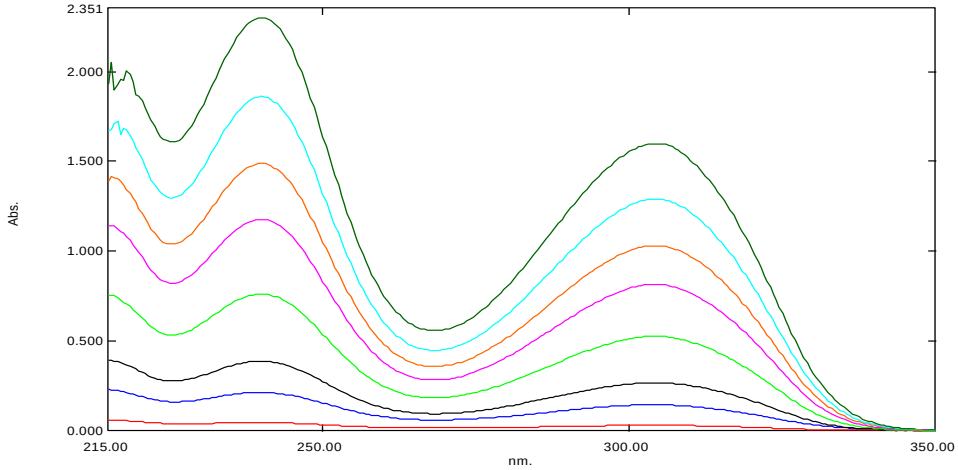

Fig. 2B: Absorption spectrum of CTB in sodium acetate buffer (pH 4.0)

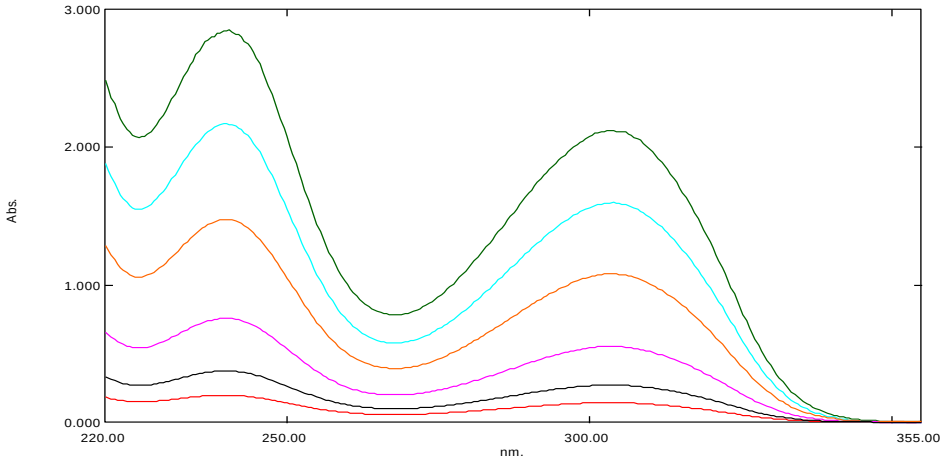

Fig. 2C: Absorption spectrum of CTB in phosphate buffer (pH 7.0)

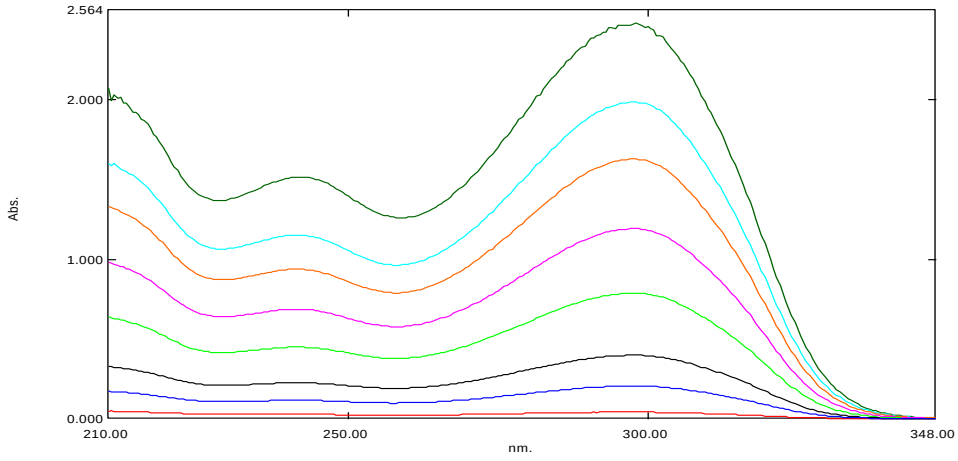

Fig. 2D: Absorption spectrum of CTB in borate buffer (pH 9.0)

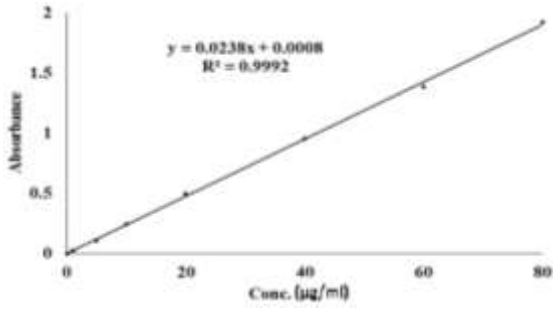

Fig. 3A: Calibration curve of CTB in 0.1 N HCl

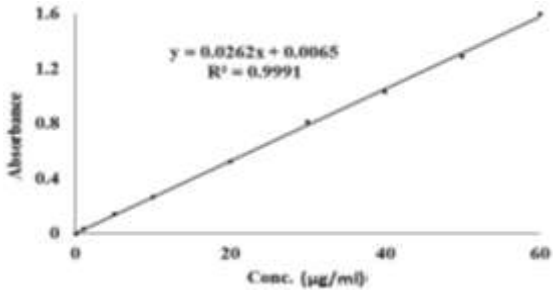

Fig. 3B: Calibration curve of CTB in sodium acetate buffer (pH 4.0)

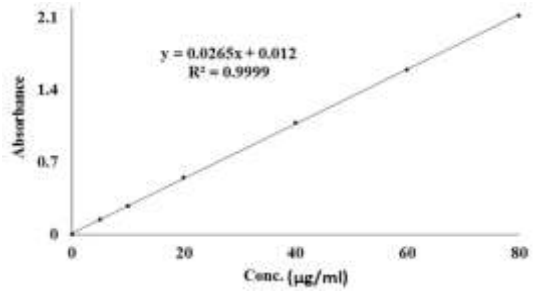

Fig. 3C: Calibration curve of CTB in phosphate buffer (pH 7.0)

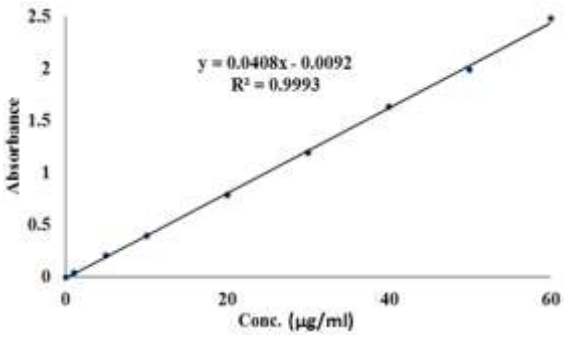

Fig. 3D: Calibration curve of CTB in borate buffer (pH 9.0)

HPLC method development and optimization

Initially, the pure drug sample solutions were analyzed using a mobile phase consisting of TBHS: acetonitrile (30:70, v/v) at a flow rate of 0.8 ml/min. Under these conditions, a sharp peak was observed at 2.089 min with fronting and the same was continued even though the mobile phase composition was changed to 45:55 or 35:65 or the flow rate was changed to 0.9 ml/min. So finally the mobile phase was totally changed to 0.1% acetic acid: acetonitrile (35:65, v/v) with a flow rate of 0.5 ml/min under which peaks were well resolved with good symmetry with a retention time of 3.26 min. Therefore, the mobile phase containing 0.1% acetic acid: acetonitrile (35:65, v/v) was chosen as the best chromatographic response for the entire study.

Forced degradation studies

CTB standard and tablet powder was found to be quite stable under dry heat conditions. Major decomposition was seen on exposure of CTB drug solution to acidic (81.17%), alkaline (83.67%), oxidation (26.66%), thermal (21.44%) and photolytic (9.54%) degradations indicating that the drug is highly resistant towards the above degradations (table 3). An extra peak was observed at 2.699 min for acidic reaction, at 2.621 min and 2.892 min for alkaline reaction, at 2.620 min and 4.312 min for thermal and at 2.628 min for the oxidation reaction. Typical chromatograms of standard and degradations studies of CTB are showed in fig. 4A, 4B, 4C, 4D, 4E and 4F.

Table 3: Results of degradation studies

Degradation studies	*Mean peak area±SD (RSD)	*Mean drug recovered (%)±SD (RSD)	*Mean drug decomposed (%)±SD (RSD)
Standard Drug	8873190.33±19453.70 (0.22)	-	-
Acid	1663184.33±15863.07 (0.95)	18.75±0.18 (0.98)	81.25±0.18 (0.23)
Alkaline	1451970.33±12129.83 (0.84)	16.37±0.13 (0.80)	83.63±0.13 (0.16)
Oxidation	6496443.33±20476.04 (0.32)	73.25±24 (0.33)	26.75±0.24 (0.91)
Thermal	6960681.67±4453.33 (0.06)	78.49±0.08 (0.10)	21.51±0.08 (0.37)
U. V	8023375.00±9048.32 (0.11)	90.47±0.05 (0.06)	9.53±0.05 (0.53)

*Each value is average of three determinations [±SD (RSD)]

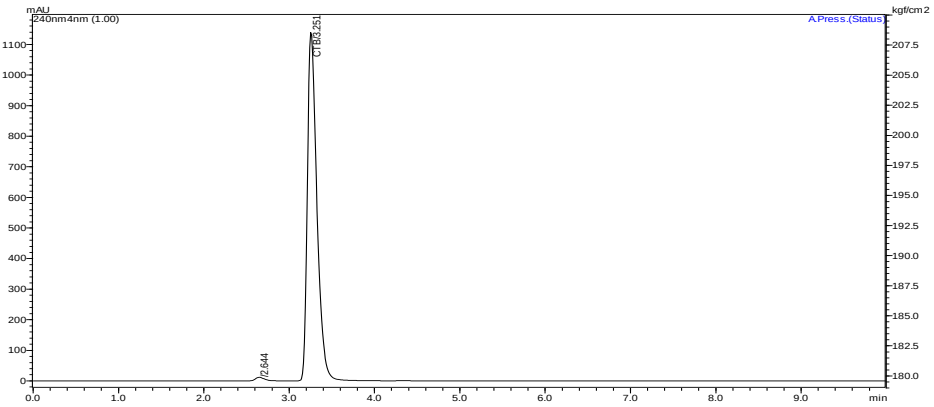

Fig. 4A: Typical chromatogram of standard of CTB

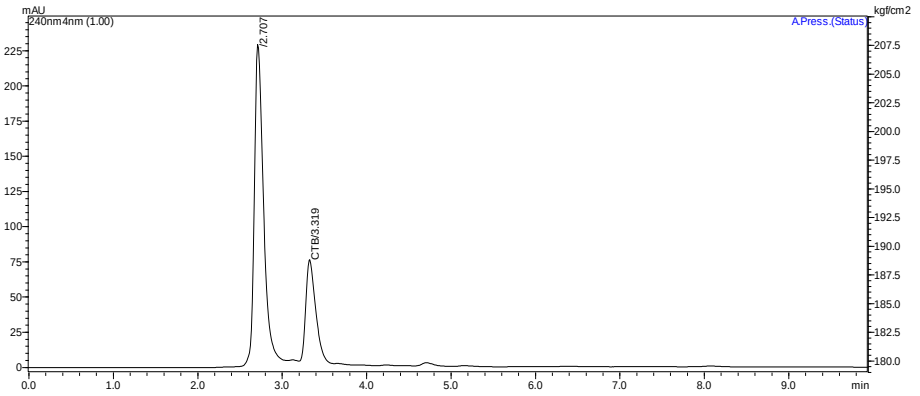

Fig. 4B: Typical chromatogram of acid degradation of CTB

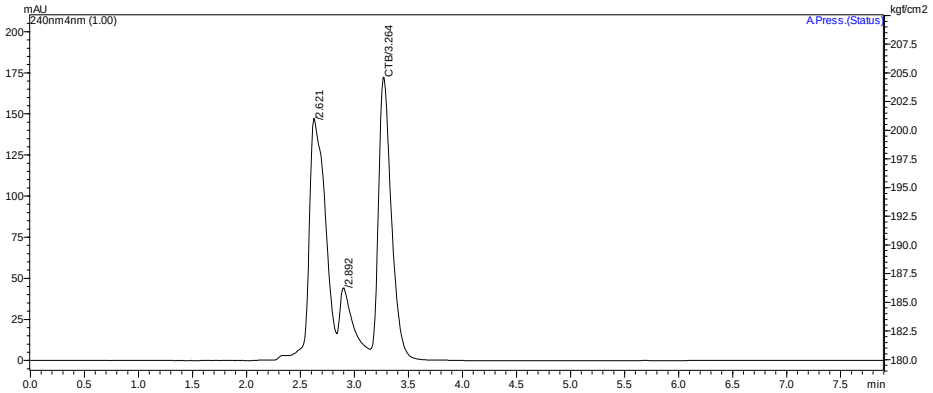

Fig. 4C: Typical chromatogram of base degradation of CTB

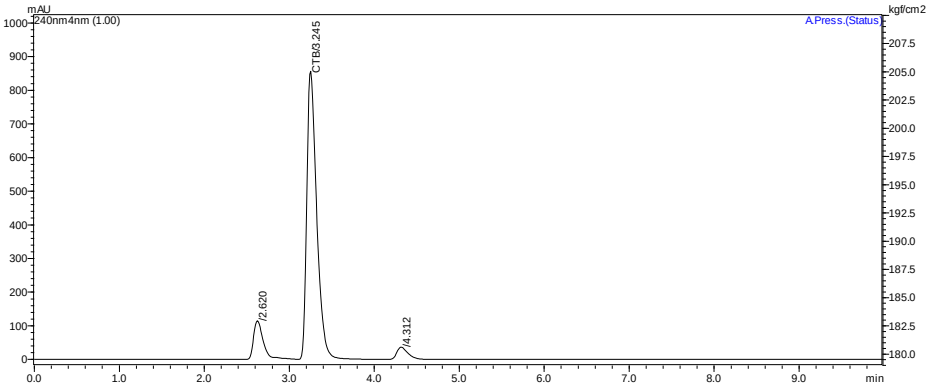

Fig. 4D: Typical chromatogram of thermal degradation of CTB

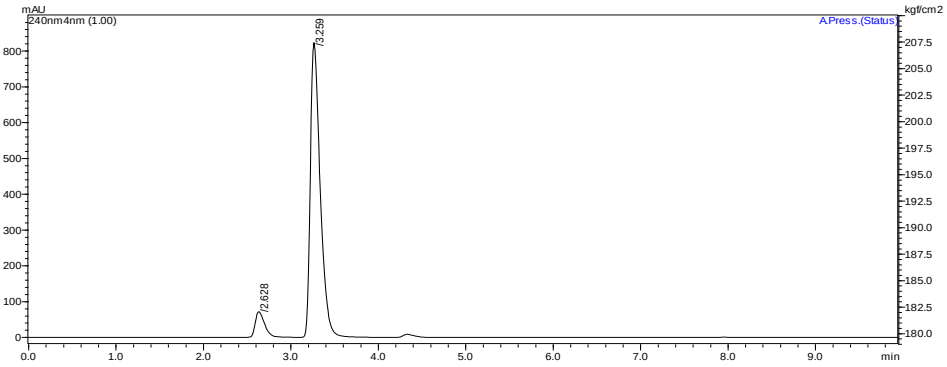

Fig. 4E: Typical chromatogram of oxidation degradation of CTB

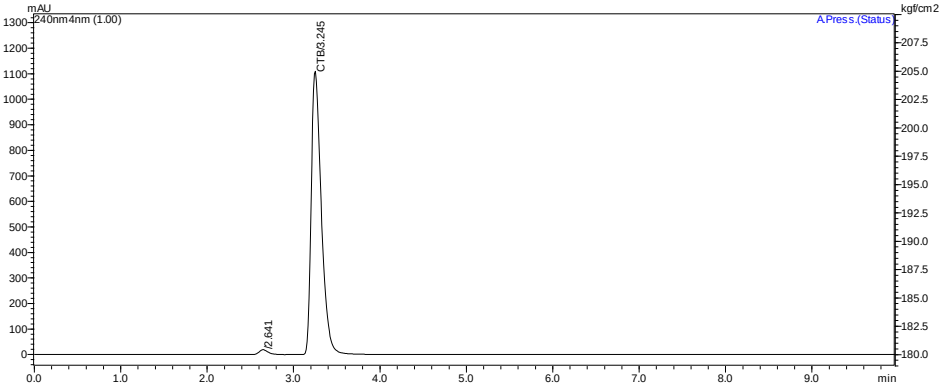

Fig. 4F: Typical chromatogram of protolytic degradation of CTB

Method validation

System suitability

The system suitability test was performed to ensure that the complete testing system was suitable for the intended application. The parameters measured were peak area, retention time, tailing factor, capacity factor and theoretical plates. In all measurements the peak area varied less than 2.0%, the average retention time was 3.26±0.02 min. The capacity factor was more than 2, theoretical plates were 3500 (more than 2000) and the tailing factor was 1.594 (less than 2) for the CTB peak. The LOQ was found to be 0.02354 µg/ml and the LOD was found to be 0.07162 µg/ml

Linearity

The typical chromatograms for CTB obtained from the bulk and extracted marketed formulations. The calibration curve for CTB was linear over the concentration range 0.5–150 µg/ml.

The data for the peak area of the drug corresponding to the concentration was treated by linear regression analysis (table 4) and the regression equation for the calibration curve was found to be y = 86247x+60749 with a correlation coefficient of 0.9994 which nearly equals to unity. The calibration curves obtained were shown in fig. 5. The % RSD range was 0.14-0.50.

Table 4: Linearity results of CTB

Conc. (µg ml^{-1})	Mean area±SD		RSD (%)
	Mean area	SD	
0.5	56321	182.48	0.324
1	84488	239.10	0.28
5	464641	2309.27	0.50
10	941821	3060.92	0.33
20	1755501	4810.07	0.27
50	4485992	6370.11	0.14
100	8893822	22234.56	0.25
150	12825530	60279.99	0.47

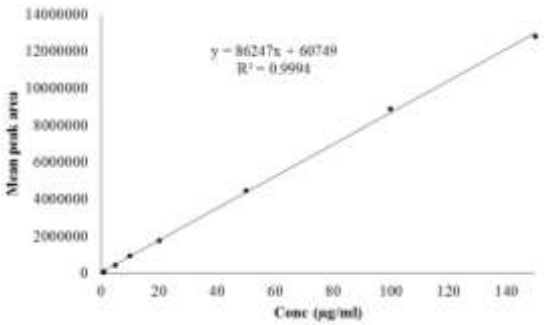

Fig. 5: Calibration curve of CTB in 0.1% acetic acid: acetonitrile

Precision

The precision of the method was determined by repeatability (intraday precision) and intermediate precision (inter-day precision) of the CTB standard solutions. Repeatability was calculated by assaying three samples of each at three different concentration levels (20, 50 and 100 µg/ml) on the same day. The inter-day precision was calculated by assaying three samples of each at three different concentration levels (20, 50 and 100 µg/ml) on three different days. The % RSD range was obtained as 0.18-1.07 and 0.72-1.39 for intra-day and inter-day precision studies respectively (table 5).

Because the stability of standard solutions can also affect the robustness of analytical methods, the stability of standard solutions of the drug substance used in this method was tested over a long period of time (48 h). One portion of a standard solution was kept at room temperature, and the other portion was stored under refrigeration at approximately 4°C and the content of these solutions were regularly compared with that of freshly prepared solutions. No change in drug concentrations was observed for solutions stored under refrigeration. But it is recommended that the sample and standard solutions must, therefore, be freshly prepared in amber colored flasks to protect from light.

Accuracy

The method accuracy was proved by the recovery studies. A known amount of CTB standard (100 µg/ml) was added to aliquots of samples solutions and then diluted to yield total concentrations as 90, 100 and 110 µg/ml as described in table 5. The assay was repeated over 3 consecutive days. The resultant % RSD was in the range 0.38-1.08 (<2.0 %) with a recovery 100.42-101.39 %.

Robustness

The robustness of an analytical procedure refers to its ability to remain unaffected by small and deliberate variations in method

parameters and provides an indication of its reliability for routine analysis [20]. The robustness of the method was evaluated by assaying the same sample under different analytical conditions deliberately changing from the original condition. The detection wavelength was set at 238 and 242 nm (±2 nm), the ratio of percentage of acetic acid: acetonitrile in the mobile phase was applied as 33:67 and 37:63 (±2 %, v/v), the flow rate was set at 0.4 and 0.6 ml/min (±0.1 ml/min). The results obtained (table 6) from assay of the test solutions were not affected by varying the conditions and were in accordance with the results for original conditions. The % RSD value of assay determined for the same sample under original conditions and robustness conditions was less than 2.0% (0.12-0.62) indicating that the developed method was robust.

Table 5: Precision and accuracy

Precision			
Conc. (µg/ml)	Intra-day precision *Mean peak area±SD (%RSD)	Inter-day precision *Mean peak area±SD (%RSD)	
20	1775623.00±17426.16 (0.98)	1748594.00±24305.13 (1.39)	
50	4477307.00±7860.14 (0.18)	4470316.33±31967.72 (0.72)	
100	8804916.33±94368.59 (1.07)	8844759.33±119575.70 (1.35)	
Accuracy			
Conc. (µg/ml)	*Mean peak area±SD (% RSD)	Drug found (µg/ml)	*Recovery (%)
90	7876212.33±47141.95 (0.60)	90.62	100.69
100	8804916.33±94368.59 (1.07)	101.39	101.39
110	9587919.00±36055.51 (0.38)	110.46	100.42

*Each value is average of three determinations

Table 6: Robustness results of CTB

Parameter	**Condition**	***Mean peak area**	***Mean peak area**	**SD**	**RSD**
Flow rate (±0.1 ml/min)	0.4	8886535	8896003.33	10726.66	0.12
	0.5	8893822			
	0.6	8907653			
Detection wavelength (±2 nm)	238	8866545	8879855.00	15282.28	0.17
	240	8896544			
	242	8876476			
Mobile phase composition (water: acetonitrile) (±2 % v/v)	33-67	8887646	8924681.33	55713.28	0.62
	35-65	8897644			
	37-63	8988754			

*Each value is average of three determinations

Table 7: Assay of commercial formulations

Brand	**Labeled amount (mg)**	***Amount obtained (mg)**				**% Recovery***			
		Method				**Method**			
		A	**B**	**C**	**D**	**A**	**B**	**C**	**D**
CACIT	500	499.45	494.45	496.15	495.26	99.89	98.89	99.23	99.05
CAPEGARD	500	497.45	495.15	499.12	498.45	99.49	99.03	99.82	99.69
CAPGET	500	494.45	490.23	492.45	494.23	98.89	98.04	98.49	98.84

*Each value is average of three determinations

Selectivity/specificity

The specificity of the developed method was determined by injecting sample solutions (100 µg/ml) which were prepared by forcibly degrading under stress conditions such as heat, light, oxidative agent, acid, and base under the proposed chromatographic conditions. The stability indicating the capability of the method was established from the separation of CTB peak from the degraded samples. The degradation of CTB was found to be very similar for both the tablets and standard.

Analysis of commercial formulations (tablets)

The proposed method was applied for the determination of CTB in tablets CACIT 500 (500 mg), CAPGET (500 mg)and the results show 98.98-99.32 % recovery (table 7) indicates that the method is selective for the assay of CTB without interference from the excipients used in these tablets.

CONCLUSION

The proposed stability-indicating HPLC method was validated and applied for the determination of CTB in pharmaceutical dosage forms. The method was found to be accurate, precise, robust and specific as the peak drug elution did not interfere with any degradants during the forced degradation studies and therefore the proposed method can be successfully applied to perform the analysis of the samples during the studies.

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CONFLICTS OF INTERESTS

The authors declare that they have no conflicts of interest

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