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Spectrophotometric determination of Capecitabine in Pharmaceutical Formulations

Medikondu Kishore[1]*, M.Jayaprakash[2], T.Vijayabhaskarareddy[3]

[1]Department of Post-Graduation Chemistry, SVRM College and Research center, Nagaram, Guntur (District), Andhra Pradesh, India-522268

[2]Executive,NatcoResearchCentre,B-13,IndustrialEstate,Sanathnagar,Hyderabad Andhra Pradesh, India-500018

[3]Jr.Manager, QualityAssurance, DrReddysLaboratories, Bachupally-FTO-III, Hyderabad Andhra Pradesh, India.

***Corres. Author :medikissi@gmail.com**

Abstract: Rapid, simple and sensitive spectrophotometric methods are presented for the determination of Capecitabine. The methods are based on their oxidation and precipitation reactions. In both methods the reactions can be monitored spectrophotometrically by measuring the absorbance of the produced complexes at 520 and 560 nm. The proposed methods have permitted the quantification of Capecitabine over linearity in the range of 10-120 mg/ml and its percentage recovery was found to be 99.65-99.93 %.
Key words: Capecitabine, spectrophotometric methods, Precipitation reactions, statistical analysis, recovery studies.

INTRODUCTION:

Capecitabine (N4-pentoxycarbonyl-5'-deoxy-5-fluorocytidine) (CPTB) is a fluoropyrimidine carbamate with antineoplastic activity and it is in a class drugs known as anti metabolites. The chemical structure of Capecitabine was shown in Figure 1. Capecitabine is used in the treatment of metastatic breast and colorectal cancers. 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 [14-15]. 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.Extensive pharmacokinetic studies have been performed on capecitabine and its metabolites [1,2] based on phases II and III trials [2–4]. Marked inter-patient variability was observed during these studies, although pharmacokinetic parameters were not predictive of either toxicity or response to treatment[2]. A very few physico- chemical methods appeared in the literature for the assay of CPTB in biological fluids and pharmaceutical formulations. Most of them are based on HPLC [10,11,13,14,17-22] and LC-UV[5,8], LC–MS[7], LS-MS/MS[6,9,12,23], MB[24] methods have been developed over the recent years to study capecitabine and its metabolites. The method is fully validated for both preclinical and clinical studies and can therefore be the basis for further preclinical and clinical studies with capecitabine. The analytically useful functional groups in CPTB have not been fully exploited for designing suitable visible spectrophotometric methods and so still offer a scope to develop visible spectrophotometric methods with better sensitivity, selectivity, precision and accuracy. The author has

made some attempts in this direction and succeeded in developing two methods for the determination of CPTB using appropriate reagents such as I_2/PMAP-SAc (M_1), TA/PMAP-Cr (VI) (M_2). A reported UV spectrophotometric method has been adopted for the determination of CPTB in pharmaceutical formulations (tablets) and used as reference method to compare the results obtained by the proposed methods.

EXPERIMENTAL

Instruments used: An Elico made UV-Visible digital spectrophotometer with 1cm matched quartz cells were used for the spectral and absorbance measurements. An Elico LI-120 digital pH meter was used for pH measurements.

Preparation of standard drug solutions: 1 mg/ml solution was prepared by dissolving 100 mg of pure CPTB in 100 ml of distilled water and this stock solution was diluted step wise with distilled water to get the working standard solutions of concentration of 40μg/ml

Preparation of reagents: All the chemicals and reagents used were of analytical grade and solutions were prepared in triply distilled water, isopropyl alcohol or chloroform.

Method M_1: *I_2 solution* (E.Merck; 0.089%, 3.5×10^{-3} M): Prepared by dissolving 89 mg of Iodine and 830mg of KI in 100 ml of distilled water and standardised iodometrically.

PMAP solution (Loba; 2%, 5.807×10^{-2}M): Prepared by dissolving 2g of p-N-methyl aminophenol sulphate in 100ml of distilled water.

SAc solution (Sd-fine; 0.4%, 2.309×10^{-2}M): Prepared by dissolving 400mg of sulphanilic acid in 100ml of distilled water

Hydrochloric acid (E.Merck, 1M): Prepared by diluting 217.5ml of concentrated HCI to 500ml with distilled water and standardised. Twenty ml of this standard solution was further diluted to 100 ml with distilled water to obtain 1M HCI solution

Method M_2: *TA solution* (Loba 0.2%, 1.17×10^{-3}M); Prepared by dissolving 200mg of Tannic acid in 100 ml of distilled water.

PMAP solution (Loba, 0.3%, 8.71×10^{-3}M): Prepared by dissolving 300mg of P-N-methyl amino phenol sulphate in 100 ml of distilled water

Cr (VI) solution (BDH, 0.3%, 1.01×10^{-2}M): Prepared by dissolving 300mg of Potassium dichromate in 100ml of distilled water

Buffer solution (pH-3): Prepared by diluting a mixture of 250ml of 0.2M Potassium acid phthalate and 204 mg of 0.1M HCl to 1L with distilled water and the pH was adjusted to 3.0

Recommended procedures: ***M_1***: Aliquots of standard CPTB solution (0.5-3.0 ml, 40 μg/ml) were delivered into a series of centrifuge tubes. Then 2 ml of (1M) HCI and 2 ml of I_2 were added successively. The volume was made up to 7ml with distilled water and kept aside for 15 min and centrifuged for 5 min. The precipitate was collected through filtration and subsequently washed with 2 ml of distilled water. The filtrate and washings were collected in a 25 ml-graduated tube. Then 3.0 ml of PMAP and 2.0 ml of SAc solutions were added successively and the volume was made up to the mark with distilled water. The absorbance was measured after 25 min at 520 nm against distilled water. A blank experiment was also carried out omitting the drug. The decrease in absorbance and in turn the drug concentration was obtained by subtracting the absorbance of the test solution from the blank and the amount of CPTB was calculated from Beer's law plot.

M_2: Aliquots of standard drug solution (0.5-3.0 ml 400 μg/ml) were delivered in to a series of centrifuge tubes and the volume in each tube was adjusted to 3.0 ml with 0.01 N HCl. Then 1.0 ml of Tannic acid was added and centrifuged for 5 min. The precipitate was collected through filtration and subsequently washed with 2.0 ml of distilled water. The filtrate and washings were collected in a 25ml-graduated test tube. Then 15ml of pH 3.0 buffer and 1.5 ml of PMAP solution were successively added. After 2 min, 2.0 ml of Cr (VI) solution was added and the volume was made up to the mark with distilled water. The absorbance was measured after 5 min at 560 nm against distilled water. A blank experiment was also carried out omitting the drug. The decrease in absorbance and in turn drug concentration was obtained by subtracting the absorbance of the test solution from the blank. The amount of drug was calculated from Beer's law plot.

For pharmaceutical formulations: An accurately weighed portion of tablet content equivalent to about 100 mg of CPTB was transferred into a 100 ml volumetric flask. Added about 80 ml of warm distilled water and shaken well for about 20 min. The contents were diluted with distilled water up to the mark and mixed thoroughly. The solution was filtered and the filtrate was evaporated to dryness. The residue was

used for the preparation of sample solution as under standard solution preparation. These solutions were analyzed as under procedures described for bulk solutions.

Reference Method[25]: An accurately weighed amount of formulation (tablets powder) equivalent to 100 mg was dissolved in a few ml of ethyl alcohol, evaporated to dryness and dissolved made up to 100 ml. 50ml of this filtrate was further diluted to 100 ml with distilled water to obtain to a concentration of 500 μg/ml. It was further diluted step wise with distilled water to get the concentration of 25 μg/ml. Aliquots of CPTB solution 1.0-5.0 ml, 25 μg/ml were taken into a series of 5ml calibrated tubes and made up to the mark with distilled water. The absorbance of each solution was measured at 250nm against distilled water. The concentration of the drug was computed from its calibration graph.

RESULTS AND DISCUSSION

Spectral Characteristics: In order to ascertain the optimum wavelength of maximum absorption (λ_{max}) of the colored species formed in the above methods, specified amounts of CPTB were taken and colors were developed separately by following the above procedures. The amounts of CPTB present in total volume of colored solutions were 50μg/ml (M_1), 4μg/ml (M_2). The absorption spectra were scanned on a spectrophotometer in the wavelength region of 340 to 900 nm against similar reagent blank. The reagent blank absorption spectrum of each method was also recorded against distilled water. The absorption curves of the colored species in each method show characteristics absorption maximum.

Optimum conditions fixation in procedures: The optimum conditions for the color development of methods were established by varying the parameters one at a time, keeping the others fixed and observing the effect produced on the absorbance of the colored species. The following experiments were conducted for this purpose and the conditions so obtained were incorporated in recommended procedures. The optimum conditions established for both methods were given in Table 1a&1b.

Table 1a. Optimum conditions established in methods M_1

Parameter	Optimum range	Conditions in procedure	Remarks
λ_{max} (nm)	515-525	520	
Effect of volume (3.5×10^{-3}M) of iodine for the complete precipitation of CPTB	1.8-2.2 ml	2.0 ml	1.8ml of iodine was required for the complete precipitation of CPTB at upper Beer's law limits.
Volume of (1M) HCl	1.50 – 2.5 ml	2.0 ml	The development of color was slow with higher or lower acidity. 1.5-2.5ml was found to be best for attaining the high sensitivity.
Volume (5.807×10^{-2}M) of PMAP	3.0 – 4.0 ml	3.0 ml	3.0ml of p-N methylaminophenol sulphate was necessary for attaining the highest sensitivity.
Volume (2.309×10^{-2}M) of Sac	1.5-3.5 ml	2.0 ml	Lower volumes of sulphanilic acid delayed the attainment of maximum color intensity but gave longer period of stability, higher volumes speeded up color development but the duration of stability period was shortened.
Stability period of color after final dilution	25-48 min	25 min	The charge transfer complex possesses two components, PMBQMI (acceptor and oxidizing agent) and SAC (donor and reducing agent). So the stability of complex was less due to slow redox reaction.

Table 1b. Optimum conditions established in methods M_2

Parameter	Optimum range	Conditions in procedure	Remarks
λ_{max} (nm)	550-570	560	
Effect of acidity and volume of tannic acid (1.17×10^{-3}M) for precipitation	0.008-0.012 N	0.01 N HCl	Decrease in the volume lead to low absorbance values. If the volume of tannic acid is increased abnormally, the precipitate formed partially dissolves in it by producing erratic results.
	0.8-1.2 ml	1.0 ml	
Effect of metol volume and color development of the filtrate	1.2-1.8 ml	1.5 ml	1.2 – 1.8 ml of (8.71x 10^{-3}M) PMAP were found to be adequate for maximum color development.
Nature of oxidant on color development in combination with metol.	Cr (VI)	Cr (VI)	Oxidants such as Ce(IV), IO_4^-, $[Fe(CN)_6]^{3-}$, Fe (III). When used instead of Cr (VI) did not produce prominent colour.
	1.5 - 2.5 ml	2.0 ml	1.5 – 2.5 ml of (1.01x 10^{-2}M) Cr(VI) were necessary for maximum colour development.
Effect of time for maximum color development	2 - 5 min	5 min	2 min before and 5 min after addition of Cr(VI) gave maximum absorbance.
pH and volume of buffer on color development	13 - 17 ml	15 ml	15ml of buffer of p^H=3 is necessary for getting constant and reproducible absorbance values.
	pH 2.8-3.3	pH = 3	
Effect of order of addition of reagents	Buffer, Metol, Cr VI	Buffer, Metol, Cr VI	The absorbance is decreased if the order of addition is changed.
Nature of solvent for final dilution.	Water	Water	

Optical Characteristics: In order to test whether the colored species formed in above methods adhere to Beer's law, the absorbances at appropriate wavelength of a set of solutions containing varying amounts of CPTB and specified of amounts of reagents were recorded against the corresponding reagent blanks. The Beer's law plots of these recorded graphically. Beer's law limits, molar absorptivity, Sandell's sensitivity and optimum photometric range for CPTB in each method were calculated. Least square regression analysis was carried out for getting the slope, intercept and the correlation coefficient values (Table 2)

Precision: The precision of the proposed methods was ascertained from the absorbance values obtained by actual determination of six replicates of a fixed amount of CPTB in total solution. The percent relative standard deviation and percent range of error (at 0.05 and 0.01 confidence limits) were calculated for the proposed methods (Table 2).

Accuracy: To determine the accuracy of each proposed method, different amounts of bulk samples of CPTB within the Beer's law limits were taken any analyzed by the proposed method. The results (percent error) are recorded in Table 2.

Table 2: Optical, regression characteristics, precision and accuracy of the proposed methods

Parameter	M_1	M_2
λ_{max} (nm)	520	560
Beer's law limits (µg/ml)	25-150	10-60
Detection limit (µg/ml)	7.081	2.565
Molar absorptivity (1.mol/cm)	8.862×10^{2}	2.628×10^{3}
Sandell's sensitivity (µg/cm^2/0.001 absorbance unit)	0.6155	0.3012
Optimum photometric range (µg/ml)	40-125	126-250
Regression equation (Y=a+bc) slope (b)	0.0101	0.012077
Standard deviation on slope (S_b)	5.452×10^{-3}	1.5021×10^{-4}
Intercept (a)	8.249×10^{-3}	6.25×10^{-3}
Standard deviation on intercept (S_a)	4.520×10^{-3}	4.983×10^{-3}
Standard error on estimation (S_e)	4.310×10^{-3}	4.751×10^{-3}
Correlation coefficient (r)	0.9989	0.9997
Relative standard deviation (%)*	0.2041	1.359
% Range of error (confidence limits) 0.05 level	1.06	1.563
0.01 level	1.36	2.450
% error in Bulk samples**	0.10	0.102

*Average of three determinations, ** Average of six determinations

Interference studies: The effect of wide range of excipients and other active ingredients usually present in the formulations for the assay of CPTB in methods under optimum conditions were investigated. The commonly used excipients and other active ingredients usually present in formulations did not interfere even if they were present in amount than they usually exist.

Analysis of formulations: Commercial formulations (tablets) containing CPTB were successfully analyzed by the proposed methods. The values obtained by the proposed and reference methods for formulations were compared statistically with F and t tests and found not to differ significantly. The results are summarized in Table 3. Percent recoveries were determined by adding standard drug to penalized formulations. The results of the recovery experiments by the proposed methods are also listed in Table 3.

Table 3: Assay of CPTB in Pharmaceutical Formulations

Pharmulations*	Amount taken (mg)	Amount found by proposed Methods**		Reference method	Percentage recovery by proposed methods***	
		M_1	M_2		M_1	M_2
Tablet I	25.00	24.53 ± 0.42 F = 2.621 t = 1.417	24.69 ± 0.53 F = 1.646 t = 0.8302	24.98± 0.68	99.93± 0.14	99.71± 0.99
Tablet II	25.00	24.65 ± 0.32 F= 4 t = 0.9020	24.70 ± 0.40 F = 2.56 t = 0.6661	24.90± 0.64	99.65± 0.44	99.89± 0.98
Tablet III	25.00	24.60 ± 0.52 F = 2.4866 t = 0.7755	24.75±0.18 F = 2.918 t = 0.6928	24.95± 0.82	99.73± 0.75	99.81± 0.92
Tablet IV	25.00	24.20 ± 0.31 F = 4 t = 2.417	24.93±0.42 F = 1.653 t = 1.335	25.3± 0.54	99.85± 0.62	99.75± 0.99

*Tablets from four different pharmaceutical companies, **Average ± standard deviation of six determinations, the t-and F-test values refer to comparison of the proposed method with the reference method. Theoretical values at 95% confidence limit, F = 5.05, t = 2.57, ***Recovery of 10 mg added to the reanalyzed pharmaceutical formulations (average of three determinations).

Chemistry of the colored species: The chemistry of the colored species formed in each one of the proposed methods for the assay of CPTB has been presented in scheme 1 and 2.

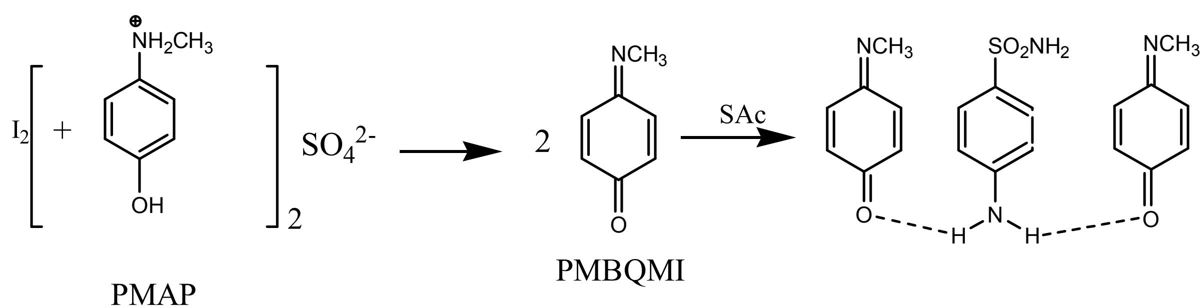
M_1: The method involves two steps. First step is the quantitative precipitation of CPTB with iodine. Second step is the formation of colored product between the unreacted iodine in the filtrate and the PMAP-SAc. The probable sequence of step reactions based on analogy are presented in the **scheme 1.**

M_2: The method involves quantitative precipitation of CPTB with tannic acid. (Step I). The liberated tannic acid from the precipitate on treatment with acetone was determined with PMAP-Cr VI at pH 3.0. Tannic acid contains gallic acid units. It is probable that colored species originate through the involvement of PMBQMI (forms in situ from PMAP – Cr VI) and gallic acid unit in tannic acid in the formation of a charge transfer complex. The probable sequences of reaction based on analogy are presented in **scheme 2.**

SCHEME 1

Step I: CPTB + I_2 → CPTB – I_2 (Precipitate) + I_2 (unreacted)

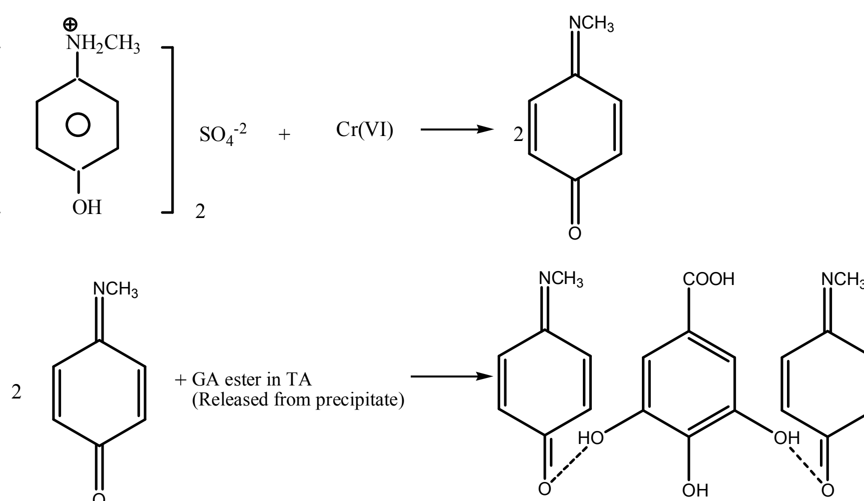
Step II



SCHEME 2

Step I: CPTB + TA → CPTB - TA (adduct Precipitate) + T.A (unreacted)

Step II



CONCLUSIONS

The proposed methods exploit the various functional groups in CPTB molecule. The concomitants, which do not contain the functional groups chosen in the present investigation, do not interfere in the color development by proposed method. Thus the proposed method is simple, sensitive or selective with reasonable precision and accuracy and constitutes better alternatives to the reported ones in the assay of CPTB in bulk form and pharmaceutical formulations.

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