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Novel derivative spectrophotometric methods for the quantification of Capecitabine

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ABSTRACT

For the assay of Capecitabine four new first derivative spectrophotometric methods have been developed in pharmaceutical dosage forms. First derivative spectrophotometric methods were developed in 0.1N HCl (Method A), sodium acetate (Method B), phosphate buffer pH 7.0 (Method C) and Borate buffer pH 9.0 (Method D). Linearity was observed over the concentration range 1-80, 1-60, 5-80 and 1-60 µg/mL for Method A, B, C and D respectively. The proposed derivative spectrophotometric methods were validated and can be applied for the determination of Capecitabine in pharmaceutical formulations (Tablets).

KEY WORDS: Capecitabine, 0.1N HCl, Sodium acetate, Phosphate buffer, Borate buffer, Derivative spectroscopy, Validation.

1. INTRODUCTION

Capecitabine is a fluoropyrimidine carbamate with antineoplastic activity and it is in a class drugs known as antimetabolites. It is a chemotherapeutic agent administered orally which is used in the treatment of metastatic breast and colorectal cancers. Capecitabine 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 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. Chemically it is 5'-deoxy-5-fluoro-N-[(pentyloxy) carbonyl] - cytidine with empirical formula of $C_{15}H_{22}FN_3O_6$ and the molecular weight of 359.35 g/moL (Indian Pharmacopoeia, 2010; United States of Pharmacopeia, 2008).

Literature review reveals that few analytical methods have been evoked for the estimation of Capecitabine by spectrophotometric (Sreenivasa Rao, 2012; Prashanthi, 2014; Kumbhar, 2013; Rao, 2014; Jothieswari, 2014; Kandimalla, 2012; Naveen Kumar, 2013; Patel, 2015), HPLC (Raju, 2011; Jayaseelan, 2010; Sreevatsav, 2013; Macula, 2012; Devanaboyina, 2013; Ravisankar, 2013; Nalluri, 2014; Venkateswara Rao, 2008; Edla, 2012), Mass spectroscopy (Christoph, 2004) and visible spectroscopy (Medikondu, 2011) methods were reported. In present study the authors were developed a sensitive, accurate and reliable method for the estimation of Capecitabine in bulk and pharmaceutical dosage forms. So, the authors have made an attempt to develop spectrophotometric methods in four different buffer solutions for the determination of Capecitabine in ophthalmic solutions and validated as per ICH guidelines.

2. MATERIALS AND METHODS

2.1. Instrumentation: A double beam UV-VIS spectrophotometer (UV-1800, Shimadzu, Japan) connected to computer loaded with spectra manager software UV Probe was employed with spectral bandwidth of 1nm and wavelength accuracy of 0±0.3 nm with a pair of 10 mm matched quartz cells. All weights were taken on electronic balance (Shimadzu).

2.2. Chemicals and reagents: Analytical grade Methanol (Merck), Disodium Phosphate (Na_2HPO_4) (Merck), Monopotassium phosphate (KH_2PO_4) (Merck), Boric Acid, Sodium hydroxide, Glacial acetic acid were used. Capecitabine was obtained as gift sample from Mylan Laboratories limited was used.

2.3. Preparation of 0.1N HCL: For preparation of 0.1N HCL, 8.5 mL of HCL is diluted with 1000ml of distilled water.

2.4. Preparation of sodium acetate buffer pH 4.0: For 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 100ml volumetric flask and made up the volume with HPLC grade water.

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

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

2.7. Preparation of stock and sample solutions: The standard solution of Capecitabine was prepared by dissolving accurately about 25 mg of the Capecitabine in methanol in a 25 mL volumetric flask. The stock solution was further diluted with 0.1N HCl, sodium acetate, phosphate buffer and Borate buffer as per the requirement for method A, B, C and D respectively.

2.8. Procedure for preparation of calibration curve: A series of Capecitabine solutions 1-80, 1-60, 5-80 and 1-60

µg/mL were prepared in 0.1N HCl, sodium acetate, phosphate buffer and Borate buffer for method A, B, C and D respectively and scanned (200-400 nm) against their reagent blank. The detection of absorption maxima i.e. λ_{max} were converted in to first derivative spectra by the inbuilt software of the instrument for further investigation for methods A, B, C and D. The amplitude was chosen from the first derivative spectra for further analytical calculations for all the stated methods.

A graph was drawn by taking the concentration of the drug solutions on the x-axis and the corresponding derivative absorbance on the y-axis for all the methods.

2.9. Assay of marketed formulations of Capecitabine: Capecitabine is available as tablets with brand names CACIT 500 (500mg; BIOCHEM limited, India.), CAPEGARD (500 mg; CIPLA Ltd, India) CAPGET (500mg; GLS PHARMA Ltd, India) and procured from the local pharmacy store.

To perform the assay of QC samples of Capecitabine twenty tablets were procured from the local pharmacy store and the contents were extracted with 0.1N HCl, sodium acetate, phosphate buffer and Borate buffer for method A, B, C and D respectively and the dilutions were made from this stock as per the requirement and the percentage recovery was calculated.

2.10. Precision and accuracy: Precision study was performed by taking the derivative absorbance of six replicates (20 µg/mL) were measured for Method A, B, C and D and the % RSD was calculated. Accuracy of the method was evaluated by the percent recovery studies. This study was performed by adding 80%, 100%, and 120% of pure drug solutions to a constant concentration of extracted formulation (pre-analysed formulation) solution and the % RSD was calculated.

3. RESULTS AND DISCUSSION

For the determination of Capecitabine four new first derivative spectrophotometric methods were developed in 0.1N HCl, sodium acetate, phosphate buffer and Borate buffer. The resulting derivative spectra were shown in Figure 2, 3, 4 and 5. The derivative absorbance observed at the amplitude was chosen for method A (232.34 - 250.62 nm), B (232.09–250.05nm), C (232.08–251.09nm) and D (250.20–279.50nm) against concentration for the construction of the calibration curve, along with zero crossing points at for method A (226.03, 240.20, 268.20, 305.11 nm), method B (225.04, 239.87, 268.20, 304.78 nm), method C (225.37, 239.87, 267.87, 303.79 nm) and method D (226.67, 242.17, 258.65, 297.86 nm) therefore the amplitude was selected for further calculations.

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 methods A, B, C and D with regression equations y = 0.0023x – 0.0006, y = 0.0029x + 0.0002, y = 0.0026x + 0.0017 and y = 0.0011x - 0.0002 respectively. The calibration curves obtained were shown in Figure 6A, 6B, 6C and 6D.

The % RSD in precision studies were found to be 0.342, 0.145, 0.485 and 0.124 for method A, B C and D respectively (RSD <2%) indicating that the method is precise. The % RSD in accuracy studies were found to be 0.221, 0.324, 0.548 and 0.144 for method A, B C and D respectively (RSD <2%) indicating that the method is more accurate.

The percentage recovery (Table 1) was found to be in the range of 98.89-99.89, 98.04-99.03, 98.49-99.82 and 98.84-99.69 for method A, B, C and D respectively (RSD <1.0 %) indicating that the proposed methods can be applied for the determination of pharmaceutical formulations successfully.

Table.1.Linearity for the derivative spectrophotometric methods of Capecitabine

Conc. (µg/mL)	Absorbance at λ (nm)					
	Method A			Method B		
	232.34	250.62	Amplitude	232	250	Amplitude
1	0	0.001	0.001	0.001	0.002	0.003
5	0.003	0.006	0.009	0.005	0.009	0.014
10	0.008	0.015	0.023	0.011	0.018	0.029
20	0.02	0.029	0.049	0.022	0.035	0.057
30	-	-	-	0.035	0.054	0.089
40	0.038	0.051	0.089	0.044	0.069	0.113
50	-	-	-	0.055	0.086	0.141
60	0.049	0.091	0.14	0.067	0.106	0.173
80	0.07	0.114	0.184	-	-	-

Table.2.Linearity for the derivative spectrophotometric methods of Capecitabine

Conc. (µg/mL)	Absorbance at λ (nm)					
	Method C			Method D		
	232	251	Amplitude	250.5	279.5	Amplitude
1	-	-	-	0	0.001	0.001
5	0.005	0.009	0.014	0.002	0.004	0.006
10	0.01	0.017	0.027	0.003	0.008	0.011
20	0.021	0.034	0.055	0.006	0.016	0.022
30	-	-	-	0.01	0.023	0.033
40	0.042	0.066	0.108	0.013	0.032	0.045
50	-	-	-	0.017	0.039	0.056
60	0.061	0.098	0.159	0.022	0.046	0.068
80	0.078	0.128	0.206	-	-	-

Table.3. Assay of marketed formulations of Capecitabine (Tablets)

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

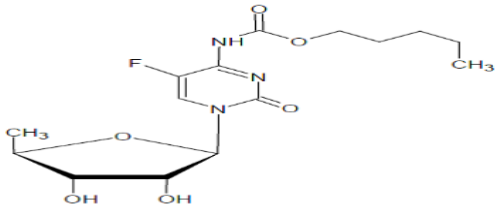

Figure. 1. Chemical structure of Capecitabine

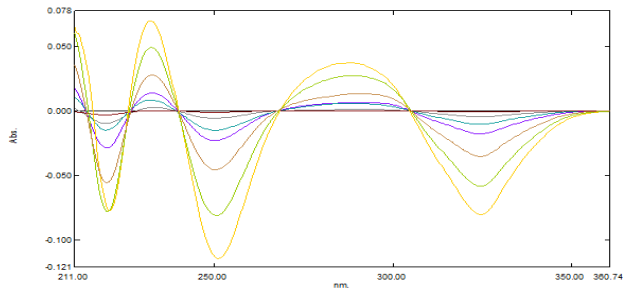

Figure. 2. Overlay first derivative spectrum of Capecitabine in 0.1N HCl

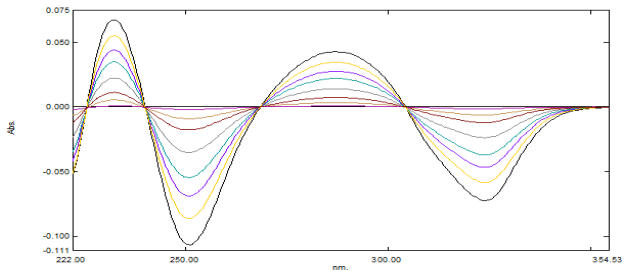

Figure. 3. Overlay first derivative spectrum of Capecitabine in sodium acetate pH 4.0

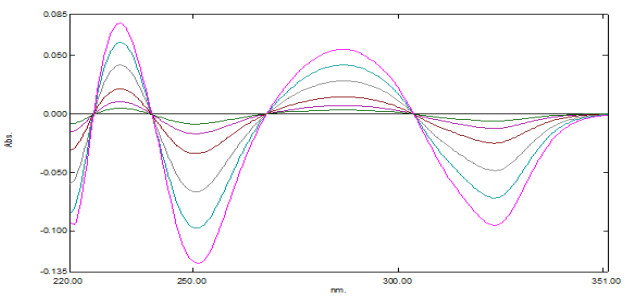

Figure. 4. Overlay first derivative spectrum of Capecitabine in phosphate buffer pH 7.0

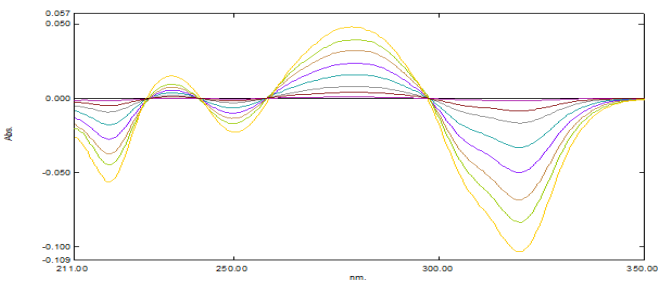

Figure. 5. Overlay first derivative spectrum of Capecitabine in Borate buffer (pH 9.0)

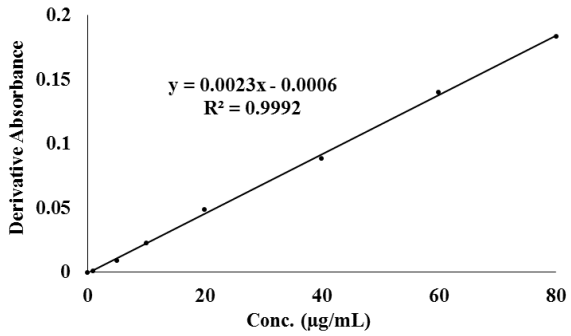

Figure.6A.Calibration curves of Capecitabine 0.1N HCl

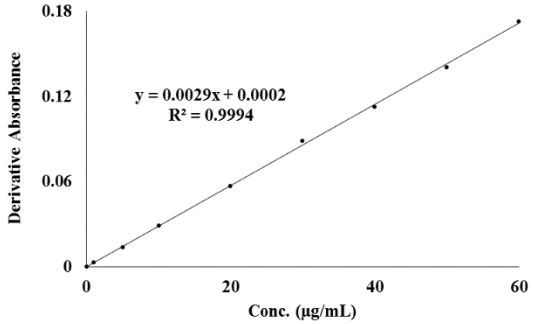

Figure.6B.Calibration curves of Capecitabine Sodium Acetate pH 4.0

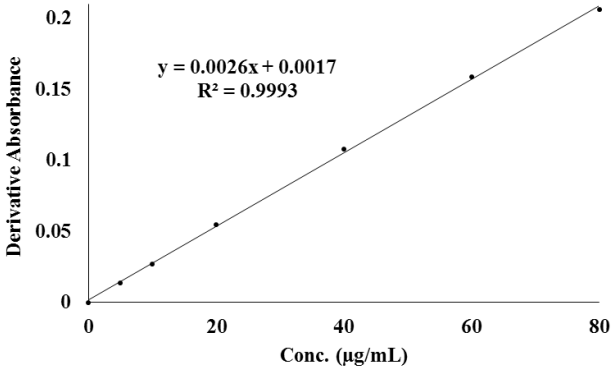

Figure.6C.Calibration curves of Capecitabine Phosphate buffer pH. 7.0

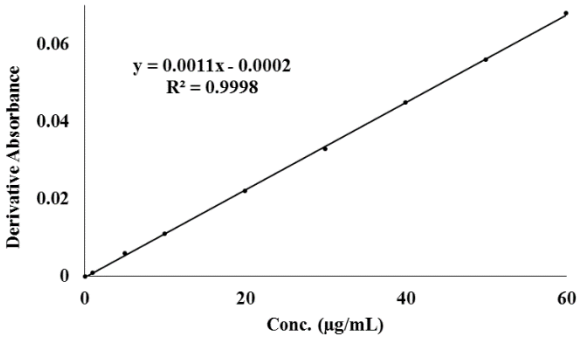

Figure.6D.Calibration curves of Capecitabine Borate buffer pH 9.0

4. CONCLUSION

The analytical techniques developed for the determination of Capecitabine are simple, precise and accurate and can be applied for the pharmaceutical formulations successfully.

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