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Der Pharmacia Sinica, 2015, 6(4):19-23

Der Pharmacia Sinica
ISSN: 0976-8688
CODEN (USA): PSHIBD

Development and validation of pH independent spectroscopic method for determination of Capecitabine

Shailja H. Patel* and Paresh U. Patel

Department of Quality Assurance, Shree S. K. Patel College of Pharmaceutical Education and Research, Ganpat University, Ganpat Vidyanagar, Mehsana, Gujarat, India

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ABSTRACT

A simple, sensitive and pH independent spectrophotometric method has been developed for the determination of capecitabine in pharmaceutical formulations. The method is based on the measurement of absorbance at isosbestic point. Isosbestic point for capecitabine was observed at 256.3 nm and absorbances were measured at same wavelength. The method is found to be linear in the range of 10–20 µg/mL for Capecitabine with R^2= 0.9987 (n=6). The proposed method is simple, rapid and gives accurate and precise results. Market formulations of capecitabine were analyzed with the proposed method. Results obtained are in good agreement with the labeled amount of capecitabine (99.7105± 0.2047, n=3) in tablet forms. The proposed method can be satisfactorily applied for estimation of capecitabine in pharmaceutical dosage form. The method can also be applied for estimation of capecitabine in aqueous medium irrespective of pH of solution.

Key words: UV spectrophotometry, Isosbestic point, pH independent, capecitabine

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INTRODUCTION

Capecitabine is a prodrug that is selectively tumour-activated to its cytotoxic moiety, fluorouracil, by thymidine phosphorylase, an enzyme found in higher concentrations in many tumors compared to normal tissues or plasma. Fluorouracil is further metabolized to two active metabolites, 5-fluoro-2'-deoxyuridine 5'-monophosphate (FdUMP) and 5-fluorouridine triphosphate (FUTP), within normal and tumour cells. These metabolites cause cell injury by two different mechanisms. First, FdUMP and the folate cofactor, N5-10-methylenetetrahydrofolate, bind to thymidylate synthase (TS) to form a covalently bound ternary complex. This binding inhibits the formation of thymidylate from 2'-deaxyuridylate.[1]Chemically it is a (1-(5-Deoxy-beta-D-ribofuranosyl)-5-fluoro-1,2-dihydro-2-oxo-4-pyrimidinyl)-carbamic acid pentyl ester (figure 1). It is white to off-white crystalline powder, odourless powder and practically insoluble in water (2.05 mg/L), slightly soluble in alcohol, soluble in chloroform[2]. It is official in Indian Pharmacopoeia[2], United State Pharmacopoeia[4]. Literature survey reveals HPLC[5,6,7] and UV spectrophotometry[8,9] and colorimetry[10] and HPTLC[11] methods for estimation of CAP in single dosage form.

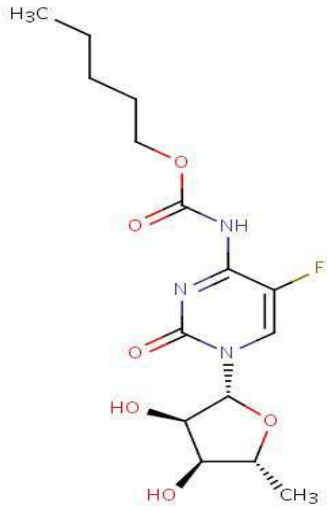

Figure 1: Chemical structure of Capecitabine (CAP)

MATERIALS AND METHODS

A shimadzu model 1600 (Japan) double beam UV/Visible spectrophotometer with spectral width of 2 nm, wavelength accuracy of 0.5 nm and a pair of 10 mm matched quartz cell was used to measure absorbance of all the solutions. Spectra were automatically obtained by UV-Probe system software. A Sartorius CP224S analytical balance (Gottingen, Germany), an ultrasonic bath (Frontline FS 4, Mumbai, India), Digital pH meter (LI 712 pH analyzer, Elico Ltd., Ahmedabad) was used in the study. CAP bulk powder was kindly gifted by was kindly supplied as a gift sample from Intas Pharma Pvt. Ltd., Ahmedabad. Tablet of Capecitabine was purchased from local pharmacy.

Method

In pH independent spectrophotometric method the isosbestic point of drug solutions in different pH were measured. (Figure 2). For this measurement, equimolar solution of Capecitabine was prepared separately in Phosphate Buffer pH 3.0 as well as in Ammonia Buffer pH 10.9 at a concentration of 20 µg /ml. They were scanned in the wavelength range of 200-400 nm. The isobestic points was recorded at 256.3 nm There was no change in isobestic points, which reveals that there was no interference by additives.

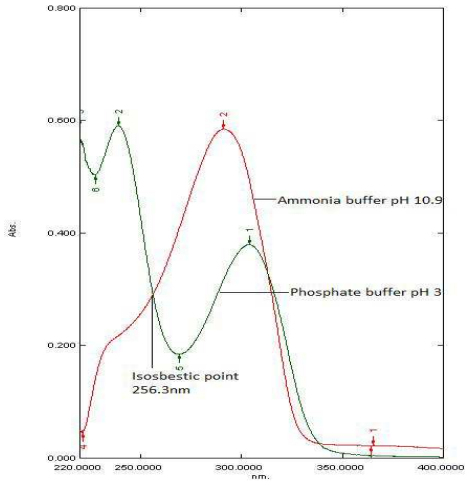

Figure 2: Overlay spectra of CAP in Phosphate buffer pH 3 and in Ammonia buffer pH 10.9

A standard stock solution of Capecitabine (100 µg/ml) was prepared by dissolving 10 mg of pure drug powder to 100 ml volumetric flask separately with Phosphate buffer pH 3 and Ammonia buffer pH 10.9.Aliquots of standard stock solution of Capecitabine was suitably diluted with Phosphate buffer pH 3 and Ammonia buffer pH 10.9 to obtain the final concentration in the range of 10-20 µg/ml. The solution was scanned in the range of 200 nm to 400 nm against water as a blank, to obtain the absorbance. The absorbance is measured at 256.3 nm. The calibration curve was prepared by plotting concentration of Capecitabine vs. Absorbance of solution at isosbestic point.

METHOD VALIDATION

The proposed method was validated according to the International Conference on Harmonization (ICH) guidelines [12]

Linearity (Calibration curve):

Calibration curve (linearity)

Calibration curves were plotted over a concentration range of 10-20 µg/ml for CAP. Accurately measured standard working solutions of CAP (1, 1.2, 1.4, 1.6, 1.8 and 2.0 ml) were transferred to two sets of a series of 10 ml of volumetric flasks separately and set-1 is diluted to the mark with Phosphate buffer pH 3 and set-2 is diluted with Ammonia buffer pH 10.9. Absorbance of all the solutions was measured at 256.3 nm against water as blank. Calibration curves were constructed by plotting absorbance vs. concentration of CAP and the regression equations were calculated.

Accuracy (% Recovery)

The accuracy of the proposed method was determined by calculating recoveries of CAP by standard addition method. Known amounts of standard solutions of CAP were added at 50%, 100% and 150% levels to prequantified solutions of CAP (20 µg/ml).

Repeatability

The precision of the instrument was checked by repeated scanning and measuring the absorbance of solutions (n=6) of CAP(18 µg/ml) without changing the parameters of the proposed method. The results are reported in terms of percentage relative standard deviation (%RSD).

Intermediate precision

The intraday and interday precision of the proposed method was determined by analyzing the corresponding responses 3 times on the same day and on 3 different days over a period of 1 week for 3 different concentrations of standard solutions of CAP(14, 16 and 18 µg/ml). The results are reported in terms of percentage relative standard deviation (%RSD).

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

The limit of detection (LOD) and limit of quantification (LOQ) of the proposed method were calculated by using the following equations as per ICH guideline.

$$\mathbf{LOD = 3.3 \times \sigma / S}$$

$$\mathbf{LOQ = 10 \times \sigma / S}$$

Where, σ = the standard deviation of the response, S = slope of the calibration curve

Estimation of Capecitabine in its dosage form

For analysis of Capecitabine in tablet dosage form, twenty tablets were accurately weighed and powdered. A quantity of accurately weighed the tablet powder equivalent to 50 mg of Capecitabine was transferred to 2 sets of 100 ml volumetric flask containing 60 ml Phosphate buffer pH 3 and Ammonia buffer pH 10.9, sonicated for 10 min. Finally volume was made up to the mark with buffer solutions and further shaken for 15 min for complete extraction of from its matrix. Above solution filtered through whatman filter paper No.42 and diluted up to mark with methanol. Aliquot of above prepared sample solution was suitably diluted with buffer solutions to obtain solution of Capecitabine (20 µg/ml) and analyzed by pH independent spectrophotometric method.

RESULTS AND DISCUSSION

The proposed method was found to be simple, sensitive, rapid, accurate, precise and economic for the routine simultaneous estimation of two drugs. The linearity ranges for both drugs were found to be 10-20 µg/ml.

Characteristic parameters for regression equation and correlation are given in Table 1. Precision was calculated as repeatability ((% RSD) and intra and inter day variation (% RSD) for both the drugs. Accuracy was determined by calculating the recovery, and the mean was determined. The LOD and LOQ were found to be 0.662 and 2.00µg/ml, respectively for CAP, indicates sensitivity of the proposed method. The method was successfully used to determine the amounts of CAP present in dosage forms. The results obtained are in good agreement with the corresponding labelled amount. By observing the validation parameters, the method was found to be sensitive, accurate and precise and hence it can be employed for the routine analysis CAP in pharmaceutical dosage form.

Table 1: Regression analysis data and summary of validation parameters for the proposed method

Parameters	pH independent spectrophotometric method CAP
Wavelength	256.3
Beer's Law Limit	10-20 µg/ml
Regression equation (y = mx + c)	y=0.015x + 0.069
Slope	0.015
Intercept	0.069
Correlation coefficient (R2)	0.998
LOD*a* (µg/ml)	0.66
LOQ*b* (µg/ml)	2
Accuracy (% recovery, n = 6)	99.90±0.2772
Repeatability (% RSD*c*, *n* = 6)	0.2907
Precision (% RSD, n = 3)	
Intraday	0.5442-0.6900
Interday	0.6069-0.8048

RSD = Relative standard deviation. [b]LOD = Limit of detection. [c]LOQ = Limit of quantification

Table 2: Analysis of CAP by proposed method

Dosage form	Label claim (mg)	Amount found (mg)	% Label claim ± S. D. (n=6)
1	**500**	498.55	99.67233±0.13711

CONCLUSION

The proposed dual wavelength method was found to be linear between the range of 10-20 µg/ml for CAP. The mean percentage recovery was found 99.90% for Capecitabine at three different levels of standard additions. The precision (repeatability, intra-day and inter-day) of methods were found within limits (RSD <2%).

It could be concluded from the results obtained in the present investigation that the proposed method for the estimation of Capecitabine from its pharmaceutical dosage form is simple, rapid, accurate, precise and economical and can be used, successfully in the quality control of pharmaceutical formulations and other routine laboratory analysis.

Acknowledgement
The authors are thankful to Intas Pharma Pvt. Ltd., Ahmedabad, India for providing gift sample of CAP for research. The authors are highly thankful to Shree S. K. Patel College of Pharmaceutical Education & Research, Ganpat University, Ganpat Vidyanagar, Mehsana, Gujarat, India for providing all the facilities to carry out the work.

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