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DEVELOPMENT AND VALIDATION OF SPECTROPHOTOMETRIC METHOD FOR SIMULTANEOUS DETERMINATION OF TEMOZOLOMIDE AND CAPECITABINE IN SYNTHETIC MIXTURE

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ABSTRACT

The present manuscript describe simple, sensitive, rapid, accurate, precise and economical spectrophotometric method for the simultaneous determination of temozolomide and capecitabine in synthetic mixture. The method is based on the simultaneous equations for analysis of both the drugs using double distilled water as solvent. Temozolomide has absorbance maxima at 328 nm and Capecitabine has absorbance maxima at 240 nm in double distilled water. The linearity was obtained in the concentration range of 2-20 µg/ml and 2-20 µg/ml for temozolomide and capecitabine, respectively. The concentrations of the drugs were determined by using simultaneous equations at both the wavelengths. The mean recovery was 99.85 ± 0.65 and 100.97 ± 0.44 for temozolomide and capecitabine, respectively. The method was found to be simple, sensitive, accurate and precise and was applicable for the simultaneous determination of temozolomide and capecitabine in synthetic mixture. The results of analysis have been validated statistically and by recovery studies.

KEYWORDS: Capecitabine, Temozolomide, Recovery, Simultaneous equations method, Validation.

INTRODUCTION

Temozolomide (TEM) (Figure 1) is chemically 3-methyl-4-oxoimidazol[5,1-d][1,2,3,5]tetrazine-8-carboxamide; $C_6H_6N_6O_2$[1] used as an anticancer agent[2] belongs from

alkylating agent class. It is official in IP and USP. IP[3] and USP[4] describe liquid chromatography method for its determination. Literature survey reveals spectrophotometric,[5] HPLC,[6] colorimetric[7] and LC/MS[8] methods for the determination of temozolomide in single dosage form and HPLC[9] method for combined dosage forms. Capecitabine (CAP) (Figure 2) is chemically 1-(5-Deoxy-beta-D-ribofuranosyl)-5-fluoro-1,2-dihydro-2-oxo-4-pyrimidinyl)-carbamicacid pentyl ester,[10] is also an anti-metabolites which is a class of anticancer drug.[11] The combination of TEM and CAP has been shown to be effective in the metastatic endocrine carcinomas of pancreas.[12] The combination was generally more effective than temozolomide.[12] Capecitabine is official in IP and USP. IP[13] and USP[14] describe liquid chromatography method for its determination. Literature survey discloses spectrophotometric,[15] HPLC,[16] colorimetric,[17] HPTLC[18] methods for determination of capecitabine in single dosage form and HPLC[19] method for combined dosage forms. This combination is not official in any pharmacopoeia, so no official method is available for the determination of these two drugs in combined dosage forms. Literature survey reveals no spectrophotometric method for the simultaneous determination of TEM and CAP in combined dosage form. The present manuscript explain simple, sensitive, accurate, precise, rapid and economic zero order derivative spectrophotometric method for simultaneous determination of temozolomide and capecitabine in synthetic mixture.

MATERIALS AND METHODS

Apparatus

A shimadzu double beam UV/Visible spectrophotometer model 1700 (Japan) with spectral width of 2 nm, wavelength accuracy of 0.5 nm and a pair of 10 mm matched quartz cell was appointed to measure absorbance of all the solutions. Spectra were automatically acquired by UV-Probe system software. A Sartorius CP224S analytical balance (Gottingen, Germany), an ultrasonic bath (Frontline FS 5, Mumbai, India) was appointed in the study.

Reagents and Materials

TEM and CAP bulk powder was kindly gifted by Torrent Research Centre, Ahmedabad, Gujarat, India. Synthetic mixture of Capecitabine and Temozolomide was made in laboratory by incorporating commonly used excipients. Double distilled water was used in the study.

Preparation of standard stock solutions

An accurately weighed quantity pure powder of TEM (10 mg) and CAP (10 mg) were transferred to a separate 100 ml volumetric flask and dissolved and diluted to the mark with

double distilled water to obtain standard solution having concentration of TEM (100 µg/ml) and CAP (100 µg/ml).

Methodology

Standard drug solutions having concentration 10ug/ml was scanned separately in the range of 200 nm to 400 nm. Maximum absorbance was noticed at 328 nm and 240 nm by Temozolomide and Capecitabine respectively. So these two wavelength was preferred as an analytical wavelength. In this method, the absorbance of the solutions were measured at the λ_{max} of both the drugs. The criteria are that the ratios $[(A_2/A_1) / (ax_2/ax_1)]$ and $[(ay_2/ay_1) / (A_2/A_1)]$ should lie outside the range 0.1-2.0. For this measurement, the standard solutions of TEM and CAP (10 µg/ml) were scanned separately in the range of 200-400 nm against double distilled water as a blank. Data were recorded at an interval of 1 nm. Figure 3 indicates the overlain spectra of the two drugs. Absorbance was measured at selected wavelengths i.e. 328 nm and 240 nm absorption maxima for TEM and CAP respectively. The absorbance and Absorptivity values at the particular wavelengths were calculated and substituted in the following equation to get the concentration

$C_x = (A_2\ Ay_1 – A_1\ Ay_2) / (Ay_1\ Ax_2 – Ay_2\ Ax_1)$ ----- (1)

$C_y = (A_1\ Ax_2 – A_2\ Ax_1) / (Ay_1\ Ax_2 – Ay_2\ Ax_1)$ ------ (2)

Where, A_1, A_2= Absorbances of mixture at λ_1 & λ_2 respectively,

ax_1 = Absorptivity of TEM at 328 nm,

ax_2 = Absorptivity of TEM at 240 nm.

ay_1 = Absorptivity of CAP at 328 nm.

ay_2 = Absorptivity of CAP at 240 nm

Validation of the proposed method

The proposed method was validated according to the International Conference on Harmonization (ICH) guidelines.[20]

Linearity (Calibration curve)

The calibration curves were plotted over a concentration range of 2-20 µg/ml for TEM and 2-20 µg/ml for CAP. Accurately measured standard solutions of TEM (0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4, 1.6, 1.8 and 2.0 ml) and CAP (0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4, 1.6, 1.8 and 2.0 ml) were transferred to a series of 10 ml of volumetric flasks and diluted to the mark with double distilled water. The absorbances of the solutions were measured at 328 and 240nm against as

blank. The calibration curves were assembled by plotting absorbances versus concentrations and the regression equations were calculated.

Method precision (repeatability)

The precision of the instrument was examined by repeated scanning and measurement of absorbance of solutions (n = 6) for TEM and CAP (10 µg/ml for TEM and 10 µg/ml for CAP) without changing the parameter of the suggested spectrophotometric method.

Intermediate precision (reproducibility)

The intraday and interday precision of the suggested 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 TEM and CAP (8, 10, 12 µg/ml for TEM and 8, 10, 12 µg/ml for CAP). The result was reported in terms of relative standard deviation (% RSD).

Accuracy (recovery study)

The accuracy of the method was determined by calculating recovery of TEM and CAP by the standard addition method. Known amounts of standard solutions of TEM and CAP were added at 50, 100 and 150 % level to prequantified sample solutions of TEM and CAP (2.5 µg/ml for TEM and 5 µg/ml for CAP). The amounts of TEM and CAP were determined by applying acquired values to the respective regression line equations. The experiment was repeated for three times.

Limit of detection and Limit of quantification

The limit of detection (LOD) and the limit of quantification (LOQ) of the drug were derived by calculating the signal-to-noise ratio (S/N, i.e., 3.3 for LOD and 10 for LOQ) using the following equations designated by International Conference on Harmonization (ICH) guidelines[20].

$LOD = 3.3 \times \sigma/S$

$LOQ = 10 \times \sigma/S$

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

Analysis of TEM and CAP in combined tablet dosage form

A quantity of synthetic mixture equivalent to 5 mg of TEM and 10 mg of CAP was transferred to a 100 ml volumetric flask. Double distilled water (50 ml) was added to the

flask and sonicated for 20 minutes. The solution was filtered through Whatman filter paper No. 41 and the volume was adjusted up to the mark with double distilled water. This solution is wonted to contain 50 µg/ml of TEM and 100 µg/ml of CAP. From this solution (1.0 ml) was taken in to a 10 ml volumetric flask and the volume was adjusted up to mark with double distilled water to get a final concentration of TEM (5 µg/ml) and CAP (10 µg/ml). The responses of the sample solution were measured at 328 nm and 240nm for determination of TEM and CAP, respectively. The amounts of the TEM and CAP present in the sample solution were calculated by fitting the responses into the regression equation for TEM and CAP in the suggested method.

RESULTS AND DISCUSSION

Simultaneous equations method, the primary requirement for developing a method for analysis is that the entire spectra should follow the Beer's law at all the wavelength, which was fulfilled in case of both these drugs. The two wavelengths were used for determination of the drugs were 328 nm (λ-max of TEM) and 240 nm (λ-max of CAP) at which the calibration curves were constructed for both the drugs. The overlain UV absorption spectra of TEM (328 nm) and CAP (240 nm) showing in double distilled water is shown in Figure 3.

The validation parameters were studied at all the wavelengths for the suggested method. Accuracy was determined by calculating the recovery, and the mean was determined (Table 1). The method was successfully used to determine the amounts of TEM and CAP present in the synthetic mixture. The results obtained were in good agreement with the corresponding labeled amount (Table 2). Precision was calculated as repeatability and intra and inter day variations (% RSD) for both the drugs. Optical characteristics and summary of validation parameters for method is given in (Table 3). By observing the validation parameters, the method was found to be simple, sensitive, accurate and precise. Hence the method can be employed for the routine analysis of these two drugs in synthetic mixture.

Table 1: Recovery data of proposed method

Drug	Level	Amount taken (µg/ml)	Amount added (%)	% Mean recovery ± S.D. (n = 3)
TEM	I	2.5	50	99.60 ± 0.80
	II	2.5	100	99.86 ± 0.75
	III	2.5	150	100.11 ± 0.40
CAP	I	5	50	100.73 ± 0.75
	II	5	100	101.2 ± 0.26
	III	5	150	101.0 ± 0.30

Table 2: Analysis of TEM and CAP by proposed method

Synthetic mixture	Label claim (µg)		Amount found (µg)		% Label claim ± S. D. (n = 5)	
	TEM	CAP	TEM	CAP	TEM	CAP
I	250	500	250.82	502.2	100.33 ± 0.91	100.4 ± 0.46

Table 3: Regression analysis data and summary of validation parameters for the proposed method.

PARAMETERS	TEM		CAP	
	At 328 nm	At 240 nm	At 328 nm	At 240 nm
Beer's Law limit (µg/ml)	2-20		2-20	
Regression equation (y = mx + c) Slope (m) Intercept (c)	y = 0.0457x + 0.0004 0.0457 0.0004	y = 0.0232x + 0.0036 0.0232 0.0036	y = 0.0054x + 0.0017 0.0054 0.0017	y = 0.0342x + 0.0015 0.0342 0.0015
Correlation Coefficient (R^2)	0.9993	0.9993	0.9956	0.9995
Method precision (Repeatability) (% RSD, n = 6),	0.11	1.23	1.46	0.44
Interday (n = 3) (% RSD)	0.28 – 1.03	0.32 – 0.80	0.87 – 1.26	0.13 – 0.21
Intraday(n = 3) (% RSD)	0.17 – 0.25	0.20 – 0.62	0.87 – 1.24	0.42 – 0.81
LOD (µg/ml)	0.0316	0.1073	0.0907	0.0661
LOQ (µg/ml)	0.0958	0.3254	0.275	0.2005
Accuracy (Mean % Recovery ± S.D) (n = 3)	99.85 ± 0.65		100.97 ± 0.44	
% Assay ± S.D. (n = 5)	100.33 ± 0.91		100.4 ± 0.46	

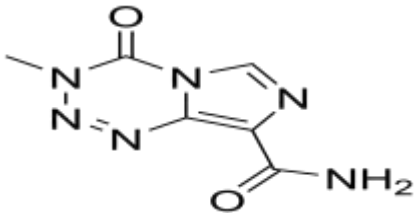

Fig. 1 Chemical structure of TEM

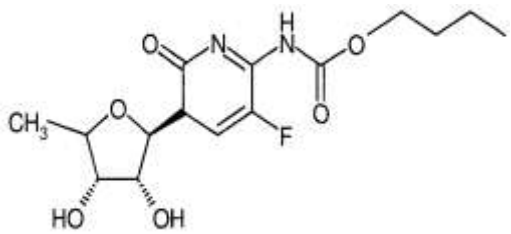

Fig. 2 Chemical structure of CAP

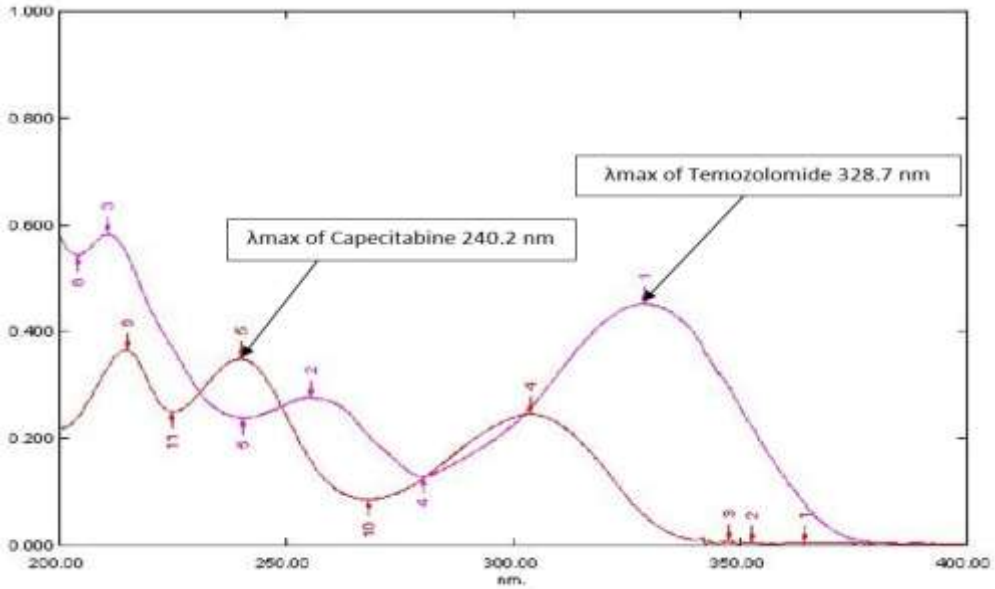

Fig. 3 Overlain absorption spectra of TEM (328 nm) and CAP (240 nm) (10 µg/ml each) showing in double distilled water.

CONCLUSION

Based on the results, obtained from the analysis of described method, it can be concluded that the method has linear response in the range of 2-20 µg/ml and 2-20 µg/ml for TEM and CAP, respectively. The result of the analysis of synthetic mixture by the suggested method is highly reproducible and reliable. The results obtained were in good agreement with the corresponding labeled amount. The additives usually present in the synthetic mixture of the assayed sample did not interfere with determination of TEM and CAP. The method can be used for the routine analysis of the TEM and CAP in synthetic mixture without any interference of excipients.

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