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RESEARCH ARTICLE

Development and Validation of Method of Analysis for Temozolomide and Capecitabine in Synthetic Mixture by Simultaneous Equation Method

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ABSTRACT

Temozolomide and Capecitabine are anti – cancer drugs available as tablets and capsules. Official method of analysis is liquid chromatography. Analysis of these medicinal products in the synthetic mixture was carried out spectrophotometrically using simultaneous equation method. It was found to be in agreement with the label claim, and the method was found to fulfill the mandates of validation parameters.

KEYWORDS

Temozolomide, Capecitabine, Simultaneous Equation Method

INTRODUCTION

Temozolomide is an anti – cancer drug with imidazotetrazine as an alkylating agent. It is orally active and undergoes rapid chemical conversions, within the body, depending upon the ambient pH. The alkylation at the O6 position of the guanine group of the molecule results in anti – cancer activity. It is accentuated with the alkylation at the N7 situation of the molecule. (1)

Capecitabine is a fluorouracil resemblance moiety and brings about its anti – cancer activity by interfering with RNA synthesis and production of fraudulent RNA. This process results in a metabolic error and thereby inhibition of cell division process.

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Temozolomide is available in formulations like tablets and capsules. Capsules are official in IP 2014. Official methods for analysis of the drug in I.P. 2014 as well as U.S.P. 2013 is liquid chromatography. (2.3) Capecitabine is available as tablets and capsules. Tablets are official in I.P. 2014 as well as U.S.P. 2013. Both I.P. 2014 and U.S.P. 2013 have proposed liquid chromatography as a formal method for analysis of the molecule. (4,5)

Other methods reported to carry out analysis of Temozolamide include RP – HPLC and Colorimetry. Spectroscopy is employed to a lesser degree, as compared to the above two methods. (7,8,9,10) Similarly, Capecitabine is reported to be analyzed by Colorimetry, Spectrophotometry, HPLC, HPTLC, and RP – HPLC.(11,12,13)

However, a study of an analytical method for analysis of temozolomide and capecitabine by Simultaneous Equation Method has not been found in the literature so far available. Since chances for the availability of a combination of these drugs is reasonably possible, development

of an analytical method for this mixture may be thought for.

MATERIALS & METHODS

Temozolomide and Capecitabine were obtained as gift samples from Torrent Research Centre, Ahmedabad. Synthetic mixtures were prepared by incorporation of various excipients, commonly used. The water utilized for the study was double distilled. Standard Stock Solutions of Temozolomide and Capecitabine containing 100 micrograms per ml of API were prepared.

Assay

Aqueous solutions of containing 5 mg of TEM and 10 mg of CAP was diluted with 50 ml of double distilled water and sonicated for 20 mins. This was followed by filtration across a Whatman filter paper no. 41. The volume of filtrate was adjusted to the mark by adding requisite double distilled water. This solution is contained 50 µg/ml of TEM and 100 µg/ml of CAP. A final concentration of TEM (5 µg/ml) and CAP (10 µg/ml) was subjected to spectrophotometry 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 & DISCUSSION

1. Method Development

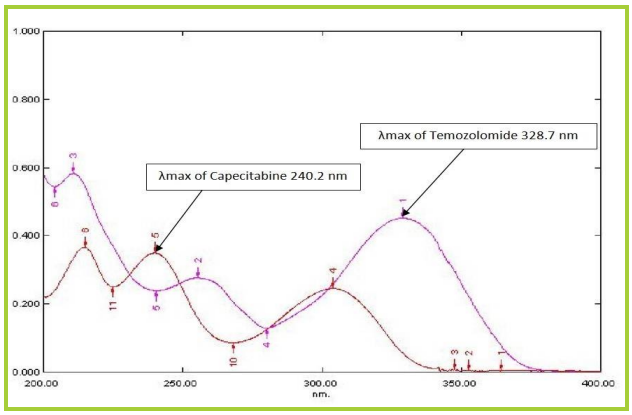

Figure : 1 Overlain absorption spectra of TEM (328 nm) and CAP (240 nm) (10 µg/ml each) showing in double distilled water

Standard solutions of TEM and CAP were separately prepared and subjected to scanning in the wavelength range of 200 – 400 nm. Double distilled water was used as a blank. The solutions were found to have a maximum absorbance at 328 nm and 240 nm respectively. Thus, these two wavelengths were selected for analysis. (Figure 1)

2. Validation of the proposed method

a) Linearity and Range

A Linear correlation was obtained between absorbance versus concentrations of TEM and CAP in the range of 2-20 µg/ml for both. The linearity of the calibration curve was validated by the high values of correlation coefficient of regression (Figures. 2,3,4,5)

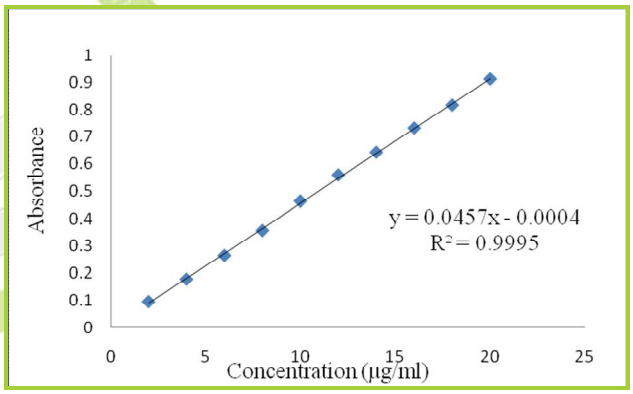

Figure : 2 Calibration curve of Temozolomide at 328 nm

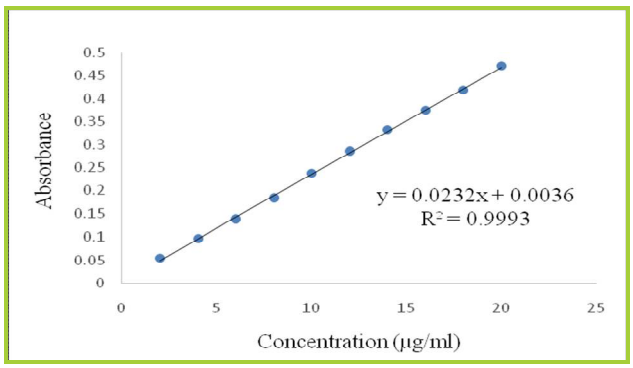

Figure : 3 Calibration curve of Temozolomide at 240 nm

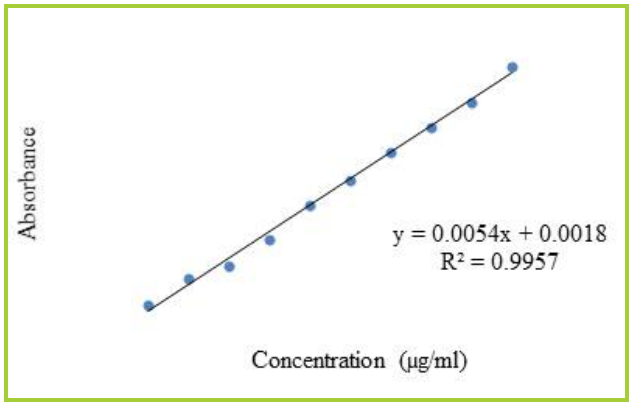

Figure : 4 Calibration curve of Capecitabine at 328 nm

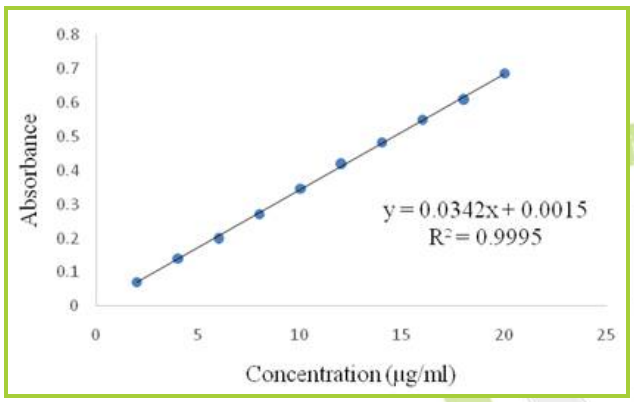

Figure : 5 Calibration curve of Capecitabine at 240 nm

b) Precision

1. Method Precision (Repeatability)

The RSD values for TEM were found to be 0.11 and 1.23 % at 328 nm and 240 nm, respectively. The RSD values for CAP were found to be 1.46 and 0.44 % at 328 nm and 240 nm, respectively (Table 1). The relative standard deviation is less than 2 %, which indicates that the proposed method is repeatable.

2. Intermediate Precision (Reproducibility)

The RSD values of TEM for interday were 0.28 – 1.03 % and 0.32 – 0.80 % at 328 nm and 240 nm, respectively. The RSD values of CAP for interday were 0.87 – 1.26 % and 0.13 – 0.21 % at 328 nm and 240 nm, respectively. Where, the RSD values of TEM for intraday were 0.17 – 0.25 % and 0.20 – 0.62 % at 328 nm and 240 nm, respectively. The RSD values of CAP for intraday were 0.87 – 1.24 % and 0.42 – 0.81 % at 328 nm and 240 nm, respectively. Variations for TEM and CAP, reveal that the proposed method was precise (Table 5.1.4).

Table : 1 Precision (Repeatability) data for TEM and CAP

Sr. No.	TEM (10 µg/ml)		CAP (10 µg/ml)	
	At 328 nm	At 240 nm	At 328 nm	At 240 nm
1	0.464	0.234	0.054	0.345
2	0.465	0.240	0.056	0.348
3	0.465	0.238	0.056	0.345
4	0.465	0.240	0.056	0.345
5	0.464	0.234	0.056	0.348
6	0.465	0.240	0.056	0.345
MEAN	0.465	0.237	0.056	0.346
S.D.	0.0005	0.0029	0.0008	0.0015
% RSD	0.11	1.23	1.46	0.44

3. LOD and LOQ

LOD and LOQ values for TEM were found to be 0.031 and 0.107 µg/ml, and 0.095 and 0.325 µg/ml, at 328 nm and 240 nm, respectively. Where, LOD and LOQ values for CAP were found to be 0.090 and 0.066 µg/ml, and 0.275 and 0.200 µg/ml, at 328 nm and 240 nm, respectively (Table 5.1.4). This data shows that

method is sensitive for the determination of the TEM and CAP.

4. Accuracy (% Recovery)

The recovery experiments were performed by the standard addition method. The mean recoveries were 99.85 ± 0.65 and 100.97 ± 0.44 % for TEM and CAP, respectively. The little value of standard deviation indicates that the proposed method is accurate. Results of recovery study are shown in Table 2.

Table : 2 Recovery data for the 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

5. Assay

Table : 3 Assay results for the synthetic mixture (TEM and CAP) using proposed method (n = 5)

Sample No.	Label claim (mg)		Amount found (mg)		% Assay	
	TEM	CAP	TEM	CAP	TEM	CAP
1	250	500	253.3	502	101.32	100.4
2	250	500	252.75	501.5	101.1	100.3
3	250	500	251	498.6	100.4	99.72
4	250	500	254	502.5	101.6	100.5
5	250	500	251.8	501.5	100.72	100.3
Mean			252.57	501.2	101.028	100.2
SD			1.190378	1.52	0.476151	0.30

Table : 4 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 = 0.0457 x + 0.0004 0.0457 0.0004	y = 0.0232 x + 0.0036 0.0232 0.0036	y = 0.0054 x + 0.0017 0.0054 0.0017	y = 0.0342 x + 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)	101.0 ± 0.47		100.2 ± 0.30	
% Assay ± S.D. (n = 5)	100.33 ± 0.91		100.4 ± 0.46	

The proposed validated methods were successfully applied to determine TEM and CAP in their synthetic mixture. Results are given in Table 4.3. No interference of the excipients with the absorbance of interest appeared; hence the proposed method is applicable for the routine simultaneous determination of TEM and CAP in the synthetic mixture.

CONCLUSION

The results of the analysis were found to be in sync with the label claim, and in compliance with the requisites of the validation parameters. The excipients did not interfere with the analysis of TEM and CAP. Thus, this can be introduced as a simple, fast and precise methods for routine analysis of the title drugs in the synthetic mixture.

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