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DEVELOPMENT AND VALIDATION OF HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHIC METHOD FOR SIMULTANEOUS ESTIMATION OF TEMOZOLOMIDE AND CAPECITABINE IN SYNTHETIC MIXTURE

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ABSTRACT

The present manuscript describe simple, sensitive, rapid, accurate, precise and economical High Performance Thin Layer Chromatographic (HPTLC) method for the simultaneous determination of Temozolomide and Capecitabine in synthetic mixture. In HPTLC method two drugs were spotted and development of plate was carried out. Plate was scanned and peak areas were measured densitometrically at 316 nm for quantitation of TEM and CAP. The linearity was achieved in the concentration range of 100-350 ng/spot for both Temozolomide and Capecitabine. The concentrations of the drugs were determined by using regression line equations. From results of recovery studies it can be concluded that there are no interferences from the excipients. The method was found to be simple, sensitive, accurate and precise. So, it can be 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: Temozolomide, Capecitabine, HPTLC method, Synthetic mixture, 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 TEM 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 TEM.[12] CAP is official in IP and USP. IP[13] and USP[14] describe liquid chromatography method for its determination. Literature survey reveals spectrophotometric[15], HPLC[16], colorimetric[17], HPTLC[18] methods for determination of CAP 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 describes simple, sensitive, accurate, precise, rapid and economic zero order derivative spectrophotometric method for simultaneous determination of TEM and CAP in synthetic mixture.

MATERIALS AND METHODS

Apparatus

- A Camag HPTLC system (Switzerland) with Linomat V automatic sample applicator and Camag TLC Scanner III.
- Camag (Muttenz, Switzerland) flat bottom and twin-trough flat-bottom TLC developing chamber (20 × 10 cm).
- Pre-coated silica gel aluminum plate 60 $F_{254,}$ (20 × 10 cm; E. Merck, Darmstadt, Germany).
- Analytical balance (CP224S, Sartorius, and Gottingen, Germany).
- UV cabinet with dual wavelength UV lamp (254 nm & 316 nm)
- Camag win-CATS software.
- Hamilton syringe (100 µl)
- Ultrasonic bath (Frontline ultrasonic bath, Mumbai, India).
- Volumetric flasks and pipettes.

REAGENTS AND MATERIALS

- TEM and CAP standard powder. (Torrent Research Centre, Ahmedabad, Gujarat, India.)
- Methanol AR grade as solvent (S.D. Fine Chemical Ltd., Mumbai, India.).

Chromatographic conditions

The estimation was performed using following chromatographic conditions

- **Stationary phase**

Precoated silica gel aluminum plate $60F_{254}$, (20 cm × 10 cm, prewashed by methanol.

- **Mobile phase:** Butanol: Water: Glacial Acetic Acid (5 :2 :1, v/v/v)
- TLC Chamber saturation Time: 20 min at room temperature
- Application rate: 0.1 µl/s
- Scanner band width: 6 mm
- Distance from the plate edge : 10 mm
- Distance from the bottom of the plate : 10 mm
- Slit dimension: 6 mm × 0.45 mm
- Scanning speed: 20 mm/s
- Detection: Densitometrically at 316 nm using a UV detector

Preparation of standard stock solutions

Precisely measured portion of TEM (10 mg) and CAP (10 mg) was transferred to a separate 10 ml volumetric flask. Then methanol (5 ml) was added in the flask and sonicated for 20 min. Volume was made up to the mark with methanol to obtain standard solution which having concentrations of TEM (1000 µg/ml) and CAP (1000 µg/ml). From above solutions, 0.5 ml of each was transferred to a 10 ml volumetric flask and volume was fixed up to mark with methanol to obtain standard solution having concentrations of TEM and CAP (50 µg/ml).

Preparation of synthetic mixture

Synthetic mixture (2000 mg) of TEM (250 mg) and CAP (500 mg) was prepared in laboratory using generally used excipients (1250 mg) like Lactose, Talc, Magnesium Stearate.

Preparation of sample solution

A quantity of the synthetic mixture equivalent to 2.5 mg of TEM and 5 mg of CAP was transferred to a 10 ml volumetric flask. Then methanol (5 ml) was added into the flask and

sonicated for 20 min. The volume was fixed up to the mark with methanol after the sonication. An aliquot of this solution (1 ml) was transferred in to a 10 ml volumetric flask and the volume was fixed up to mark with methanol.

Determination of the analytical wavelengths

The solution of TEM and CAP were applied to silica gel $60F_{254}$ HPTLC plates (20 cm × 10 cm) by means of applicator under the chromatographic condition specified before. The plate was developed in a twin-trough chamber previously saturated for 20 min with the mobile phase. HPTLC plate was dried in a current of air. Scanning was carried out in the reflectance-absorption mode using a UV detector in the range of 200-400 nm. Both drugs displays good response at 316 nm, hence 316 nm was preferred as quantification wavelength.

Validation of the proposed method[20]

Calibration curve (linearity)

Calibration curve were constructed over a concentration range of 100-350 ng/spot for TEM and CAP both. For this, 2, 3, 4, 5, 6, 7 µl of 50 µg/ml of stock solution of TEM and CAP was spotted in band width 8 mm using Hamilton 100 µl syringe on precoated silica gel aluminum plate 60 F_{254} using automatic application device. Linear ascending development was executed in 20 × 10 cm twin trough glass chamber saturated with the mobile phase for 20 min. The plate was taken from the chamber, subsequently dried in a current of air and densitometric scanning was carried out on Camag TLC scanner III in the reflectance-absorption mode at 316 nm and operated by win-CATS software. Peak areas were reported for all the peaks. The calibration curve for TEM and CAP were constructed by plotting peak area versus concentration (ng/spot) equivalent to each spot.

Range

Range is the interval between upper and lower concentration (value) of analyte in sample for which it has been demonstrated that the analytical method has appropriate level of precision accuracy and linearity. The linear response was observed over a range of 100-350 ng/spot for TEM and CAP both separately.

Accuracy (% recovery)

The accuracy of the method was determined by calculating recoveries of TEM and CAP by the standard addition method. Predetermined amounts of standard solution of TEM and CAP

were added at 80%, 100% and 120% levels to prequantified sample solutions of TEM and CAP.

Method precision (Repeatability)

The precision of the method was examined by repeated scanning and measuring the peak area of solutions (n = 6) of TEM (100 ng/spot) and CAP (100 ng/spot) without changing the parameters of the suggested Method. The results are documented in terms of relative standard deviation (% RSD).

Intermediate precision (Reproducibility)

The intraday and interday precision of the suggested method was evaluated by analyzing the equivalent responses 3 times on the same day and on 3 different days over a period of 1 week and for 3 different concentrations of sample solutions of TEM (100, 150, and 200 ng/spot) and CAP (100, 150 and 200 ng/spot). The results are documented in terms of relative standard deviation (% RSD).

Limit of detection (LOD) & Limit of quantitation (LOQ)

The limit of detection (LOD) and the limit of quantification (LOQ) for the suggested method were estimated using following equations:

$LOD = 3.3 \times \sigma/S$

$LOQ = 10 \times \sigma/S$

Where, σ = the standard deviation of the response and

S = slope of the calibration curve.

Determination of TEM and CAP in synthetic mixture

The peak area of final sample solution was measured densitometrically at 316 nm for quantitation of TEM and CAP. The amount TEM and CAP present in the sample solutions were determined by fitting the response into the respective regression line equation for TEM and CAP.

RESULTS AND DISCUSSION

In HPTLC method, the peak areas were measured at 316 nm. For this measurement, the solutions of TEM and CAP were prepared separately in methanol having concentration of 50 ng/µl for both. The R_f values were found to be 0.40 ± 0.0041 and 0.60 ± 0.000 for TEM and CAP respectively. Peak areas were recorded for all the peaks. This peak area was employed

for the determination of TEM and CAP. Typical Densitogram of TEM and CAP show in Figure 3. The concentrations of the solutions were calculated by regression line equations by applying obtained values of peak areas.

The validation parameters were studied at all the wavelengths for the proposed 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 combined dosage form.

Table 1: Recovery data for the proposed method.

DRUG	LEVEL	Amt. Present (ng/spot)	Amt. added (ng/spot)	% Mean Recovery ± SD
TEM	I	75	60	100.07 ± 1.71
	II	75	75	99.63 ± 1.39
	III	75	90	101.47 ± 0.58
CAP	I	150	120	100.56 ± 1.48
	II	150	150	99.82 ± 1.42
	III	150	180	101.02 ± 0.27

* *Mean % Recovery ± SD of three observations.*

Table 2: Determination of drugs by proposed method.

Sr. No.	Label claim (mg)		Amount found (mg)		% Label claim ± S. D. (n = 3)	
	TEM	CAP	TEM	CAP)	TEM	CAP
I	250	500	250.6	499.8	100.38 ± 0.72	99.97 ± 0.38

Table 3: Regression determination data and summary of validation parameters.

PARAMETERS	TEM	CAP
Wavelength (nm)	316	
Beer's law limit (ng/spot)	100 - 350	
Regression Equation Y= mx + c	y = 14.364x + 472.84	y = 7.304x + 156.9
Slop (m)	14.364	7.304
Intercept (c)	472.84	156.9
Correlation coefficient (r^2)	0.9986	0.9996
Method precision Repeatability (n=6, % RSD)	0.96	1.32

Interday precision (n=3, % RSD)	0.35 - 0.90	0.51 - 0.79
Intraday precision (n=3, % RSD)	0.44 - 1.15	0.66 - 1.04
LOD (ng/spot)	5.90	5.14
LOQ (ng/spot)	17.88	15.59
% Recovery ± SD (n=3)	100.3 ± 1.22	100.4 ± 1.05
Assay ± SD (n=5)	99.9 ± 1.01	100.7 ± 0.72

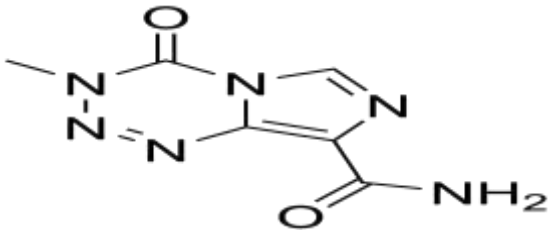

Fig. 1: Chemical structure of Temozolomide (TEM).

Fig. 2: Chemical structure of Capecitabine (CAP).

All tracks at wavelength 316 nm

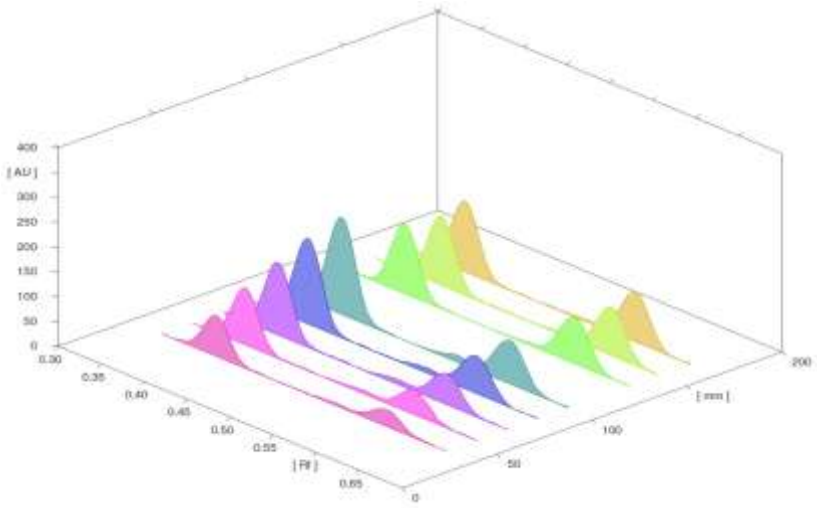

Fig. 3: 3D Densitogram of TEM and CAP at 316 nm.

CONCLUSION

Depend on the results, obtained from the analysis using defined method, it can be interpreted that the method has linear response in the range of 100 - 350 ng/spot for TEM and CAP both. The result of the analysis of synthetic mixture by the suggested method is extremely

reproducible and reliable and is in good agreement with label claim of the drugs. The additive present in the combined synthetic mixture did not interfere in the analysis. So the method can be used for the routine analysis of drugs in combined synthetic mixture.

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