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Simultaneous Estimation of Gefitinib and Capecitabine by UV-Spectrophotometric Method

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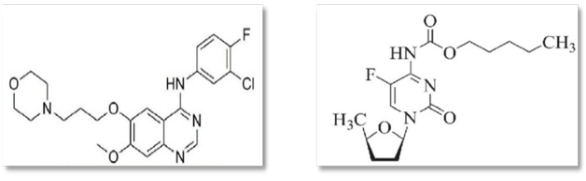
Abstract:

A UV spectrophotometric method has been developed for the simultaneous determination of Gefitinib and Capecitabine. The spectroscopic method for estimation of Gefitinib and Capecitabine employed simultaneous estimation method using Methanol as solvent. Gefitinib has absorbance maxima 302 nm and Capecitabine has absorbance maxima 333 nm and both these drugs obey Beer's law in concentration range of 2-12 µg/ml for Gifitinib and 2-12 µg/ml for Capecitabine. The recovery studies ascertained the accuracy of the proposed method and the results were validated as per ICH guidelines. The results were found satisfactory and reproducible.

Keywords: Gefitinib; Capecitabine; Simultaneous estimation; UV-Spectrophotometer and ICH guidelines.

INTRODUCTION

Gefitinib (GFT) (**Figure 1a**) is chemically N-(3-chloro-4-fluorophenyl)-7-methoxy-6-[3-(morpholin-4-yl) propoxy] quinazolin-4-amine used in the treatment of different types of cancer by selectively targeting cells. It decreases the intracellular phosphorylation of tyrosine kinases linked with transmembrane cell surface receptors, including the epidermal growth factor receptor (EGFR TK) [1, 2]. Capecitabine (CCB) (**Figure 1b**) is chemically N-pentoxycarbonyl-5-deoxy-5-flurocytidine, the oral prodrug of flurouracil (FU), used widley for the treatment of metastatic breast cancer and stage III and IV colorectal cancers [3]. After oral administration and intestinal absorption, CCB is metabolized to 5-deoxy-5-fluorocytidine (5-DFCR) by carboxylesterase. 5-DFCR is converted to 5-deoxy-5-fluorouridine by cytidine deaminase and finally to FU by thymidine phosphorylase in the normal or tumorous tissue [4-7].



(a) (b)

Figure 1: Chemical structure of **(a)** Geftinib **(b)** Capecitabine

MATERIALS AND METHODS

Materials

Capecitabine, Gefitinib are gift samples obtained from Hetero Drugs Pvt. Ltd., Hyderabad and used as the working standard. All other chemicals and reagents used were of analytical grade.

Instrumentation

A double beam UV-VIS spectrophotometer (UV-1700, Shimadzu, Japan) connected to computer loaded with spectra manager software UV Probe was used. The spectra were obtained instrumental parameters as follows: Wavelength range: 200-400 nm. All weights were taken on an electronic balance (Model Shimadzu AUX 120).

Preparation of standard stock solution and calibration curve

The standard stock solutions of GFT and CCB were prepared by dissolving 100 mg of each drug in methanol, and the final volume was adjusted with the same solvent in 100 ml of a volumetric flask to get a solution containing 1000 µg/ml of each drug. Working standard solutions of 10 µg/ml were scanned in the entire UV range of 200-400 nm to determine the λmax. The λmax of GFT and CCB is 302 and 333 nm respectively. Six working standard solutions with a concentration of 2-12 µg/ml were prepared in methanol from stock solution. The absorbance of resulting solutions were measured at their respective λmax and isobestic point, plotted a calibration curve to get the linearity and regression equation [8].

Validation of analytical method

The analytical performance characteristics tested during method validation i.e. % recovery, precision, ruggedness and sensitivity.

Simultaneous equations method

For the formulation of simultaneous equations both wavelengths 302 nm which is the λmax of GFT and 333 nm is the λ max of CCB were selected. Using a mixed standard solution GFT showed linearity values in the range of 2-12 µg/ml and 2-12 µg/ml for CCB. The method is applicable for the sample containing two drugs,

each drug of which absorbs at the λ max of the other drug. The constructed two equations depend upon the fact that the absorbance of a mixture of GFB and CCB at 303 nm and 333 nm is the sum of the absorbances at their respective wavelengths. The absorptivity coefficients of each drug were determined at two wavelengths. The concentrations of both the drugs present in the mixture were measured using the simultaneous equation with the formulae and the isobestic point is shown in the **Figure 2** at 313 nm [9].

Cx =A2ay1–A1ay2/ax2ay1–ax1ay2 ---------- **(1)**

Cy =A1ax2 -A2ax1/ax2ay1 - ax1ay2 ---------- **(2)**

Cx is the concentration of OME in the working sample solution.

Cy is the concentration of DOM in the working sample solution (mixture).

A1 = absorbance of mixture at 302 nm.

A2 = absorbance of the mixture at 333 nm.

ax1 = the absorptivity of GFT at 302 and ax2 = the absorptivity of GFB at 333 nm.

(ax1 =38.24, ax2 =34.92).

ay1 is the absorptivity of CCB at 333 nm and ay2 are absorptivity of DOM at 333 nm.

(ay1 =51.75, ay2=62.11).

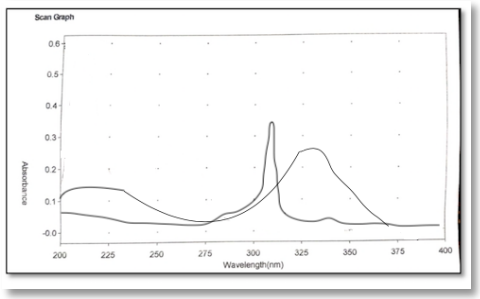


Figure 2: Overlain spectra of GFT and CCB

Determination of Absorptivity Value

The absorptivity value of GFT and CCB from each solution was calculated using following formula and the results were presented in **Tables 1and 2**. Developed method was validated as per ICH guidelines [10, 11, 12]. Absorptivity = Absorbance/concentration (gm/100 ml).

RESULTS & DISCUSSIONS

Method Development

The solution of GFT and CCB in methanol was found to exhibit maximum absorption at 302 nm and 333 nm respectively after scanning on the UV-Vis spectrophotometer which was reported as λmax in the literature and the procured drug sample of GFB, CCB complies with the reference spectra **(Figure 4, 5)**.

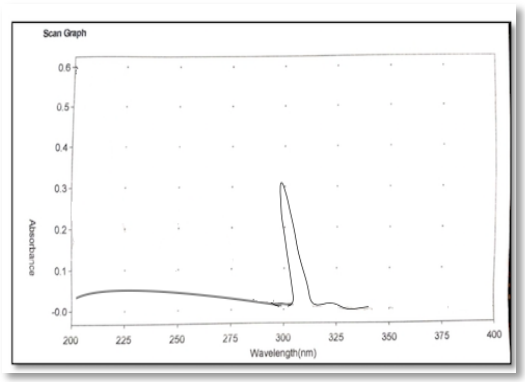


Figure 4: UV Spectra of GFT

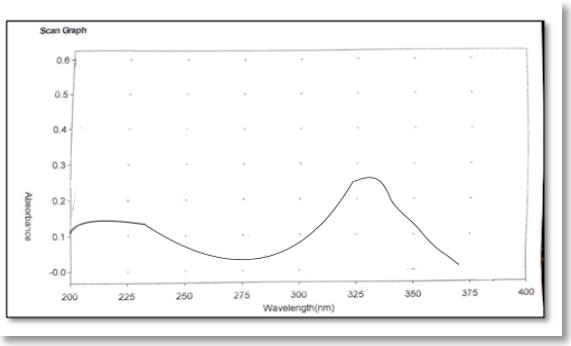


Figure 5: UV Spectra of CCB

Table 1: Absorptivity value for GFT

Concentration (µg/mL)	Absorbance at 333 nm	Absorptivity at 333 nm	Absorbance at 302 nm	Absorptivity at 302 nm
2	0.004	0.002	0.12	0.06
4	0.008	0.002	0.19	0.0475
6	0.012	0.002	0.25	0.04166667
8	0.016	0.002	0.32	0.04
10	0.02	0.002	0.39	0.039
12	0.025	0.00208333	0.46	0.03833333

Table 2: Absorptivity value for CCB

Concentration (µg/mL)	Absorbance at 333 nm	Absorptivity at 333 nm	Absorbance at 302 nm	Absorptivity at 302 nm
2	0.058	0.029	0.011	0.0055
4	0.061	0.01525	0.017	0.00425
6	0.066	0.011	0.028	0.00466667
8	0.07	0.00875	0.038	0.00475
10	0.075	0.0075	0.042	0.0042
12	0.078	0.0065	0.051	0.00425

Validation of analytical method

Linearity

Accurately weighed GFT and CCB (100 mg) was dissolved in 100 ml of methanol to obtain working standard of 100 µg/ml. Aliquots were pipetted from the stock solution of drug and were transferred to 10 ml volumetric flask, the final volume was adjusted with methanol so, that concentration of 2-12 µg/ml could be made. Absorbance of the above solution were taken at 302 nm and 333 nm respectively by using UV-Vis spectrophotometer (UV-1700, Shimadzu, Japan) against the blank solution prepared in the same manner without adding the drug. A graph of absorbance vs concentration was plotted (**Figure 6, 7**) and R2 for both the drugs was found in the range of 0.996 - 0.999.

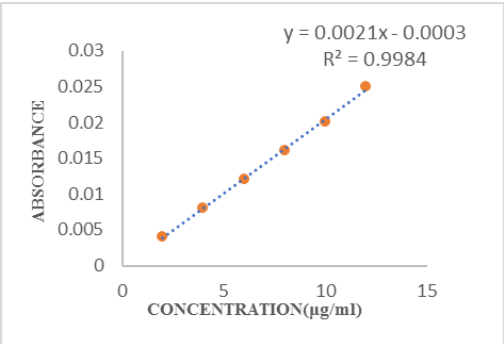


Figure 6: Calibration curve of GFT

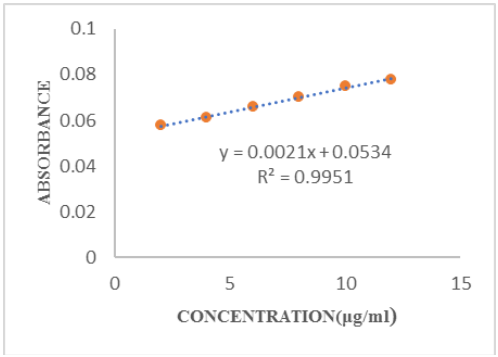


Figure 7: Calibration curve of CCB

Limit of Detection and Limit of Quantitation

Limit of detection (LOD) is the lowest amount of an analyte that can be determined but not necessarily as an exact value. Limit of quantification (LOQ) is the lowest amount of an analyte in a sample that can be quantitatively estimated with suitable precision and accuracy (12). The LOD and LOQ were separately analyzed which is based on calibration. The standard deviation of y intercept of regression line may be used. The given concentration was used for this and the absorbance was taken and standard deviation and slope of both the drugs were calculated using following formula. Results were shown in **Table 3**.

LOD = 3.3 X σ/s, LOQ= 10 X σ/s

Where,

σ =Standard deviation of y intercept of regression lines

S =Slope of calibration curve

Table 3: Linearity study

	Std. deviation	Slope	LOD	LOQ
CCB	0.007141	0.0021	10.2	34
GFT	0.115674	0.0677	5.6	17.07

Accuracy (% Recovery)

Recovery study is performed by standard addition method by adding the known amount of GFT, CCB (Working standard) at two different concentration levels i.e 60%, 80%, 100% of assay concentration and % recovery for both the drugs were calculated. Results were reported in **Table 4**.

Table 4: Recovery study

	Amount found (µg/ml)	% Recovery
CCB	4.2	70
	7.6	126
	9	112.5
GFT	4.1	102.5
	6.7	111.6
	8.2	102.5

Precision

Intra-day precision was determined at two different concentrations 2 mg/ml, 3 mg/ml containing GFT and CCB, for three times in the same day. Inter-day variability was assessed using above mentioned three concentrations on three different days, over a period of one week. Results were reported in **Table 5**.

Table 5: Precision study

	Inter-Day	Intra-Day
CCB	0.0707±0.015	0.073±0.001
GFT	0.3367±0.0153	0.336±0.02

Note: All values expressed in mean ±SD, n=3;

Ruggedness

From stock solution, sample solution containing GFT and CCB (2 µg/ml) was prepared and analyzed by two different analysts using similar operational and environmental conditions (**Table 6**).

Table 6: Ruggedness study

	Values	%RSD
CCB	0.070±0.0012	1.71
GFT	0.032±0.0043	1.3

Note: All values expressed in mean ±SD, n=3;

CONCLUSION

The proposed UV spectrophotometric method was found very simple, rapid and economical. The method is validated in compliance with ICH guidelines is suitable for estimation of GFT and CCB with excellent recovery, precision and linearity.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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