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Original Article

NEW STABILITY INDICATING METHOD DEVELOPMENT AND VALIDATION OF CAPECITABINE AND DOCETAXEL IN BULK AND PHARMACEUTICAL DOSAGE FORM BY USING RP-HPLC

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ABSTRACT

Objective: The current investigation was pointed at developing and progressively validating novel, simple, responsive and stable RP-HPLC method for the measurement of active pharmaceutical ingredients of Capecitabine and Docetaxel.

Methods: A simple, selective, validated and well-defined stability that shows gradient RP-HPLC methodology for the quantitative determination of Capecitabine and Docetaxel. The chromatographic strategy utilized Inertsil ODS column of dimensions 250x4.6 mm, 5 micron, using isocratic elution with a mobile phase of acetonitrile and water (50:50). A flow rate of 1 ml/min and a detector wavelength of 220 nm utilizing the PDA detector were given in the instrumental settings. Using the impurity-spiked solution, the chromatographic approach was streamlined. Validation of the proposed method was carried out according to an international conference on harmonization (ICH) guidelines.

Results: LOD and LOQ for the two active ingredients and their impurities were established with respect to test concentration. The calibration charts plotted were linear with a regression coefficient of R^2>0.999, means the linearity was within the limit. Recovery, specificity, linearity, accuracy, robustness, ruggedness were determined as a part of method validation and the results were found to be within the acceptable range.

Conclusion: The proposed method to be fast, simple, feasible and affordable in assay condition. During stability tests, it can be used for routine analysis of production samples and to verify the quality of drug samples during stability studies.

Keywords: Capecitabine, Docetaxel, RP-HPLC, Development, Validation

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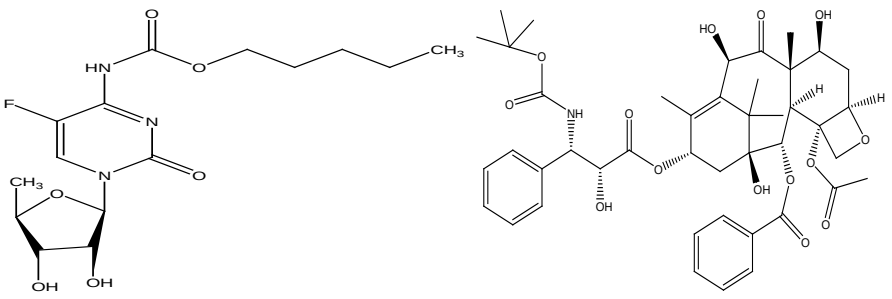
INTRODUCTION

Capecitabine, commonly known as Xeloda, is a chemotherapy [1, 2] medication that is used to treat breast cancer [3, 4], gastric cancer [5], and colorectal cancer [6]. In the treatment of breast cancer, it's usually coupled with docetaxel. It is taken by mouth. The most common side effects include abdominal discomfort [7], vomiting, diarrhoea, weakness, and rashes. Side effects include blood coagulation problems [8], allergic reactions [9], heart problems such cardiomyopathy [10], and low blood cell counts. It is not recommended for people with kidney disorders [11]. The infant may be damaged if this product is used during pregnancy. Capecitabine is converted to 5-fluorouracil (5-FU) inside the body, which is how it functions. It is a fluoropyrimidine, which contains 5-fluorouracil and tegafur [12]. Colorectal cancer (as neoadjuvant therapy with radiation, adjuvant therapy, or for metastatic cases), Breast cancer (metastatic or as monotherapy/combotherapy; this is licenced as a second-line treatment in the UK), Gastric cancer (off-label in the US; this is a licenced indication in the UK), and Oesophageal cancer [13]. (off-label in the US; this is a licenced indication in the UK).

Docetaxel (DTX or DXL) is a chemotherapeutic medication used to treat cancer. Taxotere and other trade names are used to market it. This includes breast cancer, head and neck cancer [14, 15], stomach cancer, prostate cancer [16], and non-small-cell lung cancer [17]. It can be used alone or with other chemotherapy medicines. A slow injection into a vein is used to administer it. Hair loss, cytopenia (low blood cell counts), numbness, shortness of breath, vomiting, and muscle pains are all frequent side effects of this medication. Allergies and the possibility of cancer are two more serious side effects. Side effects are more common in people with hepatic problems [18]. It is possible that using it while pregnant is detrimental to the foetus. The taxane class of medicines includes docetaxel. It works by interfering with the normal action of microtubules, which prevents cell division [19]. Breast, lung, prostate, stomach, head and neck, and ovarian cancers are all treated with docetaxel, a chemotherapy medication. According to clinical evidence [20], docetaxel has cytotoxic effects against breast, colorectal, lung, ovarian, prostate, liver, renal, gastric, and head and neck cancers, as well as melanoma. In hormone-refractory prostate cancer [21], docetaxel extends life expectancy and improves overall quality of life. The goal of this study is to use RP-HPLC to develop and validate methods for Capecitabine and Docetaxel.



A **B**

Fig. 1: Structure of (A) Capecitabine and (B) Docetaxel

MATERIALS AND METHODS

Chemicals

Acetonitrile, HPLC-grade ortho phosphoric acid, water, were purchased from Merck India Ltd, Mumbai, India. APIs of Capecitabine, Docetaxel standards were procured from Dr. Reddy's laboratory, Hyderabad.

The instrumentation

Waters alliance liquid chromatography (model 2695) monitored with empower 2.0 data handling system and a detector of photo diode array (model 2998) was used for this study [22, 23].

Method optimization

To optimize the chromatographic conditions, different ratios of phosphate buffer and the acetonitrile in the mobile phase with isocratic and gradient mode was tested. However the mobile phase composition was modified at each trial to enhance the resolution and also to achieve acceptable retention times. Finally 0.1% OPA buffer and acetonitrile with isocractic elution was selected because it results in a greater response of active pharmacy ingredients. During the optimization of the method various stationary phases such as C_8, C_{18} phenyl and amino, inertsil ODS columns were tested. From these trials the peak shapes were relatively good with a inertsil ODS column of 250 x 4.6 mm, 5 µ with a PDA detector. The mobile phase flow rate has been done at 220 nm in order to obtain enough sensitivity. By using above conditions we get retention times of Capecitabine and Docetaxel were about 3.483 and 4.076 min with a tailing factor of 1.05 and 1.08. The number of theoretical plates for Capecitabine and Docetaxel were 5218, 6784 which indicate the column's successful output the % RSD for six replicate injections was around 0.15%, 0.24%. The proposed approach suggests that it is extremely precise. According to ICH guidelines, the method established was validated.

Validation procedure

According to ICH Q2 (R1) guidelines [24, 25], analytical parameters such as system appropriateness, precision, specificity, accuracy, linearity, robustness, LOD, LOQ, forced deterioration, and stability were validated.

Preparation of buffer

1 L of HPLC grade water was taken and filter through 0.45 µ filter paper.

Chromatographic conditions

The HPLC analysis was performed on reverse phase HPLC system with isocratic elution mode using a mobile phase of acetonitrile and water and Inertsil ODS column (250x4.6 mm, 5 µ) column with a flow rate of 1 ml/min.

Diluent

Mobile phase was used as diluent.

Preparation of the standard stock solution

For standard stock solution preparation, add 70 ml of diluents to 150 mg of Capecitabine and 40 mg of Docetaxel taken in a 100 ml volumetric flask and sonicate for 10 min to fully dissolve the contents and then make up to the mark with diluent.

Preparation of standard solution

1 ml of solution is drawn from the above normal stock solution into a 10 ml volumetric flask and diluted up to the level.

Preparation of sample solution

Take the Capecitabine sample weight equivalent to 150 mg and the Docetaxel sample weight equivalent to 40 mg into a 100 ml volumetric flask and add 70 ml of diluents and sonicate for 10 min to fully dissolve the contents and then make up the mark with diluent. This solution is filtered into a device using a 0.45µ nylon syringe in a vial.

RESULTS AND DISCUSSION

The main analytical challenge during development of a new method was to separate active Pharma ingredients. In order to provide a good performance the chromatographic conditions were optimized.

System suitability

In System suitability injecting standard solution and reported USP tailing and plate count values are tabulated in table 1 [26].

Table 1: Results of system suitability

System suitability parameter	Acceptance criteria	Drug name	
		Capecitabine	Docetaxel
USP Plate Count	NLT 2000	5218	6784
USP Tailing	NMT 2.0	1.05	1.08
USP Resolution	NLT 2.0	-	4.25
% RSD	NMT 2.0	0.15	0.24

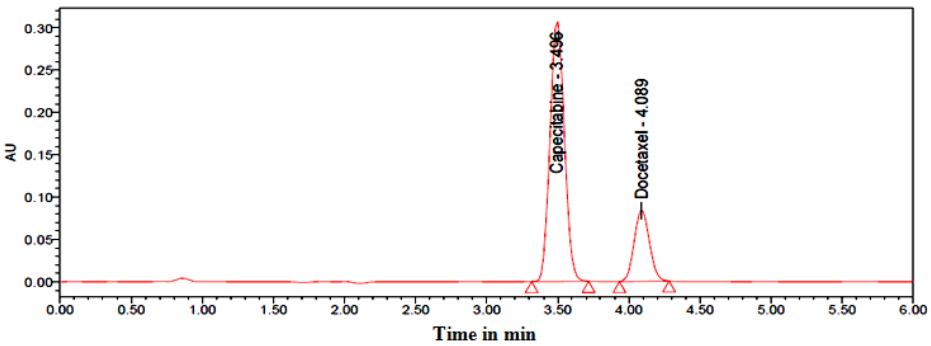


Fig. 2: Chromatogram of system suitability

Specificity

In this test method placebo, sample and standard solutions were analyzed individually to examine the interference. The below fig. shows that the active ingredients were well separated from blank and their excipients and there was no interference of placebo with the principal peak. Hence the method is specific.

Linearity

The area of the linearity peak versus different concentrations has been evaluated for Capecitabine, Docetaxel, as 10,25,50,100,125,150 percent respectively. Linearity was performed in the range of 15-225µg/ml of Capecitabine and 4-60µg/ml of Docetaxel. The correlation coefficients achieved greater than 0.999 for all.

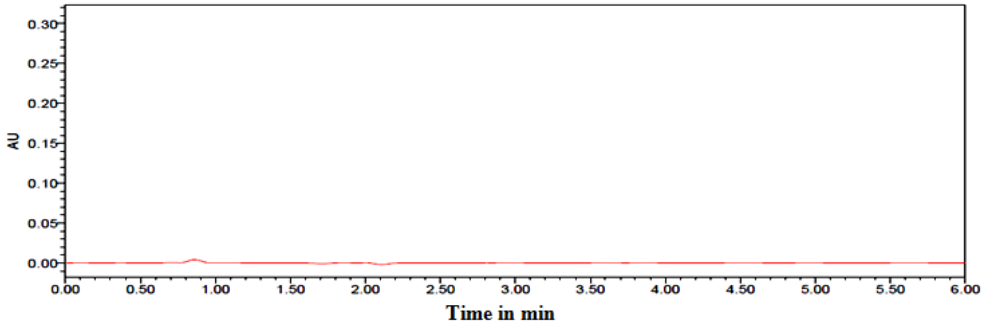
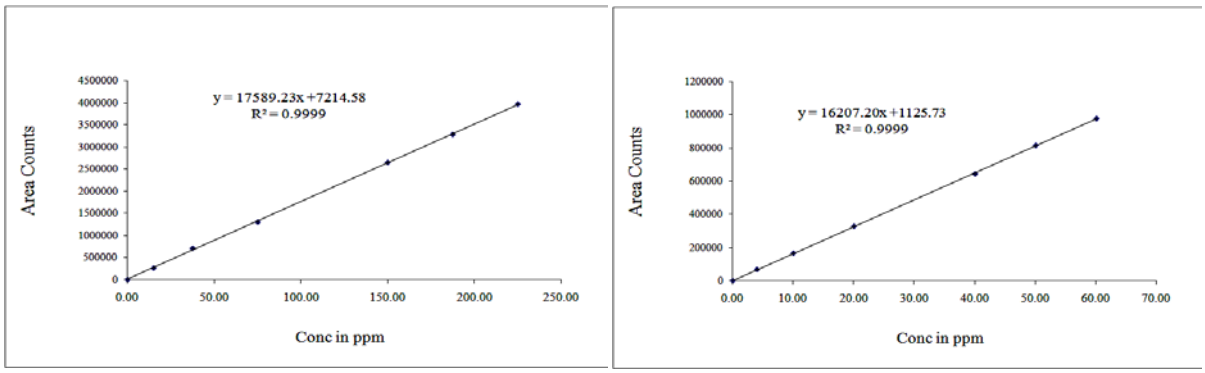


Fig. 3: Chromatogram of blank

Table 2: Linearity of capecitabine and docetaxel

S. No.	Conc. µg/ml	Capecitabine area count	Conc. µg/ml	Docetaxel area count
1	15.00	258931	4.00	66921
2	37.50	708643	10.00	164529
3	75.00	1302567	20.00	326928
4	150.00	2653415	40.00	642371
5	187.50	3286934	50.00	813624
6	225.00	3976582	60.00	975632
Correl coef		0.99990		0.99996
Slope		17589.23		16207.20
intercept		7214.58		1125.73



A **B**

Fig. 4: Calibration plots of (A) Capecitabine (B) Docetaxel

Accuracy

In this method, Accuracy was conducted in triplicate by analyzing active pharma ingredient sample solution at three kinds of concentration levels of 50, 100 and 150% of each at a specified limit. Percentage recoveries were measured and found to be within the limit. The accuracy and reliability of the developed method were established. The percentage recovery values were found to be in the range of 98.74%-99.98% for Capecitabine and 98.54-99.728% for Docetaxel. The results are given in table 3, 4 and 5.

Precision

In method precision study prepare six different samples in the concentration of Capecitabine (150 ppm) and Docetaxel (40 ppm) are injected into HPLC system. Capecitabine %assay found to be in the range of 99.75%-100.75% and Docetaxel %assay found to be in range of 98.23%-100.01. These results are given below table 4.

Intraday precision

Six replicates of a sample solution containing Capecitabine (150µg/ml) and Docetaxel (40µg/ml) were analysed on the same day. Peak areas were calculated, which were used to calculate mean, SD and %RSD values.

Interday precision

Also called Intermediate precision. In this six replicates of a sample solution containing Capecitabine (150µg/ml) and Docetaxel (40µg/ml) were analysed on a different day. Peak areas were calculated which were used to calculate mean, SD and %RSD values. The present method was found to be precise as the RSD values were less than 2% and also the percentage assay values were close to be 100%. The results are given in table 5.

LOD and LOQ

The LOD concentrations for Capecitabine are 0.188 µg/ml and s/n values is 6 and Docetaxel 0.05 µg/ml and s/n value 4. The LOQ concentration for Capecitabine 0.62 µg/ml and their s/n values are 25 and Docetaxel their 0.165 µg/ml and s/n value is 24. The method is validated as per the US FDA guidelines [27].

Robustness

The conditions of the experiment were designed to test the robustness of established system intentionally altered, such as flow rate, mobile phase in organic percentage in all these varied conditions. Robustness results for Capecitabine and Docetaxel found to be within the limit and results are tabulated in table 7.

Table 3: Results of accuracy

S. No.	% Level	Capecitabine % recovery	Docetaxel % recovery
1	50	99.98	99.72
2	100	99.63	99.10
3	150	98.74	98.54
mean		99.45	99.12
SD		0.64	0.59

Mean+SD (n=3)

Table 4: Intraday precision results of capecitabine and docetaxel

Capecitabine				Docetaxel		
S. No.	Conc. (µg/ml)	Area counts	% Assay as is	Conc. (µg/ml)	Area counts	% Assay as is
1		2653102	99.99		658874	99.63
2	150	2674513	100.72	40	655321	98.31
3		2623050	99.63		654382	100.01
4		2631204	99.75		654763	99.85
5		2675843	100.48		653285	98.23
6		2663215	100.15		656498	98.65
% RSD	0.84			0.298		
mean	100.12			99.11		
SD	0.421			0.807		

Mean+SD (n=6)

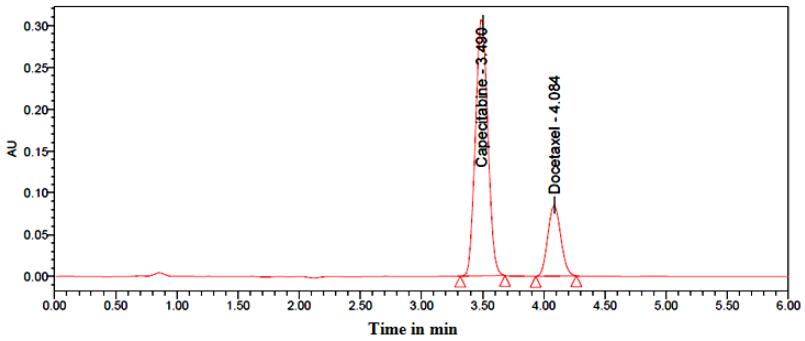


Fig. 5: Chromatogram of sample

Table 5: Inter-day outcomes of accuracy of capecitabine and docetaxel

Capecitabine				Docetaxel		
S. No.	Conc. (µg/ml)	Area counts	% Assay as is	Conc. (µg/ml)	Area count	% Assay as is
1		2648531	100.64		648579	100.17
2	150	2657482	100.12	40	643258	100.09
3		2635962	100.38		643982	100.32
4		2685471	100.52		643251	100.45
5		2665392	100.16		643985	100.47
6		2653244	100.47		647821	100.52
%RSD	0.63			0.37		
Mean	100.38			100.34		
SD	0.206			0.175		

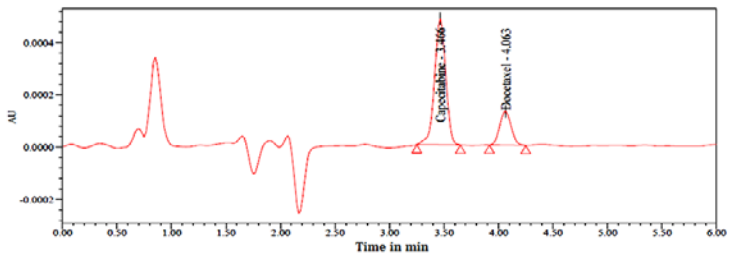
Mean+SD (n=6)

Table 6: LOD and LOQ for capecitabine and docetaxel

Capecitabine				Docetaxel			
LOD		LOQ		LOD		LOQ	
Concentration	s/n	Concentration	s/n	concentration	s/n	Concentration	s/n
0.188µg/ml	6	0.62µg/ml	25	1.818µg/ml	4	0.165µg/ml	24

Table 7: Robustness data of capecitabine and docetaxel

Parameter name	% RSD	
	Capecitabine	Docetaxel
Flow minus (0.8 ml/min	0.46	0.76
Flow plus (1.2 ml/min)	0.77	0.94
Organic minus (-10%)	1.21	0.38
Organic plus (+10%)	1.86	1.04



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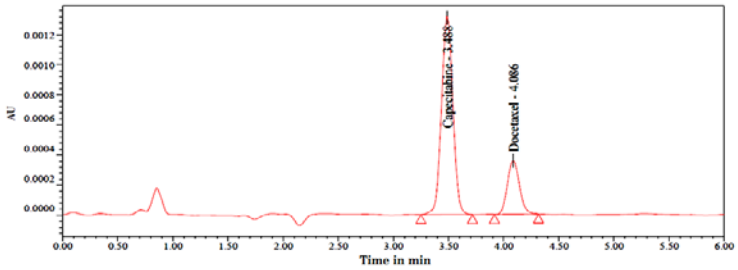

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Fig. 6: Chromatogram of (A) LOD and (B) LOQ

Stability

The standard and sample solution was kept at room temperature and at 2-8 °C up to 24 h. Then these solutions were pumped into the device and calculate the % of deviation from initial to 24 h [28]. There was no significant deviation observed and confirmed that the solutions were stable up to 24 h percentage of the assay was not quite 2%. There is no effect in storage conditions for Capecitabine and Docetaxel drugs. The results are given below table 8.

Table 8: Stability results of capecitabine and docetaxel

Stability	Capecitabine		Docetaxel	
	Purity	% of deviation	Purity	% of deviation
Initial	99.99	0.01	99.98	0.02
6 h	99.55	0.48	99.64	0.36
12 h	99.13	0.87	99.14	0.86
18 h	98.76	1.24	98.83	1.17
24 h	98.42	1.58	98.52	1.48

Degradation studies

The Docetaxel and Capecitabine sample was subjected into various forced degradation conditions to effect partial degradation of the drug. Studies of forced degradation [29] have carried out to find out that the method is suitable for products of degradation [30, 31]. In addition, the studies provide details about the conditions during which the drug is unstable, in order that the measures are often taken during formulation to avoid potential instabilities [32].

Acid degradation

Acid degradation was done at 1N HCl and degradation was formed 12.41% for Capecitabine and 13.22% for Docetaxel.

Alkali degradation

Alkali degradation was done at 1N NaOH and degradation was formed 12.36% for Capecitabine and 13.48% for Docetaxel.

Peroxide degradation

Peroxide degradation was done at 20% hydrogen peroxide and degradation was formed 13.47% Capecitabine and 15.42% for Docetaxel.

Reduction degradation

In reduction degradation, 11.59% Capecitabine and 12.54% Docetaxel degradation was observed.

Thermal degradation

In thermal degradation the sample was degraded to 10.63% of Capecitabine and 11.52% of Docetaxel.

Degradation of hydrolysis

In hydrolysis degradation the sample was degraded to 9.67% of Capecitabine and 10.47% of Docetaxel.

All degradation results are tabulated in table 9.

Table 9: Forced degradation results of capecitabine and docetaxel

Degradation condition	Capecitabine		Docetaxel	
	% Assay	% Deg	% Assay	% Deg
Acid degradation	87.59	12.41	86.78	13.22
Alkali degradation	87.64	12.36	86.52	13.48
Peroxide degradation	86.53	13.47	84.58	15.42
Reduction degradation	88.41	11.59	87.46	12.54
Thermal degradation	89.37	10.63	88.48	11.52
Hydrolysis degradation	90.33	9.67	89.53	10.47

CONCLUSION

We present in this article simple, selective, validated and well-defined stability that shows gradient RP-HPLC methodology for the quantitative determination of Capecitabine and Docetaxel. All the products of degradation formed during the stress conditions and the related active pharma ingredients are well separated and peaks were well resolved from each other and separate with an appropriate retention time indicating that the proposed method to be fast, simple, feasible and affordable in assay condition. Therefore the developed method during stability tests, it can be used for routine analysis of production samples and to verify the quality of drug samples during stability studies.

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AUTHORS CONTRIBUTIONS

All authors have contributed equally.

CONFLICTS OF INTERESTS

Declared none

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