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A Validated Stability Indicating UPLC Method for Simultaneous Determination of Related Substances, and Degradation Products of Cabazitaxel Drug Substance and its Pharmaceutical Injection Forms

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Abstract

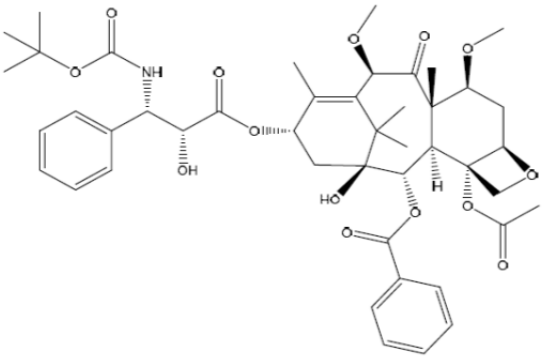
A simple, sensitive and fast analytical method for simultaneous determination of cabazitaxel and their synthetic impurities was developed and validated by using ultra performance liquid chromatography (UPLC). The separation was achieved on a Acquity UPLC BEH shield RP18 (100mm x 2.1mm, i.d., 1.7µm) at 30°C, with a mobile phase consisting of potassium dihydrogen phosphate/methanol/acetonitrile, (75/15/10, v/v/v) as mobile phase A/ and acetonitrile/water, (80/20, v/v) as mobile phase B at a flow rate of 0.5mL min^{-1} within 12.0min. The detection was made at a wavelength of 228nm by using photo diode array (PDA) detector. No interference peaks from excipients and relative retention time indicated the specificity of the method. The calibration curve showed correlation coefficients (r) > 0.99 calculated by linear regression and analysis of variance (ANOVA). The limit of detection (LOD) and limit of quantitation (LOQ), respectively, were 0.002 mg/mL and 0.006 mg/mL. Intraday and interday relative standard deviations (RSDs) were < 2.0 (drugs) and < 10% (degradation products) as well as the comparison between two different analysts, which were calculated by *f* test.

Keywords:
Cabazitaxel, UPLC, method development, Forced degradation, Stability indicating.

INTRODUCTION

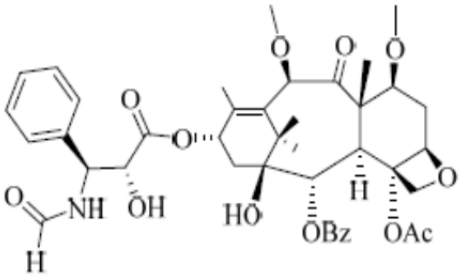
The drug substance cabazitaxel (CABZ; Fig 1), benzenepropanoic acid, β-[[(1,1-dimethyethoxy)carbonyl]amino]-α-hydroxy-,(2aR,4S,4aS,6R,9S,11S,12S,12aR,12bS)-12b-(acetyloxy)-12-(benzoyloxy)- 2a,3,4,4a,5,6,9,10,11,12,12a,12b dodecahydro-11-hydroxy-4,6-dimethoxy-4a,8,13,13-tetramethyl-5-oxo-7,11-methano-1H-cyclodeca[3,4] benz [1,2-b] oxet-9-yl ester, (α R,β S)-, Cabazitaxel is an antineoplastic agent belonging to the taxane class which is prepared by semi-synthetic methods with a precursor extracted from yew needles [3, 4]. Cabazitaxel binds to and stabilizes tubulin, resulting in the inhibition of microtubule depolymerization and cell division, cell cycle arrest in the G2/M phase, and the inhibition of tumor cell proliferation. Unlike other taxane compounds, this agent is a poor substrate for the membrane associated with multidrug resistance (MDR), P-glycoprotein (P-gp) efflux pump and may be useful for treating multidrug-resistant tumors for the treatment of hormone-refractory prostate cancer. A literature survey revealed that few analytical methods, such as spectrophotometry, HPLC, have been reported for the determination of cabazitaxel. Stability indicating RP-HPLC method for the determination of cabazitaxel [1], Quantification of cabazitaxel in human plasma by liquid chromatography/triple quadrupole mass spectrometry [2], Determination of cabazitaxel in rat whole bold on dry blood spots [5], New spectrophotometric methods for the quantitative estimation of cabazitaxel in formulations [6], All the reported literature methods were useful only in the estimation of cabazitaxel content in human plasma and dosage forms, but not for the determination of impurities present in cabazitaxel drug substance.

Furthermore, there is no stability-indicating UPLC method reported in the literature that can adequately separate and quantify all the synthetic and degradation impurities of cabazitaxel drug substance in a short run time. It is, therefore, felt necessary to develop a new stability indicating UPLC method for the related substance determination of cabazitaxel. We intend to adopt for a faster chromatographic technique UPLC for the current study. An attempt was made to determine whether UPLC can reduce analysis times without compromising the resolution and sensitivity. Hence, a stability indicating reverse phase UPLC method was developed for the quantitative determination of cabazitaxel and its six synthetic impurities, namely impurity A, B, C, D, E and F. This method was successfully validated according to the ICH guidelines [14]. The chemical structure, names of cabazitaxel and its impurities A, B, C, D, E and F were depicted in (Figure 1).

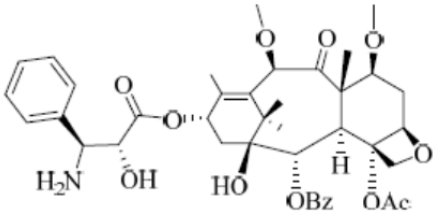


Structure of Cabazitaxel

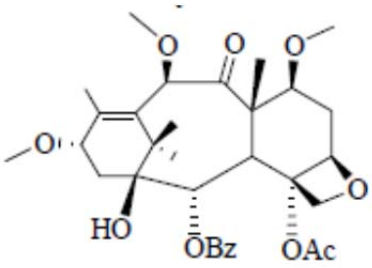
Benzenepropanoic acid, β-[[(1,1-dimethyethoxy)carbonyl]amino]-α-hydroxy-2aR,4S,4aS,6R,9S,11S,12S,12aR,12bS)-12b-(acetyloxy)-12-(benzoyloxy)-2a,3,4,4a,5,6,9,10,11,12,12a,12b-dodecahydro-11-hydroxy-4,6-dimethoxy-4a,8,13,13-tetramethyl-5-oxo-7,11-methano-1H-cyclodeca[3,4] benz [1,2-b] oxet-9-yl ester, (α R,β S)-



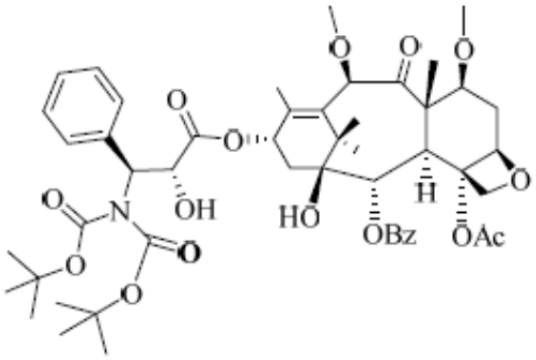
Structure of Impurity A



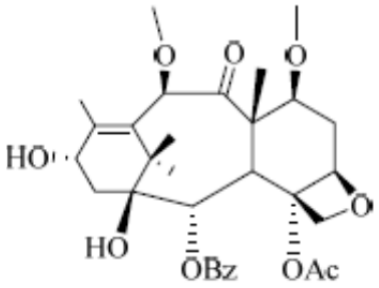
Structure of Impurity B



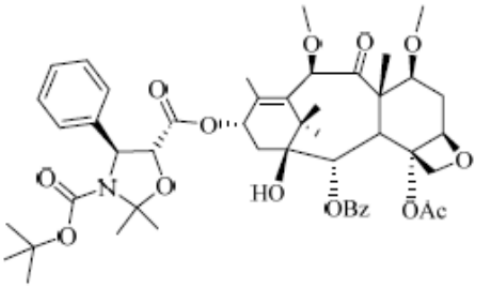
Structure of Impurity C



Structure of Impurity D



Structure of Impurity E



Structure of Impurity F

Fig 1 Chemical structure of Cabazitaxel and its synthetic impurities

EXPERIMENTAL

Materials and Reagents

Cabazitaxel standards and samples were supplied by Macleod's pharmaceuticals, mumbai, India. Commerically available JEVTANA (cabazitaxel) in 60mg/1.5mL injection was used for the injectable form analysis. The HPLC grade acetonitrile, analytical-grade potassium dihydrogen phosphates were procured from merck, HPLC grade methanol were purchased from Merck (Darmstadt, Germany). Water was prepared in-house by using a Millipore milli-Q-water purification system (Millipore corporate headquarters, Billerica, MA).

Chromatographic Conditions and Equipment

UPLC was performed using a Waters acquity system (Waters corporation, Milford MA 01757) equipped with binary solvent delivery pump, an auto sampler and tunable UV detector (waters). The chromatographic separation was performed using a Waters acquity UPLC BEH shield RP18 (100mm x 2.1mm, 1.7µm) column. The separation was achieved using a gradient method. Mobile phase A

contained a mixture of 20mM $KH_2PO_4.H_2O$, methanol and acetonitrile 75:15:10 (v/v/v), respectively, mobile phase B contained a mixture of acetonitrile and water (80:20, v/v) respectively.

The flow rate of the mobile phase was 0.5mL/min. The UPLC gradient program was set as: time (min) / % mobile phase B: 0.01/0, 5.0/50, 7.0/80, 8.0/90, 9.0/90 and 12.0/0. The column temperature was maintained at 30°C, and the detection was monitored at a wavelength of 228nm. The injection volume was 10.0µL.

Preparation of standard solutions

A solution of cabazitaxel standard (1000µg/mL) was prepared by dissolving an appropriate amount of the drug in acetonitrile: water and methanol at a ratio of 50:30:20 (v/v/v), respectively. Mixed and individual stock solutions (100µg/mL) of the impurities (denoted impurity A to impurity F) were prepared in acetonitrile: water and methanol at a ratio of 50:30:20 (v/v/v).

Preparation of sample solutions

A sample solution of cabazitaxel (1000µg/mL) was prepared by dissolving an appropriate amount of the drug in acetonitrile: water and methanol at a ratio of 50:30:20 (v/v/v), respectively or by diluting the appropriate quantity of formulation (liquid injection) in acetonitrile: water and methanol at a ratio of 50:30:20 (v/v/v) and filtering the solution.

Specificity: Forced Degradation Studies

Stress studies of the drug's substances were utilized for the identification of the possible degradation products and for the validation of the stability indicating analytical procedures. It is the ability of the analytical method to measure the analyte response in the presence of its degradation products. The specificity of the developed UPLC method for cabazitaxel was carried out in the presence of its six synthetic impurities. Stress studies were performed at an initial concentration of 1000µg/mL of cabazitaxel drug substance. Intentional degradation was attempted by the stress condition of acid (1N HCl at room temperature), base (0.01N NaOH at room temperature), hydrolysis (70°C), oxidation (3% H_2O_2 at room temperature), Thermal degradation (60°C for 7d), Photo degradation sample has been exposed to light providing on overall illumination of not less than 1.2 million lux hours and an integrated near ultraviolet energy of not less than 200 watt hours/square meter to evaluate the ability of the proposed method to separate cabazitaxel from its degradation products for the hydrolysis, acid, base and oxidation studies, the study periods were 4h, 24h, 12h and 24h, respectively.

The purity of the peaks obtained from the stressed samples was verified using the photo diode array (PDA) detector. The purity angle was within the purity threshold limit obtained in all the stressed samples and demonstrated the analyte peak homogeneity. An assay of stressed samples was performed by comparison with reference standards. An assay was also calculated for the cabazitaxel sample by spiking all six impurities at the specification level (i.e., 0.15%).

Method Validation

Linearity of Response [Calibration curve]

The linearity was determined by the calibration curve obtained using seven standard solutions in the concentration range of 500 to 2000 µg/mL (cabazitaxel). Triplicate determinations at each concentrations level were performed. The range (interval between lower and upper concentrations in the sample) of the appropriate amount of samples was determined. The slope and other statistics of calibration curves were calculated by linear regression and analysis of variance (ANOVA) [15-17].

Limit of Detection (LOD) and Limit of Quantification (LOQ)

The LOD and LOQ were determined based on standard deviation among responses and slopes of the regression equation of the calibration curve [14].

Precision

The intra-day and inter-day precisions were determined by analyzing the standard and sample solutions. For this evaluation, six standard solutions and ten sample solutions replicates at 100% of the test concentration were prepared in different days by different analysts. The statistical data were obtained from F test [15, 16]

Accuracy

The accuracy was evaluated by adding known amounts of standard solutions in the sample solution. The accuracy was evaluated at three concentrations levels, which were from 80 to 120% of cabazitaxel as well as their synthetic impurities. The recovery experiments were performed in triplicate and data were determined by dividing the value obtained for the sample prepared with the added standard, by the amount added, and the multiplied by 100%.

Robustness

A robustness study was performed by means of an experimental design and multivariate analysis using Modde 5.0 software (Umetrics, Umea, Swedan). In this study, 3 factors (column temperature, percent of mobile phase B, flow rate) were investigated. A two-level full factorial design was applied with a number of runs is equal to $2^k + n$, where k is the number of factors and n is the number of center points. Thus, 11 experiments including 3 centre points were performed in duplicate. The lower and higher values for each factor in the design are given in Table 3. The statistical relationship between a response y and the experimental variables xi, xj…. is as follows:

$$y = \beta 0 + \beta_i x_i + \beta_j x_j + \beta_{ij} x_i x_{j+ \ldots} + E$$

where the βs are the regression coefficients and E is the overall experimental error. The linear coefficients β_i and β_j describe the quantitative effect of the experimental variables in the model while the cross coefficient β_{ij}, measures the interaction effect between the variables i and j.

RESULTS AND DISCUSSION

Method Development and Optimization

The main aim of this study was to develop a selective and sensitive method for analysis of cabazitaxel and its impurities. The maximum absorption wavelength of the reference drug solution, process bi-products, and forcibly degraded drug solutions is 228nm, which is the

intersecting value obtained from the ultra violet absorption spectra, Hence, 228nm was selected as the detection wavelength for LC analysis. The blend containing 1000µg/mL of cabazitaxel and 10µg/mL of each impurity (six) was prepared in the mixture of acetonitrile: water and methanol (50:30:20 v/v/v). Cabazitaxel spiked solution were subjected to separation by reverse-phase UPLC on a Waters Acquity BEH C18, 50mm x 2.1mm, 1.7µm column with mobile phase A contained a mixture of 20mM $KH_2PO_4.H_2O$, methanol and acetonitrile 75:15:10 (v/v/v), respectively, mobile phase B contained a mixture of acetonitrile and water (80:20, v/v) respectively. Flow rate was set at 0.5mL/min. The UPLC gradient program time (min) / % mobile phase B was set as 0.01/0, 5.0/50, 7.0/80, 8.0/90, 9.0/90 and 12.0/0. Column temperature was maintained at 30°C (Trial-1). In this trail one Imp-C peak merging with Imp-D and other unknown impurity co-eluting with Imp-A (Resolution <1.5). Efforts were made to separate the peaks closely eluting pair of compounds, In order to increase the resolution between these pairs of compounds, mobile phase A buffer percentage concentration was increased from 50 to 80 in the initial gradient step. With this increased buffer composition the retention time of cabazitaxel was increased but separation between Imp-C and Imp-D was not achieved. Efforts were made to separate the pairs of compound on Waters Acquity BEH C18, 100mm x 2.1mm, 1.7µm column. The chromatographic conditions of trial-1 were employed in this trial. With the increase in column length Imp-A and its adjacent peak were separated (Resolution >2) but the resolution between Imp-C and Imp-D was not improved, various trials were made by changing the gradient compositions but none of the trial could serve the purpose. It was decided to change the column chemistry and Waters Acquity BEH SHIELD RP18, 100mm x 2.1mm, 1.7µm column was used with the conditions mentioned in trial 1. It was found that all the peaks were separated with a resolution greater than 2.

System suitability parameters were evaluated for cabazitaxel and its six impurities. Tailing factor for all the impurities and cabazitaxel was found less than 1.5. USP resolution (Rs) of cabazitaxel and six synthetic impurities was greater than 2.0 for all pairs of compounds. The chromatograms representing cabazitaxel spiked with the impurities A, B, C, D, E and F at 0.15% was shown in (Figure 2).

Validation of the Method

System suitability

A standard solution was injected six times into the UPLC system. The system was deemed to be suitable because the resolution between cabazitaxel drugs and their respective synthetic impurities was greater than 1.5, the reproducibility of 6 injections of each compound peak area produced a relative standard deviation (RSD) lower than 3.0% and the tailing factors were higher than 0.98, with these results, it can be concluded that the chromatographic system is perfectly adjusted to perform analysis.

Calibration curve

The calibration curve was linear by analyzing standard solutions at seven concentration levels. The correlation coefficient (r) were > 0.99 (Table 2 and 3). Therefore, the drugs and their synthetic impurities presented good linearity. The ANOVA for linearity of the drugs and their degradation products is also presented in Tables 2 and 3, respectively. The distribution variable (F) value for lack of fit was smaller than the tabulated F value of 95% confidence, accordingly to the ANOVA test, the UPLC method showed no lack of fit.

Limit detection (LOD) and limit of quantification (LOQ)

According to the International Conference on Harmonization (ICH) recommendations [14], the approach based on the standard deviation [SD] of the response and the slope was used for determining the detection and quantitation limits. These results also proved the sensitivity of the proposed analytical method.

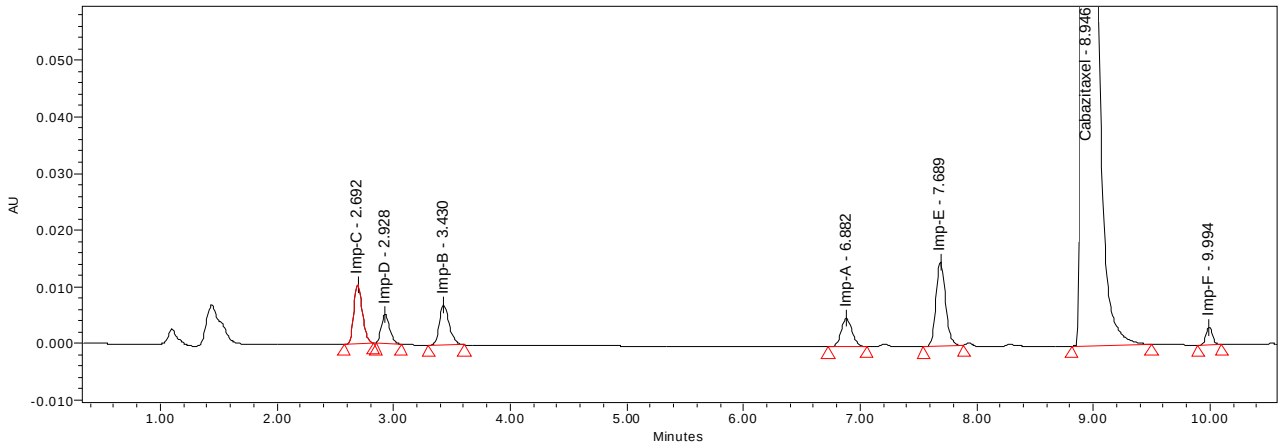


Fig 2 UPLC PDA analysis of spiked sample solution of cabazitaxel column: Acquity UPLC BEH shield RP18 (100mm x 2.1mm, 1.7µm, waters), mobile phase: phosphate buffer-acetonitrile-methanol (75:15:10, v/v), column temperature 30°C; flow rate: 0.5mL/min. Injection volume 10µL, UV detection 228nm.

Precision

The precision was determined by repeatability and intermediate precision levels. For repeatability and intermediate precisions, 6 independent samples containing the synthetic impurities were performed on two different days by two different analysts. The RSD was <2% (drugs) and <10% (degradation products), respectively (Table 3); these results indicated the precision of the proposed analytical method. The F test was also applied in order to compare the variability between the analysts and sample variations; the computed F value for all compounds did not exceed the F critical value (4.03). Thus, it can be concluded that there is no difference between the variances obtained.

Accuracy

According to the ICH guidelines, the standard solution addition should be done in a range from 80 to 120% of the nominal concentration. The accuracy of the method was evaluated at three concentration levels. Triplicate determinations were made at each concentration level. The accuracy was expressed as percentage of standard recovered from sample matrix. The mean recoveries of investigated oncologic drugs and their degradation products were found to be in the range of 98.00 and 102.00%, indicating good accuracy for the chromatographic method Table 1.

Table 1 Method Validation Data

Parameter	Cabazitaxel	Imp-A	Imp-B	Imp-C	Imp-D	Imp-E	Imp-F
Limit of Detection (µg/mL)	0.002	0.001	0.001	0.002	0.002	0.001	0.001
Limit of Quantification (µg/mL)	0.006	0.003	0.003	0.005	0.006	0.003	0.003
Precision (%RSD)							
(Level-1) 80% (n=6)	3.5	4.6	4.9	4.5	4.2	4.3	2.6
(Level-2) 100% (n=6)	2.2	3.2	3.8	2.9	4.3	3.7	2.4
(Level-3) 120% (n=6)	1.8	2.4	3.4	2.4	1.8	2.7	3.4
Accuracy (% Recovery)							
LOQ (n=3)	-	103.4	101.8	104.5	102.8	103.9	99.7
50% (n=3)	99.5	101.2	99.1	102.2	99.4	100.4	99.4
100% (n=3)	98.3	98.1	99.5	100.7	99.8	100.7	98.6
150% (n=3)	99.4	99.1	98.3	101.9	98.6	102.9	101.8
Robustness (Resolution)							
Actual flow 0.50mL/min	2.3	3.5	4.9	2.9	3.1	3.8	4.7
Different flow 0.45mL/min	2.9	4.1	4.7	2.6	3.5	4.2	4.9
Different flow 0.55mL/min	2.3	3.2	4.2	2.1	2.6	3.4	4.1
Column temperature 25°C	2.5	3.8	4.9	2.7	2.9	3.8	4.7
Column temperature 35°C	2.1	3.2	4.2	2.1	2.6	3.4	4.1

Table-2 Linear regression and ANOVA statistical data in the analysis of Cabazitaxel

Statistical Parameters	Cabazitaxel	Imp-A	Imp-B	Imp-C
Concentration range (µg mL-1)	50.0 – 200.0	5.0 – 50.0	5.0 – 50.0	5.0 – 50.0
Regression equation	y=0.054 + 0.0125	y=0.036 + 0.0123	y=0.058 + 0.0119	y=0.064 + 0.0115
Correlation coefficient (r)	0.9990	0.9994	0.9995	0.9998
Standard error	0.6134	2.798	1.533	1.423
F	3446.982	1656.252	3,548.457	6409.927
SS (residual)	1.505	21.324	9.404	8.108
MS (residual)	0.376	7.831	2.351	2.027
SS (regression)	12999.9	12970.18	12992.1	12993.39
MS (regression)	12975.9	12812.15	12902.5	12798.45
Lower 95%	-0.433	-8.2032	-2.196	-1.775
Upper 95%	2.435	5.196	-4.952	4.852

Table-2 (Continued) Linear regression and ANOVA statistical data in the analysis of Cabazitaxel

Statistical Parameters	Imp-D	Imp-E	Imp-F
Concentration range (µg mL-1)	5.0 – 50.0	5.0 – 50.0	5.0– 50.0
Regression equation	y=0.032 + 0.0132	y=0.042 + 0.0138	y=0.039 + 0.0145
Correlation coefficient (r)	0.9994	0.9995	0.9998
Standard error	3.271	2.465	2.4500
F	1210.549	2135.556	2161.845
SS (residual)	42.81919	6.076	24.0118
MS (residual)	10.7048	24.306	6.0029
SS (regression)	12958.68	12977.19	12977.49
MS (regression)	12929.48	12472.12	12173.54
Lower 95%	-6.7117	-6.6009	-.2.576
Upper 95%	8.6159	5.1196	8.6768

Table-2 (continued) Chromatographic parameter settings applied in the robustness investigation, corresponding low (-) central (0) and high (+) levels

Parameter	Low value (-)	Central value (0)	High value (+)
Temperature (°C)	48	50	52
%B in mobile composition in gradient	9	10	11
Flow rate	33	35	37

Table-3 F test two-sample for variances of Cabazitaxel

Cabazitaxel	Variable 1	Variable 2
Mean	7.528	8.093
Varience	0.052	0.025
Observations	10	10
Df	9	9
F	1.46	2.54
P (F <=f_ one-tail 0.256	-	
F critical one-tail 4.036	-	

Df = degree of freedom
F = distribution variable

Robustness study

Variations in LC operating conditions were made to demonstrate the robustness of the method. The effect of small changes of the most relevant chromatographic parameters on the results was investigated by means of an experimental design. The different chromatographic parameter settings in the design and the factors investigated are described in the experimental section. The resolutions (Rs) between the main component and the two most closely eluting impurity peak (Imp C and Imp D) were investigated as response variables. The resolution between Imp C – Imp D as Rs 2. The plots consists of bars which correspond to the regression coefficients. The bars denoted by one variable represent the regression coefficient for the linear effect of the particular variable and the bars denoted by variable 1 * variable 2 represent the interaction between the two variables concerned (Eq. (1). When the error bars (α=0.05, 95% confidence interval) include zero, the variation of the response caused by changing the variable is smaller than the experimental error and the effect is considered not to be significant. A positive regression coefficient stands for a positive effect on the resolution, while a negative regression coefficient indicates a negative effect, The magnitude of an effect is proportional to its respective coefficient, from the regression coefficient plots, it is observed that in the ranges examined the three parameters did not have a significant effect on the resolutions and no significant interactions were found. Hence it can be concluded that the method is robust in the range examined.

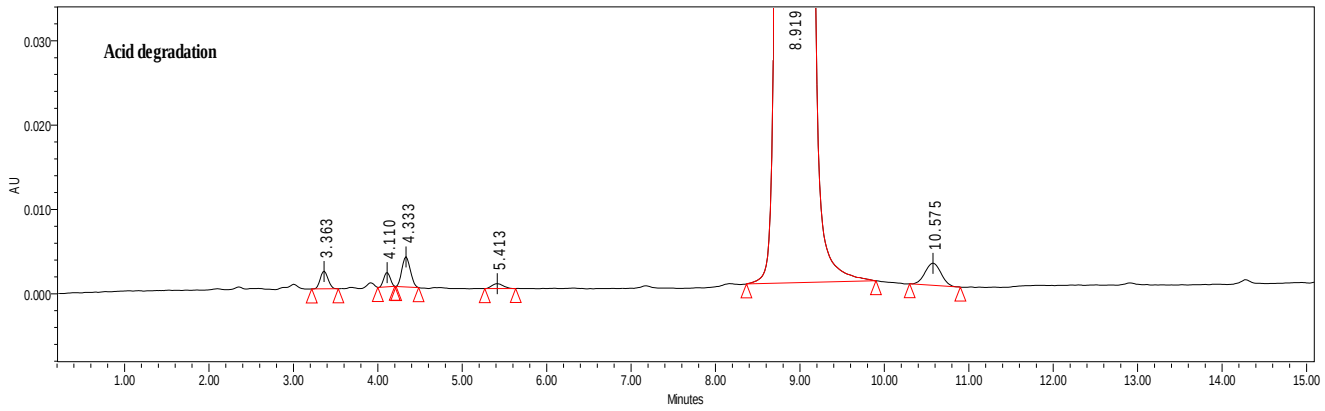
Stability solutions

To demonstrate the stability of standard working and sample solutions during analysis, both solutions were analyzed over a period of 24 h while being stored at refrigerator (± 4°C). The results showed that the retention times and peak areas of the drugs as well as their synthetic impurities remained almost unchanged and no significant degradation was observed during this period, suggesting that these solutions were stable for at least 2 days when stored in a refrigerator at ±4°C, which was sufficient for the whole analytical process.

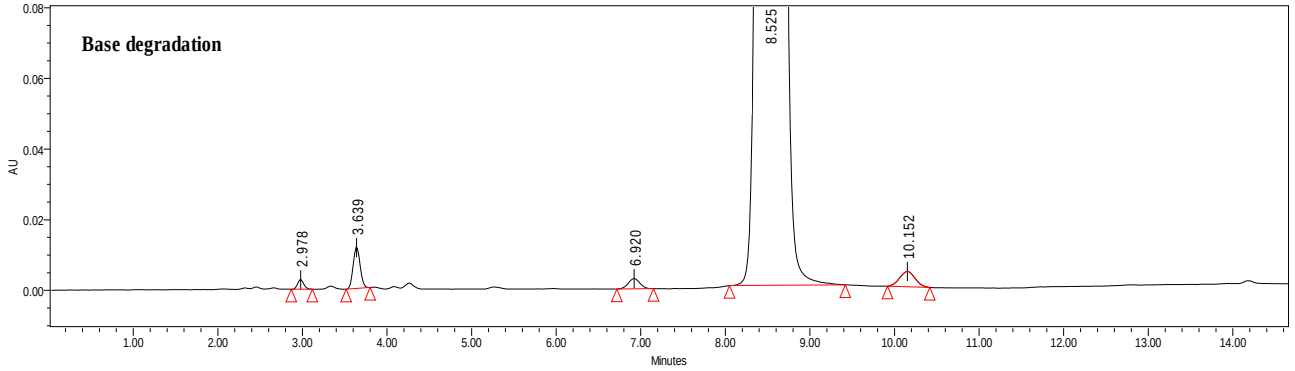
Specificity: Forced Degradation Studies

Stress studies of the drug substance were utilized for the identification of the possible degradation products and for the validation of the stability-indicating analytical procedures. If the ability of the analytical method to measure the analyte response in the presence of its degradation products. UPLC conditions using a PDA detector to monitor the homogeneity and purity of the cabazitaxel peak, degradation was not observed when cabazitaxel was subjected to light and heat conditions. Significant degradation was observed when the drug was subjected to hydrolysis (water at 70°C for 24r) and oxidative hydrolysis (3% H2O2 at room temperature for 12hr), base (0.01N NaOH at room temperature for 24hr), and acid (1N HCl at room temperature for 4hr).

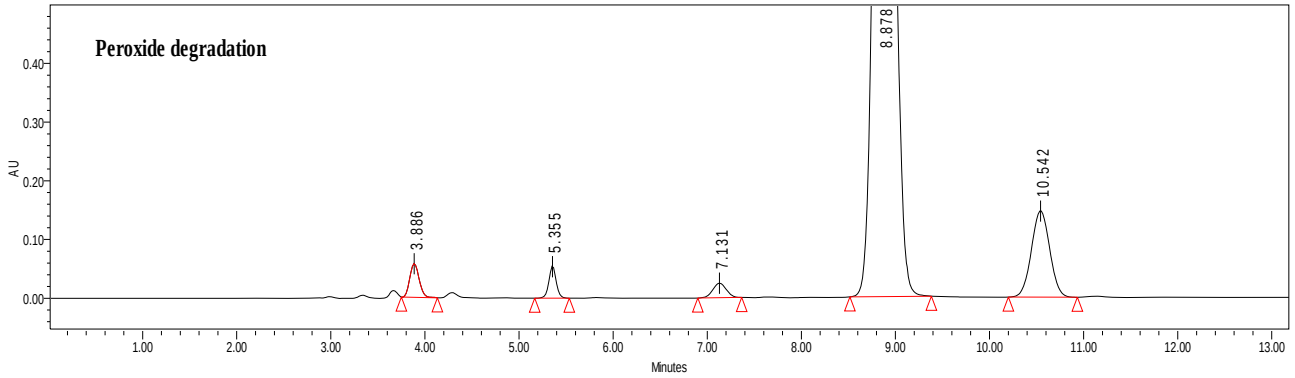
The chromatograms were checked for the appearance of any extra or overlapping peaks. Peak purity of these samples under stressed condition was verified using a PDA detector. The purity of the principle and other chromatographic peaks was found to be satisfactory and the results from force degradation studies are presented in Table 4. Degradation chromatograms were shown in (Figure 3).



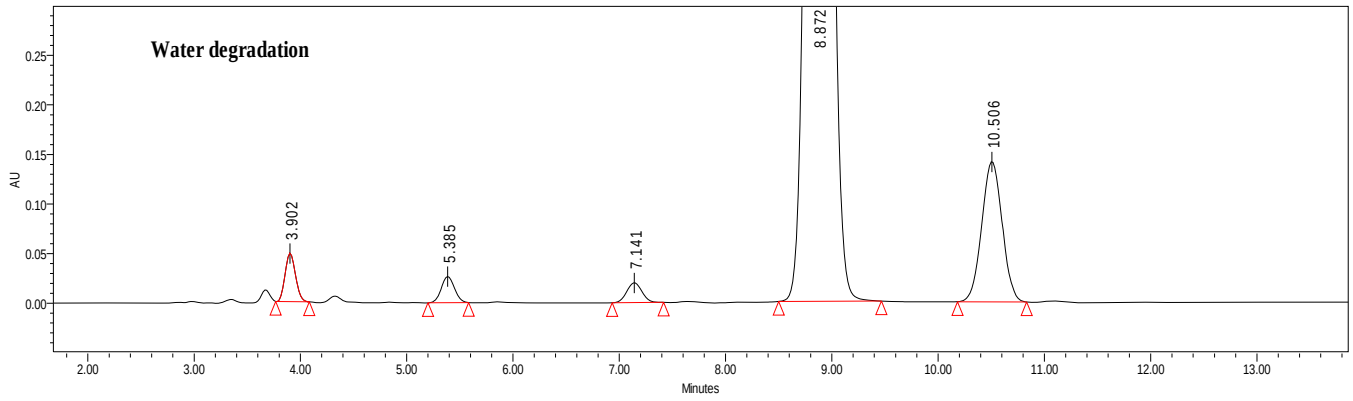
(A)


Base degradation
AU
Minutes
2.978
3.639
6.920
8.525
10.152

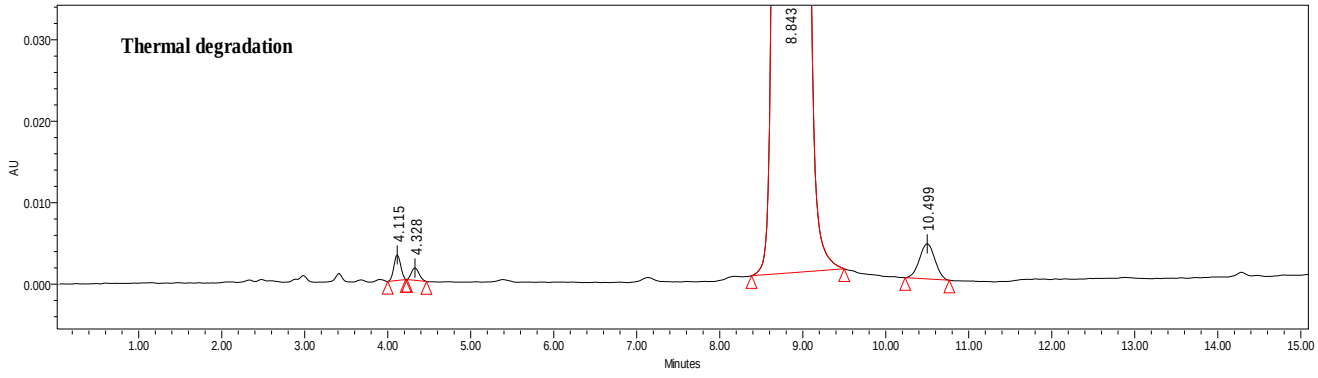
(B)


Peroxide degradation
AU
Minutes
3.886
5.355
7.131
8.878
10.542

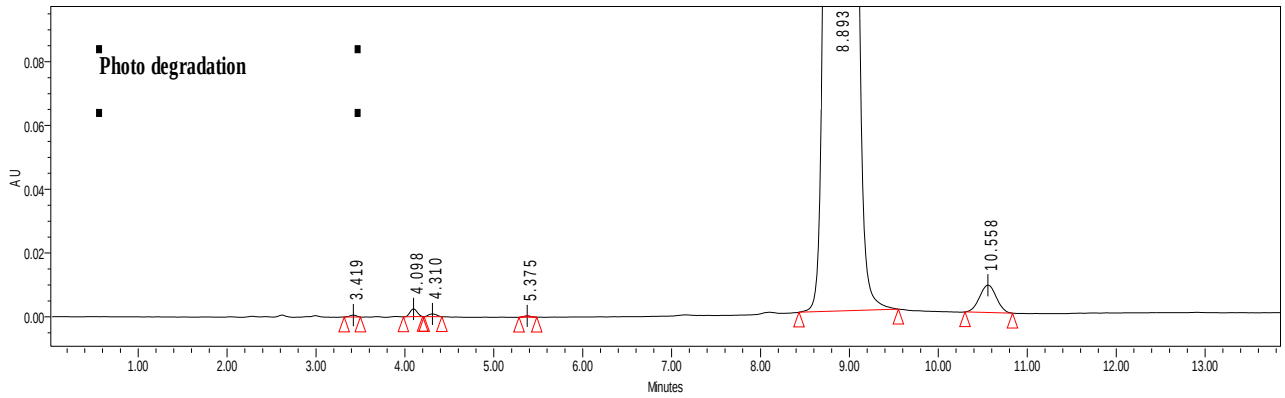
(C)


Water degradation
AU
Minutes
3.902
5.385
7.141
8.872
10.506

(D)


Thermal degradation
AU
Minutes
4.115
4.328
8.843
10.499

(E)



(F)

Fig 3 Specificity study chromatograms (A) Acid degradation; (B) Base degradation; (C) Peroxide degradation; (D) Hydrolysis degradation; (E) Thermal degradation (F) Photo degradation.

Table 4 Summary of Forced Degradation Results

Degradation Condition	% Degradation from Related substances by UPLC Remarks/Observation Degradation	Assay (%w/w)	Mass Balance (%Assay+%Degradation products of Related substances)	Remarks
1N HCl at room temperature for 4 h (acid hydrolysis)	5.4	94.5	99.9	Significant degradation observed
0.01N NaoH at room temperature for 24hr (base hydrolysis)	8.2	91.2	99.4	Significant degradation observed
3% H2O2 at room temperature for 12h (oxidation hydrolysis)	7.3	92.4	99.7	Significant degradation observed
Water at 70°C for 24 h (water hydrolysis)	6.2	94.2	100.4	Significant degradation observed
Thermal degradation Heat at 60°C for 10 d	2.3	97.1	99.4	No significant degradation observed
Photo degradation UV light (NLT 200 watt/h)	1.2	98.4	99.6	No Significant degradation observed
White light (1.2 million lux h)	1.4	98.5	99.9	No significant degradation observed

CONCLUSION

The rapid gradient RP-UPLC method developed for quantitative analysis of cabazitaxel drug and their synthetic impurities was found to be efficient, accurate and sensitive. The excipient has no interference in the UPLC analysis and the method is simple, using a minimum number of reagents. The speed of analysis and its low cost make the method suitable for routine quality control analysis.

REFERENCES

1. M.Mathrusri Annapurna, K. Pramadvara, B. Venkatesh and G. Sowjanya, Stability indicating RP-HPLC method for the determination of Cabazitaxel, *Indo American Journal of Pharmaceutical research*, 2013; 3: 9262-9269.
2. Peter de Bruijn, Anne-Joy M. de Graan, Annemieke Nieuweboer.Ron H.J Mathijssen, Mei-Ho-Lam, et al, Quantification of cabazitaxel in human plasma by liquid chromatography/triple-quadrupole mass spectrometry. A practical solution for non-specific binding. *Journal of pharmaceutical and biomedical analysis*, 2012; 59: 117-122.
3. Paller, C.J; Antonarakis, E.S. Cabazitaxel; a novel second-line treatment for metastatic castration resistant prostate cancer. *Drug Des.Devel.Ther* , 2011; 5: 117-124.
4. Wilkes, G.M. Cabazitaxel, a taxane for men with hormonerefractory metastatic prostate cancer. *Oncology* , 2010; 24 : 46-48.
5. V.Jagannath Patro, Nageshwara Rao. R and N.K.Tripathy, LC-MS/MS Determination of Cabazitaxel in Rat whole blood on Dry blood spots, *Scientific Reports,* 2012; 1 : 1-4.
6. Gudisa Kishore, New spectrophotometric methods for the quantitative estimation of Cabazitaxel in formulations, *International journal of Research and Reviews in pharmacy and Applied science* 2012; 2 : 950-958.
7. A. Kort, M.J.Hillebrand, G.A Cirkel, et al., Quantification of cabazitaxel, its metabolite Docetaxel and the determination of the demethylated metabolites RPR112698 and RPR123142 an docetaxel equivalents in human plasma by liquid chromatography tandem mass spectrometry, *J.Chromatogr. B Analyt Technol Biomed. Life Sci*, 2013; 15: 117-123.
8. Agarwal N, Sonpavde G, Sartor O, Cabazitaxel for the treatment of castration-resistant prostate cancer. *Future Oncol*, 2011; 7 : 15-24.
9. ICH Q1A (R2) Stability Testing of New Drug Substances and Products, USFDA-Federal Register, USA < http://www.ich.org , 2003.

10. ICH Q2(R1), Validation of Analytical Procedures; Text and Methodology, USFDA-Federal Register, USA < http://www.ich.org, 2005.
11. Swartz, M.E. UPLC, An Introduction and Review. *J.Liq.Chromatogr.R.T* , 2005; 28: 1253-1263.
12. Wren, S.A.C; Tchelitcheff, P, Use of ultra-performance liquid chromatography in pharmaceutical development. *J.Chromatogr. A*, 2006; 1119: 140-146.
13. The United States Pharmacopeia, Thirty-fifth Edit, Validation of Compendial Methods, Section <1225>, Rockville, 2012.
14. International Conference on Harmonization, ICH Topic Q2, Validation of Analytical Procedures; Text and Methodology. Available from: < http://www.ich.org, (R1) 2005.
15. S. Weisberg, Applied Linear Regression, second ed., Wiley, New York 1985.
16. N.Draper, H.Smith, Applied Linear Regression, second ed., Wiley, New York, 1998.
17. H. Toutenburg, Statistical Analysis of Designed Experiments, second ed., Spinger, New York 2002.
18. P.L. Garcia, E.Buffoni, F.P. Gomes, J.L.V. Quero, Wide spectra of quality control, in : I.Akyar (Ed.), Tech, Rijeka, 2011; pp, 3-20.