

A Validated Stability-indicating Liquid Chromatographic Method for Determination of Cabazitaxel-A Novel Microtubule Inhibitor

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Abstract

Cabazitaxel is used to treat advanced prostate cancer. Cabazitaxel is a semi-synthetic derivative of the natural taxoid 10-deacetyl baccatin III with potential antineoplastic activity. Cabazitaxel is also known as XRP6258, a semi-synthetic taxane from a single diastereoisomer of 10-deacetyl baccatin III derived from the needles of various Taxus species. A stability-indicating high performance liquid chromatographic technique was developed for the determination of Cabazitaxel in bulk and pharmaceutical formulations. Chromatographic separation was performed on Shimadzu Model CBM-20A/20 Alite, using Zorbax SB-C18 column (150 mm×4.6 mm i.d., 3.5 µm particle size) with a mixture of 0.1% ortho phosphoric acid and methanol (20:80, v/v) as mobile phase with a flow rate of 1.0 ml/min. Cabazitaxel was subjected to stress conditions (acidic, alkaline, oxidation photolytic and thermal degradations) and the method were validated as per ICH guidelines.

Keywords: Cabazitaxel; RP-HPLC; Stability-indicating; ICH

Introduction

Abazitaxel is used to treat advanced prostate cancer that is no longer responding to hormone therapy. It is also being studied for use against other kinds of cancer. It interferes with microtubules, which are part of the internal structure that cells need when they are dividing. This leads to cell death. Because cancer cells divide faster than normal cells, they are more likely than normal cells to be affected by this drug. Cabazitaxel is a semi-synthetic derivative of a natural taxoid used for the treatment of hormone-refractory prostate cancer [1]. Unlike other taxane compounds, this agent is a poor substrate for the membrane-associated, multidrug resistance (MDR), P-glycoprotein (P-gp) efflux pump and may be useful for treating multidrug-resistant tumors. 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.

Cabazitaxel has been approved in the US by the Food and Drug Administration (FDA) [2] in June 2010 and in Europe by the European Medicines Agency (EMA) in January 2011 in combination with prednisone for the treatment of patients with castration resistant metastatic prostate cancer whose disease progresses after docetaxel treatment [3], based on the results of the TROPIC trial investigating Cabazitaxel plus prednisone versus mitoxantrone plus prednisone following docetaxel failure [4]. Cabazitaxel chemically known as (2aR,4S,4aS,6R,9S,11S,12S,12aR,12bS-12bS)-12b-acetoxy-9-(((2R, 3S)-3-((tert-butoxycarbonyl) amino)-2-hydroxy-3-phenyl propanoyl) oxy)-11-hydroxy-4,6-dimethoxy 4a,8,13,13-tetramethyl-5-oxo-2a,3,4,4a,5,6,9,10,11,12,12a,12b-dodecahydro-1H-7,11-methanocyclodeca benzo [1,2-b] oxet-12-yl benzoate is chemotherapy drug with molecular formula C₄₅H₅₇NO₁₄ and molecular weight 835.93 g/mol (Figure 1) [5,6]. Cabazitaxel penetrates the blood-brain barrier. Cabazitaxel is currently being investigated in the setting of metastatic breast cancer progressing after taxane or anthracycline based chemotherapeutic regimens [7,8].

Very few analytical methods have been reported for the determination of Cabazitaxel such as spectroscopic techniques [9], HPLC [10,11], LC-MS/MS [12-14] in biological fluids. At present the

authors have proposed a stability indicating RP-HPLC method for the determination of Cabazitaxel in presence of its degradation products.

Experimental

Chemicals and reagents

Cabazitaxel standard (purity 99%) was obtained from Dr. Reddy's Laboratories Ltd. (Visakhapatnam, India). Methanol (HPLC grade),

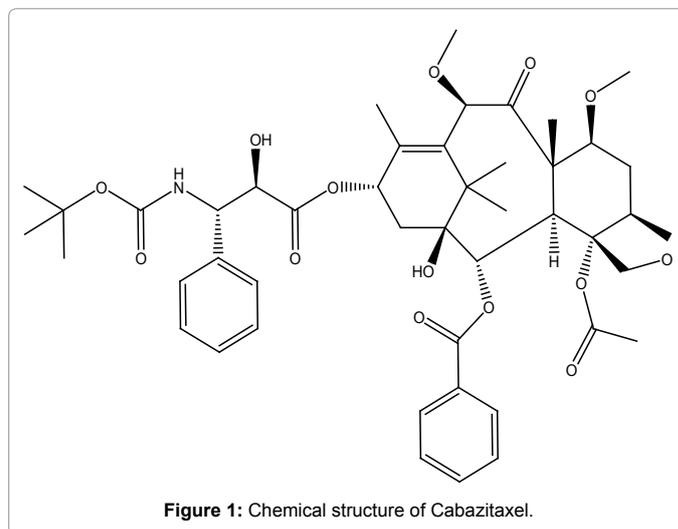


Figure 1: Chemical structure of Cabazitaxel.

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Polysorbate 80, Ethanol (HPLC grade), Sodium hydroxide (NaOH) and Hydrochloric acid (HCl), orthophosphoric acid and Hydrogen peroxide (H₂O₂) were purchased from Merck (India). All chemicals were of analytical grade and used as received.

Cabazitaxel is available as infusion with brand name Jevtana[®] (Sanofi-Aventis, Malaysia) with label claim of 60 mg of drug (60 mg cabazitaxel in 1.5 mL polysorbate 80 with diluent approximately 5.7 mL of 13% (w/w) ethanol in water for injection).

Instrumentation

Chromatographic separation was achieved by using Zorbax SB-C18 column (150 mm×4.6 mm i.d., 3.5 μm particle size) for HPLC system of Shimadzu Model CBM-20A/20 Alite, equipped with SPD M20A prominence photodiode array detector (maintained at 25 °C) and LC Solutions 1.25 software.

Chromatographic conditions

Isocratic elution was performed using 0.1% orthophosphoric acid: methanol (20:80, v/v) as mobile phase. The overall run time was 10 min. with flow rate 1.0 ml/min with UV detection at 210 nm. 20 μL of sample was injected into the HPLC system.

Preparation of stock solution

Cabazitaxel stock solution (1000 μg/ml) was prepared by weighing accurately 25 mg of Cabazitaxel in a 25 ml volumetric flask with mobile phase. Working standard solutions were prepared on daily basis from the stock solution with mobile phase and filtered through 0.45 μm membrane filter prior to injection.

Method validation

The method was validated for system suitability, linearity, limit of quantitation (LOQ), limit of detection (LOD), precision, accuracy, selectivity and robustness [15].

Linearity test solutions for the assay method were prepared from a stock solution at different concentration levels (0.1–200 μg/ml) of the assay analyte concentration and 20 μL of each solution was injected into the HPLC system and the peak area of the chromatogram obtained was noted. The calibration curve was plotted by taking the concentration on the x-axis and the corresponding peak area on the y-axis. The data was treated with linear regression analysis method.

The limit of quantification and limit of detection were based on the standard deviation of the response and the slope of the constructed calibration curve (n=3), as described in ICH guidelines Q2 (R1) [15].

Precision was evaluated at three concentration levels (20, 50 and 100 μg/ml) against the qualified reference standard. The intra-day precision study was carried out by doing 3 independent assays for each concentration of Cabazitaxel and the mean as well as the % RSD were calculated for each concentration. The inter-day precision study was performed on three different days i.e. day 1, day 2 and day 3 at three different concentration levels (20, 50 and 100 μg/ml) and each value is the average of three determinations. The % RSD of three obtained assay values on three different days was calculated.

Standard addition and recovery experiments were conducted to determine the accuracy of the method. The study was carried out in triplicate at three different levels (80, 100 and 120%) and the percentage recovery was calculated. The accuracy study was performed by adding known amount of the standard (8, 10 and 12 μg/ml) to the samples which was prepared in our laboratory using the excipients. The study

was carried out in triplicates and the percentage recoveries were calculated according to table 3.

The robustness of the assay method was established by introducing small changes in the HPLC conditions which included wavelength (208 and 212 nm), percentage of methanol in the mobile phase (78 and 82%) and flow rate (0.9 and 1.1 ml/min) and each value (Table 4) is the average of three determinations. Robustness of the method was studied for 100 μg/ml of Cabazitaxel.

As the marketed formulations were not available in India the drug was mixed with some of the common excipients used in the laboratory and extracted with the mobile phase. The percentage recovery was calculated from the calibration curve.

Forced degradation studies

Forced degradation studies were performed to evaluate the stability indicating properties and specificity of the method [16]. All solutions for stress studies were prepared at an initial concentration of 1 mg/ml of Cabazitaxel and refluxed for 30 min at 60°C and then diluted with mobile phase. 1.0 mg/ml Cabazitaxel solution was exposed to acidic degradation with 0.1 M HCl for 20 min at 60°C the stressed sample was cooled, neutralized and diluted with mobile phase. Similarly stress studies were conducted in alkaline conditions with 0.01 M NaOH at 60°C for 20 min. and neutralized after cooling with proper dilution with mobile phase. Oxidative stress studies were performed using 30% H₂O₂ and thermal stress studies were conducted in thermostat at 60°C for 20 min. 20 μL solution of each of these solutions which were exposed to forced degradation studies were injected into the HPLC system and the chromatograms were recorded from which the percentage recovery as well as the degradants were studied.

Results and Discussion

Method optimization

Initially the stressed samples were analyzed using a mixture of 0.1% ortho phosphoric acid: methanol (45:55, v/v) with a flow rate of 1.0 ml/min in which the peak shape was not symmetrical. Then the mobile phase composition was slightly modified with 0.1% ortho phosphoric acid: methanol (20:80, v/v) where a sharp peak was eluted at 4.241 ± 0.02 min without tailing (UV detection 210 nm) which was taken as the best chromatographic response for the entire study. The typical chromatogram of the blank as well as the drug solution was shown in Figure 3 respectively. The performance characteristics of the present stability indicating liquid chromatographic method was compared and discussed with the previously published methods in Table 1.

Method validation

Cabazitaxel has shown linearity 0.1-200 μg/ml (Table 2) (% RSD 0.11-0.54) with linear regression equation

$$y=21017x+3853.9 \quad (r^2=0.9999)$$

The LOQ was found to be 0.0967 μg/ml and the LOD was found to be 0.0319 μg/ml (Figure 2).

The % RSD range was obtained as 0.23-1.43 and 0.21-0.51 for intra-day and inter-day precision studies respectively and 98.95-99.43% of recovery was observed in the accuracy studies with % RSD 0.28-0.60 (<2.0%) (Table 3) indicating that the method is precise and accurate.

The robustness of an analytical procedure refers to its ability to remain unaffected by small and deliberate variations in method parameters and provides an indication of its reliability for routine

Mobile phase/Reagent	λ (nm)	Linearity ($\mu\text{g/ml}$)	Remarks	Reference
Phosphate buffer: Acetonitrile (30:70, v/v)	230	0.1-150	HPLC	[10]
Sodium acetate buffer: Acetonitrile (30:70, v/v)	234	0.1-250	HPLC	[11]
Ammonium hydroxide and methanol (83:17, v/v) (pH 3 \pm 0.1)	275	2-20	LC-MS	[12]
Acetonitrile: Ammonium acetate (80:20, v/v)	236	2.49-99.60	(LC-MS/MS) (dried blood spots)	[13]
Acetonitrile: Ammonium formate (gradient)	362	(10-100) 10^3	(LC-MS) Human plasma	[14]
o-phosphoric acid : Methanol (20:80, v/v)	210	0.1-200	Stability indicating HPLC (PDA detector)	Present work

Table 1: Comparison of the previously published liquid chromatographic methods with the present method.

Conc. ($\mu\text{g/ml}$)	*Mean peak area \pm SD	*RSD (%)
0.1	2552 \pm 587	0.23
1	22629 \pm 70.15	0.31
5	111635 \pm 112.80	0.11
10	207597 \pm 871.91	0.42
20	410587 \pm 1149.64	0.28
50	1085699 \pm 4885.65	0.45
100	2109177 \pm 4218.35	0.20
150	3156873 \pm 9786.31	0.31
200	4198887 \pm 22673.99	0.54

*Mean of three replicates

Table 2: Linearity of Cabazitaxel.

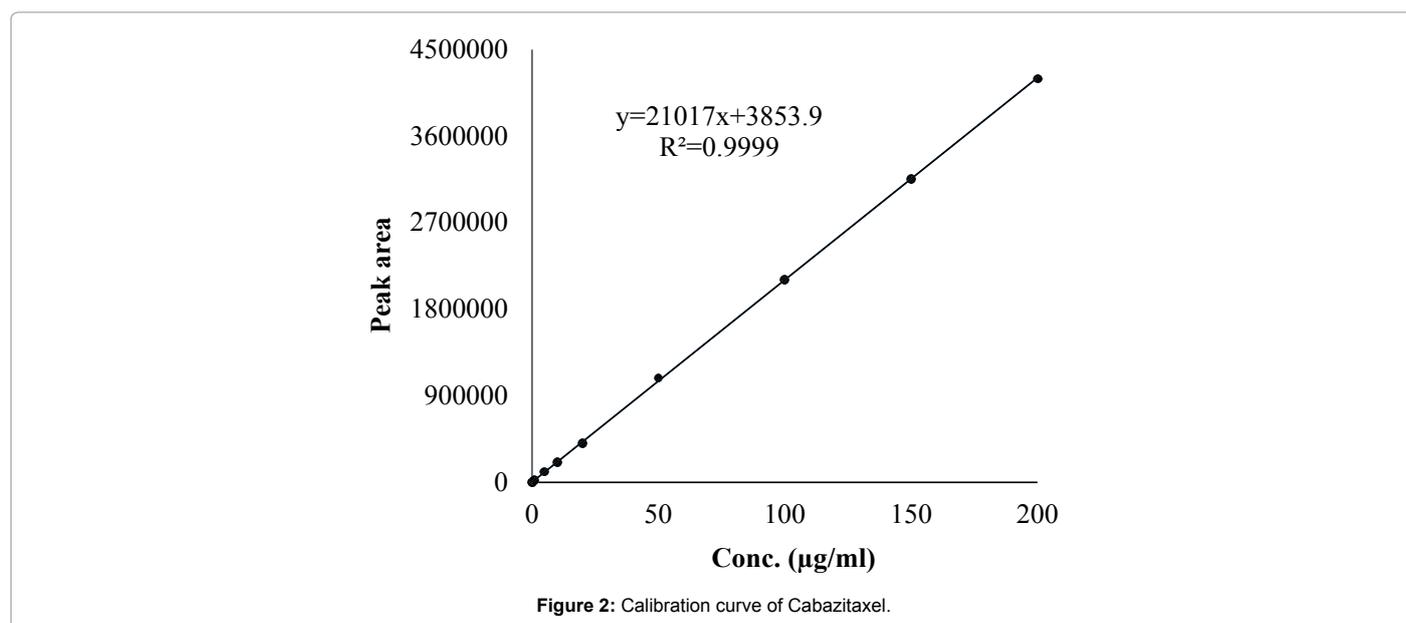


Figure 2: Calibration curve of Cabazitaxel.

analysis. The results obtained (Table 4) from assay of the test solutions were not affected by varying the conditions and were in accordance with the results for original conditions. The % RSD was less than 2.0% (0.19-1.66) indicating that the proposed method is robust.

As the marketed formulation was not available the drug was mixed with different excipients available in the laboratory and then extracted using the mobile phase. The proposed method was applied to the laboratory prepared formulation and the percentage recovery was calculated as 97.84.

Forced degradation studies

The stability indicating capability of the method was established from the separation of Cabazitaxel peak from the degraded samples. The

typical chromatograms obtained from the forced degradation studies were shown in Figure 3. Cabazitaxel has shown 19.48% degradation with degradant at 1.736 min during acidic stress indicating that the drug is sensitive towards acidic environment. The amino group present in the drug structure may be highly responsible for this degradation. Cabazitaxel has undergone complete decomposition with degradants 1.667 min, 1.992 min and 2.571 min at during alkali stress indicating that the drug is very much sensitive towards alkaline condition. In other degradations the drug has undergone decomposition slightly (<15.0%). Decomposition was seen on exposure of Cabazitaxel solution to acidic (19.48%), alkaline (99.72%), oxidative (0.17%) thermal (1.03%) and photolytic (3.02%) conditions (Table 5). It is concluded that Cabazitaxel is very much sensitive towards alkaline conditions.

The present stability-indicating method for the determination of

Conc. (µg/ml)	Intra-day precision		Inter-day precision	
	*Mean peak area ± SD (% RSD)		*Mean peak area ± SD (% RSD)	
20	410508.33 ± 961.20 (0.23)		410331.33 ± 867.24 (0.21)	
50	1069037.33 ± 15310.37 (1.43)		1084725.33 ± 5525.22 (0.51)	
100	2114961.00 ± 9778.37 (0.46)		2110788.33 ± 5377.64 (0.25)	
Accuracy				
Spiked conc. (µg/ml)	Total conc. (µg/ml)	Mean peak area ± SD (% RSD)	Drug Found (µg/ml)	% Recovery*
8 (80%)	18	378301.00 ± 1042.36 (0.28)	17.82	98.98
10 (100%)	20	421803.00 ± 1587.67 (0.38)	19.89	99.43
12 (120%)	22	461385.33 ± 2765.64 (0.60)	21.77	98.95

*Mean of three replicates

Table 3: Precision and accuracy studies of Cabazitaxel.

Parameter	Condition	*Mean peak area ± SD (% RSD)	*Assay (%)
Flow rate (± 0.1 ml/min)	0.9	2102325.00 ± 14560.41 (0.69)	99.86
	1.0		
	1.1		
Detection wavelength (± 2 nm)	208	2103174.33 ± 3932.91 (0.19)	99.90
	210		
	212		
Mobile phase composition (± 2%, v/v)	18:82	2093672.33 ± 34806.61 (1.66)	99.45
	20:80		
	22:78		

*Mean of three replicates

Table 4: Robustness study of Cabazitaxel.

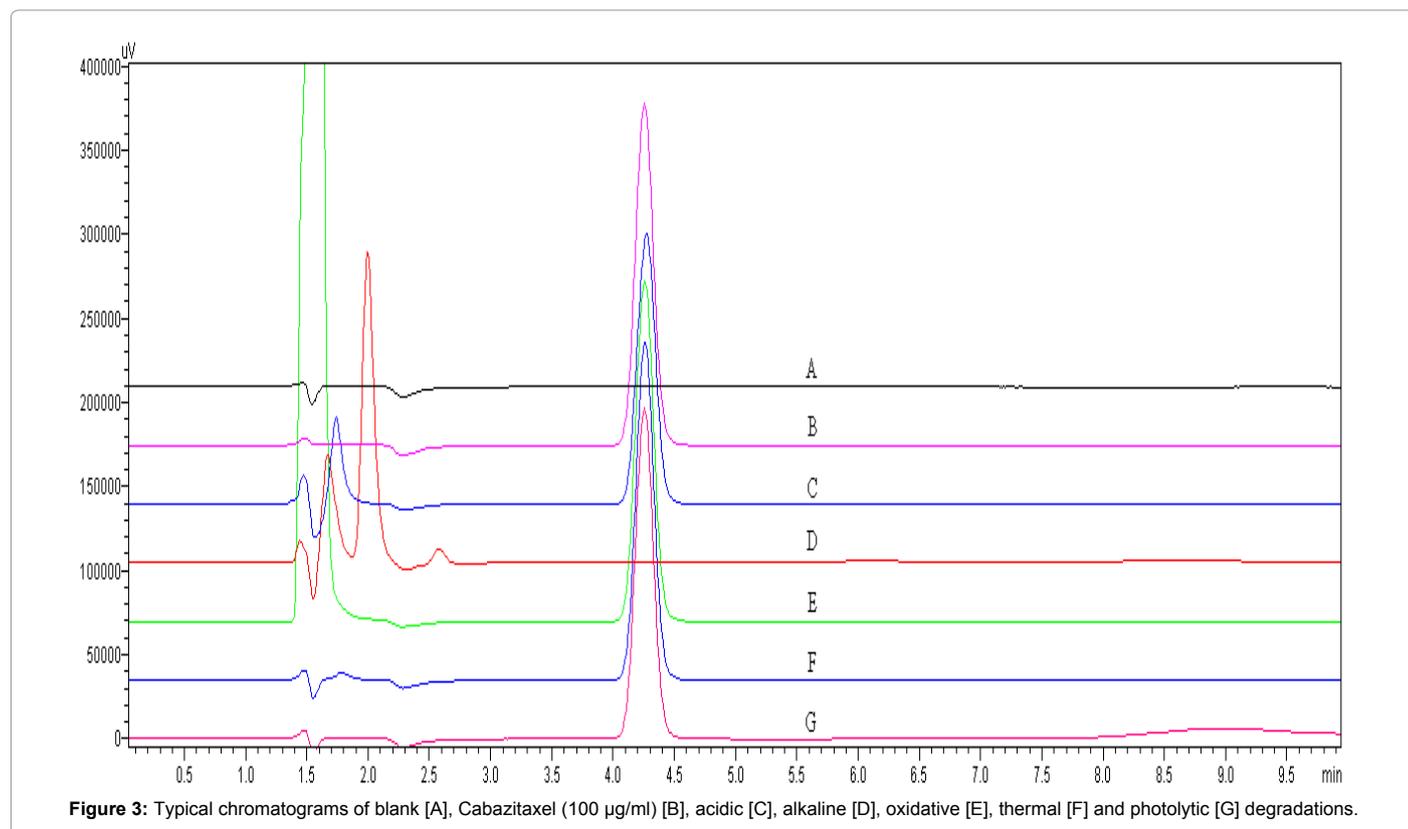


Figure 3: Typical chromatograms of blank [A], Cabazitaxel (100 µg/ml) [B], acidic [C], alkaline [D], oxidative [E], thermal [F] and photolytic [G] degradations.

Cabazitaxel in pharmaceutical formulations is specific because the drug peak was well separated even in the presence of degradation products. The system suitability parameters for the Cabazitaxel peak shows that the theoretical plates were more than 2000 and the tailing factor was less than 2 (or <1.5-2.0) (Table 5).

Conclusion

The proposed stability-indicating liquid chromatographic method for the determination of cabazitaxel is economical as acetonitrile was replaced with methanol and the method can be applied for the assay of pharmaceutical dosage forms. Cabazitaxel is very much sensitive

Stress Condition	*Mean peak area	*Drug recovered (%)	*Drug decomposed (%)	Theoretical plates	Tailing factor
Standard (Untreated)	2109177	100	-	3517.939	1.064
Acidic	1698271	80.52	19.48	3392.511	1.039
Alkaline	5807	0.28	99.72	-	-
Oxidative	2105689	99.83	0.17	3415.160	1.038
Thermal	2087474	98.97	1.03	3521.359	1.053
Photolytic	2045504	96.98	3.02	3477.122	1.049

*Mean of three replicates

Table 5: Forced degradation studies of Cabazitaxel.

towards the alkaline conditions and it can be very easily undergoes decomposition.

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