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Original Article

Analytical Stress Degradation Studies of Cabazitaxel (A Semi synthetic Natural Taxoid) using Liquid Chromatography

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ABSTRACT

Cabazitaxel, a semi-synthetic derivative of the natural taxoid used to treat advanced prostate cancer. A simple validated stability-indicating liquid chromatographic method has been developed for the determination of Cabazitaxel in pharmaceutical formulations using Shimadzu Model CBM-20A/20 AliteHPLC system equipped with PDA detector and Zorbax SB-C18 column (150 mm × 4.6 mm i.d., 3.5 µm particle size) with a mixture of tetra butyl ammonium hydrogen sulphateand acetonitrile (30:70, v/v) as mobile phase (UV detection 231 nm; flow rate 1.2 ml/min). Stress conditions studies such asacidic, alkaline, oxidation photolytic and thermal degradations have been performed and the method was validated as per ICH guidelines.

Key words: Cabazitaxel, RP-HPLC, Stability-indicating, validation, ICH guidelines.

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INTRODUCTION

Cabazitaxel is a taxane type of chemotherapy drug which is a semi-synthetic derivative of a natural taxoid used to treat advanced hormone-refractory prostate cancer that is no longer responding to hormone therapy.[1] Cabazitaxel chemically known as (2aR, 4S, 4aS, 6R, 9S, 11S, 12S, 12aR, 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 with molecular formula $C_{45}H_{57}NO_{14}$ and molecular weight of 835.93 g/mol (Figure 1). Cabazitaxel mainly interferes with microtubules, a part of the internal structure that cells need when they are dividing which leads to cell death. Cabazitaxel has been approved in the US by the Food and Drug Administration (FDA)[2-3] and in Europe by the European Medicines Agency (EMA) in combination with prednisone for the treatment metastatic prostate cancer.[4-6]

Few analytical methods have been reported for the determination of Cabazitaxel using spectrophotometry,[7] HPLC,[8-11] LC-MS/MS[12-14] in biological fluids. The present aim of the authors was to develop simple, fast and cost effective stability indicating RP-HPLC method for the determination of Cabazitaxel in presence of its degradation products and to validate as per ICH guidelines.

MATERIALS AND METHODS

Chemicals and reagents

Cabazitaxel standard was obtained as a gift sample from Dr. Reddy's Labs (India). Acetonitrile (HPLC grade), Tetra butyl ammonium hydrogen sulphate (TBHS), Sodium hydroxide (NaOH) and Hydrochloric acid (HCl), and Hydrogen peroxide (H_2O_2) 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.

Instrumentation

Chromatographic method was performed on HPLC system of Shimadzu Model CBM-20A/20 Alite, equipped with SPD M20A prominence photodiode array detector. Method was achieved by using Zorbax SB-C18 column (150 mm × 4.6 mm i.d., 3.5 µm particle size) as stationary phase, maintained at 25°C.

Preparation of tetra butyl ammonium hydrogen sulphate (10 mM)

Accurately 3.3954 g of tetra butyl ammonium hydrogen sulphate (TBHS) was weighed and transferring in to a 1000 ml volumetric flask and dissolved with HPLC grade water. The solution was sonicated for 15 mins and then filtered using 0.45 Millipore membrane filter.

Preparation of stock solution

The stock solution (1000 µg/ml) was prepared by accurately weighing 25 mg of Cabazitaxel standard and transferred to a 25 ml volumetric flask. Dissolved using 10 ml of acetonitrile and finally make up the volume with acetonitrile. 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.

Chromatographic conditions

The chromatographic separation was achieved by an isocratic elution of TBHS: acetonitrile (30:70, v/v) as mobile phase, with a flow rate of 1.2 ml/min. UV detection was done at 231 nm. 20 µL of sample was injected into the HPLC system. The overall run time was 10 min.

Method Validation

The developed method has been validated for the validation parameters such as linearity, precision, accuracy, limit of quantitation (LOQ), limit of detection (LOD), selectivity and robustness as per prescribed ICH guidelines.[16]

Linearity

Linearity of the developed method was performed by preparing a series of solutions (0.1–150 µg/ml) from the stock solution and injected in to

the HPLC system. The same was repeated in triplicate. The average peak areas of the chromatograms obtained were noted and plotted against concentration in the calibration curve. 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 studies

The intra-day precision studies were carried out at three different concentration levels (20, 50 and 100 µg/ml) on the same day and the % RSD was calculated. The inter-day precision study was also performed at three different concentration levels (20, 50 and 100 µg/ml) on three different days i.e. day 1, day 2 and day 3 and the % RSD was calculated.

Accuracy

The accuracy of the assay method was performed by standard addition and recovery experiments at three concentration levels (80, 100 and 120%) i.e., 40, 50 and 60 µg/ml of pure drug solution was added to the previously analysed formulation solution (50 µg/ml) and then injected into the HPLC system and the percentage recoveries were calculated.

Robustness studies

Robustness study was performed at 100 µg/ml of Cabazitaxel by incorporating small changes in the parameters such as flow rate (1.1 and 1.3 ml/min), wavelength (229 and 233 nm) and mobile phase composition (acetonitrile; 68 and 72%).

Forced degradation studies

The Specificity of the developed stability indicating method were evaluated by performing forced degradation studies.[16] The Cabazitaxel sample was exposed to different stress conditions i.e., acidic, alkaline, oxidation photolytic and thermal degradations.

In acidic degradation 1 ml of 1 N HCl was added to 1.0 mg/ml Cabazitaxel solution and heated at 80°C for 30 min and then the stressed sample was cooled, neutralized prior dilution with mobile phase. Similarly, alkaline degradation study was conducted at 1.0 mg/ml Cabazitaxel solution to which 1 ml 0.01 N NaOH was added and heated at 80°C for 30 min. and neutralized after cooling prior dilution with mobile phase. Oxidative stress studies were conducted using at 1.0 mg/ml to which 1 ml of 30 % H_2O_2 added and thermal stress studies were conducted in thermostat at 80°C for 30 min and cooled prior dilution with mobile phase. In photolytic degradation study 1.0 µg/ml of cabazitaxel was exposed in the UV cabinet to the short and long UV radiation and diluted with mobile phase.20 µL solution of each of these solutions were filtered prior injection in to the HPLC system.

Cabazitaxel marketed formulation is not available in India and therefore the drug was formulated with excipients as per the specification in our laboratory, then extracted and diluted as per the requirement. The percentage recovery was computed from the calibration curve.

RESULTS AND DISCUSSION

Method optimization

The main aim of the work is to separate the Cabazitaxel from its degradation products for which various initially various mobile phases and columns were tried to achieve better resolution and separation conditions. Among those a mixture of TBHS: acetonitrile (40: 60, v/v) with 1.0 ml/min flow rate has given a broadpeak at 4.168 min. Hence the mobile phase composition and flow rate were slightly modified as TBHS: acetonitrile (30:70, v/v) and flow rate 1.2ml/min where a sharp peak was eluted at 3.271 ± 0.02 min and therefore taken as the best chromatographic response for the entire study (Figure 3A). The previously published methods were compared with the present developed stability indicating liquid chromatographic method the performance characteristics in Table 1.

Method Validation

The method was validated for linearity, limit of quantitation (LOQ), limit of detection (LOD), precision, accuracy, robustness and selectivity as per the ICH guidelines.

Linearity

The method shows linearity over a concentration range of 0.1-150 µg/ml (Table 2) (% RSD 0.11-0.42) with linear regression equation y=29178x - 6.1506 (r^2=0.9992) (Figure 2). The LOQ was found to be 0.0103 µg/ml and the LOD was found to be 0.0034 µg/ml.

Accuracy

In the accuracy studies of the proposed analytical method the % recovery at three different concentrations (80, 100 and 120 %) was found to be 98.30-98.81 % with the % RSD of 0.33-0.50 (Table 3).

Table 1: Comparitive study of performance characteristics of the present proposed method with the previously published methods

Mobile phase (v/v)	λ (nm)	Linearity (µg/ml)	Method	Ref
Sodium dihydrogen phosphate buffer: Acetonitrile	230	0.025-1.5	HPLC	9
Phosphate buffer: Acetonitrile (30:70)	230	0.1-150	HPLC	10
Methanol: 0.1% Phosphoric acid (20:80)	210	0.1-200	HPLC	11
Sodium acetate buffer: Acetonitrile (30:70)	234	0.1-250	HPLC	12
Acetonitrile: Ammonium acetate (80:20)	236	2.49-99.6	(LC-MS/MS)	13
Ammonium hydroxide: methanol (83:17)	275	2-20	LC-MS	14
Acetonitrile: Ammonium formate	362	0.01-0.1	LC-MS	15
TBHS: acetonitrile (30: 70)	231	0.1-150	Stability indicating HPLC (PDA detector)	Present work

Table 2: Linearity of Cabazitaxel

Conc. (µg/ml)	*Mean peak area ± SD	RSD (%)
0.1	2875±3.16	0.11
1	28654±71.64	0.25
5	149874±629.47	0.42
10	278207±306.03	0.11
20	535874 ± 1875.56	0.35
50	1556873±5760.43	0.37
100	2873937±6035.27	0.21
150	4380456±7884.82	0.18

*Mean of three replicates.

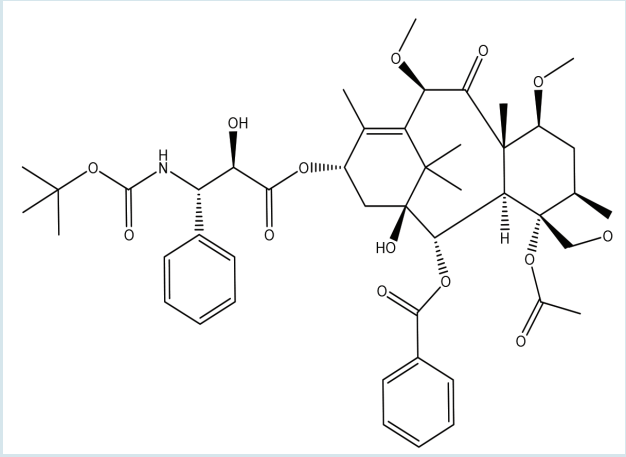

Figure 1: Chemical structure of Cabazitaxel

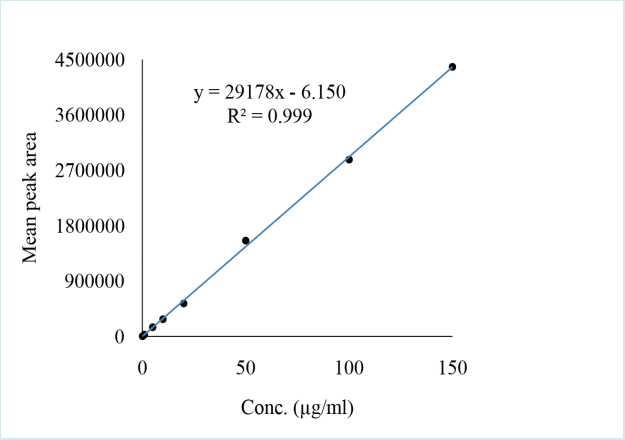

Figure 2: Calibration plot of Cabazitaxel

Table 3: Accuracy studies of Cabazitaxel

Spiked conc. µg/ml)	Total conc. (µg/ml)	*Conc. found (µg/ml) ± SD	%RSD	SEM	%Recovery
8 (80 %)	18	17.79±0.0582	0.33	0.1866	98.81
10 (100 %)	20	19.72±0.0985	0.50	0.2543	98.59
12 (120 %)	22	21.63±0.0995	0.46	0.2619	98.30

*Mean of three replicates.

Table 4: Precision studies of Cabazitaxel

Conc. (µg/ml)	Intra-day precision			Inter-day precision		
	*Conc. obtained (µg/ml) ± SD	%RSD	SEM	*Conc. obtained (µg/ml) ± SD	%RSD	SEM
20	19.99±0.0569	0.28	0.0328	19.82±0.1593	0.80	0.0919
50	49.78±0.2264	0.45	0.1307	49.62±0.3351	0.68	0.1935
100	99.77±0.2468	0.25	0.1425	99.55±0.3919	0.39	0.2263

*Mean of three replicates.

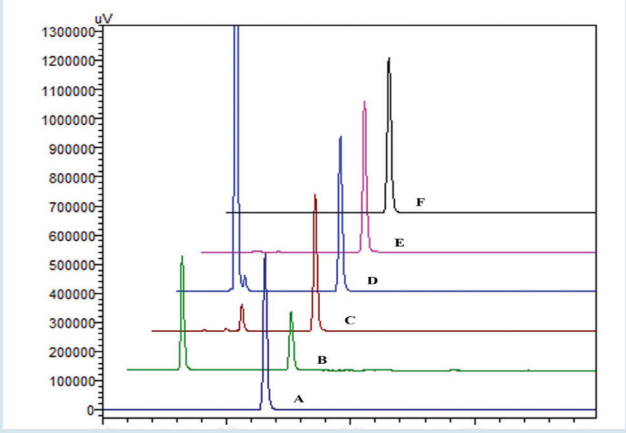

Figure 3: Overlay chromatogram of Cabazitaxel [A] standard [B] acidic [C] basic [D] oxidation [E] thermal [F] photolytic degradations

Precision studies

In the precision studies the %RSD was found to be in a range of 0.28-0.45 (Intra-day) and 0.39-0.80 (Inter-day) (Table 4).

Robustness

In robustness studies slight changes were made in flow rate, detection wavelength, mobile phase compositionetc. The chromatographic responses were given in Table 5. The % RSD obtained was 0.007-0.187 (< 2.0%) indicating that the proposed method is robust.

Forced degradation studies

The specificity and the stability indicating capability of the established method judged by the separation of the main drug peak from the other degradation peaks. The typical chromatograms of the forced degradation samples w.r.t. to the standard Cabazitaxel drug solution (100 µg/ml) were shown in Figure 3A-3F. Cabazitaxel during acidic degradation has undergone 63.20 % degradation with degradants at 1.097 mins indicating that the drug is more sensitive to acidic conditions. The amino group present in the structure of Cabazitaxel may be responsible for the acidic

Table 5: Robustness study of Cabazitaxel

Parameter (condition)	*%Assay ± SD (%RSD)	SEM	*Rt ± SD (%RSD)	SEM
Flow rate (± 0.1 ml.min^{-1})				
1.1	100.66±0.0078 (0.0078)	0.0045	3.327±0.002 (0.046)	0.0009
1.3	98.53±0.0335 (0.340)	0.0193	3.172±0.003 (0.079)	0.0015
Detection wavelength (± 2 nm)				
229	98.53±0.1843 (0.1870)	0.1064	3.272±0.001 (0.018)	0.0003
233	100.00±0.0599 (0.0559)	0.0323	3.272±0.001 (0.018)	0.0003
Mobile phase composition (±2v/v)				
32:68	96.73±0.0525 (0.0543)	0.0303	3.294±0.003 (0.076)	0.0015
28:72	98.54±0.1167(0.1185)	0.0674	3.257±0.003 (0.077)	0.0015

*Mean of three replicates

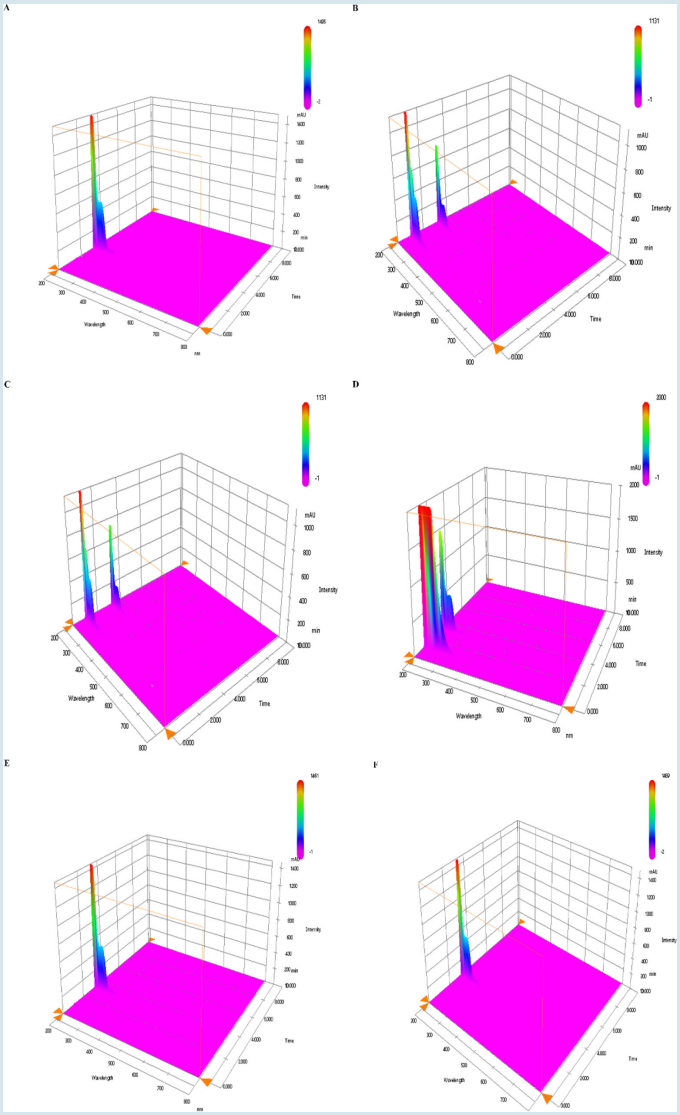

Figure 4: 3D chromatographs of Cabazitaxel (100 µg/ml) [A] standard, [B] acidic, [C] basic, [D] oxidative, [E] thermal and [F] photolytic degradations.

Table 6:Forced degradation studies of Cabazitaxel

Stress Conditions	Retention time (Rt)	Peak area	*Drug recovered (%)	*Drug decomposed (%)	Extra peaks	Theoretical plates (N)	Tailing factor	Capacity Factor(k')	Resolution (R)	Peak Purity Index	Single Point Threshold	
Standard drug (Untreated)	3.271	2873937	100	-	-	6578.252	1.262	-	-	1	0.999960	
Acidic degradation	3.297	1057654	36.80	63.20	1.097	7485.835	1.271	2.005	15.119	1	0.999878	
Alkaline degradation	3.28	2442093	84.97	15.03	1.8	7276.304	1.266	0.822	11.023	1	0.999958	
Oxidative degradation	3.288	2788768	97.04	2.96	1.178 1.360	7359.692	1.261	1.791	15.308	1	0.999961	
Thermal degradation	3.275	2749395	95.67	4.33	-	7205.17	1.257	-	-	1	0.999960	
Photolytic degradation	3.27	2862825	99.61	0.39	-	6565.333	1.255	-	-	1	0.999961	

*Mean of three replicates

degradation. During basic stress condition Cabazitaxel has undergone 15.03% degradation with degradant at 1.80 min showing that the drug is sensitive towards basic environment. In the drug structure the amino group may be highly responsible for these degradations. In oxidative degradation the H_2O_2 peak was observed at 1.178 min and undergone degradation of 2.96% with the degradation peak at 1.360 mins (Table 6). From these stress degradation studies conducted for Cabazitaxel it is concluded that drug is much sensitive towards acidic and alkaline conditions. 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). The 3D chromatographs were shown in Figure 4 which represents the selection of the wavelength and also the specificity of the drug from the degradation peaks.

As the injection is not available in India, the proposed method was applied to the laboratory prepared injection and the percentage recovery was found to be 98.75%.

CONCLUSION

The proposed stability-indicating RP-HPLC method can be applied for the quantification of Cabazitaxelin pharmaceutical formulations even in the presence of degradation products. The method was validated as per ICH guidelines.

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CONFLICT OF INTEREST

The authors have no conflict of interest.

ABBREVIATION USED

RP-HPLC: Reverse phase high performance Liquid chromatography; **CBZ:** Cabazitaxel; **TBHS:** Tetra butyl ammonium hydrogen sulphate; **LC-MS:** Liquid chromatography–Mass spectrometry; **ICH:** International conference on harmonization; **RSD:** Relation standard deviation; **LOD:** limit of detection; **LOQ:** limit of quantification.

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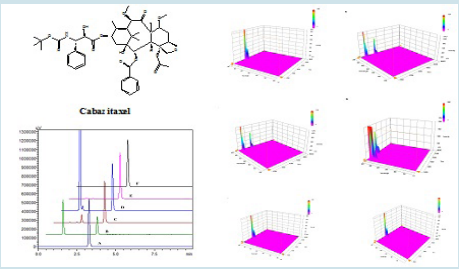
PICTORIAL ABSTRACT



SUMMARY

- A new stability-indicating RP-HPLC method was developed for the quantification of Cabazitaxel in pharmaceutical formulations in presence of degradation products using a mixture of tetra butyl ammonium hydrogen sulphate and acetonitrile as mobile phase and validated as per ICH guidelines.

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