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New Liquid Chromatographic Method (Stability Indicating) for the determination of Cabazitaxel – An Anti-Cancer Agent

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ABSTRACT

Cabazitaxel is used for the treatment of prostate cancer. A stability-indicating high performance liquid chromatographic technique was developed and validated for the determination of Cabazitaxel in the presence of its degradants. Chromatographic elution was performed on Shimadzu Model CBM-20A/20 Alite, equipped with Zorbax SB-C18 column (150 mm × 4.6 mm i.d., 3.5 µm particle size) with a mixture of phosphate buffer (pH 7.0) and acetonitrile as mobile phase having a flow rate of 0.8 ml/min. The method follows Beer-Lambert's law over a concentration range 0.2-200 µg/ml ($y = 32288x + 20229$) ($r^2 = 0.9994$). Cabazitaxel was exposed to stress conditions (acidic, alkaline, oxidation, thermal and photolytic degradations) and it was observed that Cabazitaxel is more sensitive towards alkaline conditions. The proposed method was validated as per ICH guidelines and it can be successfully applied for the determination of Cabazitaxel in pharmaceutical formulations and for kinetic studies.

KEY WORDS: Cabazitaxel, Anti-cancer, Stability-indicating, RP-HPLC, Validation, ICH Guidelines.

1. INTRODUCTION

Cabazitaxel, a next generation taxane used to treat metastatic castration-resistant prostate cancer (Neil, 2006). The anti-tumour activity has been shown to promote stabilisation of microtubules, blocking tumour cell division (Jordan, 2004). Cabazitaxel has been approved by the Food and Drug Administration in June 2010 in the US and by the European Medicines Agency in Europe in January 2011 for the patients with castration resistant metastatic prostate cancer whose disease progresses after docetaxel treatment (Oudard, 2011) (Assessment Report, 2011) in combination with prednisone. It is a dimethyloxy derivative of Docetaxel. The extra methyl groups provide cabazitaxel an uncommon capability among chemotherapy agents i.e. the ability to 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 (Pivot, 2008), (Villanueva, 2011). 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 ($C_{45}H_{57}NO_{14}$) with molecular weight 835.93 g/mol. (Figure 1) (Sanofi-Aventis, 2006), (Cheetham, 2013).

Few analytical methods such as spectrophotometry (Kishore, 2012), RP-HPLC (Mathrusri, 2013; 2014) and LC-MS/MS (Chengyan, 2015; Kort, 2013; Jagannath, 2012; Peter, 2012) techniques were reported for the determination of Cabazitaxel. In the present the authors have proposed a robust and selective stability indicating liquid chromatographic method for the determination of Cabazitaxel in presence of its degradants.

2. MATERIALS AND METHODS

2.1. Chemicals and reagents: Cabazitaxel was obtained as a gift sample from Dr. Reddy's Laboratories Ltd., (India). All chemicals are of AR grade. Acetonitrile (HPLC grade), Potassium Dihydrogen Phosphate (HPLC grade), Hydrogen peroxide (H2O2), Hydrochloric acid (HCl), and Sodium hydroxide (NaOH) were purchased from Merck (India) and used as received. Cabazitaxel is available as infusion with brand name Jevtana® (Sanofi-Aventis, Malaysia) with label claim of 60 mg of drug.

2.2. Instrumentation: Shimadzu Model CBM-20A/20 Alite HPLC system equipped with SPD M20A prominence photodiode array detector and Zorbax SB-C18 column (150 mm × 4.6 mm i.d., 3.5 µm particle size) was used for chromatographic separation.

2.3. Chromatographic conditions: Isocratic mode of elution was selected and phosphate buffer (pH 7.0) and acetonitrile (20:80, v/v) mixture was used as mobile phase with flow rate 0.8 ml/min. UV detection was carried out at 210 nm. The overall run time was 10 min.

2.4. Preparation of stock solution: Stock solution (1000 µg/ml) was prepared by dissolving about 25 mg of Cabazitaxel in a 25 ml volumetric flask with acetonitrile. Working standard solutions were prepared from the stock solution with mobile phase as per the requirement and filtered through 0.45 µm membrane filter.

2.5. Preparation of Phosphate buffer solution (pH 7.0): The phosphate buffer was prepared by transferring 50.0 ml of 0.2 M potassium dihydrogen phosphate in a 1000 ml volumetric flask, add 29.1ml of 0.2 M sodium hydroxide and dilute with HPLC grade water to 1000 ml.

2.6. Validation: The developed method was validated as per ICH (2005) prescribed validation parameters such as linearity, precision, accuracy, limit of quantitation (LOQ), limit of detection (LOD), selectivity and robustness.
2.6.1. Linearity: Linearity of the assay method was conducted by preparing a series of solutions (0.2–200 µg/ml) from the stock solution and injected in to the HPLC system (n=3). The average peak area of the chromatograms obtained was plotted against concentration to construct 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) (ICH, 2005).
2.6.2. Precision: The intra-day precision studies were carried out at three different concentration levels (10, 50 and 100 µg/ml) and the % RSD was calculated. The inter-day precision study was also performed on three different days i.e. day 1, day 2 and day 3 and the % RSD was calculated.
2.6.3. Accuracy: The accuracy of the assay method was performed by standard addition and recovery experiments and evaluated in triplicate at three concentration levels (80, 100 and 120%), and the percentage recoveries were calculated. The study was carried out in triplicate at 90, 100 and 110 µg/ml. The percentage recovery was calculated in each case.
2.6.4. Robustness: Robustness studies of Cabazitaxel (100 µg/ml) were performed by incorporating small changes in the parameters such as pH of buffer (6.9 and 7.1), wavelength (208 and 212 nm), composition of mobile phase (78 and 82%) and flow rate (0.7 and 0.9 ml/min).
2.6.5. Limit of quantification and Limit of detection: 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) (ICH guidelines, 2005). Sensitivity of the method was established with respect to limit of detection LOD and LOQ for analytes.
2.6.6. Forced degradation studies: The stability indicating properties and specificity of the method were evaluated by performing forced degradation studies (ICH, 2003). All solutions for stress studies were prepared at an initial concentration of 1 mg/ml of Cabazitaxel and refluxed for 30 min at 70 ºC and then diluted with mobile phase.

Acidic degradation was conducted by exposing 1.0 mg/ml Cabazitaxel solution to 1ml of 1 N HCl for 30 min at 70 ºC and then the stressed sample was cooled, neutralized, diluted with mobile phase and filtered.

Similarly alkaline stress degradation study was conducted in alkaline conditions with 0.1ml 0.1 N NaOH at 70 ºC for 30 min. and neutralized after cooling prior dilution with mobile phase and finally filtered before injection in to the HPLC system.

Oxidative stress studies were conducted using 1 ml of 30 % H_2O_2 and thermal stress studies were conducted in thermostat maintained at 70 ºC for 30 min.

Photolytic degradation study was performed by exposing the drug solution to UV light (365 nm) in UV chamber for 3 hours and later diluted with mobile phase as per the requirement.

20 µL solution of each of these solutions were injected in to the HPLC system and the peak area of the chromatograms was noted for the calculation of percentage recoveries as well as the degradants.

Cabazitaxel marketed formulation is not available in India and therefore the drug was formulated with some of the common available excipients in our laboratory, extracted with the mobile phase and the percentage recovery was calculated from the calibration curve.
2.6.7. Assay of marketed formulations (Tablet): Cabazitaxel marketed formulation is not available in India and therefore the drug was formulated with some of the common available excipients in our laboratory, extracted with the mobile phase and the percentage recovery was calculated from the calibration curve.

3. RESULTS AND DISCUSSION

3.1. Method development and optimization: Initially various mobile phases and columns were tried to achieve better resolution and separation conditions. Among those the stressed samples were analyzed using a mixture of Phosphate buffer (pH 7.0): acetonitrile (40:60, v/v) with a flow rate of 0.6 ml/min in which the peak shape of the obtained peak was not symmetrical. Then the mobile phase composition was modified to Phosphate buffer (pH 7.0): acetonitrile (20:80, v/v) where a peak was eluted at 3.153 ± 0.02 min which is sharp without tailing (UV detection 210 nm) which was taken as the best chromatographic response for the entire study. The representative chromatogram of the drug solution was shown in Figure 3. The present developed stability indicating liquid chromatographic method was compared and discussed with the previously published methods for the performance characteristics in Table 1.
3.2. Method Validation: The method was validated for system suitability, linearity, limit of quantitation (LOQ), limit of detection (LOD), precision, accuracy, selectivity and robustness (ICH guidelines, 2005).
3.2.1. Linearity: Cabazitaxel has shown linearity over a concentration range of 0.2–200 µg/ml (Table 2) (% RSD 0.11-0.47) with linear regression equation y = 32288x + 20229 (r^2 = 0.9994) (Figure 2). The LOQ was found to be 0.0413 µg/ml and the LOD was found to be 0.0124 µg/ml.

3.2.2. Accuracy: The method accuracy was proved by the recovery test at three different concentrations (80, 100 and 120 %). A known amount of standard (5 µg/ml) were added to aliquots of sample solutions and then diluted to yield the total concentrations of 9, 10 and 11 µg/ml as described in Table 3. The % RSD was found to be 0.29-1.59.
3.2.3. Precision studies: For the precision studies the % RSD range was found to be 0.1-0.37 (Intra-day) and 0.60-1.36 (Inter-day) which was within the limit (<2%). Table 3 indicates the results representing that the method is precise and accurate.
3.2.4. Robustness: Slight changes in flow rate, detection wavelength, mobile phase composition etc. affects the chromatographic response such as retention time, tailing factor and theoretical plates etc. and the results were given in Table 4. The % RSD obtained was 0.58-1.52 (< 2.0%) indicating that the proposed method is robust.
3.3. Forced degradation studies: The stability indicating capability of the method was established to judge the specificity of the method. The typical chromatograms obtained for the forced degradation studies were shown in Figure 3C-3G. Cabazitaxel has completely undergone basic degradation with degradants at 1.514 and 1.856 mins showing the drug is very much sensitive to basic conditions. The basic degradation was performed using 1 ml of 0.1 N NaOH and no drug peak was observed indicating that the entire drug has undergone degradation. Later the same experiment was performed by using the least concentration of NaOH but the same thing was repeated. The carbonyl groups may be responsible for the basic degradation. During acidic stress Cabazitaxel has undergone 32.40 % degradation with degradant at 1.502, 1.624 and 2.270 min showing that the drug is sensitive towards acidic environment. In the drug structure the amino group may be highly responsible for this degradation. In oxidative degradation the H_2O_2 peak was observed at 1.792 min and the degradation peak at 1.443 mins showing that the drug is sensitive to oxidation. Cabazitaxel solution was seen decomposing on exposure to acidic (32.40 %), alkaline (100 %), oxidative (15.16 %) thermal (37.67 %) and photolytic (17.20 %) conditions (Table 5). From these stress degradation studies conducted for Cabazitaxel it is concluded that drug is much sensitive towards 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).
3.4. Analysis of commercial formulations: The proposed method was applied to the laboratory prepared injection and the percentage recovery was found to be 98.03% (Figure.3).

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

Mobile phase/Reagent	λ (nm)	Linearity (µg/ml)	Method	Reference
Sodium acetate buffer: Acetonitrile (30:70)	234	0.1-250	HPLC	(Mathrusri, 2014)
Sodium dihydrogen phosphate buffer : Acetonitrile	230	$(2.5–150)10^{-2}$	HPLC (Impurity profile)	(Chengyan, 2015)
0.1% ortho phosphoric acid: methanol (20:80)	210	0.1-200	HPLC	(Mathrusri, 2014)
Ammonium hydroxide: acetonitrile (83:17)	275	2-20	LC-MS	(Kort, 2013)
Phosphate buffer (pH 5): Acetonitrile (30:70)	230	0.1-150	HPLC	(Mathrusri, 2013)
Acetonitrile: Ammonium acetate (80:20)	236	2.49-99.60	LC-MS/MS (dried blood spots)	(Jagannath, 2012)
Acetonitrile: Ammonium formate (gradient mode)	362	$(10-100)\ 10^{-3}$	LC-MS (Human plasma)	(Peter, 2012)
Phosphate buffer (pH 7): Acetonitrile (20: 80)	210	0.2-200	HPLC (Stability indicating)	Present work

Table.2. Linearity of Cabazitaxel

Conc. (µg/ml)	*Mean peak area ± SD	RSD (%)	Conc. (µg/ml)	*Mean peak area ± SD	RSD (%)
0.2	6257± 13.14	0.21	50	1687524 ± 5737.58	0.34
1	33587 ± 110.84	0.33	100	3364532± 8411.33	0.25
5	149874 ± 614.48	0.41	150	4754126± 5229.54	0.11
10	345687 ± 449.39	0.13	200	6487545± 30491.46	0.47
20	685874± 2812.08	0.41			

*Mean of three replicates

Table.3. Precision and accuracy studies of Cabazitaxel

Conc. (µg/ml)	Intra-day precision	Inter-day precision
	* Mean peak area ± SD (%RSD)	* Mean peak area ± SD (% RSD)
10	345971.00±799.76 (0.23)	341494.00±4078.30 (1.19)
50	1684183.33±2893.75 (0.17)	1664069.67±22553.68(1.36)
100	3359407.33±12420.01 (0.37)	2096391.33±20052.22 (0.60)

Accuracy

Spiked conc. (µg/ml)	Total conc.	* Mean peak area ± SD (% RSD)	Drug Found (µg/ml)	% Recovery
40 (80%)	90	2871329.00±45296.45 (1.58)	88.30	98.11
50 (100%)	100	3246228.33±9394.17 (0.29)	99.91	99.91
60 (120%)	110	3537210.67±10869.53 (0.31)	108.93	99.02

*Mean of three replicates

Table.4. Robustness study of Carbazitaxel

Parameter	Condition	*Mean peak area ± SD (% RSD)	*Assay (%)
Flow rate (± 0.1 mL/min)	0.7	3347422.33± 21353.98 (0.64)	99.49
	0.8		
	0.9		
Detection wavelength (± 2 nm)	208	3366895.00± 19483.27 (0.58)	100.07
	210		
	212		
Mobile phase composition (± 2 %, v/v)	18:82	3340834.00± 50772.73 (1.52)	99.30
	20:80		
	22:78		
pH (± 0.1 unit)	6.9	3349896.33± 45631.16 (1.36)	99.56
	7.0		
	7.1		

*Mean of three replicates

Table.5. Stress degradation studies of Cabazitaxel

Stress Condition	*Drug recovered(%)	*Drug decomposed(%)	Peaks observed (mins)	Theoretical Plates	Tailing factor
Standard (Untreated)	100	-	4.175	6288.171	1.387
Acidic degradation 1 ml 0.1N HCl, 70°C, 30 mins	67.60	32.40	4.186, 1.502, 1.624 2.270	6888.530	1.386
Alkaline degradation 0.1 ml 0.1N NaOH, 70°C, 30 mins	-	100	1.514, 1.856	-	-
Oxidative degradation 1 ml 30% H_2O_2, 70°C, 30 mins	84.84	15.16	4.177, 1.544	6940.968	1.393
Thermal degradation 70°C, 30 mins	62.33	37.67	4.164	6810.025	1.394
Photolytic degradation 365 nm, 3 hours	82.80	17.20	4.169	6480.845	1.386

*Mean of three replicates

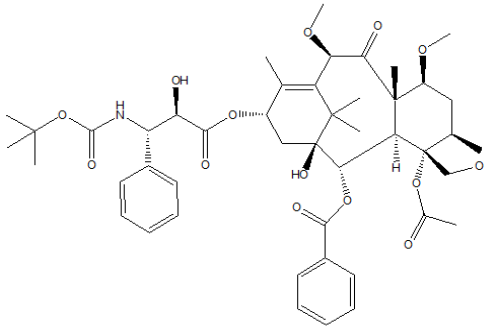

Figure.1. Chemical structure of Cabazitaxel

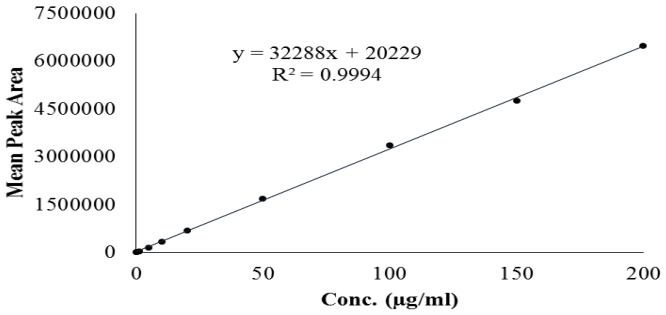

Figure.2. Calibration curve of Cabazitaxel

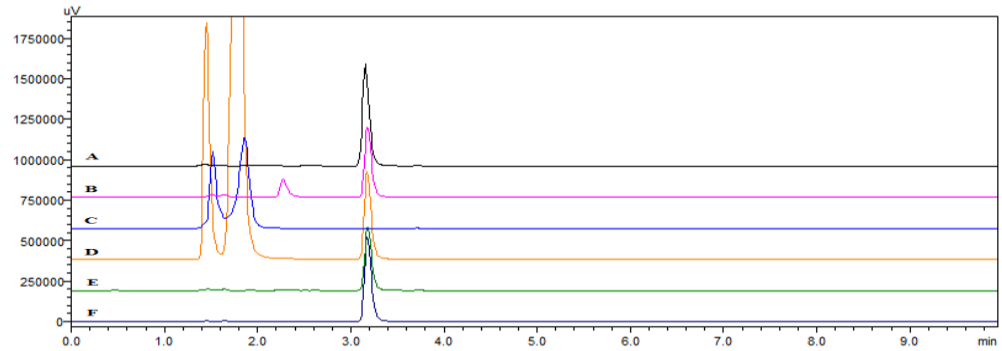

Figure.3. Typical chromatograms of Cabazitaxel (100 µg/ml) [A], acidic [B], alkaline [C], oxidative [D], thermal [E], and photolytic [F] degradations

4. CONCLUSION

The proposed stability-indicating liquid chromatographic method is simple, robust and economical and validated and it can be applied for the determination of Cabazitaxel in pharmaceutical dosage forms as well as biological fluids. The present proposed stability-indicating for the quantification of Cabazitaxel is specific because the drug peak was well separated even in presence of degradation products.

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