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**RESEARCH ARTICLE**

# Development and Validation of a Stability-Indicating Liquid Chromatographic Method for the Assay of Cabazitaxel

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**Abstract:** A validated stability-indicating high-performance liquid chromatographic technique was developed for the determination of cabazitaxel. Chromatographic separation was performed on Shimadzu Model CBM-20A/20 Alite, using a mixture of sodium acetate buffer (pH 4.0) and acetonitrile (30:70, 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 was validated as per ICH guidelines.

**Keywords:** Cabazitaxel, RP-HPLC, Stability-indicating, ICH

## Introduction

Cabazitaxel is a semi-synthetic derivative of a natural taxoid[1] used for the treatment of hormone-refractory prostate cancer. 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. It is a microtubule inhibitor and the fourth taxane to be approved as a cancer therapy. Cabazitaxel has been approved in the US by the Food and Drug Administration (FDA) in June 2010[2] 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 trail 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)-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, 12*b*-dodecahydro-1*H*-7, 11-methanocyclodeca[5-6] benzo [1, 2-b] oxet-12-yl benzoate is chemotherapy drug with molecular formula $C_{45}H_{57}NO_{14}$ and molecular weight 835.93 g/mol (Figure 1). 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 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].

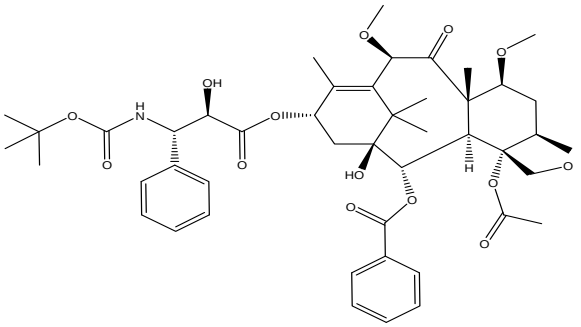

**Figure 1.** Chemical structure of Cabazitaxel

Very few analytical methods have been reported for the determination of cabazitaxel such as spectroscopic techniques[9], HPLC[10], LC-MS/MS in dry blood spots and human plasma[11-13]. As per the literature available till date there was no RP-HPLC method reported. So, at present the authors have developed a stability indicating RP-HPLC method for the determination of cabazitaxel in presence of its degradation products.

# Experimental

Cabazitaxel standard was obtained from Reddy's Labs (India). Acetonitrile (HPLC grade), Sodium hydroxide (NaOH) and Hydrochloric acid (HCl), Glacial acetic acid and Hydrogen peroxide ($H_2O_2$) were purchased from Merck (India).

Cabazitaxel is available as infusion with brand name Jevtana® (Sanofi-Aventis, Malaysia) with label claim of 60 mg of drug. All chemicals were of analytical grade and used as received.

### *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 ºC.

### *Chromatographic conditions*

Isocratic elution was performed using sodium acetate buffer: acetonitrile (30:70, 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.

### *Preparation of sodium acetate buffer solution (pH 4.0)*

The buffer solution (pH - 4.0) was prepared by mixing 28.6 mL of glacial acetic acid with 10 mL of 50% (w/v) NaOH in a 1000 mL volumetric flask, dissolving and diluting to volume with HPLC grade water.

### *Method validation*

The method was validated for system suitability, linearity, limit of quantitation (LOQ), limit of detection (LOD), precision, accuracy, selectivity and robustness[14]. Linearity test solutions for the assay method were prepared from a stock solution at different concentration levels (0.1-250 µg/mL) of the assay analyte concentration and 20 µL of each solution was injected in to 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 study*

The intra-day precision of the assay method was evaluated by carrying out 9 independent assays of a test sample of Cabazitaxel at three concentration levels (20, 50 and 100 µg/mL) against a qualified reference standard. The %RSD of three obtained assay values at three different concentration levels was calculated. 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.

The accuracy of the assay method was evaluated in triplicate at three concentration levels (50, 100 and 150%) and the percentage recoveries were calculated. Standard addition and recovery experiments were conducted to determine the accuracy of the method for the quantification of Cabazitaxel in the drug product. The study was carried out in triplicate at 150, 200 and 250 µg/mL. The percentage recovery in each case was calculated.

The robustness of the assay method was established by introducing small changes in the HPLC conditions which included wavelength (232 and 236 nm), percentage of acetonitrile in the mobile phase (68 and 72%) and flow rate (0.9 and 1.1 mL/min). Robustness of the method was studied using six replicates at a concentration level of 100 µg/mL of cabazitaxel.

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 and 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[10]. All solutions for stress studies were prepared at an initial concentration of 1 mg/mL of Cabazitaxel and refluxed for 30 min at 60 ºC 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 ºC 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 ºC for 20 min and neutralized after cooling with proper dilution with mobile phase. Oxidative stress studies were performed using 30 % $H_2O_2$ and thermal stress studies were conducted in thermostat at 60 ºC for 20 min. 20 µL solution of each of these solutions which were exposed to forced degradation studies were injected in to 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*

The performance characteristics of the present stability indicating liquid chromatographic method was compared and discussed in detail with the previously published methods in Table 1. Initially the stressed samples were analyzed using a mixture of sodium acetate buffer (pH 4.0): acetonitrile (50:50, v/v) with a flow rate of 0.8 mL/min in which the peak symmetry was not satisfactory. The flow rate was changed to 1.0 mL/min and again the drug sample was injected in to the loop where a sharp peak was eluted with tailing. Finally the mobile phase composition was modified as sodium acetate buffer (pH 4.0): acetonitrile (30:70, v/v) and a sharp peak were eluted at retention time 3.823 ± 0.03 min. (UV detection at 210 nm) which was chosen as the best chromatographic response for the entire study.

### *Method validation*

Cabazitaxel shows linearity over a concentration range of 0.1-250 µg/mL (Table 2) with % RSD 0.14-0.51. The linear regression equation was found to be y = 26523x + 971.45 ($r^2$ = 0.9993). The LOQ was found to be 0.0738 µg/mL and the LOD was found to be 0.0244 µg/mL.

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

| S. No. | Method /Reagent | λ nm | Linearity µg/mL | Remarks | Ref. |
|---|---|---|---|---|---|
| 1. | Phosphate buffer: Acetonitrile (30:70, v/v) | 230 | 0.1-150 | Stability indicating method | [10] |
| 2. | 10 mM ammonium hydroxide and methanol (83:17, v/v) (pH = 3 ± 0.1) | 275 | 2-20 | Very narrow linearity range | [11] |
| 3. | Acetonitrile: ammonium acetate (20 mM) (80:20, v/v) | 236 | 2.49-99.60 | Very narrow linearity range | [12] |
| 4. | Phosphate buffer: Acetonitrile: Methanol (40:40:20, v/v) | 362 | (10-100) $10^3$ | Mixture of solvents | [13] |
| 5. | Tetra butyl ammonium hydrogen sulphate : Methanol (20: 80, v/v) | 210 | 0.1-250 | Wide linearity range Stability indicating method | Present work |

The % RSD range was obtained as 0.10-0.26 and 0.55-0.68 for intra-day and inter-day precision studies respectively and 99.55-99.94% of recovery was observed in the accuracy studies with % RSD 0. 12-0.64 (<2.0%) (Table 3) indicating that the method is precise and accurate.

**Table 2.** Linearity of Cabazitaxel

| Conc. µg/mL | *Mean peak area ± SD | RSD, % |
|---|---|---|
| 0.1 | 2788±9.03 | 0.32 |
| 1 | 28357±80.25 | 0.28 |
| 5 | 144728±719.30 | 0.50 |
| 10 | 283098±920.07 | 0.33 |
| 20 | 514448±1409.59 | 0.27 |
| 50 | 1353855±1922.47 | 0.14 |
| 100 | 2567180±6417.95 | 0.25 |
| 150 | 4028658±18934.69 | 0.47 |

**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) |
| 20 | 514096.67±636.41 (0.13) | 510790.67±3465.79 (0.68) |
| 50 | 1350126.67±3515.91 (0.26) | 1345668.67±7369.41 (0.55) |
| 100 | 2567327.00±2513.28 (0.10) | 2547848.00±16967.82 (0.67) |

| Accuracy | | | | |
|---|---|---|---|---|
| Spiked conc. µg/mL | Total conc. µg/mL | *Mean peak area ± SD (% RSD) | Drug found, µg/mL | % Recovery |
| 50 (50%) | 150 | 3961402.33±36731.44 (0.12) | 149.32 | 99.55 |
| 100 (100%) | 200 | 5299109.00±7685.98 (0.15) | 199.76 | 99.88 |
| 150 (150%) | 250 | 6627715.67±42467.24 (0.64) | 249.85 | 99.94 |

**Mean of three replicates*

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 analysis[14]. 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% (0.53-1.22) indicating that the proposed method is robust.

**Table 4.** Robustness study of Cabazitaxel

| Parameter | Condition | *Mean peak area | *Mean peak area ± SD (% RSD) | *Assay, % |
|---|---|---|---|---|
| Flow rate (± 0.1 mL/min) | 0.9 | 2548571 | 2563665.67±13680.34 (0.53) | 99.86 |
| | 1.0 | 2567180 | | |
| | 1.1 | 2575246 | | |
| Detection wavelength (± 2 nm) | 232 | 2569874 | 2559906.00±14992.64 (0.59) | 99.71 |
| | 234 | 2567180 | | |
| | 236 | 2542664 | | |
| Mobile phase composition (± 2 %, v/v) | 28:72 | 2512335 | 2541052.00±27514.01 (1.08) | 98.98 |
| | 30:70 | 2567180 | | |
| | 32:68 | 2543641 | | |
| pH (± 0.1 unit) | 4.9 | 2524563 | 2559051.00±31227.39 (1.22) | 99.68 |
| | 5.0 | 2567180 | | |
| | 5.1 | 2585410 | | |

**Mean of three replicates*

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 representative chromatogram of Cabazitaxel was shown in Figure 2A. The proposed method was applied to the laboratory prepared formulation and the percentage recovery was calculated as 98.57.

### *Forced degradation studies*

The stability indicating capability of the method was established from the separation of cabazitaxel peak from the degraded samples. Cabazitaxel has shown 33.17% degradation

with two degradants at 1.472 min and 1.944 min during acidic stress indicating that the drug is more sensitive towards acidic environment. The amino group present in the drug structure may be highly responsible for this degradation. 22.15% of cabazitaxel has also undergone decomposition with a degradant at during oxidation indicating that the drug is sensitive towards oxidation. In other degradations the drug has undergone decomposition slightly (< 15.0%). Typical chromatograms obtained from the stressed samples were shown in Figure 2B-2F. A slight decomposition was seen on exposure of cabazitaxel solution to acidic (33.17%), alkaline (14.32%), oxidative (22.15%) thermal (14.50%) and photolytic (6.74%) conditions (Table 5).

The present stability-indicating method for the determination of cabazitaxel in pharmaceutical formulations is specific because the drug peak was well separated even in the presence of degradation products. Also, the overall data demonstrated that the excipients and the degradation products did not interfere with the cabazitaxel peak. 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).

**Table 5.** Forced degradation studies of cabazitaxel

| Stress Condition | *Mean peak area | *Drug recovered, % | *Drug decomposed, % | Theoretical plates | Tailing factor |
|---|---|---|---|---|---|
| Standard (Untreated) | 2567180 | 100 | - | 5975 | 0 |
| Acidic | 1715519 | 66.83 | 33.17 | 7326 | 0 |
| Alkaline | 2199683 | 85.68 | 14.32 | 7389 | 1.436 |
| Oxidative | 1998488 | 77.85 | 22.15 | 7687 | 1.336 |
| Thermal | 2195016 | 85.50 | 14.50 | 7609 | 1.326 |
| Photolytic | 2394235 | 93.26 | 6.74 | 7377 | 1.319 |

**Mean of three replicates*

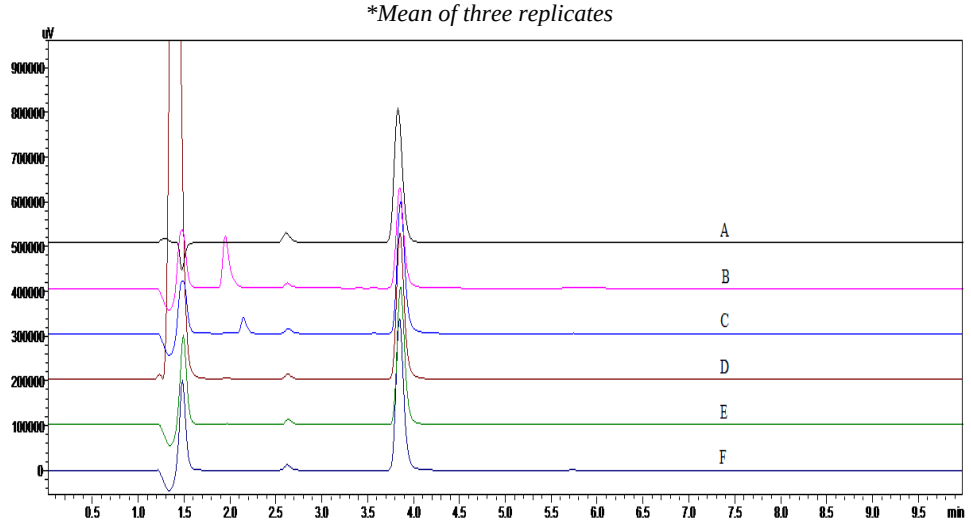


**Figure 2.** Typical chromatograms of Cabazitaxel (100 µg/mL) [A], acidic [B], alkaline [C], oxidative [D], thermal [E] and photolytic [F] degradation

## Conclusion

The proposed stability-indicating HPLC method was validated as per ICH guidelines and applied for the determination of cabazitaxel in pharmaceutical dosage forms and can be successfully applied to perform long-term and accelerated stability studies of cabazitaxel formulations.

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