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Research Article

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RP-HPLC method for the determination of cabazitaxel in bulk and injection dosage form

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ABSTRACT

An RP-HPLC method has been developed and validated for the quantification of cabazitaxel in injection dosage form. The desired chromatographic separation was achieved on the Inertsil ODS 3V (150 mm × 4.6 mm, 5 µm) HPLC column using isocratic elution at 254 nm detection wavelength. The optimized mobile phase consists of mixture of 0.1M NaH_2PO_4 and methanol in the ratio of 60:40 (v/v). The method showed linearity over the range of 24-72 µg/ml with regression coefficient value of 0.9999. The limits of detection and quantitation were found to be 0.052 µg/ml and 0.175 µg/ml, respectively. The recovery for all of the components was in the range of 100.394-100.693% with percent relative standard deviation of less than 1%. The proposed method was successfully applied for the quantitative determination of cabazitaxel in injection dosage form.

Keywords: Cabazitaxel, Jevtana, Chromatography, Validation, Analysis

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INTRODUCTION

Cabazitaxel is a microtubule inhibitor approved by Food and Drug Administration (U.S) for the treatment of hormone-refractory prostate cancer [1-4]. Chemically, it is described as (2aR,4S,4aS,6R,9S,11S,12S,12aR,12bS-12bS)-12b-acetoxy-9-(((2R,3S)-3-((tert-butoxycarbonyl)amino)-2-hydroxy-3-phenylpropanoyl) 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 (Figure 1). Cabazitaxel has potential antineoplastic activity and is a semi-synthetic derivative of taxoid called 10-deacetylbaccatin III.

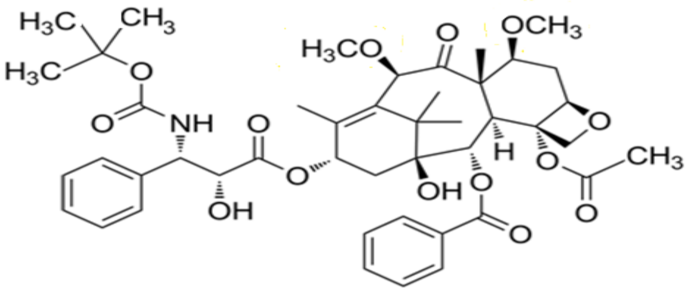

Figure 1: Chemical structure of cabazitaxel

A whole literature survey of analytical methods for the quantification of cabazitaxel is done. Five visible spectrophotometric methods using napthaquinone sulphate, haemtoxylin, 2,2 bipyridine, 4-amino phenazone and isonicotanic hydrazide as chromogenic reagents [5] have been reported by Kishore for the quantification of cabazitaxel in formulations. Ultra performance liquid chromatography (UPLC) method was developed and validated for simultaneous determination of cabazitaxel and their synthetic impurities [6]. Liquid chromatography tandem mass spectrometry (LC-MS/MS) has been used to quantify cabazitaxel in pharmaceuticals in rat whole blood [7] and human plasma [8,9]. The reported spectrophotometric methods suffer from disadvantages like heating, long analysis time, less sensitivity, lack precision and selectivity [5]. Though the UPLC [6] and LC-MS/MS [7-9] are sensitive and have short analysis time, they require sophisticated instrumentation. Furthermore, LC-MS/MS [7-9] methods were applied only to biological samples.

RP-HPLC technique is certainly the most widely used technique for the assay of drugs in bulk, pharmaceutical formulations and biological samples. Few RP-HPLC methods, according to our literature survey, were developed and validated for the quantification of cabazitaxel in pure and in pharmaceutical formulations [10-15]. The reported RP-HPLC methods [10–15] have some drawbacks in terms of sensitivity, increased retention time (>2.5 min), increased run time (>8 min), more flow rate (>1.0 ml/min), precision and accuracy. System suitability, one of the important validation parameter is not reported in most of the reported methods. The more flow rate, run time and retention time increases the utilization of solvents which inturn increases the cost of analysis. The main aim of the present investigation is to develop and validate a simple, economical, sensitive, selective, robust, and reproducible RP-HPLC method for quantitative determination of cabazitaxel in pure and injection dosage forms.

EXPERIMENTAL SECTION

Instrumentation:

The Waters 2695 alliance with binary HPLC pump equipped with Waters 2998 PDA detector was used for method development and method validation. The output signal was monitored and processed by using Waters Empower2 software.

Materials:

Cabazitaxel bulk drug was made available from Lara drugs pvt Ltd., Hyderabad. Commercially available cabazitaxel injection dosage form (Jevtana, Sanofi-Aventis, Malaysia; labeled to contain 60 mg/1.5 ml) were used for the dosage form analysis. Sodium dihydrogen phosphate (Sd. Fine Chemicals Ltd., Mumbai) and methanol (Merck India Ltd., Mumbai) were of analytical and HPLC grade, respectively. Milli-Q-water was used all throughout the experiment. Orthophoshoric acid of analytical reagent grade is from Sd. Fine Chemicals Ltd., Mumbai.

Mobile phase preparation:

The mobile phase used was mixture of 0.1 M phosphate buffer (pH 3.5) and methanol in the ratio of 60:40 *v/v*. For the preparation of 0.1 M phosphate buffer, 11.99 g of sodium dihydrogen phosphate was dissolved into 1000 ml of water and mixed well. Finally pH was adjusted to 3.5 with orthophosphoric acid (85%) and degassed for 20 min in sonicator. Before use, it was filtered through 0.45 µm millipore membrane filter paper.

Chromatographic conditions:

Isocratic elution of mobile phase was set at a flow rate of 1.0 ml/min and the injection volume was 10 µl. The analytical column used was Inertsil ODS 3V (150 mm × 4.6 mm, 5 µm) at 30 ^{o}C temperature. The detection was carried out at a wavelength of 254 nm for a run time of 8 min.

Preparation of standard solutions:

Stock solution of cabazitaxel was prepared at a concentration of 1.0 mg/ml. Accurately weigh and transfer 100 mg of cabazitaxel into a 100 ml volumetric flask. Added 10 ml of methanol and sonicated for 10 minutes to dissolve it completely and make up to the volume with the mobile phase. The working standard solutions were prepared in the concentration range of 24-72 µg/ml by appropriate dilution of the stock solution with mobile phase.

Sample solution preparation:

Jevtana injection dosage kit contains 60 mg of cabazitaxel in 1.5 ml of polysorbate and 5.7 ml of 13% ethanol in water for injection as diluent. The entire content of the vial was reconstituted with 5.7 ml of the diluent. The concentration of the resulting solution is 10 mg/ml of cabazitaxel. This solution was further diluted step wise with mobile phase to get a concentration of 48 µg/ml for analysis. This solution was filtered through millipore membrane filter before analysis.

Validation of the method:

The RP-HPLC method was validated in terms of system suitability, selectivity, linearity, limit of detection, limit of quantification, precision, accuracy and robustness according to ICH guidelines [16].

RESULTS AND DISCUSSION

Optimization of the chromatographic conditions:

During preliminary work, different HPLC columns [Thermo scientific, C18 (150 mm × 4.6 mm, 5 µm) and Inertsil ODS 3V (150 mm × 4.6 mm, 5 µm)] with different temperatures were tested in order to optimize the conditions of analysis. The best column efficiency and peak shape were achieved on an Inertsil ODS 3V (150 mm × 4.6 mm, 5 µm) maintained at a temperature of 30 ^oC. Different trails were performed using different proportions of 0.1M K_2HPO_4, 0.1 M Na_2HPO_4 and 0.1 M NaH_2PO_4 at different pH with combination of methanol. Mobile phase with different flow rates were also tested. The mobile phase containing mixture of 0.1 M NaH_2PO_4 (pH 3.5) and methanol (60:40 *v/v*) at a flow rate of 1.0 ml/min was found to be satisfactory and gave symmetric and well resolved peak for cabazitaxel. Selecting 254 nm as the analytic wavelength is based on the absorption maxima shown by the drug in mobile phase. The retention time of cabazitaxel, under the optimized conditions, was found to be 2.507 minutes (Figure 2).

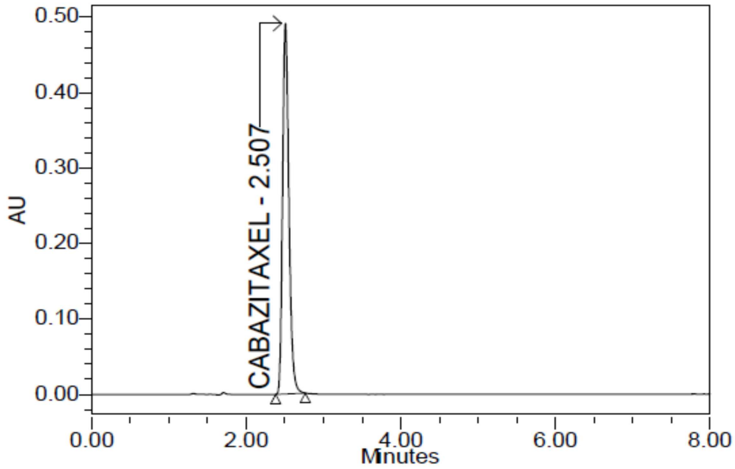

Figure 2: Chromatogram of cabazitaxel at optimized chromatographic conditions

Method validation:

System suitability:

Chromatographic parameters associated with the developed HPLC method must meet the system suitability limits before the analysis. The relative standard deviation of peak area, relative standard deviation of retention time, USP plate count and USP tailing factor for cabazitaxel peaks was evaluated using a solution containing 48 µg/ml of cabazitaxel. All the results (Table 1) assure the satisfactoriness of the proposed method for routine analysis of cabazitaxel.

Table 1: System suitability results

Parameters	Value	Recommended limits
Retention time	2.507(%RSD – 0.440)	RSD ≤2
Peak area	2814486(%RSD – 0.439)	RSD ≤2
USP plate count	4709	> 2000
USP tailing factor	1.26	≤ 2

Selectivity:

The selectivity study was assessed to verify the absence of interference by the excipients commonly found the formulations and components of mobile phase. For this study, standard cabazitaxel solution (48 µg/ml), sample solution (48 µg/ml) and mobile phase blank solution were injected into the chromatographic system. The chromatograms were recorded and are shown in Figures 3-5. The chromatogram demonstrated the selectivity of the proposed method, since there were no peaks at the retention time of cabazitaxel in the chromatograms of placebo blank and mobile phase blank. The retention time of cabazitaxel in standard and sample solutions is same.

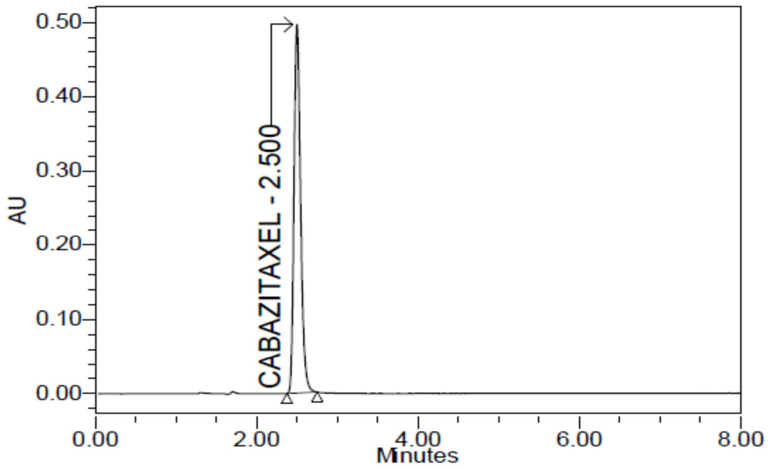

Figure 3: Chromatogram of standard cabazitaxel solution

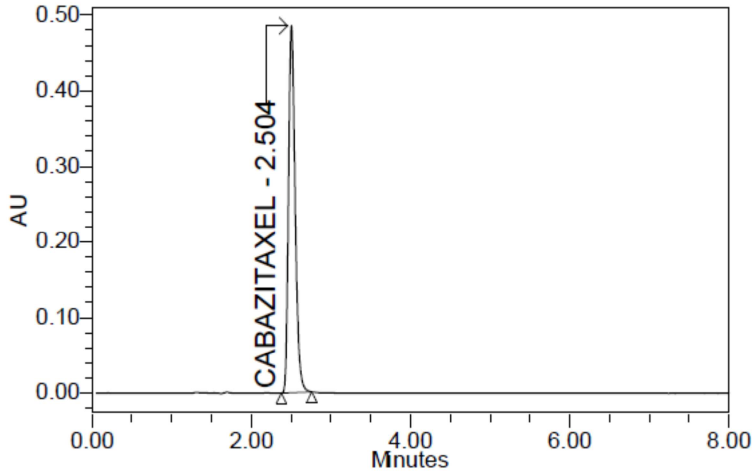

Figure 4: Chromatogram of cabazitaxel sample solution

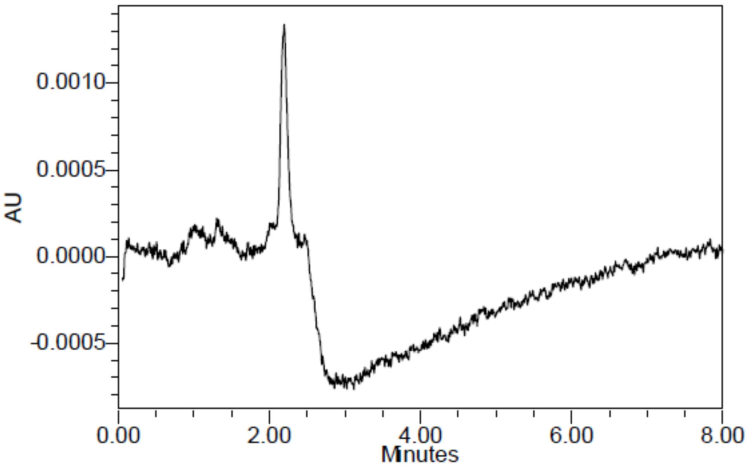

Figure 5: Chromatogram of mobile phase blank

Linearity:

Linearity standard solutions for the proposed HPLC method were prepared from cabazitaxel stock solution at five concentration levels (24, 36, 48, 60 and 72 µg/ml). The peak area versus concentration data of analyte was treated by least-squares linear regression analysis. The linear curve was obtained over the concentration range from 24 µg/ml to 72 µg/ml and the correlation coefficient obtained was 0.9999. The regression equation was: $y = 58597x + 967.7$. Figure 6 shows the linearity curve of cabazitaxel. The results showed that a good correlation is found between the peak area and concentration of cabazitaxel.

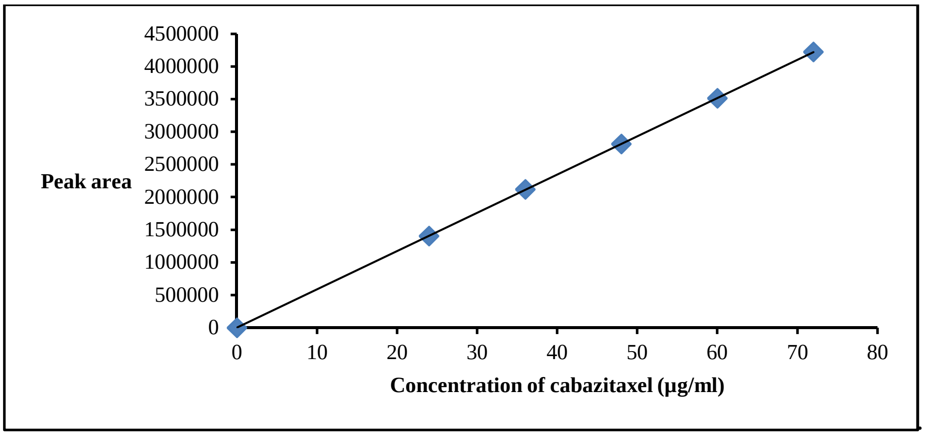

Figure 6: Linearity curve of cabazitaxel

Limit of detection (LOD) and limit of quantitation:

The limit of detection (LOD) and limit of quantitation (LOQ), which represents the sensitivity of the method, were calculated based on the signal-to-noise ratio. LOD and LOQ were experimentally verified by five injections of cabazitaxel at the LOD and LOQ concentrations. LOD and LOQ values were found to be 0.052 µg/ml and 0.175 µg/ml, respectively which indicated the adequate sensitivity of the method.

Precision and accuracy:

The precision and accuracy of the method was checked by injecting cabazitaxel standard solution 6 times at the 48 µg/ml concentration level. The precision and accuracy of the method was expressed as the percent RSD and percent recovery. The results are indicated in Table 2. The low percent RSD values and good recovery value indicated the precision and accuracy of the method, respectively.

Table 2: Precision and accuracy of the method

Concentration of drug (µg/ml)	Peak area	Statistical calculation	Assay (%)	Statistical calculation
48	2810333	**Average:** 2815458.8	99.253	**Average:** 99.434
48	2812398		99.326	
48	2815642		99.441	
48	2816588	**% RSD:** 0.086	99.474	**% RSD:** 0.086
48	2813923		99.380	
48	2818743		99.550	

Recovery studies:

The proposed method's accuracy was further evaluated in triplicate using three concentration levels (50, 100 and 150%) on the sample solutions. Standard addition and recovery experiments were conducted on the real sample solutions to determine the accuracy of the method. The percentage of recoveries for cabazitaxel was calculated. Table 3 summarizes the accuracy results, expressed as percent recovery. The method showed good recovery.

Table 3: Results of recovery studies

Spiked Level (%)	**Concentration of drug (µg/ml)**		**Recovery (%)**	**Mean (%)**
	Added	**Found**		
50	23.760	23.906	100.614	100.693
50	23.760	23.951	100.803	
50	23.760	23.917	100.662	
100	47.520	47.680	100.338	100.394
100	47.520	47.659	100.271	
100	47.520	47.793	100.574	
150	71.280	71.582	100.424	100.456
150	71.280	71.700	100.589	
150	71.280	71.532	100.354	

Robustness

The method robustness was established at a concentration of 48 µg/ml cabazitaxel. In order to measure the method robustness, the HPLC parameters were deliberately varied. The studied parameters were: column temperature (±2°C) and flow rate (±0.1). The system suitability parameters were measured to demonstrate the robustness of the method. The results (Table 4) indicated that the small change in the conditions did not significantly affect the system suitability. Therefore, the method is robust.

Table 4: Method robustness results

Parameter varied	Peak area	USP plate count	USP Tailing
Flow rate – 0.9 ml/min	3523167	4437	1.29
Flow rate – 1.1 ml/min	3328056	4093	1.25
Column temperature-29°C	3511060	4414	1.29
Column temperature-31°C	3328798	4092	1.25

CONCLUSION

An isocratic RP-HPLC method was successfully developed for the estimation of cabazitaxel in bulk and in its injection dosage form. The results of method validation have proven that the developed method is linear, sensitive, precise, accurate and robust. The selectivity data proved that the developed method is selective for cabazitaxel and free from the interference of the components of mobile phase and excipients in injection dosage form. The short run time (8 min) enables rapid determination of the cabazitaxel.

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