RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF CABAZITAXEL IN PHARMACEUTICAL DOSAGE FORMS

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ABSTRACT

A simple, selective, precise, and stability indicating RP-HPLC method has been developed and validated for analysis of Cabazitaxel, a chemotherapy medication used to treat different types of cancer. The chromatographic separation was achieved on a Agilent ZORBAX Eclipse Plus C18 column with Methanol: Acetonitrile: water (40:40:20 v/v), as mobile phase at a flow rate of 0.9mL/min. Detection was performed at 237nm and a sharp peak was obtained for Cabazitaxel at a retention time of 7.12min. The calibration curve showed a good linear relationship between response and concentration with the equation y = 6816x + 67048, and the regression coefficient was 0.999 over the concentration range 20-120µg/mL. The limit of detection (LOD) and limit of quantification (LOQ) were 0.04µg/ mL and 0.15µg/mL respectively. The method was validated for accuracy, precision, reproducibility, specificity, robustness, and detection and quantification limits, in accordance with ICH guidelines. The method was successfully separated the degraded products from the stress degradation conditions. The wide linearity range, accuracy and simple mobile phase imply the method is suitable for routine quantification of Cabazitaxel with high precision.

KEYWORDS: Cabazitaxel, RP- HPLC, Stress degradation, JEVTANA© – 30mg, ICH.

INTRODUCTION

Cabazitaxel is a mitotic inhibitor used to treat different types of cancer.[1] This includes ovarian cancer, breast cancer, lung cancer, Kaposi sarcoma, cervical cancer and pancreatic cancer.[2,3] Cabazitaxel is also used for the prevention of restenosis. It was discovered in a US National Cancer Institute program at the Research Triangle Institute in 1967 when Monroe E. Wall and Mansukh C. Wani isolated it from the bark of the Pacific yew tree, Taxus brevifolia and named it taxol.[4,5] Cabazitaxel is approved in the UK for ovarian, breast and lung, bladder, prostate, melanoma, esophageal, and other types of solid tumor cancers as well as Kaposi's sarcoma.[6] It is use in the United States for the treatment of breast, pancreatic, and non-small cell lung cancers was approved by the FDA. In India the drug is sold under various brands. It is given by injection into a vein. A newer formulation, in which Cabazitaxel is bound to albumin, is sold under the trademark Abraxane. The structure of Cabazitaxel shown as figure No.1.

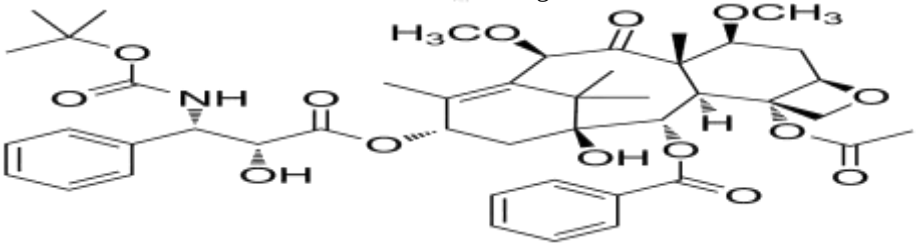

Figure 1: Structure of Cabazitaxel.

To analyze the Cabazitaxel, various analytical methods have been reported. Among them only two stability indicating liquid chromatography methods have reported. Analytical methods for Cabazitaxel includes,

Stability indicating HPLC method (7, 8), Assay method (9-12), Bio- analytical method (13-18), bio-analytical with other drug (19), UV-Spectrophotometer (20), UV- derivative Spectrophotometer (21). Now it is a challenging task to develop a RP-HPLC method is simpler in terms of mobile phase wide linearity range with a good correlation coefficient value, high precise, rapid and economic stability indicating method for estimating Cabazitaxel commercial formulations.

MATERIALS AND METHODS

Reagents and Chemicals

The reagents used in this work were methanol (HPLC grade- Lichrosolv), Acetonitrile (HPLC grade- Lichrosolv), which were procured from Merck, India. Hydrochloric acid AR

grade, sodium hydroxide AR grade, hydrogen peroxide AR grade were obtained from Rankem Pharmaceuticals Ltd. India. The water was deionised and further purified by means of Milli-Q plus water purification system, Millipore Ltd (USA). The formulation injections of Cabazitaxel (JEVTANA© – 30mg) were purchased from local pharmacy and the membrane filter was procured from Merck Milli Pour, India.

Instrumentation

Chromatography was performed using PEAK LC 7000 isocratic HPLC with PEAK 7000 delivery system, Rheodyne manual sample injector with switch (77251), Analytical column Agilent ZORBAX Eclipse Plus C18 (250mm x 4.6mm, 5µm) column, Electronic balance- DENVER (SI234), manual Rheodyne injector with a 20µL loop was used for the injection of sample. PEAK LC software was employed. UV 2301 Spectrophotometer was used to determine the wavelength of maximum absorbance.

Preparation of Solutions

A mixture of Methanol: Acetonitrile: water (40:40:20 v/v) were used as diluents in the preparation of analytical solutions. The stock solution of Cabazitaxel prepared by dissolving 10mg of standard drug into a 10mL volumetric flask to prepare 1000µg/mL .Required standard dilutions for construction of calibration curve were prepared from 1000µg/mL stock solution. The blank, system suitability mixture, 6 replicates of standard and test solution were injected separately and chromatographed under the optimized chromatographic conditions. Formulation drug sample was prepared by mixing of five formulation injections of Cabazitaxel (JEVTANA© – 30mg) sample solution. From the sample solution, an amount equivalent to 10mg standard drug was further diluted to 10mL in a volumetric flask containing little amount of mobile phase and was shaken to dissolve the drug completely and the volume made up to 10mL with sample mobile phase. Then the sample solution obtained was filtered through Nylon membrane filter paper. Sample solution having Cabazitaxel concentration of 1000µg/mL was obtained was further diluted to 60µg/mL the solution was used for the formulation assay.

Standard solution was injected in to HPLC system by altering the various method parameters like mobile phase, column, detector wavelength, flow rate of the mobile phase, P^H of the mobile phase solution etc. the optimized conditions with system suitability chromatogram was selected as method for further validation.

METHOD VALIDATION

The developed method was validated by evaluating linearity, accuracy, precision, robustness, ruggedness, detection limit, quantification limit and stability as per ICH guide lines.

Calibration curve (Linearity and Range): Linearity was performed by diluting standard stock solution to give final concentration in the range of 20 to 120µg/mL for Cabazitaxel 20µL of concentration injected. Calibration curve with concentration verses peak areas was plotted and the obtained data were subjected to regression analysis using the least squares method.

Accuracy

The accuracy of the method was carried out using one set of different standard addition methods at different concentration levels, 50%, 100% and 150%, and then comparing the difference between the spiked value (theoretical value) and actual found value.

Precision

The precision of the method was evaluated from the peak area obtained by actual determination of six

replicates of a fixed amount of the drug (60µg/mL). The precision of the assay was also determined in terms of intra- and inter-day variation in the peak areas of a set of drug solutions on three different days. The intra- and inter-day variation in the peak area of the drug solution was calculated in terms of relative standard deviation (%RSD).

Robustness

Robustness of the proposed method for Cabazitaxel was carried out by the slight variation in detector wavelength, mobile phase ratio and P^H. The percentage change in each changed condition was noted for Cabazitaxel.

Ruggedness

The test solutions were prepared as per test method and injected under variable conditions. Ruggedness of the method was studied by different analysts.

Detection limit and quantification limit

The limit of detection (LOD) and limit of quantification (LOQ) were established based on the calibration curve parameters, according to the following formulas:
LOD=3.3SD/slope
LOQ=10SD/slope

or detection limit=3.3σ/s, quantification limit=10σ/s, where σ is the standard deviation of y- intercept of regression line, and s is the slope of the calibration curve.

Forced Degradation

Forced degradation studies can be used to determine the degradation pathways and degradation products that could form during storage, and facilitate during formulation, development, manufacturing and packaging. To evaluate the stability of the drug, various stress conditions are applied to the drug like acid, base, peroxide, sunlight, UV light etc. Degradation test is performed by incubating the standard for 48 hours in different conditions.

Light (Normal and UV light)

To demonstrate the degradation of the sample, kept in open petri dish at Lab light and UV light. The sample was further analyzed after 24hours exposed sample at Lab light and UV light.

Thermal

The sample kept in a Petri dish and keep in oven at 80°C up to 24hours. After expose of the samples and prepare sample solution for injection.

Acid

100mg of sample taken in to 20 mL of 0.1 N Hydrochloric Acid and incubated for 24hours. Then 5 mL of acid hydrolyzed sample solution was neutralized with 5 mL of 0.1 N sodium hydroxide solution and make up with diluents in 25mL of volumetric flask. The above solutions inject once after system suitability solution and evaluate the degradants in chromatogram and compare with standard values.

Base

100 mg sample added in 20 mL of 0.1 N Sodium Hydroxide solution. After 48 hours to take 5 mL of Base hydrolyzed sample solution in 25 mL volumetric flask and neutralize with 5 mL of 0.1 N Hydrochloric acid solutions and make up with diluent. The above solutions inject once after system suitability solution and evaluate the degradants in chromatogram and compare with without base hydrolysis values.

Hydrogen Peroxide

For peroxide degradation 100 mg sample is incubated for in 20mL of 3% Hydrogen Peroxide for 24hours 5mL of oxidized sample solution taken into 25mL volumetric flask and make up

with diluent. The above solutions inject once after system suitability solution and evaluate the degradants in

chromatogram and compare with without oxidized (Initial) values.

Aqueous

To evaluate the drug stability in aqueous solution 100 mg sample is incubated in 20mL of aqueous solution. After 48 hours to take 5 mL of sample solution in 25 mL volumetric flask and make up with diluent. The above solutions inject once after system suitability solution and evaluate the degradants in chromatogram and compare with without aqueous values.

RESULTS AND DISCUSSION

The final decision on mobile phase composition and flow rate was made on the basis of peak shape, peak area, tailing factor, baseline drift and time required for analysis. The solvent system selected Methanol: Acetonitrile: water (40:40:20 v/v) gave good resolution of degraded product and drug peak. The system suitability tests performed verified the resolution, column efficiency and repeatability of the chromatographic system and ensured that the equipment, electronics, and analytical operations for the samples analyzed could be constituted as an integral system that can be evaluated as a whole. The proposed method conditions are presented in (Table No.1).

Table 1: Optimized chromatographic conditions.

S. No.	Condition	Results
1	Mobile phase	Methanol: Acetonitrile: water (40:40:20 v/v)
2	Pump mode	Isocratic
3	pH	5.9
4	Diluents	Mobile phase
5	Column	Agilent ZORBAX Eclipse Plus C18 (250mm x 4.6mm, 5µm) column
6	Column Temp	Ambient
7	Wavelength	237nm
8	Injection Volume	20µL
9	Flow rate	0.9mL/min
10	Run time	12min
11	Retention Time	7.12min

The proposed HPLC method of analysis was found to be precise and accurate, as depicted by the statistical data of analysis. High values of correlation coefficients and small values of intercepts validated the linearity of the calibration plots (20-120µg/mL) and obedience to Beer's laws (y = 6816x + 67048) (Table No. 2 & Figure No.2).

Table 2: Results of Linearity test.

Level	Concentration in µg/mL	Peak Area
1	20	200202
2	40	341485
3	60	472449
4	80	619135
5	100	754358
6	120	877381
	Slope: 6816 Intercept: 67048 Correlation Coefficient: 0.999	

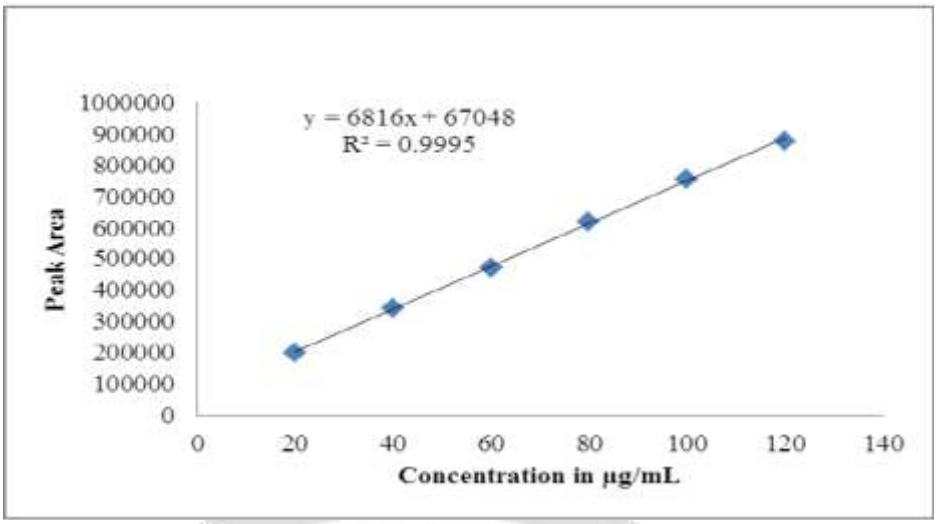

Figure 2: Calibration curve of Cabazitaxel.

The RSD of the precision and repeatability study indicate the high reproducibility of the proposed method (intraday-0.86, interday – 0.48 and ruggedness-0.85). Summary of intraday precision and interday precision studies are presented in (Table No.3).

Table 3: Intraday Precision and interday Precision studies.

S. No	Concentration in µg/mL	Peak Area	
		Intraday Precision	**Interday Precision**
1	60µg/mL	479829	471768
2		471826	473938
3		478400	470284
4		479650	471829
5		470442	471124
6		474343	476484
% RSD		0.864	0.480

The method was also found to be robust as there was no significant change in the peak area, peak shape and retention time of Cabazitaxel. The method also shows the good recovery results (99.4% -100.3%). Summary of validation results are presented in (Table No. 4).

Table 4: Summary of method validation.

Validation parameter	Results
Calibration curve	20-120 µg/mL
Intraday precision (RSD)	0.86
Interday precision (RSD)	0.48
Ruggedness (RSD)	0.85
Recovery	99.4-100.3%
Robustness (change of percentage)	0.65-1.37%
Limit of detection	0.04µg/mL
Limit of quantification	0.15µg/mL
Formulation assay in %	99.148%

Furthermore, the low values of LOD and LOQ indicate that the method can be employed over a wide

concentration range for linearity. This method is also highly sensitive and could effectively separate the drug from its degraded product. Solution of Cabazitaxel found stable at thermal condition and sensitive to other stress conditions where three degradative products are found separated in chromatogram. Stability indicating chromatograms are presented in Figure No.3 to 9 and results are presented in (Table No.5).

Table 5: Forced Degradation results.

S. No.	Condition	No additional peaks observed	Peak Area	% Obtained	% degradation
1	Standard [60µg/mL]	...	472449	100	---
2	Acidic	3	428535	90.705	9.295
3	Base	3	457324	96.799	3.201
4	Light	3	409739	86.727	13.273
5	Peroxide	3	450375	95.328	4.672
6	Thermal	2	435553	92.191	7.809
7	UV	3	436317	92.352	7.6478

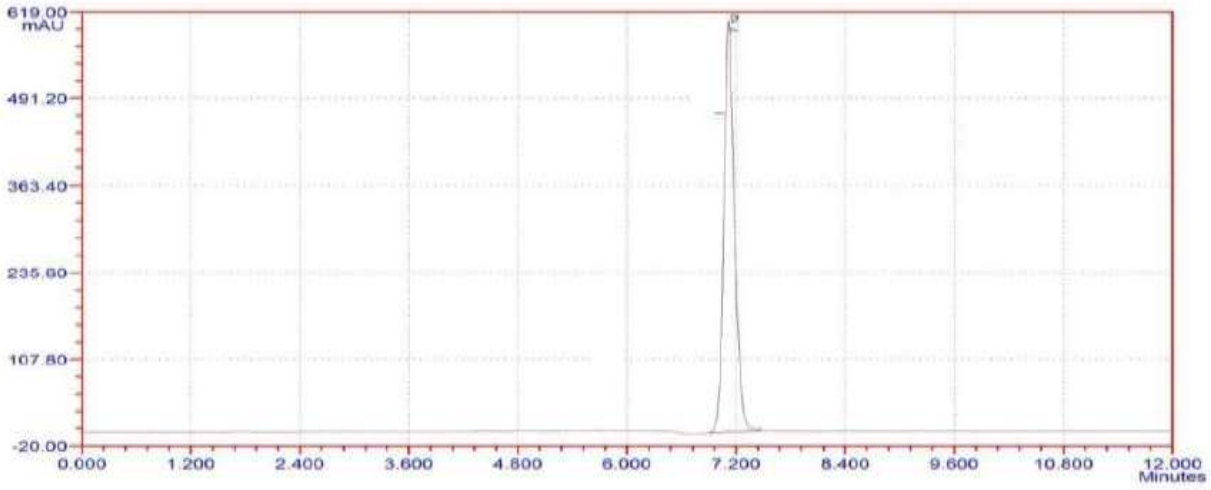

Figure 3: Standard chromatogram of Cabazitaxel.

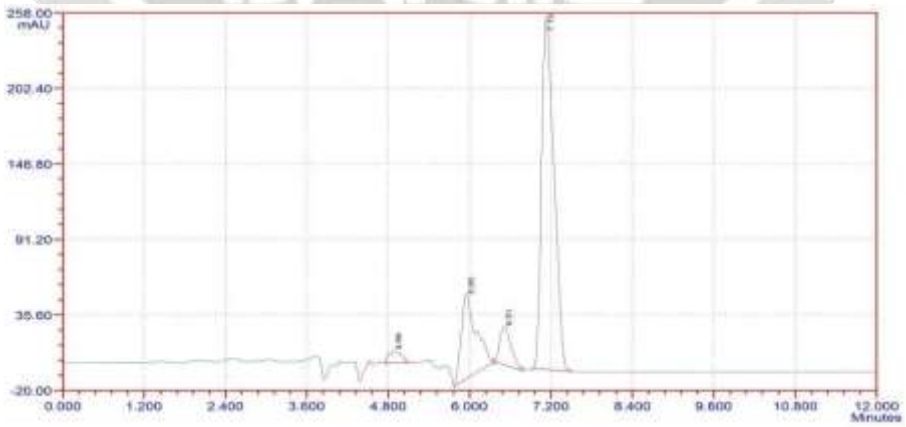

Figure 4: Acid degradation chromatogram.

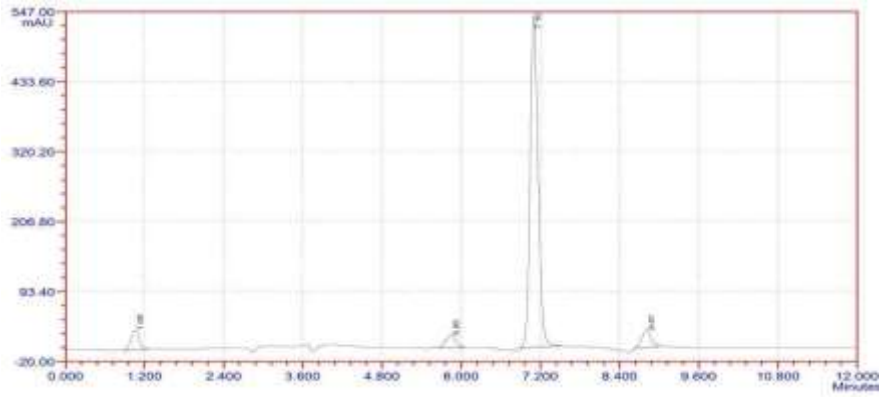

Figure 5: Base degradation chromatogram.

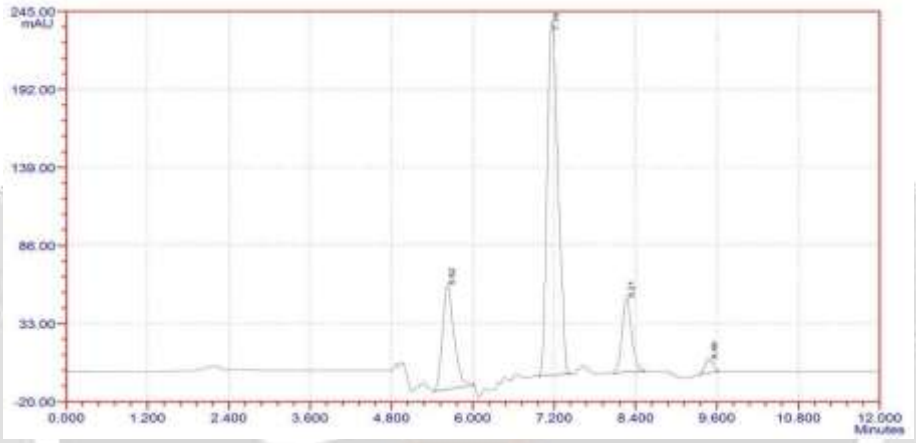

Figure 6: Light degradation chromatogram.

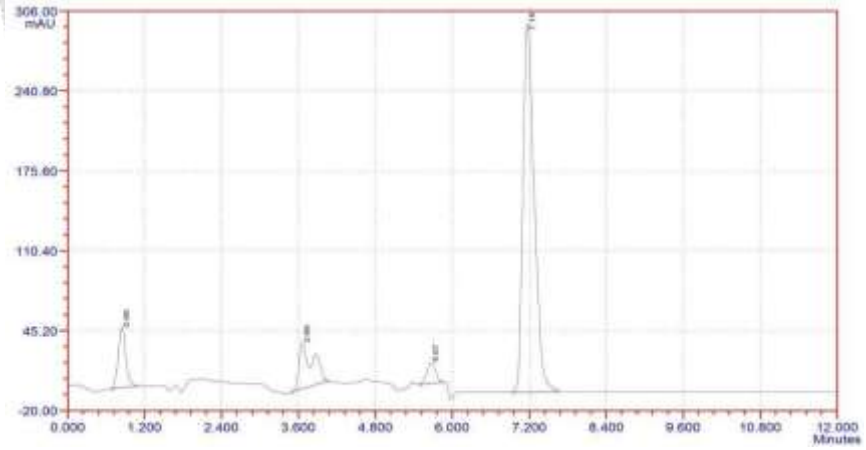

Figure 7: Peroxide degradation chromatogram.

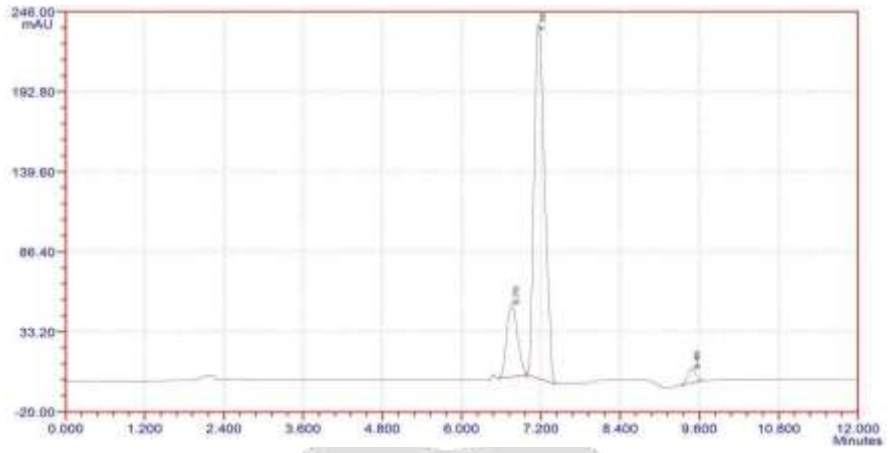

Figure 8: Thermal degradation chromatogram.

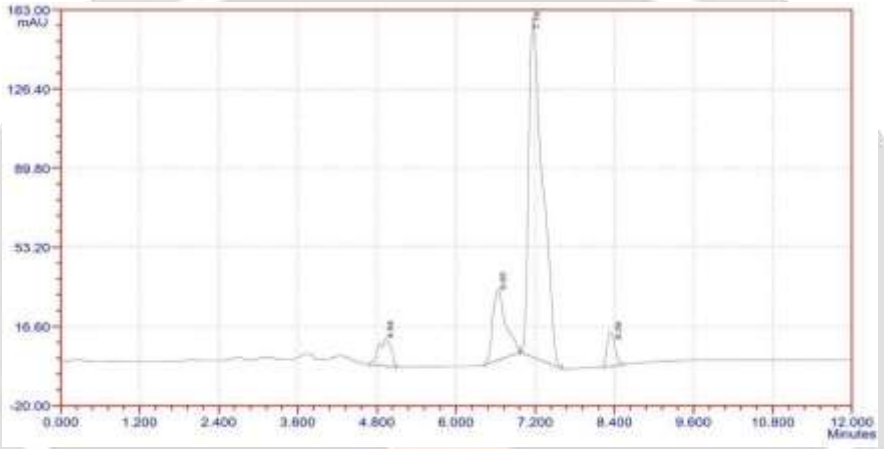

Figure 9: UV Light degradation chromatogram.

The degradation study indicated that the drug degrades as shown by the decreased areas in the peaks when compared with peak areas of the same concentration of the non-degraded drug, without giving any additional degradation peaks. Percent degradation was calculated by

comparing the areas of the degraded peaks in each degradation condition with the corresponding areas of the peaks of both the drugs under non degradation condition. Summary of degradation studies of both the drugs is given in Table 3. The proposed method was compared with the author Seemi Siddiqui et al (2012) and Ali Mohammadi et al (2009). When compared with method proposed by Seemi Siddiqui, the present proposed method was found simpler in terms of mobile phase and wide linearity range with good correlation coefficient value. The method proposed by Ali Mohammadi has very low linearity range and less precise when compared with current method. Hence the present proposed method has high analysis range and found good validation result within acceptable limit. Statistical analysis proved that method was accurate, precise, and repeatable. The developed method was found to be simple, sensitive and selective for analysis of Cabazitaxel without any interference from the excipients. The method specifically estimates both the drugs in presence of all the degradants generated during forced degradation study. Assay results for dosage form using proposed method showed 99.14% of Cabazitaxel. It can be concluded that the method separates the drugs from their degradation products; it may be employed for analysis of stability for their tablet dosage form.

CONCLUSION

The HPLC method developed is accurate, precise, reproducible, specific and stability indicating. The method is linear over a wide range, economical and utilizes a mobile phase which can be easily prepared. All these factors make this method suitable for quantification of Cabazitaxel in pharmaceutical dosage forms. It can therefore be concluded that use of the method with very high accuracy and precision. In conclusion, the

presented HPLC method is simple, selective, and reproducible.

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