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NEW SPECTROPHOTOMETRIC METHODS FOR THE QUANTITATIVE ESTIMATION OF CABAZITAXEL IN FORMULATIONS

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ABSTRACT

Cabazitaxel is a semi-synthetic derivative of a natural taxoid.We are developed 5 colorimetric method to quantify the Cabzitaxel in formulation dosages. Total work carried out in visible range only. We were applied INH, Naptha Quinone sulphate, Haemtoxylin, 2,2 Bipyridine , 4-Amino phenazone reagent to develop a color with Cabazitaxel. In results we got 99.2- 99.89 % accuracy in recovery studies.

KEYWORDS Cabazitaxel, Spectrophotometric Methods, INH, Naptha Quinone sulphate, Haemtoxylin, 2,2 Bipyridine , 4-Amino phenazone.

INTRODUCTION

Cabazitaxel is a semi-synthetic derivative of a natural taxoid.[1] It was developed by Sanofi-Aventis and was approved by the U.S. Food and Drug Administration (FDA) for the treatment of hormone-refractory prostate cancer on June 17, 2010. It is a microtubule inhibitor, and the fourth taxane to be be approved as a cancer therapy.[2]Cabazitaxel in combination with prednisone is a treatment option for hormone-refractory prostate cancer following docetaxel-based treatment. Cabazitaxel is used to treat advanced prostate cancer that is no longer responding to hormone therapy. It is also being studied for use against other kinds of cancer. Cabazitaxel is a type of chemotherapy drug known as a taxane. It interferes with microtubules, which are part of the internal structure that cells need when they are dividing. This leads to cell death. Because cancer cells divide faster than normal cells, they are more likely than normal cells to be affected by this drug. Possible side effects to this drug are , Nausea. Diarrhea, Constipation, Feeling weak, Feeling tired, Low red blood cell count (anemia)*Low blood platelet count.

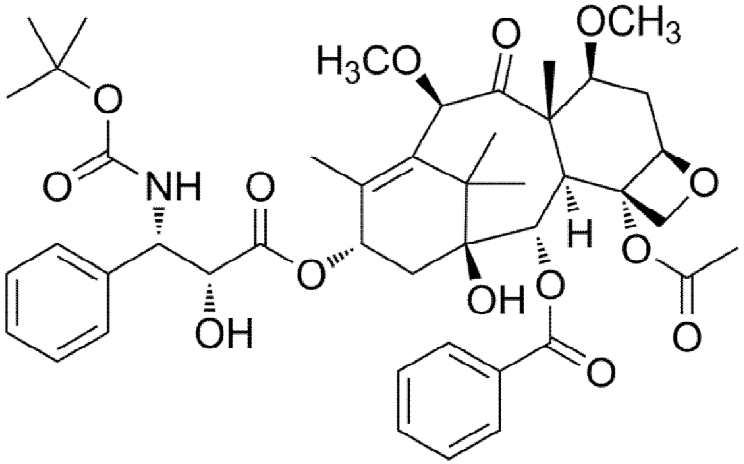

Figure.1 Structure of Cabazital

EXPERIMENTAL PROCEDURE

All chemicals used were of analytical reagent grade and distilled water was used to prepare all solutions. Double beam UV-Visible Spectrophotometer is used for measuring the absorbance's of the color formed during the analysis.

Preparation of reagents

2,2 Bipyridine : Weigh accurately 200 mg of 2,2 Bipyridine and was dissolved in 100 ml of distilled water with warming.

4-Amino Phenazone solution: Weigh accurately 500mtg of 4-Amino Phenazone and was dissolved in 100 mL of Methanol containing 1 mL of conc. HCl.

Haematoxylin : 0.2 % in methanol.

Chloramine T: 0.4 % in distill water.

Iso Nicotanic hydrazide solution: Weigh accurately 800 mg of Iso Nicotanic hydrazide and is dissolved in 100 mL of MeOH containing 1% of conc. HCl.

Naptha Quinone Sulphate: Weigh accurately 100 mg of Naptha Quinone Sulphate and was dissolved in 100 ml of distilled water.

Fe (III) solution: Accurately 250 mg of anhydrous ferric chloride was weighed and was taken in a100 ml graduated volumetric flask. It was dissolved in little amount of distilled water and the final volume was made up to the mark with distill water.

HCl solution (1N): Prepared by diluting 86 ml of conc. HCl to 1000 ml with distilled water and standardized.

Buffer pH 7: 390 ml of 0.067 M KH_2PO4 was prepared and is added to 610 ml 0.067 Na_2HPO_4 in Distill water.

NaOH Solution: Weigh accurately 20g of Sodium Hydroxide and was dissolved in 100 ml of distilled water.

Preparation of working standard drug solution

The standard Cabazitaxel (100 mg) was weighed accurately and transferred to volumetric flask (100 ml). It was dissolved properly and diluted up to the mark with methanol to obtain final concentration of 1000 µg /ml (stock solution I). 10 ml of stock solution I was diluted to 100 ml with Methanol (Stock solution II, 100 µg/ml) and the resulting solution was used as working standard solution.

METHODS

2,2 Bipyridine Method: (M1)

From the standard stock solution II of Cabazitaxel, appropriate concentration(5 to35 ppm) is pipetted out in to a 25 ml volumetric calibrated tube, 0.5 ml FeCl3 solution and 2 ml of 2,2 Bipyridine were added. The tube was heated in water bath up to 30 min. after cooling the tube 1 ml of acid was added and make up to 25 ml with distilled water. Make up to 25 ml volume. The absorbance of the formed color was measured after 5min at 470 nm against a reagent blank.

4-Amino Phenazone Method: (M2)

From the standard stock solution II of Cabazitaxel 0.5 mL of standard drug solution (10-70ppm) were transferred into a series of 10 mL calibrated tubes. Then 3.0 mL of 4-Amino Phenazone solution was added to each tube and kept aside for 15 min. Later the solution in each tube was made up to 10 ml with methanol. The absorbance was measured at 450 nm against the reagent blank.

Haematoxylin Method: (M3)

To series of 25 mL tubes 1 mL of Haematoxyline and 1mL of Chloramine T was added. To this add 10 mL of pH 7.0 buffer solution. Kept a side for 20 min. Then sample solution ranging to 2-14 µg of drug , kept in a water bath at 70 ^{0}C for 5 min. cool to room temperature and made up to 25 mL with distill water. The absorbance was measured at 740 nm against the reagent blank.

Iso Nicotanic hydrazide Method: (M4)

Aliquot of standard drug solution (0.5-30ppm) was delivered into a series of 10 ml of calibrated tubes. Then 2.0 mL of Iso Nicotanic hydrazide solution was added to each tube and heated for 10 min at 60 ^{0}C. The solution in each tube was cooled and made up to 10 mL with methanol. The absorbance was measured at 470 nm against the reagent blank.

Naptha Quinone Sulphate Method: (M5)

Aliquot of standard drug so0lution was transferred in to a series of calibrated test tubes containing 0.2ml of NaOH and 0.2 of Naptha Quinone Sulphate reagent solution was added in each tube and the contents were heated at 50^{0}c for a min and cooled for 2min ice water. This operation was performed in the dark. After cooling the contents in the tube were rinsed with 1ml of water. These rinsing were transferred in to 25ml separating funnel containing 10ml of dichloro methane and shaken immediately for 5sec. the whole organic layer from the bulk was collected from the funnel after 2min of mixing 3ml DNPH was added. It was heated for 10min at 50^{0}c by using air condenser and chilled in ice water. Then .5ml of concentrated sulphuric acid was added slowly, mixed the absorbance were measured after 5min at 500nm against the reagent blank.

Assay Procedure for Formulations

An amount of finely ground tablet powder equivalent to 100 mg of Cabazitaxel (Jevtana) was accurately weighed into a 100 ml calibrated flask, 60 ml of water added and shaken for 20 min. Then, the volume was made up to the mark with water, mixed well, and filtered using a Whatman No 42 filter paper. First 10 ml portion of the filtrate was discarded and a suitable aliquot of the subsequent portion (1000 µg mL-1 Cabazitaxel) was diluted appropriately to get suitable concentrations for analysis by proposed methods.

Method Validation

Selection of analytical concentration ranges: (linearity test)

Linearity test was evaluated by measuring the absorbance values of standard solutions. The standard stock solution of Cabazitaxel, appropriate aliquots were pipetted out in to a six or seven series of volumetric flasks and add the solutions required in required for each individual method. After color formation absorbance of each concentration was measured at wavelength found for the proposed method. Results were shown in Table: 1 and Standard graphs of linearity for proposed methods were shown below.

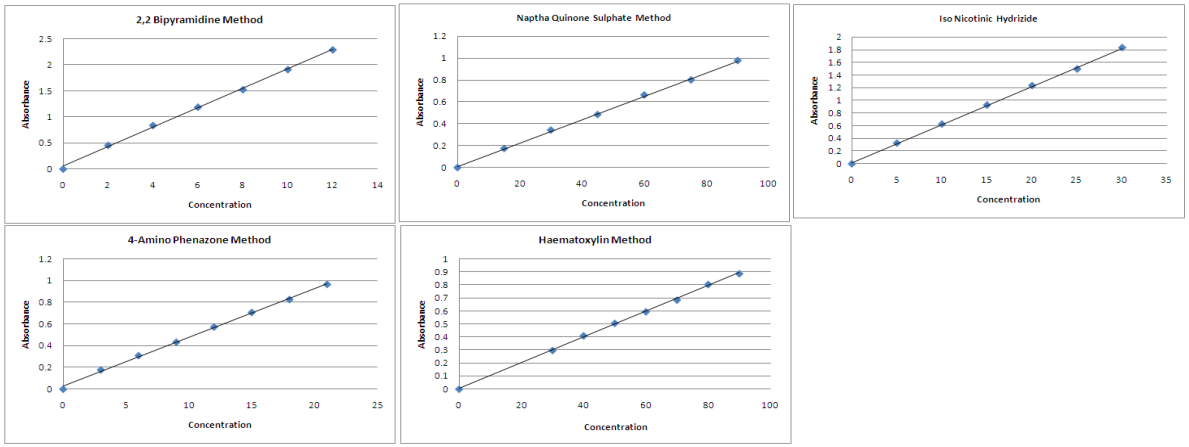

Figure 1: Calibration curves for the proposed methods.

S.NO	Parameter	M1	M2	M3	M4	M5
1	Wavelength Max	470nm	450nm	540nm	480nm	500nm
2	Concentration Range	5-35 ppm	10-70 ppm	2-14 ppm	0.5-3.5ppm	4-28 ppm
3	Correlation coefficient	0.9996	0.9997	0.9991	0.9994	0.9993
4	Slope	0.134	0.047	0.052	0.63	0.018
5	Intercept	0.048	0.007	0.021	0.005	0.005
6	RSD of Precision	0.52	0.32	0.25	0.16	0.28
7	Average recovery	99.76	99.84	99.62	99.88	99.58
8	Stability period	60min	120min	180min	420min	180min
9	LOD	0.05ppm	0.1ppm	0.01 ppm	0.02 ppm	0.25 ppm
10	LOQ	0.0025ppm	0.05ppjm	0.005 ppm	0.005ppm	0.5 ppm
8	% Assay of Formulation	99.3	99.6	99.35	99.72	99.52

Table.1 Results of developed methods

Precision

To evaluate the accuracy and precision of the methods, pure drug solution (Within the working limits) was analyzed and being repeated six times. The relative error (%) and relative standard deviation (%) were less than 2.0 and indicate the high accuracy and precision for the proposed methods (Table 2).

SNO	M1	M2	M3	M4	M5
Concentration	6PPM	12PPM	60ppm	20PPM	60PPM
1	1.189	0.571	0.596	1.231	0.661
2	1.196	0.579	0.591	1.226	0.669
3	1.188	0.577	0.605	1.235	0.665
4	1.199	0.572	0.601	1.239	0.656
5	1.196	0.566	0.593	1.221	0.654
6	1.185	0.568	0.592	1.232	0.652
RSD	0.47	0.88	0.94	0.52	1.02

Table.2 Accuracy and Precision

Recovery Studies

To ensure the accuracy and reproducibility of the results obtained, known amounts of pure drug was added to the previously analyzed formulated samples and these samples were reanalyzed by the proposed method and also performed recovery experiments. The Percentage recoveries thus obtained were given in Table 3.

Method	Recovery	Average Recovery
M1	50%	99.76
	100%	
	150%	
M2	50%	99.84
	100%	
	150%	
M3	50%	99.62
	100%	
	150%	
M4	50%	99.88
	100%	
	150%	
M5	50%	99.58
	100%	
	150%	

Table.3

Application to Analysis of Commercial Sample:

In order to check the validity of the proposed methods, Cabazitaxel was determined in commercial formulation. From the results of the determination it is clear that there is close agreement between the results obtained by the proposed methods and the label claim. These results indicating that there was no significant difference between the proposed methods and the reference methods in respect to accuracy and precision

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S.NO	Method	Formulation	Amount prepared	Amount found	% Assay
1	M1	Jevtana	10 ppm	9.57	98.17
2	M2	Jevtana	30 ppm	29.63	98.58
3	M3	Jevtana	6 ppm	5.94	98.53
4	M4	Jevtana	2 ppm	1.97	99.3
5	M5	Jevtana	16 ppm	15.96	99.25

Table.4

DISCUSSION

The results obtained method M1 were due to redox reaction followed by complex formation between the anti-oxidant and ferric chloride and 2, 2-bipyridine to form an orange colored solution that exhibited maximum absorption at 470nm against the corresponding reagent blank.
In method M2, 4-Amino phenazone react with the keto group of the drug, results forms a schiffs base. The formed schiffs base show absorbance at 450nm.

In method M3, Haematoxylin and Chloramine T react with each other in basic media and form a compounds haematin. The lone pair electrons on the hetero sulphur group of the drug forms charge transfer spectra when react with haematin. Results the solution attain color. Absorbance of the formed color was measured at 540nm.

In method M4, the keto group of the drug reacts with Iso Nicotinic hydrazode to gives a colored Hydazone. The formed color chromogen shows absorbance at 480nm.

The presence of imino group of the drug will responcible for the development of the colored complex in Naptha Quinone Sulphate Method. The imino gropu of the drug undergo nucleophilic substitution with Naptha Quinone Sulphate. Results form a colored complex. The absorbance of the formed color complex was measured at 500nm.

The linearity ranges of Cabazitaxel are found to be 5-35, 10-70ppm, 2-14, 0.5-30ppm, 4-28 ppm for M1 to M5 respectively. A linear correlation was found between absorbance and concentration of Cabazitaxel. The graphs showed negligible intercept and are described by the equation: Y = a + bX (where Y = absorbance of 1-cm layer of solution; a = intercept; b = slope and X = concentration in ug mL-1 max) .Regression analysis of the Beer's law data using the method of least squares was made to evaluate the slope (b), intercept (a) and correlation coefficient(r) for each system according to ICH guide

The accuracy of the proposed methods was further ascertained by performing Accuracy studies. The Relative standard deviations of results for the proposed were very low and the values are within the range below 2. It indicates that the high accuracy and precision for the proposed methods. The Recovery results were very close to the actual range and it revealed that co-formulated substances did not interfere in the determination.

CONCLUSIONS

Five useful micro methods for the determination of Cabazitaxel have been developed and validated. The methods are simple and rapid taking not more than 20-25 min for the assay. These spectrophotometric methods are more sensitive than the existing UV and HPLC methods, and are free from such experimental variables as heating or extraction step. The methods rely on the use of simple and cheap chemicals and techniques but provide sensitivity comparable to that achieved by sophisticated and expensive technique like HPLC. Thus, they can be used as alternatives for rapid and routine determination of bulk sample and tablets.

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