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ANALYTICAL DEVELOPMENT AND VALIDATION OF STABILITY INDICATING RP-HPLC METHOD FOR THE DETERMINATION OF RELATED SUBSTANCES AND ASSAY OF CABAZITAXEL IN CABAZITAXEL INJECTION DOSAGES FORM

Alok K Singh*[1], Dr. Amrish Chandra[2], Dr. Girendra K Gautam[3]

[1]Research Scholar, Department of Pharmacy, Bhagwant University, Ajmer.

[2]AMITY Institute of Pharmacy, AMITY University, Noida, UP, India.

[3]Associate Professor & Head, Department of Pharmacy,
Bhagwant University, Ajmer.

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***Corresponding Author**
Alok K. Singh
Research Scholar,
Department of Pharmacy,
Bhagwant University,
Ajmer, Rajasthan India.

ABSTRACT

A simple, accurate, precise, rugged, robust, linear and reproducible method was developed by RP-HPLC method for estimation of related substance and assay of cabazitaxel in Cabaxitaxel injection. A gradient RP-HPLC method was developed and validated on C-18 Column (Sunfire, 150 x 4.6 mm, 3.5 µm) using 0.05 M KH_2P0_4 and 0.2% of 1-octane sulphonic acid with pH 2.0 as mobile phase A, while for mobile phase B acetonitrile was used. The flow rate was adjusted to 1.3 ml/min, column oven temperature 30°C and the detection wavelength was 230 nm with 85 minutes run time. The retention time for cabazitaxel was found to be 13.85, 10-Dab-III impurity 2.57, Amine impurity 3.62, Detroc oxazolidine impurity 16.17, Oxazolidine protected Cabazitaxel impurity 22.73, Ditroc impurity 24.08, Ditroc oxazolidine impurity 59.01. Detection response for cabazitaxel and known impurities were found linear over a range of LOQ to 250% of the working specification limits. *Proposed method was validated for specificity, accuracy, precision, linearity, range, ruggedness & robustness. This developed method can be applicable for routine and stability quantitative analysis.*

KEYWORDS: Cabaxitaxel, Impurities, RP- HPLC, Stability indicating, Method Development and Validation.

INTRODUCTION

Cabazitaxel is an antineoplastic agent belonging to the taxane class which is prepared by semi-synthetic methods with a precursor extracted from yew needles. 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. Unlike other taxane compounds, this agent is a poor substrate for the membrane associated with multidrug resistance (MDR), P-glycoprotein (P-gp) efflux pump and may be useful for treating multidrug-resistant tumors for the treatment of hormone-refractory prostate cancer.

A literature survey revealed that few analytical methods, such as spectrophotometry, HPLC, have been reported for the determination of cabazitaxel. Stability indicating RP-HPLC method for the determination of cabazitaxel Quantification of cabazitaxel in human plasma by liquid chromatography/triple quadrupole mass spectrometry, Determination of cabazitaxel in rat whole bold on dry blood spots, New spectrophotometric methods for the quantitative estimation of cabazitaxel in formulations, All the reported literature methods were useful only in the estimation of cabazitaxel content in human plasma and dosage forms, determination of impurities present in cabazitaxel drug substance. Furthermore, there is any no stability-indicating RP-HPLC method reported in the literature that can completely separate and quantify all the potential impurities, degradation impurities and assay of cabazitaxel in cabazitxel injection in a single method.

It is, therefore, felt necessary to develop a new stability indicating showing mass balance RP-HPLC method for the related substances determination and assay of cabazitaxel in Cabaxitaxel injction. Hence, a stability indicating RP- HPLC method was developed for the quantitative determination of cabazitaxel and its six known impurities, degradation peaks in presence of excipients, namely impurity 10-Dab-III impurity, Ditroc impurity, Ditroc oxazolidine impurity, Detroc oxazolidine impurity, Amine impurity, Oxazolidine protected cabazitaxel impurity and assay in single method.

This method was successfully validated according to the ICH guidelines.

Cabazitaxel is an active ingredient of Cabazitaxel Injection. Each vial contains 60mg/1.5 ml of Cabazitaxel. An HPLC method for determination of related substance for Cabazitaxel in Cabazitaxel Injection has been developed and being validated for its suitability for routine use and testing the stability samples. This report describes the experimental data and

evaluation of data for the validation studies to be performed on the related substance method for Cabazitaxel in Cabazitaxel Injection.

MATERIALS AND METHODS

Preparation of Buffer

Dissolve 1.36 grams of KH_2P0_4 and 2g of 1-octane sulphonic acid sodium salt anhydrous in 1000 ml of milli -Q water and adjust the pH 2.0 (± 0.05) with dilute orthophosphoric acid. Filter through 0.45 micron nylon filter paper.

Preparation of mobile phase A

Prepare mixture of buffer and Acetonitrile in the ratio of 90: 10 (% v/v).

Preparation of mobile phase B

Acetonitrile.

Chromatographic Conditions

Flow rate : 1.3 mL/min
Wavelength of detection : 230 nm by UV/PDA
Column temperature : 30°C
Injection volume : 10 µL
Elution : Gradient
Run time : 85 min.
Diluent : Acetonitrile: Water (80:20 %v/v)
Blank : Diluent

Gradient programming

Time (minutes)	01	18	25	45	55	70	75	85
Mobile phase A (% v/v)	68	34	32	32	18	18	68	68
Mobile phase B (% v/v)	32	66	68	68	82	82	32	32

Preparation of Impurity stock solution

Weigh accurately 5mg of Ditroc impurity and 5mg of Oxazolidine protected cabazitaxel impurity into 5 ml volumetric flask, add 0.5ml of tetrahydrofuran to dissolve and made upto the mark with diluent.

Preparation of system suitability solution

Weigh about 20mg of Cabazitaxel working standard and transfer into a 10mL volumetric

flask, add 5mL of acetonitrile, dissolve well, then 45µL of impurity stock solution into the flask, make up to the mark with water and mix well.

Preparation of Standard Solution

Weigh about 20 mg of the Cabazitaxel working standard and transfer into 10 mL volumetric flask, add about 5ml of analytical diluent, dissolve well, then make upto the mark with analytical diluent and mix well.

Sensitivity solution

Dilute 3.0 mL of standard solution to 100 mL with diluent.

Dilute 5.0 mL of above solution to 50 mL with diluents.

Placebo solution

Weigh accurately about 1g of sample solution into 20 mL of volumetric flask. Add 3.1 mL of provided diluent for Cabazitaxel injection shake slowly and mix. Make up volume upto the mark with diluent and mix.

Preparation of Sample Solution

Weigh accurately about 1g of sample solution (equivalent to 40 mg of Cabazitaxel drug) into 20 mL of volumetric flask. Add 3.1 mL of provided diluent for Cabazitaxel injection shake slowly and mix. Make up volume upto the mark with diluent and mix.

Evaluation of System Suitability

i) Resolution between Ditroc impurity and oxazolidone protected Cabazitaxel impurity should be not less than 1.2.
ii) Signal to noise ratio of the Cabazitaxel peak in sensitivity solution is not less than 30.
iii) Tailing factor for the Cabazitaxel peak should not be more than 2.0.
iv) The % RSD for the six replicate injections of standard should not be more than 2.0.

1. SPECIFICITY

1.1 Selectivity

Experiment: A representative of Cabazitaxel standard solution, known impurities (10-Dab-III impurity, Ditroc impurity, Ditroc oxazolidine impurity, Detroc oxazolidine impurity, Amine impurity, Oxazolidine protected Cabazitaxel impurity) and sample solution of Cabazitaxel Injection were prepared as per the methodology and chromatographed the solutions along with blank/diluent and placebo using the chromatographic system described

in the methodology and a photodiode array detector.

1.2 Placebo Interference

Experiment: Diluent (Blank), placebo, standard and sample solutions were chromatographed as per methodology and evaluated for any placebo interference.

1.3 Forced Degradation Studies

1.3.1 Acid Degradation (0.1 M HCl)

Procedure: Weighed accurately about 1g of sample solution (equivalent to 40 mg of Cabazitaxel drug) into 20 mL of volumetric flask. Added 3.1 mL of provided diluent for Cabazitaxel injection and added 3 ml diluent shake slowly and mixed. Added 0.1 ml of 0.1M HCl, heated the content at 60°C for 60 minutes. Cooled to room temperature, then Neutralized the content by adding 0.1mL of 0.1M NaOH solution. Diluted up volume upto the mark with diluent and mixed.

1.3.2 Base Degradation (0.1 M NaOH)

Procedure: Weighed accurately about 1g of sample solution (equivalent to 40 mg of Cabazitaxel drug) into 20 mL of volumetric flask. Added 3.1 mL of provided diluent for Cabazitaxel injection and added 3 ml diluent shake slowly and mix. Added 0.5 ml of 0.1M NaOH, heated the content at 60°C for 5 minutes. Cooled to room temperature, and then neutralized the content by adding 0.5mL of 0.1M HCl solution. Diluted up volume upto the mark with diluent and mixed.

1.3.3 Peroxide Degradation (50 %v/v H_2O_2)

Procedure: Weighed accurately about 1g of sample solution (equivalent to 40 mg of Cabazitaxel drug) into 20 mL of volumetric flask. Added 3.1 mL of provided diluent for Cabazitaxel injection and added 3 ml diluent shake slowly and mix. Added 1ml of 50 %v/v H_2O_2 solution, heated the content at 60°C for 30 minutes. Cooled to room temperature. Dilute up volume upto the mark with diluent and mixed.

1.3.4 Thermal Degradation (60°C/20 hrs.)

Procedure: Sample exposed at 60°C for 20 hours were analyzed as per Methodology.

1.3.5 Photolytic Degradation (1.2 million Lux hours and 200 watt hours/square meter)

Procedure: Sample exposed at 1.2 million Lux hours were analyzed as per methodology.

1.3.6 Humidity Degradation (25°C/92% for 20 hrs.)

Procedure: Sample exposed at 25°C/92%RH humidity condition for at least 20 hours were analyze as per methodology.

Note

Simultaneously placebo were subjected to above stress conditions and chromatographed along with samples.

Table 1: Forced Degradation Studies for Cabazitaxel.

Sr. No	Name	Condition	RT	Purity Angle	Purity Threshold	Purity Criteria	% Degradation
1	Acid degradation	0.1 M HCl-60°C/60 min.	13.860	0.316	1.018	Pass	22.784
2	Base degradation	0.1M NaOH-60°C/5 min.	13.873	0.191	1.026	Pass	41.942
3	Peroxide degradation	50 % H_2O_2-60°C /30 min.	13.821	0.583	1.019	Pass	No degradation
4	Thermal degradation	60°C for 20hours	13.829	0.534	1.019	Pass	No degradation
5	Photolytic degradation	1.2 million Lux hours	13.839	0.645	1.015	Pass	No degradation
6	Humidity degradation	25°C/92%RH for 20 hours	13.833	0.532	1.018	Pass	No degradation

2. LOD and LOQ (Limit of Detection and Limit of Quantification)

Experiment: Based on the determination of Prediction linearity and visual observation for Cabazitaxel and known impurities, LOD and LOQ concentrations were determined and verified by precision test. RSD for six replicate injections were calculated for each analyte.

Table 2A: Precision for LOD and LOQ Response (Area).

	Cabazitaxel		10-Dab-III impurity		Ditroc impurity	
	LOD	LOQ	LOD	LOQ	LOD	LOQ
Mean of 6 injections	1543	3456	734	2295	572	1693
SD	340.217	317.620	75.368	43.319	51.254	82.233
% RSD	22.049	9.190	10.268	1.888	8.960	4.857

Table 2B: Precision for LOD and LOQ Response (Area).

	Ditroc oxazolidine impurity		Detroc oxazolidine impurity		Amine impurity	
	LOD	LOQ	LOD	LOQ	LOD	LOQ
Mean of 6 injections	429	1151	327	1270	1344	2820
SD	74.194	99.291	71.664	103.022	152.934	219.141
% RSD	17.295	8.626	21.916	8.112	11.379	7.771

Table 2C: Precision for LOD and LOQ Response (Area).

	Oxazolidine protected Cabazitaxel impurity	
	LOD	**LOQ**
Mean of 6 injections	375	586
SD	64.400	47.622
% RSD	17.173	8.127

3. LINEARITY

Experiment: A series of solutions of working/reference standards of Cabazitaxel, 10-Dab-III impurity, Ditroc impurity, Ditroc oxazolidine impurity, Detroc oxazolidine impurity, Amine impurity and Oxazolidine protected Cabazitaxel impurity were prepared over a range of LOQ to 250% of the working specification limits. Working concentration for Cabazitaxel is 2000µg/mL; the linearity range tested was between LOQ to 5000µg/mL. And Working concentration for all known impurities are 6µg/mL, the linearity range tested was between LOQ to 15µg/mL. Linearity data treated for calculation of correlation coefficient.

Table 3A: Linearity Data for Cabazitaxel and 10-Dab-III impurity.

% Concentration	**Cabazitaxel**			**10-Dab-III impurity**		
	RT*	**Conc.* (µg /mL)**	**Response (Area)**	**RT***	**Conc.* (µg /mL)**	**Response (Area)**
LOQ Level	14.366	0.165	3456	2.661	0.151	2295
Linearity-Level-50%	14.343	1041.074	8957853	2.654	2.973	39637
Linearity-Level-80%	14.328	1641.048	14036239	2.649	4.757	64140
Linearity-Level-90%	14.370	1834.461	15645483	2.660	5.351	70579
Linearity-Level-100%	14.321	2027.874	17176716	2.647	5.946	78721
Linearity-Level-110%	14.319	2209.445	19490633	2.645	6.540	87404
Linearity-Level-120%	14.304	2390.030	21299932	2.642	7.135	97064
Linearity-Level-150%	14.300	2971.255	25904784	2.643	8.919	117557
Linearity-Level-250%	14.306	4450.468	37698219	2.648	14.864	192139
Slope	**8531.59**			**12915.77**		
Intercept	**111192.46**			**2004.28**		
Correlation Coefficient	**0.9996**			**0.9996**		

Conc.* = Concentration, RT*= Retention Time

Table 3B: Linearity Data for Ditroc impurity and Ditroc oxazolidine impurity.

% Concentration	Ditroc impurity			Ditroc oxazolidine impurity		
	RT*	Conc.* (µg /mL)	Response (Area)	RT*	Conc.* (µg /mL)	Response (Area)
LOQ Level	24.807	0.307	1693	60.761	0.258	1151
Linearity-Level-50%	24.786	3.032	22928	60.715	2.934	16986
Linearity-Level-80%	24.770	4.850	37899	60.718	4.694	26289
Linearity-Level-90%	24.756	5.457	42143	60.717	5.281	29250
Linearity-Level-100%	24.756	6.063	46849	60.717	5.867	34059
Linearity-Level-110%	24.754	6.669	52555	60.717	6.454	37866
Linearity-Level-120%	24.742	7.276	57354	60.732	7.041	42664
Linearity-Level-150%	24.744	9.095	72078	60.721	8.801	54254
Linearity-Level-250%	24.768	15.158	117217	60.748	14.668	88208
Slope	7814.78			6120.91		
Intercept	-201.61			-1336.87		
Correlation Coefficient	**0.9997**			**0.9990**		

Conc.* = Concentration, RT*= Retention Time

Table 3C: Linearity Data for Detroc oxazolidine impurity and Amine impurity.

% Concentration	Detroc oxazolidine impurity			Amine impurity		
	RT*	Conc.* (µg /mL)	Response (Area)	RT*	Conc.* (µg /mL)	Response (Area)
LOQ Level	16.673	0.175	1270	3.719	0.273	2820
Linearity-Level-50%	16.647	2.986	24224	3.707	3.140	24608
Linearity-Level-80%	16.646	4.777	39438	3.693	5.051	40042
Linearity-Level-90%	16.687	5.375	44495	3.727	5.734	44891
Linearity-Level-100%	16.644	5.972	48703	3.693	6.280	50691
Linearity-Level-110%	16.646	6.569	53987	3.694	6.908	55169
Linearity-Level-120%	16.634	7.166	58804	3.688	7.536	57051
Linearity-Level-150%	16.634	8.958	73110	3.689	9.420	75353
Linearity-Level-250%	16.656	14.930	119346	3.702	15.700	121914
Slope	8004.80			7747.89		
Intercept	871.10			815.58		
Correlation Coefficient	**0.9998**			**0.9994**		

Conc.* = Concentration, RT*= Retention Time

Table 3D: Linearity Data for Oxazolidine protected Cabazitaxel impurity.

% Concentration	Oxazolidine protected Cabazitaxel impurity		
	RT*	Conc.* (µg /mL)	Response (Area)
LOQ Level	23.642	0.146	586
Linearity-Level-50%	23.607	3.282	23273
Linearity-Level-80%	23.614	5.106	37220
Linearity-Level-90%	23.655	5.835	41876
Linearity-Level-100%	23.613	6.382	45629
Linearity-Level-110%	23.616	7.112	51300
Linearity-Level-120%	23.607	7.659	57578

Linearity-Level-150%	23.610	9.574	67944
Linearity-Level-250%	23.635	15.956	114148
Slope	7177.05		
Intercept	153.41		
Correlation Coefficient	**0.9995**		

Conc.* = Concentration, RT*= Retention Time

4. ACCURACY

Experiment: Sample of Cabazitaxel Injection were spiked with known impurities, namely 10-Dab-III impurity, Ditroc impurity, Ditroc oxazolidine impurity, Detroc oxazolidine impurity, Amine impurity and Oxazolidine protected Cabazitaxel impurity at different levels between LOQ and 200% of the specification limit, in triplicate, and then sample preparation were carried out as described under methodology given in section IV.

Table 4A: Accuracy Data for 10-Dab-III impurity.

Sample	Retention Time	Response (Area)	Amount Added	Amount Recovered	% Recovery
Accuracy - LOQ-Set 1	2.595	1093	0.00154	0.00175	**113.6**
Accuracy - LOQ-Set 2	2.598	1081	0.00154	0.00173	**112.3**
Accuracy - LOQ-Set 3	2.598	1097	0.00154	0.00175	**113.6**
Mean	**113.2**				
SD	**0.751**				
%RSD	**0.663**				

Table 4B: Accuracy Data for 10-Dab-III impurity.

Sample	Retention Time	Response (Area)	Amount Added.	Amount Recovered	%Recovery
Accuracy-50 % Set-1	2.555	37527	0.0597	0.0594	**99.5**
Accuracy-50 % Set-2	2.558	38272	0.0597	0.0606	**101.5**
Accuracy-50 % Set-3	2.558	38049	0.0597	0.0602	**100.8**
Accuracy-100 % Set-1	2.556	79637	0.1195	0.1260	**105.4**
Accuracy-100 % Set-2	2.556	77347	0.1195	0.1224	**102.4**
Accuracy-100 % Set-3	2.556	78219	0.1195	0.1238	**103.6**
Accuracy-150 % Set-1	2.554	117150	0.1792	0.1854	**103.5**
Accuracy-150 % Set-2	2.552	116659	0.1792	0.1846	**103.0**
Accuracy-150 % Set-3	2.551	117925	0.1792	0.1866	**104.1**
Accuracy-200 % Set-1	2.551	149990	0.2390	0.2373	**99.3**
Accuracy-200 % Set-2	2.551	145604	0.2390	0.2304	**96.4**
Accuracy-200 % Set-3	2.550	145298	0.2390	0.2299	**96.2**
Mean	**101.3**				
SD	**2.961**				
%RSD	**2.923**				

Table 5A: Accuracy Data for Ditroc impurity.

Sample	Retention Time	Response (Area)	Amount Added.	Amount Recovered	% Recovery
Accuracy - LOQ-Set 1	24.417	1178	0.00271	0.00262	**96.7**
Accuracy - LOQ-Set 2	24.417	1158	0.00271	0.00258	**95.2**
Accuracy - LOQ-Set 3	24.400	1112	0.00271	0.00248	**91.5**
Mean	**94.5**				
SD	2.676				
%RSD	2.832				

Table 5B: Accuracy Data for Ditroc impurity.

Sample	Retention Time	Response (Area)	Amount Added.	Amount Recovered	% Recovery
Accuracy-50 % Set-1	24.042	24670	0.0556	0.05440	**97.8**
Accuracy-50 % Set-2	24.056	24007	0.0556	0.05300	**95.3**
Accuracy-50 % Set-3	24.059	23246	0.0556	0.05130	**92.3**
Accuracy-100 % Set-1	24.048	46116	0.1112	0.10170	**91.5**
Accuracy-100 % Set-2	24.048	45802	0.1112	0.10100	**90.8**
Accuracy-100 % Set-3	24.038	45911	0.1112	0.10130	**91.1**
Accuracy-150 % Set-1	24.028	71071	0.1668	0.15680	**94.0**
Accuracy-150 % Set-2	24.019	71779	0.1668	0.15830	**94.9**
Accuracy-150 % Set-3	24.003	71585	0.1668	0.15790	**94.7**
Accuracy-200 % Set-1	23.999	101189	0.2224	0.22320	**100.4**
Accuracy-200 % Set-2	23.981	99087	0.2224	0.21850	**98.2**
Accuracy-200 % Set-3	23.982	100542	0.2224	0.22180	**99.7**
Mean	**95.1**				
SD	**3.342**				
%RSD	**3.514**				

Table 6A: Accuracy Data for Ditroc oxazolidine impurity.

Sample	Retention Time	Response (Area)	Amount Added.	Amount Recovered	% Recovery
Accuracy - LOQ-Set 1	60.127	975	0.002580	**0.00276**	**107.0**
Accuracy - LOQ-Set 2	60.141	893	0.002580	**0.00253**	**98.1**
Accuracy - LOQ-Set 3	60.128	957	0.002580	**0.00271**	**105.0**
Mean	**103.4**				
SD	**4.669**				
%RSD	**4.515**				

Table 6B: Accuracy Data for Ditroc oxazolidine impurity.

Sample	Retention Time	Response (Area)	Amount Added.	Amount Recovered	%Recovery
Accuracy-50 % Set-1	59.259	20607	0.0548	0.05780	**105.5**
Accuracy-50 % Set-2	59.261	19378	0.0548	0.05440	**99.3**
Accuracy-50 % Set-3	59.251	18852	0.0548	0.05290	**96.5**
Accuracy-100 % Set-1	59.227	39493	0.1097	0.11080	**101.0**
Accuracy-100 % Set-2	59.225	39243	0.1097	0.11010	**100.4**
Accuracy-100 % Set-3	59.211	39896	0.1097	0.11190	**102.0**
Accuracy-150 % Set-1	59.190	59249	0.1645	0.16620	**101.0**
Accuracy-150 % Set-2	59.174	58583	0.1645	0.16430	**99.9**
Accuracy-150 % Set-3	59.147	57615	0.1645	0.16160	**98.2**
Accuracy-200 % Set-1	59.140	72194	0.2193	0.20250	**92.3**
Accuracy-200 % Set-2	59.109	71446	0.2193	0.20040	**91.4**
Accuracy-200 % Set-3	59.105	70779	0.2193	0.19850	**90.5**
Mean	**98.2**				
SD	**4.628**				
%RSD	**4.713**				

Table 7A: Accuracy Data for Detroc oxazolidine impurity.

Sample	Retention Time	Response (Area)	Amount Added.	Amount Recovered	% Recovery
Accuracy - LOQ-Set 1	16.450	746	0.00170	0.00193	**113.5**
Accuracy - LOQ-Set 2	16.450	704	0.00170	0.00182	**107.1**
Accuracy - LOQ-Set 3	16.450	728	0.00170	0.00189	**111.2**
Mean	**110.6**				
SD	**3.242**				
%RSD	**2.931**				

Table 7B: Accuracy Data for Detroc oxazolidine impurity.

Sample	Retention Time	Response (Area)	Amount Added.	Amount Recovered	% Recovery
Accuracy-50 % Set-1	16.220	22101	0.0581	0.0567	**97.6**
Accuracy-50 % Set-2	16.232	21835	0.0581	0.0560	**96.4**
Accuracy-50 % Set-3	16.231	22286	0.0581	0.0572	**98.5**
Accuracy-100 % Set-1	16.223	45311	0.1161	0.1162	**100.1**
Accuracy-100 % Set-2	16.228	45900	0.1161	0.1177	**101.4**
Accuracy-100 % Set-3	16.220	46710	0.1161	0.1198	**103.2**
Accuracy-150 % Set-1	16.211	68928	0.1742	0.1768	**101.5**
Accuracy-150 % Set-2	16.205	68882	0.1742	0.1767	**101.4**
Accuracy-150 % Set-3	16.194	69658	0.1742	0.1787	**102.6**
Accuracy-200 % Set-1	16.190	90786	0.2323	0.2329	**100.3**
Accuracy-200 % Set-2	16.171	86106	0.2323	0.2209	**95.1**
Accuracy-200 % Set-3	16.173	89995	0.2323	0.2309	**99.4**
Mean	**99.8**				
SD	**2.488**				
%RSD	**2.493**				

Table 8A: Accuracy Data for Amine impurity.

Sample	Retention Time	Response (Area)	Amount Added.	Amount Recovered	% Recovery
Accuracy - LOQ-Set 1	3.630	0.00250	0.00250	0.00261	**104.4**
Accuracy - LOQ-Set 2	3.637	0.00250	0.00250	0.00259	**103.6**
Accuracy - LOQ-Set 3	3.637	0.00250	0.00250	0.00248	**99.2**
Mean	**102.4**				
SD	**2.800**				
%RSD	**2.734**				

Table 8B: Accuracy Data for Amine impurity.

Sample	Retention Time	Response (Area)	Amount Added.	Amount Recovered	% Recovery
Accuracy-50 % Set-1	3.528	29379	0.0564	0.0563	**99.8**
Accuracy-50 % Set-2	3.533	29999	0.0564	0.0580	**102.8**
Accuracy-50 % Set-3	3.535	29654	0.0564	0.0571	**101.2**
Accuracy-100 % Set-1	3.533	49587	0.1127	0.1096	**97.2**
Accuracy-100 % Set-2	3.535	49588	0.1127	0.1096	**97.2**
Accuracy-100 % Set-3	3.534	49661	0.1127	0.1098	**97.4**
Accuracy-150 % Set-1	3.529	71434	0.1754	0.1672	**95.3**
Accuracy-150 % Set-2	3.527	71933	0.1754	0.1686	**96.1**
Accuracy-150 % Set-3	3.521	71799	0.1754	0.1682	**95.9**
Accuracy-200 % Set-1	3.521	86236	0.2255	0.2063	**91.5**
Accuracy-200 % Set-2	3.515	85626	0.2255	0.2047	**90.8**
Accuracy-200 % Set-3	3.514	85723	0.2255	0.2049	**90.9**
Mean	**96.3**				
SD	**3.873**				
%RSD	**4.022**				

Table 9A: Accuracy Data for Oxazolidine protected Cabazitaxel impurity.

Sample	Retention Time	Response (Area)	Amount Added.	Amount Recovered	% Recovery
Accuracy - LOQ-Set 1	23.117	529	0.00151	0.00152	**100.7**
Accuracy - LOQ-Set 2	23.165	551	0.00151	0.00159	**105.3**
Accuracy - LOQ-Set 3	23.149	576	0.00151	0.00166	**109.9**
Mean	**105.3**				
SD	**4.600**				
%RSD	**4.368**				

Table 9B: Accuracy Data for Oxazolidine protected Cabazitaxel impurity.

Sample	Retention Time	Response (Area)	Amount Added.	Amount Recovered	% Recovery
Accuracy-50 % Set-1	22.861	0.0726	0.0726	0.07220	**99.4**
Accuracy-50 % Set-2	22.869	0.0726	0.0726	0.07330	**101.0**
Accuracy-50 % Set-3	22.865	0.0726	0.0726	0.07380	**101.7**
Accuracy-100 % Set-1	22.857	0.1271	0.1271	0.12880	**101.3**
Accuracy-100 % Set-2	22.855	0.1271	0.1271	0.12430	**97.8**
Accuracy-100 % Set-3	22.843	0.1271	0.1271	0.13100	**103.1**
Accuracy-150 % Set-1	22.829	0.1997	0.1997	0.20460	**102.5**
Accuracy-150 % Set-2	22.817	0.1997	0.1997	0.19720	**98.7**
Accuracy-150 % Set-3	22.800	0.1997	0.1997	0.21090	**105.6**
Accuracy-200 % Set-1	22.796	0.2542	0.2542	0.26550	**104.4**
Accuracy-200 % Set-2	22.776	0.2542	0.2542	0.26310	**103.5**
Accuracy-200 % Set-3	22.774	0.2542	0.2542	0.26170	**103.0**
Mean	**101.8**				
SD	**2.335**				
%RSD	**2.294**				

5. PRECISION

5.1 System Precision

Experiment: Six replicate injections of the standard preparation were made into the HPLC and used the methodology given in section IV.

5.2 Method Precision

Experiment: Six sample preparations of Cabazitaxel Injection were prepared and injected into the HPLC using the method as described under methodology given in section-IV. Samples were spiked with known impurities at specification limits as the impurities levels were inadequate in the sample. The data generated is given following Tables.

Table 10: Table for System Precision.

Injection	Retention Time	Response (Area)
1	14.288	17746599
2	14.293	17894685
3	14.298	17833618
4	14.288	17896965
5	14.310	17898559
6	14.314	17842696
Mean		**17852187**
SD		**59237.191**
%RSD		**0.332**

Table 11A: Method Precision- for Day-1.

Sr. No.	10-Dab-III impurity		Ditroc impurity		Ditroc oxazolidine impurity		Detroc oxazolidine impurity	
	RT	% Impurity	RT	% Impurity	RT	% Impurity	RT	% Impurity
1	2.656	0.309	24.736	0.289	60.610	0.282	16.652	0.323
2	2.656	0.315	24.734	0.283	60.589	0.281	16.652	0.327
3	2.654	0.309	24.722	0.289	60.554	0.271	16.642	0.298
4	2.651	0.328	24.713	0.282	60.484	0.279	16.619	0.297
5	2.654	0.315	24.710	0.279	60.476	0.281	16.632	0.303
6	2.650	0.312	24.687	0.271	60.438	0.290	16.617	0.298
Mean		**0.315**		**0.282**		**0.281**		**0.308**
SD		**0.007**		**0.007**		**0.006**		**0.014**
% RSD		**2.222**		**2.482**		**2.135**		**4.545**

RT = Retention Time,

Table 11B: Method Precision- Retention time and RRT for Day-1.

Sr. No.	Amine impurity		Oxazolidine protected Cabazitaxel impurity		Highest individual unspecified impurity		Total impurities
	RT	% Impurity	RT	% Impurity	RT	% Impurity	% Impurity
1	3.738	0.288	23..564	0.350	13.416	0.105	2.077
2	3.740	0.303	23.555	0.367	13.416	0.110	2.125
3	3.737	0.295	23.539	0.352	13.404	0.107	2.071
4	3.717	0.305	23.490	0.370	13.386	0.108	2.109
5	3.731	0.292	23.501	0.355	13.996	0.105	2.081
6	3.724	0.301	23.479	0.345	13.380	0.103	2.072
Mean		**0.297**		**0.357**		**0.106**	**2.089**
SD		**0.007**		**0.010**		**0.003**	**0.022**
% RSD		**2.357**		**2.801**		**2.830**	**1.053**

RT = Retention Time,

Table 12A: Method Precision- for Day-2.

Sr. No.	10-Dab-III impurity		Ditroc impurity		Ditroc oxazolidine impurity		Detroc oxazolidine impurity	
	RT	% Impurity	RT	% Impurity	RT	% Impurity	RT	% Impurity
1	2.641	0.312	24.648	0.260	60.381	0.276	16.581	0.297
2	2.645	0.314	24.647	0.260	60.365	0.281	16.584	0.298
3	2.460	0.316	24.627	0.264	60.332	0.283	16.570	0.303
4	2.641	0.315	24.620	0.265	60.310	0.291	16.566	0.305
5	2.639	0.312	24.618	0.262	60.293	0.283	16.564	0.303
6	2.636	0.312	24.606	0.269	60.260	0.279	16.555	0.296
Mean		**0.314**		**0.263**		**0.282**		**0.300**
SD		**0.002**		**0.003**		**0.005**		**0.004**
% RSD		**0.637**		**1.141**		**1.773**		**1.333**

RT = Retention Time,

Table 12B: Method Precision- for Day-2.

Sr. No.	Amine impurity		Oxazolidine protected Cabazitaxel impurity		Highest individual unspecified impurity		Total impurities
	RT	% Impurity	RT	% Impurity	RT	% Impurity	RT % Impurity
1	3.709	0.303	23.435	0.349	13.347	0.122	2.050
2	3.714	0.304	23.432	0.348	13.348	0.120	2.054
3	3.710	0.309	23.407	0.332	13.334	0.124	2.058
4	3.711	0.305	23.398	0.356	13.330	0.125	2.092
5	3.707	0.299	23.389	0.359	13.326	0.124	2.103
6	3.701	0.302	23.375	0.337	13.316	0.121	2.042
Mean		**0.304**		**0.347**		**0.123**	**2.067**
SD		**0.003**		**0.011**		**0.002**	**0.025**
% RSD		**0.987**		**3.170**		**1.626**	**1.209**

RT = Retention Time,

6. Ruggedness

Experiment: Six sample preparations of Cabazitaxel Injection were analyzed by different analyst, using different column, on different day and using different HPLC using the method as described under methodology given in section IV, along with standard preparation. Samples were spiked with known impurities at specification limits as the impurities level was inadequate in the sample.

Table 13A: Ruggedness Data for Related Substance for Cabazitaxel.

Sr. No.	10-Dab-III impurity		Ditroc impurity		Ditroc oxazolidine impurity		Detroc oxazolidine impurity	
	Analyst -1	Analyst-2	Analyst -1	Analyst -2	Analyst-1	Analyst-2	Analyst -1	Analyst-2
1	0.309	0.309	0.289	0.289	0.282	0.282	0.323	0.323
2	0.315	0.312	0.283	0.281	0.281	0.278	0.327	0.324
3	0.309	0.309	0.289	0.289	0.271	0.271	0.298	0.298
4	0.328	0.325	0.282	0.279	0.279	0.277	0.297	0.294
5	0.315	0.312	0.279	0.276	0.281	0.278	0.303	0.300
6	0.312	0.312	0.271	0.271	0.290	0.290	0.298	0.297
Mean	**0.315**	**0.313**	**0.282**	**0.281**	**0.281**	**0.279**	**0.308**	**0.306**
SD	**0.007**	**0.006**	**0.007**	**0.007**	**0.006**	**0.006**	**0.014**	**0.014**
% RSD	**2.222**	**1.917**	**2.482**	**2.491**	**2.135**	**2.151**	**4.545**	**4.575**
Overall Mean	**0.314**		**0.282**		**0.280**		**0.307**	
Overall SD	**0.006**		**0.007**		**0.006**		**0.013**	
Overall % RSD	**1.911**		**2.482**		**2.143**		**4.235**	

Table 13B: Ruggedness Data for Related Substance for Cabazitaxel.

Sr. No.	Amine impurity		Oxazolidine protected Cabazitaxel impurity		Highest individual unspecified impurity		Total impurities	
	Analyst-1	Analyst-2	Analyst-1	Analyst-2	Analyst-1	Analyst-2	Analyst-1	Analyst-2
1	0.288	0.288	0.350	0.350	0.105	0.105	2.077	2.076
2	0.303	0.300	0.367	0.363	0.110	0.109	2.125	2.104
3	0.295	0.295	0.352	0.352	0.107	0.107	2.071	2.071
4	0.305	0.302	0.370	0.366	0.108	0.107	2.109	2.088
5	0.292	0.289	0.355	0.351	0.105	0.104	2.081	2.059
6	0.301	0.301	0.345	0.344	0.103	0.103	2.072	2.070
Mean	**0.297**	**0.296**	**0.357**	**0.354**	**0.106**	**0.106**	**2.089**	**2.078**
SD	**0.007**	**0.006**	**0.010**	**0.008**	**0.003**	**0.002**	**0.022**	**0.016**
% RSD	**2.357**	**2.027**	**2.801**	**2.260**	**2.830**	**1.887**	**1.053**	**0.770**
Overall Mean	**0.297**		**0.355**		**0.106**		**2.084**	
Overall SD	**0.006**		**0.009**		**0.002**		**0.019**	
Overall % RSD	**2.020**		**2.535**		**1.887**		**0.912**	

7. Robustness

Experiment: Diluent, standard preparation, placebo preparation and sample preparation in triplicate of the same lot (as used in 4.2) of Cabazitaxel Injection 10mg were prepared as described under methodology given in section-IV. The samples along with standard and placebo were injected under different chromatographic conditions as shown below.

7.1 Change in column oven temperature (± 5°C)

7.2 Change in flow rate (±0.2mL)

7.3 Change in Wavelength (± 2nm)

7.4 Change in Buffer pH of Mobile phase (±0.2 units)

Table 14: Table for Robustness for Cabazitaxel.

Robustness Parameter	Placebo/ Diluent interference	Retention Time Standard	R#	Retention Time of Known Impurities from Spike sample					
				*A	*B	*C	*D	*E	*F
Control Conditions	No	14.123	3.8	2.597	24.359	59.861	16.395	3.632	23.147
Low Temperature (- 5°C), 25°C	No	14.032	3.1	2.584	24.517	60.278	16.381	3.591	23.211
High Temperature (+5°C), 35°C	No	13.903	2.6	2.597	23.807	58.551	16.270	3.622	22.719
Low Flow 1.1 ml/min	No	15.098	2.7	3.027	26.082	62.115	17.527	4.143	24.778
High Flow 1.5 ml/min	No	13.132	3.0	2.273	22.982	57.826	15.447	3.233	21.763
High pH (2.2)	No	13.754	3.1	2.516	24.064	59.545	16.168	3.418	22.895
Low pH (1.8)	No	13.905	3.5	2.543	24.230	59.651	16.231	3.431	24.230
High Wavelength (232nm)	No	13.989	2.7	2.590	24.283	59.629	16.350	3.616	24.283
Low Wavelength (228nm)	No	14.006	2.8	2.592	24.315	59.669	16.360	3.619	23.041

R#= Resolution between Ditroc impurity and oxazolidone protected Cabazitaxel impurity should be not less than 1.2.;

***A=** 10-Dab-III impurity**, *B=**Ditroc impurity**, *C=** Ditroc oxazolidine impurity**, *D=** Detroc oxazolidine impurity**, *E=** Amine impurity**, *F=** Oxazolidine protected Cabazitaxel impurity**.**

8. Solution Stability in Analytical Solution

Experiment: Standard solution, Sample solution as per methodology (Control and Spike sample) were analysed initially and at different time intervals at room temperature.

Table 15: Table for Solution Stability of Standard Solution.

Sr. No.	Time Interval, Hours	Area of Cabazitaxel
1	0	17428384
2	13 Hours	17397993
3	50 Hours	17743100
Mean		**17523159**
SD		**191079.658**
%RSD		**1.090**

Table 16A: Table for Solution Stability in Control Sample Solution

Sr. No.	Time Interval, Hours	10-Dab-III impurity	Ditroc impurity	Ditroc oxazolidine impurity	Detroc oxazolidine impurity
1	0	Not detected	Not detected	Not detected	Not detected
2	13 Hours	Not detected	Not detected	Not detected	Not detected
3	50 Hours	Not detected	Not detected	Not detected	Not detected
Mean		Not applicable	Not applicable	Not applicable	Not applicable
SD		Not applicable	Not applicable	Not applicable	Not applicable
% RSD		Not applicable	Not applicable	Not applicable	Not applicable

Table 16B: Table for Solution Stability in Control Sample Solution.

Sr. No.	Time Interval in Hours	Amine impurity	Oxazolidine protected Cabazitaxel impurity	Highest individual unspecified impurity	Total impurities
1	0	0.051	Not detected	0.126	0.271
2	13 Hours	0.049	Not detected	0.124	0.275
3	50 Hours	0.056	Not detected	0.131	0.288
Mean		**0.052**	Not applicable	**0.127**	**0.278**
SD		**0.004**	Not applicable	**0.004**	**0.009**
% RSD		**7.692**	Not applicable	**3.150**	**3.237**

Table 17A: Table for Solution Stability in Spike Sample Solution.

Sr. No.	Time Interval, Hours	10-Dab-III impurity	Ditroc impurity	Ditroc oxazolidine impurity	Detroc oxazolidine impurity
1	0	0.309	0.289	0.282	0.323
2	13 Hours	0.316	0.285	0.283	0.300
3	50 Hours	0.329	0.277	0.284	0.316
Mean		**0.318**	**0.284**	**0.283**	**0.313**
SD		**0.010**	**0.006**	**0.001**	**0.012**
% RSD		**3.145**	**2.113**	**0.353**	**3.834**

Table 17B: Table for Solution Stability in Spike Sample Solution.

Sr. No.	Time Interval, Hours	Amine impurity	Oxazolidine protected Cabazitaxel impurity	Highest individual unspecified impurity	Total impurities
1	0	0.288	0.350	0.125	2.077
2	13 Hours	0.308	0.354	0.123	2.078
3	50 Hours	0.318	0.381	0.128	2.148
Mean		**0.305**	**0.362**	**0.125**	**2.101**
SD		**0.015**	**0.017**	**0.003**	**0.041**
% RSD		**4.918**	**4.696**	**2.400**	**1.951**

RESULT AND DISCUSSION

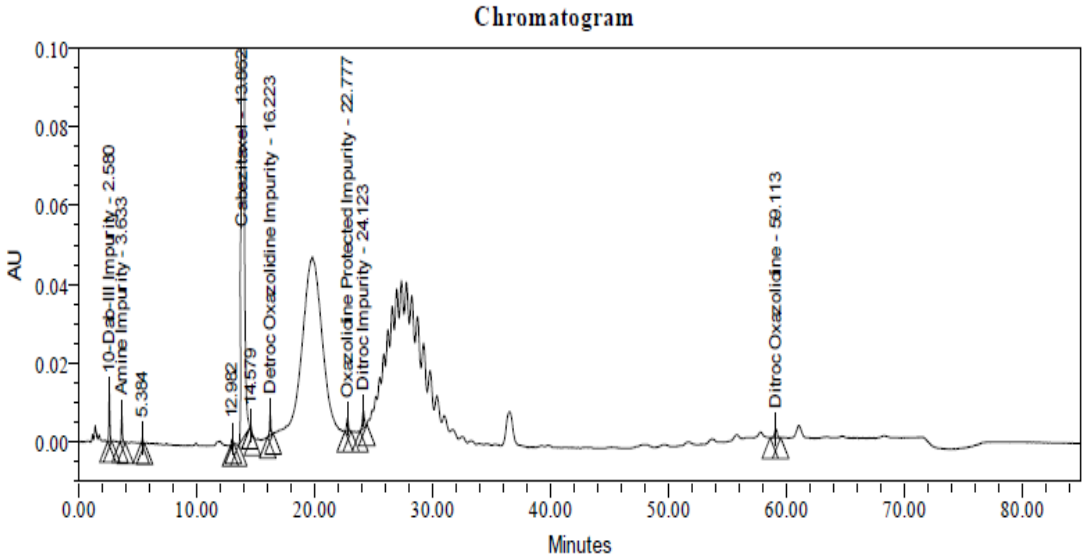
Retention time of Cabazitaxel peak and Known impurities in sample preparation is comparable with standard preparation. Peak purity passes for Cabazitaxel peak and known impurities in standard and sample preparations, No interference was observed at the retention time of Cabazitaxel and known impurities peak. Cabazitaxel peak homogeneous each degradation condition and Peak purity passes for all degradation conditions. So method was found specific, stability indicating with mass balance.

The correlation coefficients are within limits (Not less than 0.99) for 6 known impurities and Cabazitaxel. Mean recovery for Known for 6 known impurities found within limit from LOQ to 200%. RSD is 0.332% for Cabazitaxel (Limit is, RSD should not be more than 2.0% for method precision). RSD is within limit (RSD should not be more than 10.0%) for known impurities, Higher individual unspecified impurity, and total impurities for Day-1 and Day-2. The RSD of twelve results obtained from two different analysts of Cabazitaxel Injection are within limit (RSD should not be more than 10.0%).

The test method is robust for all variable conditions. No interference observed due to diluent/blank and the test method is robust for all variable conditions. No interference observed due to Diluent / blank and placebo.

Standard and sample solutions are found to be stable up to 50 hours.

SampleName: Spike sample



Peak Results

	Name	RT	RT Ratio	Area	% Area	USP Resolution	USP Plate Count	USP Tailing
1	10-Dab-III Impurity	2.580	0.186	71277	0.47		4682	1.15
2	Amine Impurity	3.633	0.262	45189	0.29	6.36	7004	1.40
3		5.384		9172	0.06	9.42	13402	1.43
4		12.982		8496	0.06	41.03	84558	0.86
5	Cabazitaxel	13.862		15021640	98.00	4.47	67559	1.17
6		14.579		7937	0.05	3.64	107445	1.54
7	Detroc Oxazolidine Impurity	16.223	1.170	42016	0.27	8.36	95099	0.94
8	Oxazolidine Protected Impurity	22.777	1.643	34341	0.22	26.74	113616	1.30
9	Ditroc Impurity	24.123	1.740	52346	0.34	4.53	94983	0.87
10	Ditroc Oxazolidine	59.113	4.264	35483	0.23	96.68	321429	0.89

Peak Purity Table

	Name	RT	Purity1 Angle	Purity1 Threshold	Purity_Criteria
1	10-Dab-III Impurity	2.580	2.348	2.801	Pass

Peak Purity Table

	Name	RT	Purity1 Angle	Purity1 Threshold	Purity_Criteria
2	Amine Impurity	3.633	3.473	4.707	Pass
3	Cabazitaxel	13.862	0.601	1.018	Pass
4	Detroc Oxazolidine Impurity	16.223	3.194	5.155	Pass
5	Oxazolidine Protected Impurity	22.777	5.428	7.130	Pass
6	Ditroc Impurity	24.123	4.673	5.790	Pass
7	Ditroc Oxazolidine	59.113	8.269	11.323	Pass

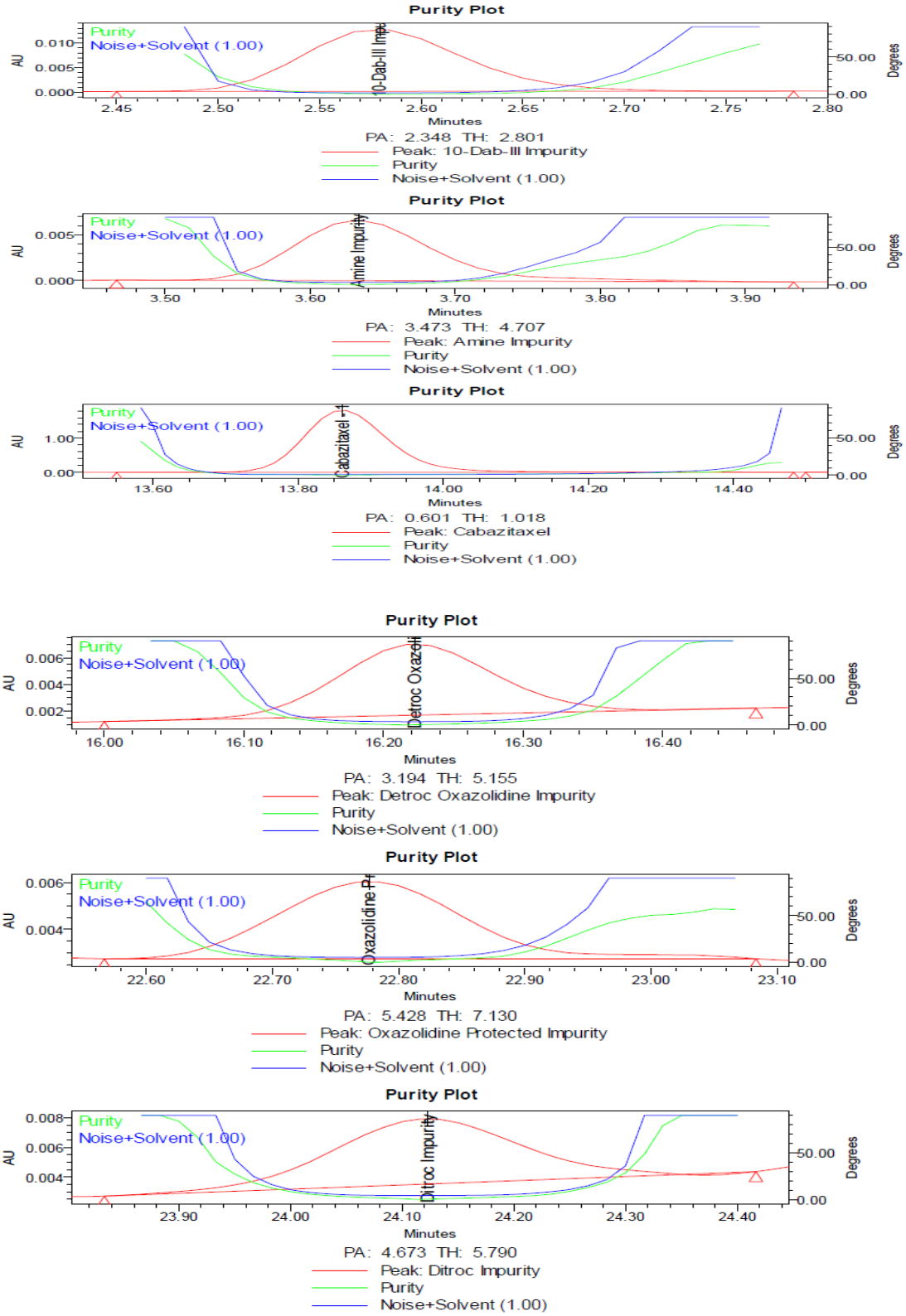
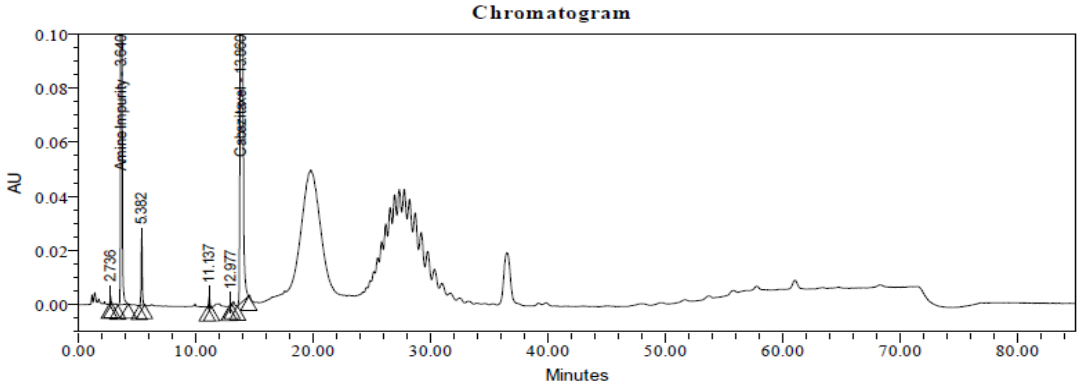


Figure 1: Spike sample (all known impurities in control sample) with peak purity data.

SampleName: Acid degradation sample



Peak Results

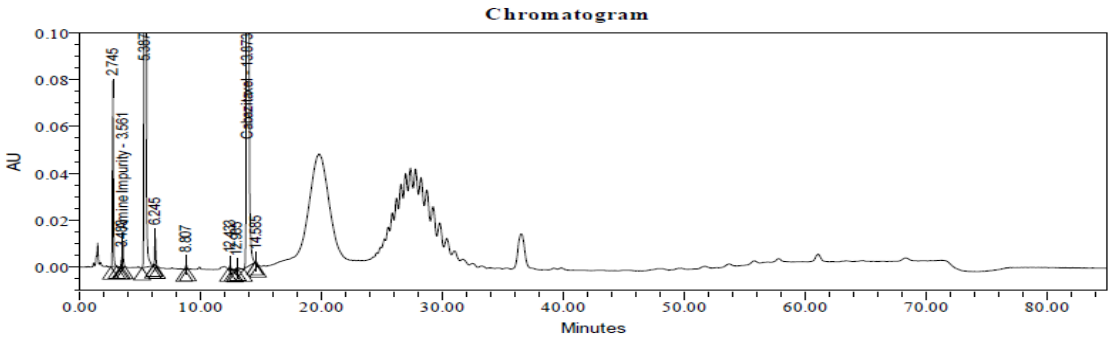
	Name	RT	RT Ratio	Area	% Area	USP Resolution	USP Plate Count	USP Tailing
1		2.736		15580	0.10		5171	0.93
2	Amine Impurity	3.640	0.263	2919648	18.52	5.63	7460	1.18
3		5.382		159743	1.01	10.14	16146	1.08
4		11.137		26401	0.17	31.13	52926	1.07
5		12.977		6153	0.04	10.10	98546	0.82
6	Cabazitaxel	13.860		12633839	80.16	4.60	69739	1.16

Peak Purity Table

	Name	RT	Purity1 Angle	Purity1 Threshold	Purity_Criteria
1	Amine Impurity	3.640	0.350	1.066	Pass
2	Cabazitaxel	13.860	0.316	1.018	Pass

Figure 2: Acid degradation sample.

SampleName: Base degradation sample



Peak Results

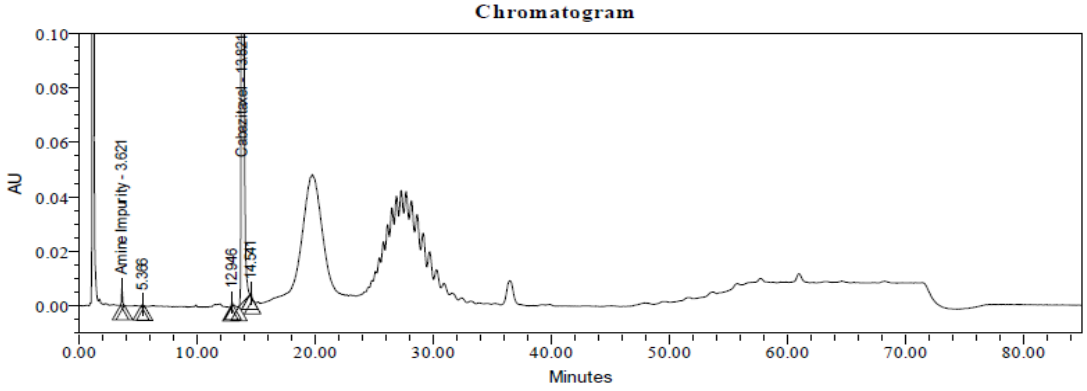
	Name	RT	RT Ratio	Area	% Area	USP Resolution	USP Plate Count	USP Tailing
1		2.745		436481	2.78		6092	1.18
2		3.483		12871	0.08			
3	Amine Impurity	3.561	0.257	66858	0.43		7247	
4		5.387		5642165	36.00	10.25	15709	1.15
5		6.245		71059	0.45	4.99	22477	1.13
6		8.807		12477	0.08	13.79	31381	0.99
7		12.433		12084	0.08	17.87	60207	1.06
8		12.985		2055	0.01	3.27	136842	0.74
9	Cabazitaxel	13.873		9411877	60.05	5.07	71635	1.16
10		14.585		4933	0.03	3.62	102710	1.53

Peak Purity Table

	Name	RT	Purity1 Angle	Purity1 Threshold	Purity_Criteria
1	Amine Impurity	3.561	0.805	1.149	Pass

Figure 3: Base degradation sample.

SampleName: Peroxide degradation sample



Peak Results

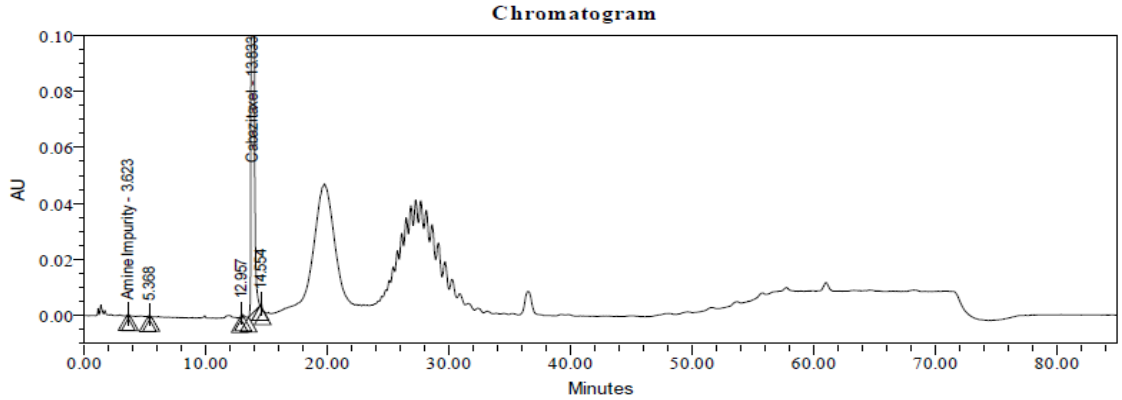
	Name	RT	RT Ratio	Area	% Area	USP Resolution	USP Plate Count	USP Tailing
1	Amine Impurity	3.621	0.262	37609	0.25		7888	1.16
2		5.366		4099	0.03	10.24	15881	1.10
3		12.946		8434	0.06	44.25	92135	0.89
4	Cabazitaxel	13.821		15202137	99.62	4.51	67059	1.19
5		14.541		8012	0.05	3.65	110086	1.45

Peak Purity Table

	Name	RT	Purity1 Angle	Purity1 Threshold	Purity_Criteria
1	Amine Impurity	3.621	3.679	4.774	Pass
2	Cabazitaxel	13.821	0.583	1.019	Pass

Figure 4: Peroxide degradation sample.

SampleName: Humidity degradation sample



Peak Results

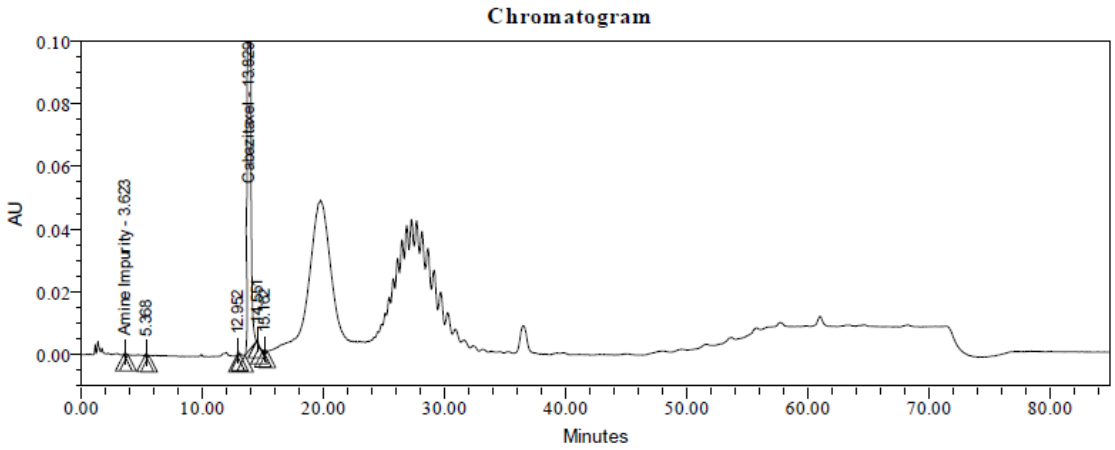
	Name	RT	RT Ratio	Area	% Area	USP Resolution	USP Plate Count	USP Tailing
1	Amine Impurity	3.623	0.262	3758	0.03		6352	1.32
2		5.368		4152	0.03	10.20	18103	0.94
3		12.957		7886	0.05	44.55	82618	0.87
4	Cabazitaxel	13.833		14871271	99.84	4.41	66660	1.18
5		14.554		7523	0.05	3.72	110388	1.39

Peak Purity Table

	Name	RT	Purity1 Angle	Purity1 Threshold	Purity_Criteria
1	Amine Impurity	3.623	23.880	33.977	Pass
2	Cabazitaxel	13.833	0.532	1.018	Pass

Figure 5: Humidity degradation sample.

SampleName: Thermal degradation sample



Peak Results

	Name	RT	RT Ratio	Area	% Area	USP Resolution	USP Plate Count	USP Tailing
1	Amine Impurity	3.623	0.262	5080	0.03		5985	1.11
2		5.368		4314	0.03	9.43	14895	1.57
3		12.952		8419	0.05	42.10	82206	1.01
4	Cabazitaxel	13.829		15299843	99.81	4.44	67043	1.18
5		14.551		7094	0.05	3.73	120157	1.57
6		15.162		4244	0.03	3.18	81026	1.02

Peak Purity Table

	Name	RT	Purity1 Angle	Purity1 Threshold	Purity_Criteria
1	Amine Impurity	3.623	24.852	31.701	Pass
2	Cabazitaxel	13.829	0.534	1.019	Pass

Figure 6: Thermal degradation sample.

CONCLUSION

The test method was validated for specificity, linearity and range, accuracy, precision, ruggedness, stability of analytical solution, system suitability and robustness, was found to meeting the predetermined acceptance criteria. The validated method is specific, linear, accurate precise, rugged and robust for determination of related substances as well as assay for Cabazitaxel in Cabazitaxel Injection. Hence this method was found stability indicating and can be introduced into routine use and testing of stability samples for the related substances as well as assay of Cabazitaxel in Cabazitaxel Injection.

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Note: Spike sample (all known impurities in control sample) with peak purity data.

Specificity study chromatograms (A) Acid degradation; (B) Base degradation; (C) Peroxide degradation (D) Hydrolysis degradation; (E) Thermal degradation (F) Photo degradation.

REFERENCES

1. M. Mathrusri Annapurna, K. Pramadvara, B. Venkatesh and G.Sowjanya, Stability indicating RP-HPLC method for thedetermination of cabazitaxel, *Indo American Journal of Pharmaceutical research*, 2013; 3: 9262-9269.
2. Peter de Bruijn, Anne-Joy M. de Graan, Annemieke Nieuweboer.Ron H.J Mathijssen, Mei- Ho-Lam, et al, Quantificationof cabazitaxel in human plasma by liquid chromatography/triplequadrupole mass spectrometry. A practical solution for non-specific binding. *Journal of pharmaceutical and biomedical analysis*, 2012; 59: 117-122.
3. Paller, C.J; Antonarakis, E.S. Cabazitaxel; a novel second-line treatment for metastatic castration resistant prostate cancer. *Drug Des. Devel. Ther*, 2011; 5: 117-124.
4. Wilkes, G.M. Cabazitaxel, a taxane for men with hormonerefractory metastatic prostate cancer. *Oncology*, 2010; 24: 46-48.
5. V. Jagannath Patro, Nageshwara Rao. R and N.K.Tripathy, LCMS/MS Determination of Cabazitaxel in Rat whole blood on Dry blood spots, *Scientific Reports*, 2012; 1: 1-4.
6. Gudisa Kishore, New spectrophotometric methods for the quantitative estimation of Cabazitaxel in formulations, *International journal of Research and Reviews in pharmacy and Applied science*, 2012; 2: 950-958.
7. A. Kort, M.J. Hillebrand, G.A Cirkel, et al., Quantification of cabazitaxel, its metabolite Docetaxel and the determination of the demethylated metabolites RPR112698 and RPR123142 an docetaxel equivalents in human plasma by liquid chromatography tandem mass spectrometry, *J. Chromatogr. B Analyt Technol Biomed. Life Sci.*, 2013; 15: 117-123.
8. Agarwal N, Sonpavde G, Sartor O, Cabazitaxel for the treatment of castration-resistant prostate cancer. *Future Oncol*, 2011; 7: 15-24.
9. ICH Q1A (R2) Stability Testing of New Drug Substances and Products, USFDA-Federal Register, USA < http://www.ich.org, 2003.
10. ICH Q2(R1), Validation of Analytical Procedures; Text and Methodology, USFDA-Federal Register, USA < http://www.ich.org, 2005.
11. Swartz, M.E. UPLC, An Introduction and Review. *J.Liq. Chromatogr. R.T*, 2005; 28: 1253-1263.
12. Wren, S.A.C; Tchelitcheff, P, Use of ultra-performance liquid chromatography in

pharmaceutical development. *J.Chromatogr. A*, 2006; 1119: 140-146.

13. The United States Pharmacopeia, Thirty-fifth Edit, Validation of Compendial Methods, Section <1225>, Rockville, 2012.
14. International Conference on Harmonization, ICH Topic Q2, Validation of Analytical Procedures; Text and Methodology. Available from: < http://www.ich.org, (R1), 2005.
15. P.L. Garcia, E.Buffoni, F.P. Gomes, J.L.V. Quero, Wide spectra of quality control, in: I.Akyar (Ed.), Tech, Rijeka, 2011; 3-20.