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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF PHOSPHATE AND DEXTROSE CONTENT BY RP-HPLC IN CABAZITAXEL INJECTION.

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Abstract:

A simple, accurate, precise, rugged, robust, linear and reproducible method was developed by RP-HPLC method for simultaneous estimation of phosphate and dextrose content which was used in the formulation of Cabaxitaxel injection dosages form. An isocratic and simple RP-HPLC method was developed and validated on column Rezex HPX-87H (300 x 7.8) mm. 0.005 M H_2SO_4 was used as mobile phase. The flow rate was adjusted to 0.6 ml/min, column oven temperature 40°C and the detector cell temperature was adjusted 45°C by using refractive index detector. *The retention time for both content (phosphate and dextrose) was kept 40 minutes. Retention time of phosphate was found about 11 minutes while dextrose was at 14 minutes. Correlation coefficient of phosphate was 0.9999 while for dextrose content was found 0.9999 from 5ppm to 800ppm. Robustness was performed by deliberate changes in chromatographic conditions, method was found robust and usable even during small variations encountered in routine analysis and stability study. Stability of phosphate and dextrose content was performed at 5°C upto 1 day and found stable. 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, Phosphate and Dextrose, RP- HPLC, Method Development and Validation.*

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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. Phosphate and dextrose contents are used in the stable formulation of Cabazitaxel injection. 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 RP-HPLC method reported in the literature that can completely separate and quantify both dextrose and phosphate content simultaneously. It is, therefore, felt necessary to develop a new RP-HPLC method which can separate both phosphate and dextrose content.

Analyte profile and physiochemical properties :

Structure:

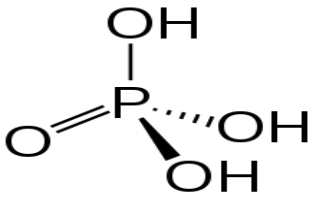

Fig. 1: Chemical structure of phosphoric acid

Synonym:
Phosphate, Ortho-phosphoric acid, Phosphoric acid.

IUPAC name :
Trihydroxidooxidophosphorus.

Chemical Formula:
H_3PO_4 or PO_4^{3-}

Molecular weight:
94.9714 g/mol

Solubility :
Freely soluble in water, methanol and ethanol.

pKa :
There are three pKa of phosphate as following.
($pK_{a1} \approx 2.12$)
($pK_{a2} \approx 7.21$)
($pK_{a3} \approx 12.67$)

Details for Dextrose:
Analyte profile and physiochemical properties :

Dextrose is a sugar usually obtained by the hydrolysis of Starch. It contains one molecule of water of hydration or is anhydrous.

Structure:

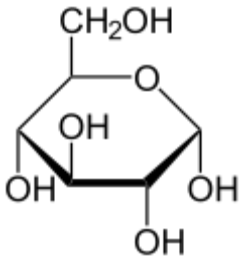

Fig.2: Chemical structure of dextrose

Synonym:
D-Glucose

IUPAC name :
D-gluco-hexopyranose

Chemical Formula:
$C_6H_{12}O_6$

Molecular weight:
180.16 g/mol

Solubility :
Highly soluble in water and acetic acid poorly soluble in methanol and ethanol.

pKa :
12.9

MATERIALS AND METHODS:

Preparation of Mobile Phase:
Transfer about 0.3 ml sulphuric acid (0.0025M) in to 1 liter of milli-Q water. Mix well and filter through 0.45µ nylon membrane filter paper and degas prior to use.

Chromatographic Conditions:
Flow rate: 0.6 mL/min
Detector: Refractive index detector
Detector temperature: 45°C
Column temperature: 40°C
Injection volume : 10 µL
Elution: Isocratic
Run time: 40 min.
Diluent: Acetonitrile: Water (70:30 %v/v)
Blank: Diluent
Detector sensitivity: 512
Column used: Rezex HPX -87H (300 x 7.8) mm

Preparation of Standard Stock Solution:
Weigh accurately about 100 mg of Dextrose and Orthophosphoric acid each WS in to a 100 ml volumetric flask. Add 30 ml of diluent and sonicate to dissolve. Allow to attain room temperature. Dilute up to the mark with diluent and mix well.

Preparation of Standard Solution:
Accurately transfer 5.0 ml of above standard stock solution in to a 20 ml volumetric flask. Dilute up to the mark with diluent and mix well.

Preparation of Sample Solution:
Weigh accurately 1.0 ml of sample in to a 10 ml volumetric flask. Add 7.0 ml of acetonitrile and sonicate to dissolve. Allow to attain room temperature. Dilute up to mark with 3.0 ml of water and mix well.

Evaluation of System Suitability:

i) Resolution between phosphate and dextrose should be not less than 3.0.
ii) Tailing factor for the phosphate and dextrose should not be more than 2.0.
iii) The % RSD for the six replicate injections of standard should not be more than 5.0.
iv) Theoretical plates for phosphate and dextrose should be not less than 5000.

1. Specificity:

1.1 Selectivity:

Experiment: A representative of Cabazitaxel standard solution 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.

1.2 Placebo and diluent Interference:

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

2. Linearity:

Experiment: A series of solutions of working/reference standards of phosphate and dextrose were prepared over a range of 2% to 300% of the working specification limits. Working concentration for phosphate and dextrose are 250µg/mL, the linearity range tested was between 5µg/mL to 800µg/mL. Linearity data treated for calculation of correlation coefficient.

Table 1: Phosphoric acid Linearity table

Sr. No.	Volume of Standard stock solution-OPA (mL)	Final Solution in Volumetric Flask (mL)	Concentration in ppm	Area
1	1.0	200	5.512	142.92
2	1.0	100	11.024	295.38
3	1.0	50	22.048	607.68
4	5.0	50	110.24	3041.83
5	5.0	20	275.6	7796.27
6	5.0	10	551.2	16014.14
7	7.5	10	826.8	23806.01
			Slope	28.92
			Intercept	-59.06
			Correlation Coefficient (r^2)	0.99996

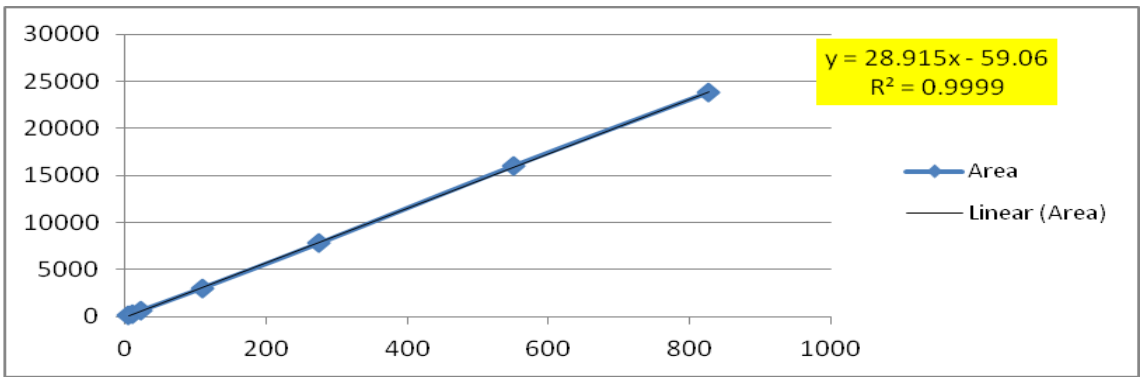

Fig. 3: Linearity graph of phosphoric acid

Table 2: Dextrose Linearity table

Sr. No.	Volume of Standard stock solution-Dextrose (mL)	Final Solution in Volumetric Flask (mL)	Concentration in ppm	Area
1	1.0	200	5.401	211.26
2	1.0	100	10.802	423.56
3	1.0	50	21.604	867.12
4	5.0	50	108.02	4221.38
5	5.0	20	270.05	10724.65
6	5.0	10	540.1	21876.8
7	7.5	10	810.15	32789.65
			Slope	40.52
			Intercept	-65.21
			Correlation Coefficient (r^2)	0.99998

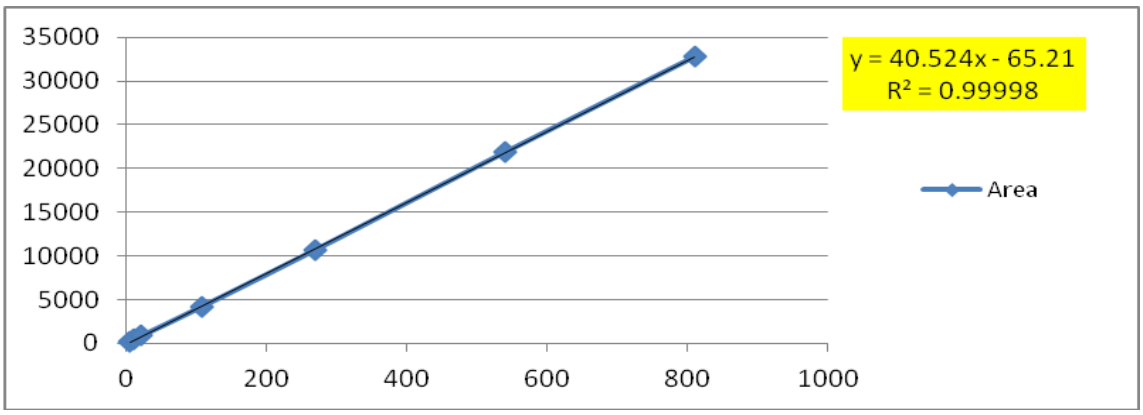

Fig. 4: Linearity graph of dextrose

3. Accuracy:

Experiment: Sample of Cabazitaxel specification limit, 50% of specification limit and 150% of specification limit, in triplicate, and then sample preparation were carried out as described under methodology.

Table 4: Phosphate content accuracy table

% Level	% Accuracy	Average Recovery	SD	%RSD
Accuracy Level 50% Set-1	97.70	**97.59**	**1.21**	**1.23**
Accuracy Level 50% Set-2	98.74			
Accuracy Level 50% Set-3	96.34			
Accuracy Level 100% Set-1	98.39	**97.28**	**0.98**	**1.01**
Accuracy Level 100% Set-2	96.51			
Accuracy Level 100% Set-3	96.96			
Accuracy Level 150% Set-1	95.46	**96.46**	**0.95**	**0.98**
Accuracy Level 150% Set-2	96.55			
Accuracy Level 150% Set-3	97.35			

Table 5: Dextrose content accuracy table

% Level	% Accuracy	Average Recovery	SD	%RSD
Accuracy Level 50% Set-1	98.73	**98.91**	**0.19**	**0.19**
Accuracy Level 50% Set-2	98.90			
Accuracy Level 50% Set-3	99.10			
Accuracy Level 100% Set-1	97.53	**97.45**	**0.47**	**0.48**
Accuracy Level 100% Set-2	97.87			
Accuracy Level 100% Set-3	96.94			
Accuracy Level 150% Set-1	94.45	**97.60**	**2.78**	**2.85**
Accuracy Level 150% Set-2	98.64			
Accuracy Level 150% Set-3	99.72			

4. Precision:

4.1 System Precision

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

Sr. No.	**Dextrose area**	**Phosphate area**
1	220000	151230
2	224069	150751
3	213476	160932
4	221654	154321
5	210015	149981
6	219890	156410
Mean	**218184.00**	**153937.50**
SD	**5325.04**	**4202.89**
%RSD	**2.44**	**2.73**

4.2 Method Precision:

Experiment: Six sample preparations of Cabazitaxel Injection were prepared and injected into the HPLC using the method as described under methodology. The data generated is given following Tables.

Precision (Phosphate content):

Table 6: Table for method Precision

Precision Samples	**Results in mg/ml**
Precision Sample Set-1	4.98
Precision Sample Set-2	5.19
Precision Sample Set-3	5.14
Precision Sample Set-4	5.10
Precision Sample Set-5	4.98
Precision Sample Set-6	5.19
Mean (mg/ml)	**5.06**
± SD	**0.09**
% RSD	**1.70**

Precision (Dextrose content):

Table 7: Table for method Precision

Precision Samples	Results in mg/ml
Precision Sample Set-1	1.37
Precision Sample Set-2	1.36
Precision Sample Set-3	1.37
Precision Sample Set-4	1.37
Precision Sample Set-5	1.38
Precision Sample Set-6	1.37
Mean (mg/ml)	**1.37**
± SD	**0.01**
% RSD	**0.46**

5. Robustness:

Experiment: Diluent, standard preparation, and sample preparation in triplicate of the same lot of Cabazitaxel Injection was prepared as described under methodology given as per sample preparation. The samples along with standard and diluent were injected under different chromatographic conditions as shown below.

- Change in column oven temperature (± 5°C)
- Change in flow rate (±0.1mL)
- Change in Buffer strength

Diluent, Standard solution and Control sample were injected as per following
Chromatographic conditions and reported the observation of standard solution.

Table 8: Table for robustness

Robustness Parameters	RT	USP Tailing	USP Plate counts	Rs
Initial Conditions: 0.005 M Sulphuric acid, Flow 0.4mL/Min, COT 40°C	12.88	1.35	8667	3.18
Flow plus (0.7 mL/min)	10.34	1.33	8236	3.06
Flow plus (0.5 mL/min)	17.20	1.36	8913	3.15
Column temp. plus (50°C)	12.94	1.37	8727	3.02
Column temp. minus (40°C)	12.86	1.34	8161	3.15
0.01 M Sulphuric acid	13.22	1.35	8122	3.13
0.0025 M Sulphuric acid	11.81	1.31	8299	3.08

Rs=Resolution between dextrose and phosphoric acid.
RT= Retention time.

Conclusion:

From above robustness study, method was found robust and usable even during small variations encountered in routine analysis and stability study.

6. Solution Stability in Analytical Solution

Experiment: Standard solution, Sample solution as per methodology was analyzed initially and at different time intervals at **5°C** (Auto sampler).

Table 9: Dextrose Standard Solution stability in analytical solution at 5°C

Time in Hrs.	Area of Inj-1	Area of Inj-2	Average Area	% Difference
Initial 0 Hrs.	220.86	221.15	221.01	NA
After 10 Hrs.	227.04	227.13	227.09	-2.75
After 24 Hrs.	226.32	226.27	226.30	-2.39

Table 10: Sample Solution stability in analytical solution at 5°C

Time in Hrs.	Area of Inj-1	Area of Inj-2	Average Area	% Difference
Initial 0 Hrs.	85.78	86.01	85.90	NA
After 10 Hrs.	85.84	88.12	86.98	-1.26
After 24 Hrs.	85.92	88.12	87.02	-1.31

Table 11: Phosphate Standard Solution stability in analytical solution at 5°C

Time in Hrs.	Area of Inj-1	Area of Inj-2	Average Area	% Difference
Initial 0 Hrs.	158.67	159.21	158.94	NA
After 10 Hrs.	161.42	163.20	162.31	-2.12
After 24 Hrs.	162.05	163.39	162.72	-2.38

Table 12: Sample Solution stability in analytical solution at 5°C

Time in Hrs.	Area of Inj-1	Area of Inj-2	Average Area	% Difference
Initial 0 Hrs.	161.51	163.25	162.38	NA
After 10 Hrs.	162.63	163.98	162.63	-0.15
After 24 Hrs.	163.71	164.41	164.06	-1.03

RESULTS AND DISCUSSION:

Retention time of phosphate and dextrose content in sample preparation is comparable with standard preparation. No interference was observed at the retention time of phosphate and dextrose with diluent and placebo peak. So method was found specific. The correlation coefficients are within limits (Not less than 0.9999) for phosphate and dextrose content. Mean recovery for phosphate and dextrose content found within limit from 50% to 150%. Standard sand sample RSD is within limit for phosphate and dextrose content l (Limit is, RSD should not be more than 5.0% for method precision and system precision). The test method is robust for all variable conditions. No interference observed due to diluent/blank. Standard and sample solutions are found to be stable up to 24 hours.

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Reference chromatograms:

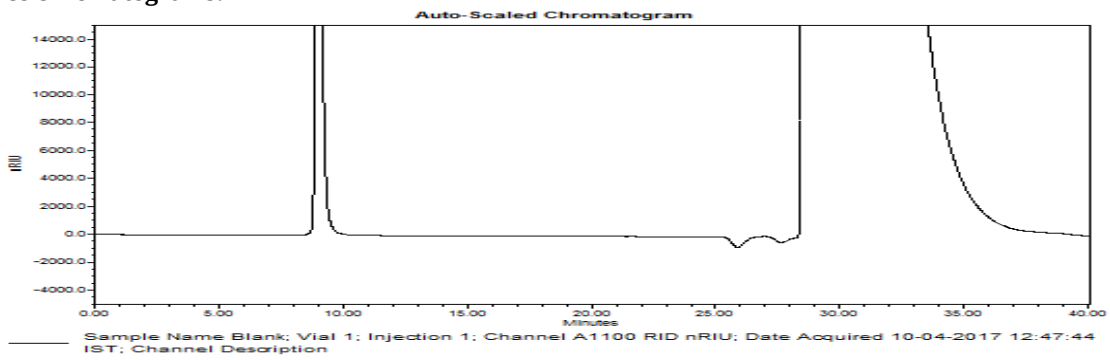
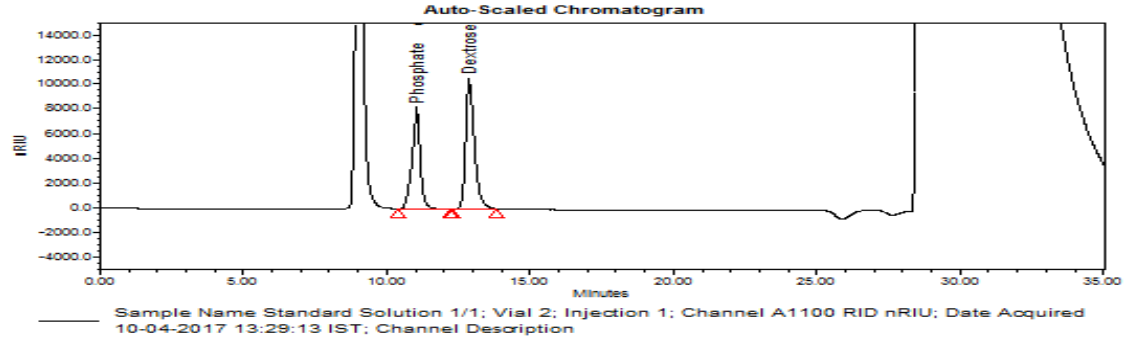
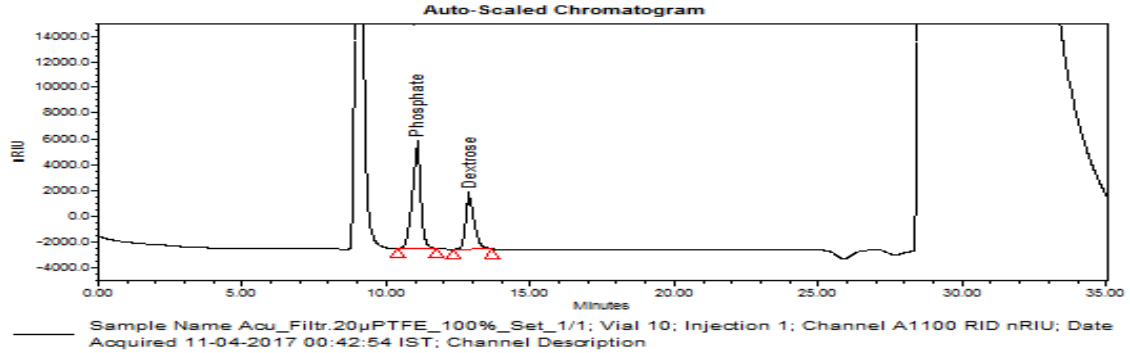


Fig. 5: Blank (Diluent)



	Name	Retention Time (min)	Calculated_Area	% Area	USP Resolution	USP Plate Count	USP Tailing
1	Phosphate	11.06	158.67	41.81		6440	0.90
2	Dextrose	12.88	220.86	58.19	3.18	8667	1.35
Sum			379.53				

Fig. 6: Mixed Standard (Phosphate and Dextrose):



	Name	Retention Time (min)	Calculated_Area	% Area	USP Resolution	USP Plate Count	USP Tailing
1	Phosphate	11.09	161.51	65.31		6310	0.88
2	Dextrose	12.89	85.78	34.69	3.10	8537	1.33
Sum			247.29				

Fig. 7: Sample Solution

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 simultaneous determination of phosphate and dextrose content in Cabazitaxel Injection. Hence this method can be introduced into routine use and testing

of stability samples.

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