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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR ESTIMATION OF DEXTROSE IN CITRATE PHOSPHATE DEXTROSE ADENINE ANTICOAGULANT SOLUTION BY HPLC-RID

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ABSTRACT

A novel approach was used to develop and validate a rapid, specific, accurate and precise high-performance liquid chromatographic (HPLC-RID) method for the estimation of dextrose in citrate phosphate dextrose adenine (CPDA) anticoagulant solution. Literature reveals different methods for the estimation of dextrose in bulk and their formulations. But there is no HPLC-RID method for the estimation of Dextrose in Citrate-Phosphate-Adenine-Dextrose (CPDA) anticoagulant solution. Hence the present plan of work is to develop a new, simple, precise & accurate method for dextrose analysis in bulk and formulation. The chromatographic separation was developed and validated on C18 column -7.8mm x 30-cm;9 µm. Degassed water was used as mobile phase. The flow rate was adjusted to 0.3 ml/min, column oven temperature 85°C and the detector cell temperature was adjusted 85°C by using refractive index detector. The retention time of dextrose was kept 30 minutes and dextrose was eluted at 20.333 minutes. The developed method offers excellent linearity in wide concentration range of (23 mg/mL – 35 mg/mL) for dextrose with r2 0.9989. Limit of detection (LOD) and limit of quantification (LOQ) for five analytes were 6.3 mg/mL and 19.1 mg/mL respectively. HPLC-RID method showed very good repeatability (RSD <5 %) and reproducibility. The developed method was successfully applied for quantification of dextrose in CPDA anticoagulant solution. The mean % recovery was achieved at 100.81%w/v. Robustness was performed by deliberate changes in chromatographic conditions and method was found to be robust. The proposed method was validated for specificity, accuracy, precision, linearity, range, ruggedness & robustness and can be applicable

for routine quantitative analysis of dextrose in citrate-phosphate-dextrose -adenine anticoagulant solution.

Keywords: HPLC-RID, ICH guidelines, method validation, Dextrose, CPDA- Citrate Phosphate Dextrose Adenine

INTRODUCTION:

Anticoagulants are given to prevent the blood from clotting or prevent existing clots from getting larger. Clots can block blood flow to the heart muscle or block blood flow to the brain. These cause a heart attack or a stroke **[1]**. Anticoagulant Citrate Phosphate Dextrose Adenine Solution is a sterile solution of sodium citrate, citric acid, sodium dihydrogen phosphate dihydrate dextrose and adenine in water for injection. Literature reveals that Raina Hadjikinova *et al*., (2017) **[1]** developed and validated HPLC-RID method for Determination of Sugars and Polyols. Ajithkumar *et al*., (2017) **[2]** developed and validated a modified polarimetric assay method for small volume samples. B. S. Chandravanshi *et al*., (2018) **[3]** studied on Improvement in Analytical Methods for Determination of Sugars in Fermented Alcoholic Beverages by HPLC-RID and HPLC-UV spectroscopy. But there is no HPLC-RID method for the estimation of Dextrose in Citrate Phosphate Dextrose Adenine (CPDA) anticoagulant solution. Hence the present plan of work is to develop and validate a new, simple, precise & accurate method for dextrose analysis in bulk and formulation by HPLC-RID. The structure of glucose was shown in **Figure 1**.

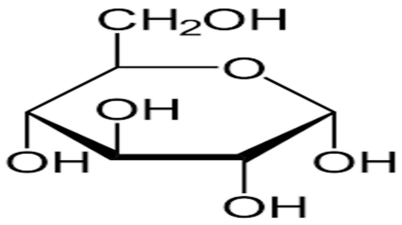


Figure 1: Chemical Structure of dextrose

MATERIALS AND METHODS:

Instrumentation:

The HPLC-RID system containing C18 (7.8-mm x 30-cm, 9µm) column with RID detector. Data acquisition, data handling and instrumentation control were performed by Empower software.

Chemicals and reagents:

The standard dextrose anhydrous, citric acid, disodium phosphate, Adenine, maltose monohydrate, maltotriose, fructose was supplied by the TERUMO PENPOL Private Limited, Puliyarakonam. Citrate phosphate dextrose adenine anticoagulant formulation was purchased from local market. HPLC grade water purchased from S.D. fine chemicals. The volumetric flask 50mL &100mL, pipette 1 ml and 20µl were purchased from Borosil. All chemicals are analytical grade.

METHOD DEVELOPMENT:

Standard solution:

Weigh 1.45gm of dextrose anhydrous RS into 50 ml volumetric flask and make up to the volume with distilled water.

Sample solution:

CPDA marketed available dosage form was procured and injected in to HPLC system for analyze the chromatographic condition.

System suitability test solution:

Dissolve 5mg of Maltose monohydrate RS, 5mg of Maltotriose RS and 5 mg of Fructose RS in water and dilute with 50 ml of distilled water.

Optimised chromatographic condition:

HPLC analyses were performed on a Shimadzu HPLC. Separations were carried on a Bio-Rad Aminex HPX-87^{0}C 9µm C18 Column 7.8-mm x 30-cm. The column temperature was set at 85^{o}C and the flow rate was 0.3 mL min-1 while using isocratic elution with distilled water. Injection volume was 20µL and detection with refractive index detector and dextrose Peak identity was confirmed by retention time at 20. 20.333mints. Five various test protocols were performed by changing temperature and flow rate of mobile phase then optimised chromatogram as per ICH guidelines **[9]**.

Method Validation:

The proposed method was validated according to Q2 (R1) guidelines. The validation parameters are system suitability, specificity, linearity, accuracy, precision, robustness, ruggedness, LOD and LOQ.

System suitability parameters: Standard solution was injected into the system for five times and the system suitability parameters (Retention time ($R_{t),}$ Theoretical plates (TP), Tailing factor (t)) were checked.

Specificity: To access the specificity of method by injecting blanks, standards and placebo were injected into the system and the obtained chromatograms are studied for any responses.

Linearity: The linearity of the method was obtained by preparing suitable aliquots of standard stock solution in different concentrations range for 23-3mg/ml (80, 90, 100, 110 and 120%). The calibration curve was obtained by plotting the peak area against the respective concentration.

Accuracy: Accuracy studied by analysis of sample solutions of 3 concentration levels such as 80%, 100 % and 120 % in triplicates as per the method for CPDA solutions. The preparation of accuracy solution shown in table1. stock solutions taken individually, injected into system triplicate and % mean recovery was calculated.

Table 1: Preparation of Accuracy solution

Active Pharmaceutical	Concentration levels

ingredients	Level-1: 80 %	Level-2: 100 %	Level-3: 120 %
Citric acid monohydrate	0.262 g	0.327 g	0.392 g
Sodium citrate dihydrate	2.104 g	2.630 g	3.156 g
Dextrose (Anhydrous)	2.320 g	2.900 g	3.480 g
Monobasic sodium phosphate	0.201 g	0.251 g	0.301 g
*Water for injection	100 mL	100 mL	100 mL

Precision/Ruggedness:

a) System Precision (Repeatability): The system precision was determined by injecting six working standard solutions. The areas of all the injections were taken and standard deviations, % Relative standard deviation (RSD) were calculated.

b) Intermediate Precision**:** The intermediate precision was determined by injecting six working standard solutions and on consecutive days by different personnel. The areas of all the injections were taken and standard deviations, %RSD were calculated.

LOD & LOQ:

Limit of detection (LOD) and Limit of quantification (LOQ) were determined by calibration curve method. Six sets of Solutions were made from the Standard solution and injected into the system.

$$LOD = \frac{3.3\ SD}{S} \qquad LOQ = \frac{10\ SD}{S}$$

Where SD = Standard Deviation of y-intercept of calibration curve

S = Mean slope of calibration curves

Robustness:

To study the robustness of the proposed method small deliberate changes in method like flow rate and temperature are made with conditions such as decreasing the flow rate to 0.2 ml/min (FM), Increasing the flow rate to 0.4 ml/min (FP), Decreasing the temperature to 75°C (TM) and increasing the temperature to 85°C (TP) and the samples were injected into the system and %RSD was calculated.

RESULT AND DISCUSSION:

Method development and Optimisation:

The analytical method development of dextrose standard solution was trial with five protocol studies. The all five test protocols were shows good asymmetry and theoretical plates was NLT 2000. But test protocol 01 showed resolution 1.36 between maltose and maltotriose. Hence trial 01 method was optimized and further validation was processed with the same method. The optimised chromatogram is shown in **Figure 2**.

Method validation

Method validation is the process to confirm that the analytical procedure employed for a specific test is suitable for its intended use. The analytical procedure shall meet the

following analytical parameters like linearity, range, accuracy, precision, specificity, system suitability and robustness. limit of detection and limit of quantification. Method validation was performed according to ICH guidelines **[8, 9]**.

System suitability

A system suitability of the method was performed before the validation, five replicate injections of the standard solution were injected and theoretical plate, asymmetry, resolution, and % RSD of peak area was determined.

Specificity

The specificity of the method was studied by injecting blank, placebo and standard preparations. Chromatograms were recorded and retention times from sample and standard preparations were compared for identification of analytes. The chromatograms for specificity are given in the **Figure 3-5**. The result shows that there is no interference between standard and sample. Hence the method was specific.

Linearity and range:

The linearity was checked by preparing and analyzing the standard preparation at five different concentration ranges of 23-35µg/ml for dextrose. The calibration curve is constructed by plotting the peak area versus the concentration. The linearity of this method was evaluated by linear regression analysis. The slope & intercept calculated for CPDA were given in **Table 2** and linearity graph shown in **Figure 6** and r2 value <0.9989. Hence, the developed method was linear.

Precision:

Standard solution was injected six times and calculated % Relative standard deviation. The %RSD acceptance limits was NMT 2% as per ICH guidelines. The result was shown in **Table 3**.

The precision was expressed as relative standard deviation (RSD% = SD/mean×100). The relative standard deviation (RSD) of the calibration standards (n = 6) for intra-day

precision (repeatability) and inter-day (intermediate) precision ranged from 3.0 to 5.5% and between 6.2 and 8.0 %, respectively **(Table 4)**. In articles for simultaneous analysis of sugars and polyols some authors found repeatability values below the maximum acceptable limits

for the validation of chromatographic methods **[12, 13, 20]**.

Intermediate precision/Ruggedness

System precision activities to be carried out by a different chemist using a different instrument and column on a different day. Calculated % RSD value was not more than 5%.

Accuracy

Accuracy shall be assessed using minimum of nine determinations over minimum of three. Analyst shall carry out the analysis of sample solutions of 3 concentration levels such as 80%, 100% and 120 % in triplicates as per the method for CPDA solutions. The mean % recovery was found to be 100.16%w/v **(Table 4)**.

LOD and LOQ:

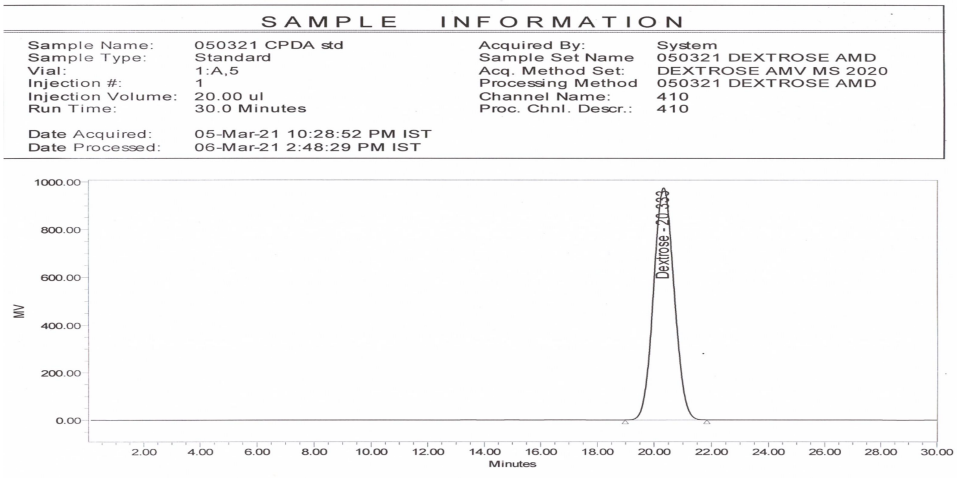
LOD and LOQ values were calculated according to the ICH guidelinesQ2 (R1). which were estimated as [standard deviation of response/slope of the regression equation] by multiplying with 3.3 and 10 respectively. The values are given in **Table 5**.

Robustness:

The robustness of the method was evaluated to demonstrate the reliability of the developed method for routine use. The robustness is studied by the evaluating effects of small but the deliberate differences in method condition. The condition was flow rate variation (0.3ml ± 0.1 ml/min) and temperature at 85 ± 5^0C. The results of robustness are given in the **Table 6**. The varied chromatographic conditions did not alter the retention time and assay values. So, it could be concluded that the developed method is robust.

Assay of marketed formulation:

The proposed method was tested by analyzing the commercially available marketed formulation. The percentage purity of dextrose in CPDA solution was found to be 98.96%w/v and results were given in the **Table 7** and **Figure 7**.



	Peak Name	RT	Area	% Area	Height
1	Dextrose	20.333	48828501	100.00	974752

Figure 2: Optimised chromatogram of Dextrose in CPDA anticoagulant solution

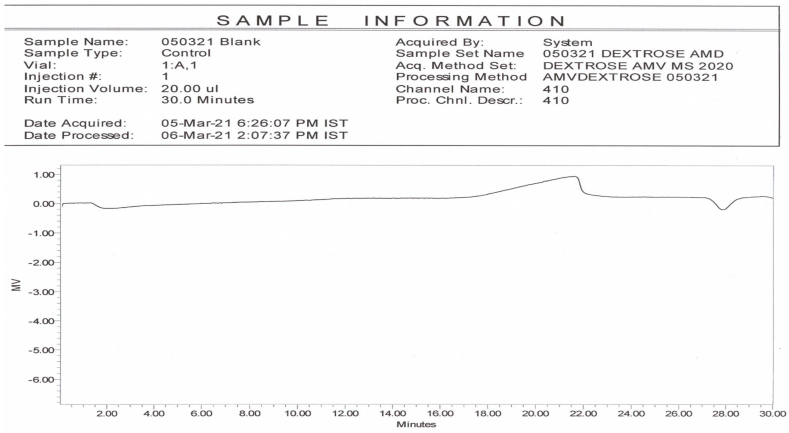


Figure 3: Blank chromatogram

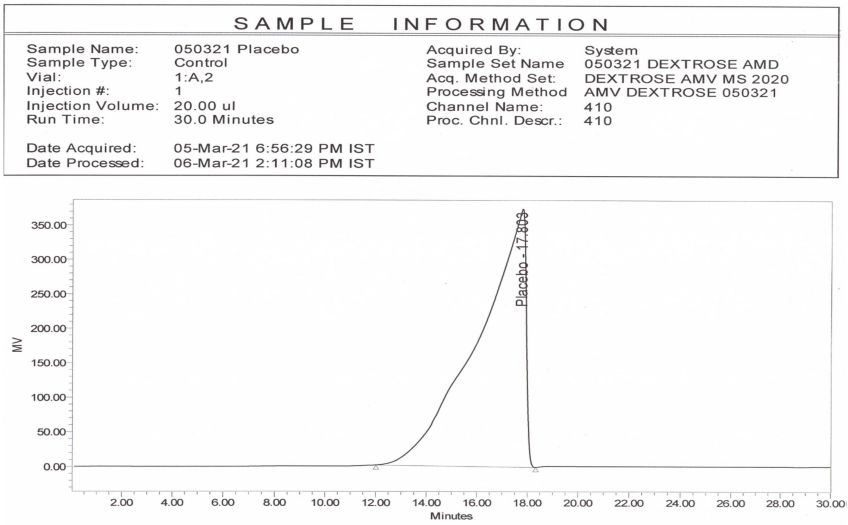


Figure 4. Placebo chromatogram

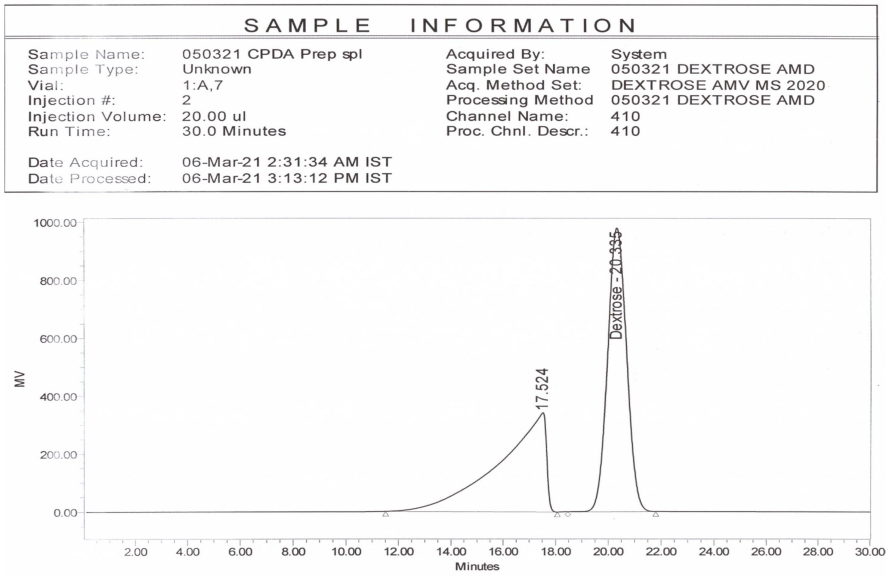


Figure 5: CPDA Sample chromatogram

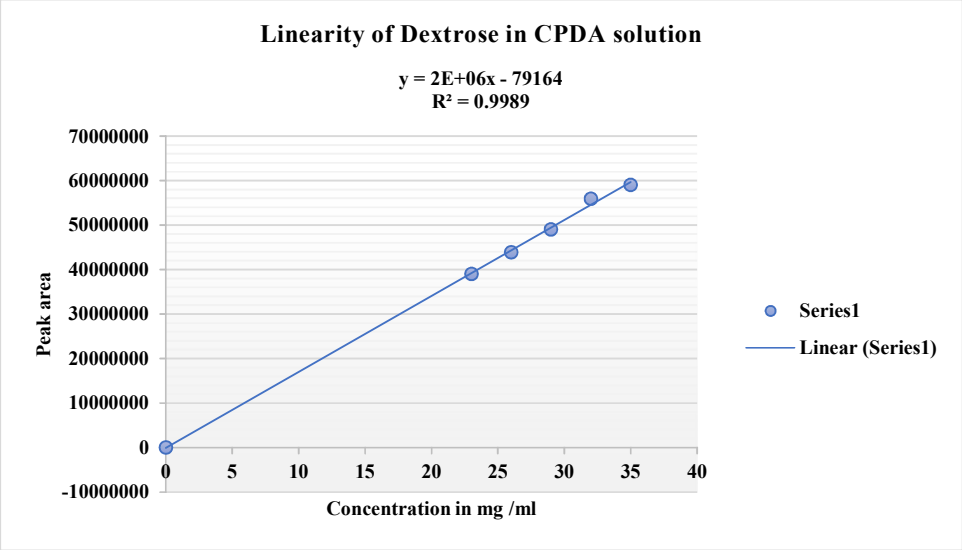


Figure 6: Linearity of Dextrose in CPDA anticoagulant solution

Table 2: Linearity data of dextrose in CPDA anticoagulant solution

Linearity level		
	Conc.(mg/ml)	CPDMean Area
1	23	39048214
2	26	43892550
3	29	49059664
4	32	55913319
5	35	59013822
Correlation coefficient		0.9989
Slope		79164
Intercept		2E+06x
Equation		Y=2E+06x - 79164

Table 3: Precision of dextrose in CPDA solution

S. No.	System Precision	
	Peak area	*RT*
1.	48558182	20.304
2.	48534347	20.305
3.	48563218	20.307
4.	48544982	20.307
5.	48568044	20.306
6.	48183494	20.292
AVG	48492044.5	20.303
SD	151664.7	0.0057
%RSD	0.312	0.028

Table 4: Accuracy study of dextrose in CPDA anticoagulant solutions

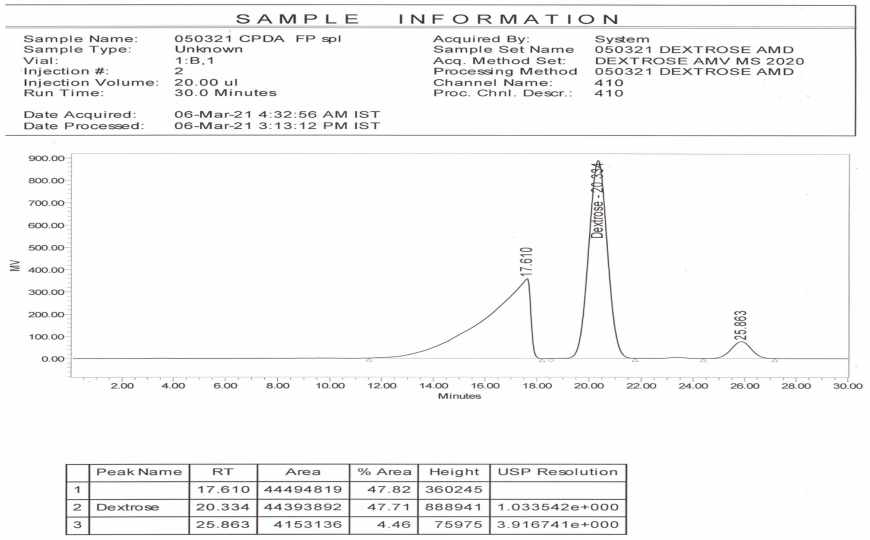
Accuracy Level in %*	Average peak area*	Theoretical conc. in mg/ml	Found conc. mg/ml	% Recovery in (% w/v)	Mean % Recovery in (% w/v)
80	39462356	23	23.2	101.06	100.16
100	48778670	29	28.8	99.42	
120	59013122	35	34.9	99.99	

Table 5: LOD and LOQ data of dextrose in CPDA anticoagulant

Parameter	LOD	LOQ
Dextrose (mg/ml) in CPDA solution	6.3	19.1

Table 6: Robustness data of dextrose in CPD solution

Condition Varied	Changed condition	Dextrose in CPDA solution	
		Area	% Assay
Flow rate	0.20	48365892	99.05
	0.30	49125698	100.71
	0.40	49036589	100.30
Temperature	80^0C	49369985	100.92
	85^0C	48936988	99.99
	90^0C	49236997	100.55
AVG		48974541	100.24
SD		429545.5	0.837
%RSD		0.877	0.835



	Peak Name	RT	Area	% Area	Height	USP Resolution
1		17.610	44494819	47.82	360245	
2	Dextrose	20.334	44393892	47.71	888941	1.033542e+000
3		25.863	4153136	4.46	75975	3.916741e+000

Figure 6: Assay of dextrose in CPDA anticoagulant solution

Table 7: Results of Assay of Dextrose in CPDA anticoagulant solution

Formulation	Parameters	Dextrose in CPDA solution
Generic formulation	Label claim	2.90gms
	Amount found	2.87gms
	Percentage purity	98.96%w/v

CONCLUSION:

In this study, isocratic, HPLC-RID methods were successfully developed and validated for the estimation of Dextrose in Citrate-Phosphate-Dextrose-Adenine solution. The proposed methods were found to be simple, accurate, precise, robust, specific and sensitive. The methods could be employed for the analysis of Dextrose in anticoagulant solution for routine analysis in quality control departments in industries, research institution, approved testing laboratories, pharmaceutics & bio-equivalence studies and clinical pharmacokinetic studies and for stability studies.

CONFLICT OF INTEREST:

The authors have no conflicts of interest regarding this investigation**.**

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