

DEVELOPMENT AND VALIDATION OF A REFRACTOMETRIC ASSAY METHOD

K. C. Ajithkumar and K. Pramod*

College of Pharmaceutical Sciences, Govt. Medical College, Kozhikode – 673008, Kerala, India.

Article Received on
26 August 2017,

Revised on 17 Sept. 2017,
Accepted on 08 Oct. 2017

DOI: 10.20959/wjpr201713-9817

*Corresponding Author

Dr. K. Pramod

College of Pharmaceutical
Sciences, Govt. Medical
College, Kozhikode –
673008, Kerala, India.

ABSTRACT

Refractometry is used to detect the refractive index of chemical substances, when light passes through it. Since every chemical substance or sample has specific refractive index at specific concentration, here we used it as an assay method and validated the method in terms of linearity and range, accuracy and precision. The detection of limit and the quantitation of limit were determined as per ICH guidelines. The method is expected to be extended in quantification of variety of samples.

KEYWORDS: ICH, linearity, refractive index, range, precision, detection limit, quantitation limit

INTRODUCTION

Refractometric analysis is the technique used to determine the refractive index of gas, liquid, or solid with response to the chemical composition of the substances and purity. It is an important tool in the field of agriculture, food industry and chemical industry. It can be used to determine the purity of drugs^[1], used for estimation of biological samples^[2], characterization of chemical such as optical active samples and viscous liquids. In agriculture it will be used for the estimation of sugar in fruit juices and wines.^[3] Refractive index will be depended by temperature and the wavelength of the light used. So the temperature should be maintained constant to avoid the chances of error in an experiment. Here we develop and validated an assay method using dextrose as test sample.

MATERIALS AND METHODS

Materials: Anhydrous dextrose was purchased from Medilise Chemicals, Kannur, India. Reagent grade I water (Millipore, Molsheim, France) was used for the study.

Refractometry: We employed (Abbemat 350, Anton Paar India Pvt. Ltd., Haryana, India) refractometer in the study.

Preparation of standard solutions: Anhydrous dextrose (25 g) was dissolved in water in a 100 mL volumetric flask and then the volume was made up with water. The dilutions of this stock solution were made by diluting the required aliquot with water to obtain standard solution in the range of 2- 25%w/v. The refractive index of the resultant solutions was determined at a wavelength of 589 nm and 20°C.

Linearity and range

The calibration curve was plotted using the concentration range of 2- 25 % w/v. The refractive index of the solutions was determined at a wavelength of 589 nm and 20°C. A calibration curve was constructed by plotting refractive index versus concentration of standard solution and the regression equation was determined. The experiment was carried out in triplicate.

Precision

Three concentrations of dextrose solution (2, 8 and 20% w/v) were prepared. The precision of the method was assessed by analyzing dextrose for repeatability and intermediate precision.

(a) Repeatability

Repeatability (intraday) was assessed by analyzing dextrose in three different concentrations (2, 8 and 20% w/v) of three times a day. The % relative standard deviation (RSD) was calculated for refractive index thus obtained, to obtain the intra-day variation.

(b) Intermediate precision

Intermediate precision (inter-day) was established by analyzing three different concentrations (2, 8 and 20% w/v) of dextrose for three different days. The % relative standard deviation (RSD) was calculated for refractive index thus obtained, to obtain the inter-day variation.

Detection and quantitation limits

The limit of quantification and limit of detection were determined according to equations (1) and (2).

$$QL = 10 \sigma / S \quad \text{----- (1)}$$

$$DL = 3.3 \sigma / S \quad \text{----- (2)}$$

where, σ is the standard deviation of the intercept of the calibration plot and S is the slope of the calibration curve.

RESULTS AND DISCUSSION

The developed refractometry method for the estimation of dextrose in water was developed and validated.

Linearity and range

The refractive index of the prepared standard solutions (2- 25 %w/v) was determined. The mean refractive index was found to be 1.335627 – 1.36353. The plot of refractive index versus concentration was linear in above concentration range 2- 25 %w/v with excellent coefficient of determination of 0.9978 ± 0.00024 . The calibration curve data for dextrose in water is displayed in Table 1 and the calibration plot is displayed in Fig. 1.

Table. 1: Calibration curve data for dextrose in water.

Concentration (% w/v)	Mean refractive index (α) \pm SD (n=3)	Regressed refractive index	R ²
2	1.335627 \pm 0.000197	1.335902	0.9978 \pm 0.00024
4	1.338377 \pm 0.0004517	1.338372	
6	1.340803 \pm 0.0002957	1.340842	
8	1.343307 \pm 0.0001504	1.343311	
10	1.345750 \pm 0.0000656	1.345781	
15	1.352337 \pm 0.0002768	1.351956	
20	1.358870 \pm 0.0000361	1.358131	
25	1.363530 \pm 0.0000985	1.364305	

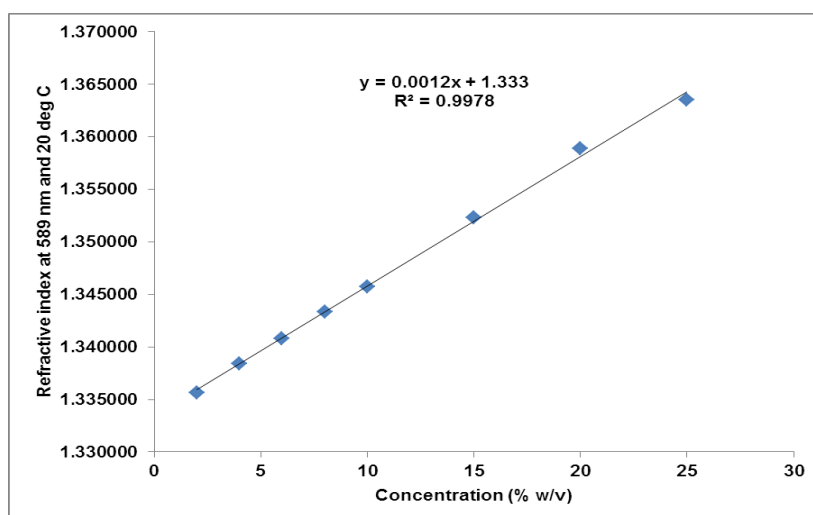


Fig. 1. Calibration plot for dextrose in water.

Precision

The precision of the method was assessed by analyzing three different concentrations (2, 8 and 20%w/v) of dextrose.

(a) **Repeatability:** Repeatability (intra-day) was assessed by analyzing three different concentrations (2, 8 and 20% w/v) of dextrose three times a day. The % RSD was calculated for refractive index thus obtained, to obtain the intraday variation and is given in Table 2.

(b) **Intermediate precision:** Intermediate precision (inter-day) was established by analyzing three different concentrations (2, 8 and 20% w/v) of dextrose for three different days. The % RSD was calculated, to obtain the inter-day variation and is given in Table 2.

The low values of % RSD for repeatability and intermediate precision suggested an excellent precision of the developed refractometric method.

Table 2: Repeatability and intermediate precision.

Concentration (% w/v)	Repeatability (n=3)		Intermediate precision (n=3)	
	Mean refractive index \pm SD(n=3)	% RSD	Mean refractive index \pm SD(n=3)	% RSD
2	1.33493 \pm 0.00051	0.0386	1.33520 \pm 0.00067	0.0500
8	1.34204 \pm 0.00108	0.0807	1.34332 \pm 0.00026	0.0191
20	1.35918 \pm 0.00042	0.0308	1.35899 \pm 0.00029	0.0211

Detection and quantitation limits

The detection limit (DL) and quantitation limit (QL) were determined as per the ICH guidelines and were found to be 0.6354 and 1.9255 % w/v respectively.

Table 3 summarizes the linear regression and validation data of refractometric method for the quantification of dextrose in water.

Table 3: Linear regression and validation data (n = 3).

Parameter	Data (Mean \pm SD)
Regression analysis	
Slope	0.00123 \pm 0.00001
Intercept	1.33343 \pm 0.00024
Regression coefficient (R ²)	0.9978 \pm 0.0003
Validation	
Range (% w/v)	2 – 25
Detection limit (DL) (% w/v)	0.6354
Quantitation limit (QL) (% w/v)	1.9255

CONCLUSIONS

A refractometric method for the quantification of dextrose was successfully developed and validated. The method was validated in terms of linearity and range and precision. The detection limit (DL) and quantitation limit (QL) were determined as per the ICH guidelines

and were found to be 0.6354 and 1.9255 %w/v respectively. The method is expected to be useful in a variety of industries where dextrose finds its application. The same approach can be extended for determination of purity of other liquid samples.

ACKNOWLEDGEMENT

K. C. Ajithkumar gratefully acknowledges Kerala State Council for Science, Technology and Environment (KSCSTE), Thiruvananthapuram, India, for sanctioning student project (No. 14/SPS 57/2016/KSCSTE, Dated 02.04.2016).

REFERENCES

1. Green MD, Nettey H, Villalva RO, Pamanivong C, Khounsaknalath L, Grande OM, Newton PN, Fernández FM, Vongsack L, Manolin O. Use of refractometry and colorimetry as field methods to rapidly assess antimalarial drug quality. *J Pharm Biomed Anal*, 2007; 43(1): 105-110.
2. Riche M. Analysis of refractometry for determining total plasma protein in hybrid striped bass (*Morone chrysops* × *M. saxatilis*) at various salinities. *Aquaculture*, 2007; 264: 279–284.
3. Barbosa EA, Silva DM, Preto AO, Verzin R. Design, construction, and performance of a real-time holographic refractometry prototype for liquid analysis. *Review of scientific instruments*, 2011; 82: 013103.
4. ICH-Guidelines Q2 (R1). *Validation of Analytical Procedures: Text and Methodology*. Geneva, Switzerland: 2005.