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Original scientific paper

Overview of the compendial methods for quantitative determination of glucose in solutions for intravenous infusion

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Abstract

This paper provides a review of the pharmacopoeial methods for determination of the content of glucose in parenteral solutions (HPLC/RID method, polarimetry and volumetric titration) and evaluation of all critical aspects of the proposed methods, with a focus on accuracy, precision, time of analysis and resources for introducing, maintenance and conducting analysis. Comparative analysis of the results from assay (ten independent determinations) of Glucose 5% *w/v* solution for intravenous infusion, obtained using all three methods, was performed. The analysis confirmed that there is no statistical difference between the testing results obtained with each method, indicating that all three methods provide valid accurate and precise results. However, the availability of the equipment and trained personnel may be the crucial factor for small laboratories for selection of the method for assay of glucose in parenteral preparations.

The obtained results and discussion on all critical aspects (accuracy, precision, time of analysis and resources for introducing, maintenance and conducting analysis) of the proposed methods, provide a valuable input to small laboratories on the suitability of these methods for quality control of Glucose 5% *w/v* solution for intravenous infusion.

Key words: Glucose, HPLC/RID, Polarimetry, Volumetry

Introduction

Glucose is (+)-d-glucopyranose, derived from starch, which can be anhydrous or hydrated with one molecule of water and is widely used as an active substance in solutions for intravenous infusion or as an excipient in different pharmaceutical preparations, especially in preparations for parenteral use. Solutions for intravenous infusions containing glucose are available at two different concentrations 50 g/L and 100 g/L in containers with different volumes 50 mL, 100 mL, 500 mL and 1000 mL.

Preparations for intraperitoneal use contain glucose in varying concentrations, ranging from 25 to 250 mmol/L (McMillin, 1990). European pharmacopoeia (Ph.Eur.), British pharmacopoeia (BP) and United States pharmacopoeia (USP) describe different monographs for quality control of glucose depending of the intended use. The determination of the content of glucose in the active substance and in the finished pharmaceutical preparations is a critical parameter for quality control. The pharmacopoeial monographs describe different methods for quantitative determination of glucose including cation - exchange liquid chromatography (LC) with

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refractive index detection (RID), volumetric redox titration or methods based on measurement of optical rotation (Ph.Eur., BP, USP).

The high-performance liquid chromatography (HPLC) method with RID is selective and accurate method for quantification of carbohydrates in different matrixes with a simple sample preparation (Bernárdez et al., 2004; Eyéghé-Bickong et al., 2012; Filip et al., 2016; Galant et al., 2015; Nováková et al., 2008; Shabir, 2003; Xu et al., 2015). This method is also used for determination of related substances, including different monosaccharides, disaccharides or degradation products, which may be present as impurities in glucose substance or in finished products. Taking into account that some laboratories are not equipped with HPLC-RID instrument, alternative methods (polarimetry or volumetric method) may be used for quantitative determination of glucose in routine quality control.

Polarimetry is a sensitive and nondestructive technique based on a measurement of the angle of optical rotation (rotation of the plane of polarization of linearly polarized light) by chiral substances and it is used as a method for identification and quantitative determination of chiral compounds. (Kvittingen and Sjursnes, 2020; McNichols et al., 2000; Pirnstill et al., 2013; Timson et al., 2003).

Volumetric analysis are considered as accurate and precise analytical procedures, but are not specific and these methods can be used in the monographs/specifications for assay in cases when the identification and purity tests are sufficiently specific and characteristic for the substance of interest. Redox titrations are commonly used for quantitative determination of sugars, based on the reducing properties of sugars. (Adams et al., 1952; Mazzuchin et al., 1970; Ph. Eur. ; Somogyi, 1945).

The aim of this study was to give an overview of the compendial methods for quantitative determination of glucose in solutions for intravenous infusion: HPLC-RID, polarimetry and volumetric method (redox titration) and to perform comparative analysis of the results obtained using these three methods. The results can be used for demonstrating suitability of each method as an alternative to the method defined in the monograph for assay of glucose in solutions for intravenous infusion in small scale production.

Material and methods

One small-scale production batch of Glucose 5% *w/v* solution for intravenous infusion, 250 mL, produced by Galenic laboratory Kumanovo, Republic of North Macedonia, was used as a sample for determination of the content of glucose, using the three methods.

Determination of Glucose using HPLC-RID

The determination of the content of glucose was performed according to the method described for the assay in the Ph. Eur. monograph for Glucose monohydrate 01/2016:0178, with slight modifications in the chromatographic conditions.

Chromatographic conditions

Agilent 1260 Infinity series HPLC system (Agilent Technologies©) equipped with a refractive index detector (RID) was used for analysis. Separation was performed using Hi-Plex Ca (Duo), 300 x 6.5 column (strong cation-exchange). Ultrapure water (conductivity 0.0055 µS/cm). was used as a mobile phase, with a flow rate of 0.4 mL/min. The column temperature was maintained at 80 °C, while temperature of RID was set to 45 °C. The volume of injection of the samples was 10 µL.

Standard solution

Working standard solutions were prepared by suitable dilution of standard substance (D-(+) Glucose with high-purity, Supelco) with purified water and further diluted to obtain concentrations of 1%, 2.5% and 5%.

Sample solution

The sample was injected in the HPLC system after being filtrated with a 0.22 µm filter, without any dilution.

Determination of Glucose using Polarimetry

The determination of the content of glucose was performed using the method described in the BP Pharmacopoeia (BP 2023) and USP Pharmacopoeia (USP42-NF37 – p.1296), using Anton Paar MCP 200 (III-22) automatic polarimeter.

Sample preparation

0.1 mL of 5 M Ammonia was added to a volume of the sample containing equivalent of 1g of glucose, and further diluted with purified water to 50 mL. The sample was mixed well, allowed to stand for 30 min and the angle of optical rotation was measured using a 2 dm tube.

Determination of Glucose using volumetric titration

Determination of glucose by volumetric titration is based on redox reaction between sodium thiosulfate and iodine, with visual determination of endpoint.

Volumetric solution

The volumetric solutions, 0.1 M Iodine VS, and 0.1 M Sodium thiosulfate VS, were standardized before use according to *European Pharmacopoeia*, 4.2.2.

Titration procedure

10 mL the of sample was diluted with a purified water in a volumetric flask of 100 mL. 5 mL of this solution were

transferred in conical flask, followed by addition of 33 mL 0.1M NaOH and 20 mL 0.1 M Iodine *VS*. The samples were mixed well and then allowed to stand for 30 min at room temperature in the dark place. Following addition of 10 mL 0.5 M H_2SO_4 the solution was titrated with the 0.1 M Sodium thiosulfate *VS*, until the brown color changed to pale yellow. After addition of a 2 mL starch solution, the titration was continued until the intense blue color disappeared.

Results and discussion

The most suitable approach for quality control of the finished products, performed in laboratories of small-scale production sites is to follow the official pharmacopeial methods. Taking into account that the European pharmacopeia is official and therefore mandatory for the quality control of starting materials and active pharmaceutical ingredients (APIs), the laboratory has adopted and verified the HPLC/RID method for assay of glucose substance. This method, with minor modifications according to general requirements for adjustment of chromatographic conditions and system suitability (Ph.Eur. 2.2.46), was used for the quantitative determination of glucose in the finished product: solution for infusion. The laboratory has additionally considered introducing polarimetry as a method for the quantification of glucose in solution for infusion, as described in USP and BP monographs.

Volumetric redox titration is no longer compendial method for quantification of glucose in solutions for infusion, but this method is still described in Ph.Eur. for assay of reducing sugars, expressed as glucose, in concentrated solutions for haemodialysis. Even though this method is not sufficiently specific, it is accurate and precise and suitable for assay of formulations, containing only glucose dissolved in water.

In this paper a comparative analyses of the results from quantitative determination of glucose using three different methods (HPLC/RID, polarimetry and volumetric titration), is shown. Ten independent determinations (n=10) were performed on samples from a small-scale production batch of Glucose 5% *w/v* solution for intravenous infusion, 250 mL, using each method. The results from comparative analysis and statistical evaluation of precision (standard deviation, S, and relative standard deviation RSD % of the assay values) and accuracy (95 % confidence interval) of the obtained testing results are shown in Table 1.

Table 1. Comparative analysis of the results from quantitative determination of Glucose 5% w/v solution for intravenous infusion using three different methods

Method	HPLC/RID	Polarimetry	Volumetric titration
Assay of Glucose (percentage of the declared content with confidence interval, n=10) [(i)]			
Average values	101.14± 0.38%	100.16± 0.43%	100.56± 0.41%
Precision			
S	0.54	0.60	0.58
Variance	0.29	0.34	0.33
RSD (%)	0.53	0.60	0.57
F - Test[(ii)], Two-Sample for Variances			
F (HPLC *vs* Polarimetry)	1.067		
F (Polarimetry *vs* Volumetry)		1.240	
F (HPLC *vs* Volumetry)			1.163
F Critical one-tail	3.179	3.179	3.179
Decision	No statistical difference	No statistical difference	No statistical difference
t - Test[(iii)], Two-Sample Assuming Equal Variances			
t (HPLC vs Polarimetry)	-3.864		
t (Polarimetry vs Volumetry)		-1.523	
t (HPLC vs Volumetry)			-2.328
t Critical one-tail	1.734	1.734	1.734
Decision	No statistical difference	No statistical difference	No statistical difference

[(i)] P (95% level of confidence)

[(ii)] $F = s_1^2/s_2^2$; $(df_1 = n_1 - 1 \; and \; df_2 = n_2 - 1)$; $\alpha = 0.005$

[(iii)] $t_{df} = [(\bar{x}_1 - \bar{x}_1) - (\mu_1 - \mu_2)] / \sqrt{s_p^2 \left(\frac{1}{n_1} + \frac{1}{n_2}\right)}$; $(df = n_1 + n_2 - 2)$; $\alpha = 0.005$

Statistical test (t - Test, Two-Sample Assuming Equal Variances and F - Test, Two-Sample for Variances) were performed in order to confirm comparability of the obtained average values and variability between results obtained using each method. The obtained t-values and F-values confirmed that there is no statistical difference between the testing results obtained with each method, indicating that all three methods provide valid accurate and precise results. All obtained results, together with the confidence interval (*p=95%*), are within the specification limits.

Additionally, we have compared the time needed for analysis of a sample using each method. For HPLC/RID method the total analysis time is approximately 30 minutes per sample. Titration requires approximately 40 minutes per sample (30 minutes required for chemical reaction between glucose and iodine and additional time for the titration). When done in sequence, the time for titration can be optimized, taking into account the waiting time *vs* titration time, but it is only possible when experienced analysts are performing testing. Similarly, for polarimetry, 30 minutes wait time is required for mutarotation, but when compared to titration, the measurements can be automated and the total analysis time is shorter. The less time consuming method is HPLC/RID, because once the samples are prepared; the sequence of analysis does not require constant work by the analysts. But taking into account the time needed for equilibration of the system and sample preparation in HPLC/RID, the time needed for analysis of the sample using all three methods is similar.

In terms of resources for introducing, maintenance and conducting analysis by the three discussed methods, volumetric method is the less expensive in terms of equipment, but analysts have to be with suitable experience for performing titration.

All aspects of the comparative discussion for suitability of different methods for assay of glucose in parenteral preparations stated above, indicate that HPLC/RID and polarimetry have advantage over the volumetric titration considering higher specificity of these methods. HPLC/RID and polarimetry, are suitable methods for assay of glucose, both methods are specific, precise and accurate and may be used as complementary methods for accurate determination of the concentration and purity of tested samples. However, the availability of the equipment and trained personnel may be the crucial factor for small laboratories for selection of the method for assay of glucose in parenteral preparations.

Conclusion

In this study, a comparative analysis of the results from assay of Glucose 5% *w/v* solution for intravenous infusion, obtained using three different compendial methods: HPLC/RID, polarimetry and volumetric titration, was performed, with a critical review of the applicability of each method.

The obtained results and discussion on all critical aspects of the proposed methods (accuracy, precision, time of analysis and resources for introducing, maintenance and conducting analysis), provide a valuable input to small laboratories on the suitability of these methods for quality control of Glucose 5% *w/v* solution for intravenous infusion.

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Резиме

Преглед на официјални методи за квантитативно определување на глукоза во раствори за интравенска инфузија

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Клучни зборови: Глукоза, HPLC/RID; Поларимеtрија, Волуметрија

Во трудот е даден преглед на фармакопејските методи за определување на содржината на глукоза во парентерални раствори (HPLC/RID метод, поларимеtрија и волуметриска титрација) и евалуација на сите критични аспекти на предложените методи, со фокус на точноста, прецизноста, времето на анализа и потребните ресурси за воведување, одржување и спроведување на анализата. Извршена е споредбена анализа на резултатите добиени со сите три методи од спроведеното испитување (десет независни определувања) на раствор од 5% *w/v* глукоза за интравенска инфузија, со којашто се потврди дека нема статистички значајна разлика помеѓу добиените резултати, што укажува дека сите три методи обезбедуваат валидни точни и прецизни резултати. Сепак, достапноста на опремата и обучениот персонал може да биде клучен фактор за малите лаборатории за избор на методот за определување на глукозата во парентерални препарати.

Добиените резултати и дискусијата за сите критични аспекти (точност, прецизност, време на анализа и ресурси за воведување, одржување и спроведување на анализа) на предложените методи, даваат значаен придонес за малите лаборатории во изборот на соодветен метод за контрола на квалитет на 5% w/v раствор на глукоза за интравенска инфузија.