Research Article

Development of sugars quantification method using polarimeter couple with UV-Visible quartz cuvette cell

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Abstract

The objective of this study was to develop the method for determination of sugars at low sample volume using UV-Visible quartz cuvette cuvett instead of polarimeter cell. This proposed method could be analysis of sugars with high sample throughput rate and appropriate for analysis of pharmaceutical formulation. This study, the simple determination method by using sample UV-Visible quartz cuvette was developed in order to analyze dextrose, sucrose and lactose. It was found that quartz cuvette was not affected from the sodium light source at wavelength of 589.0 nm and it was possible to adapt for polarimetry technique. The linear calibration curve of dextrose, sucrose and lactose standard solution using polarimeter sample tube were constructed in the concentration ranges of 2.5–15.0 % w/v. Linear regression between the angle of rotation (y) and concentration of each sample (x) were expressed as the equation of $y = 1.0941x - 0.1824$ ($r^2 = 0.9993$), $y = 1.3515x - 0.1581$ ($r^2 = 0.9998$) and $y = 1.0390x - 0.0162$ ($r^2 = 0.9997$) for dextrose, sucrose and lactose, respectively. Interestingly, the calibration curve of sugars standard solution using UV-Visible quartz cuvette were shown as $y = 0.1050x - 0.1038$ ($r^2 = 0.9990$), $y = 0.1312x - 0.1458$ ($r^2 = 0.9989$) and $y = 0.1072x - 0.0335$ ($r^2 = 0.9991$) for dextrose, sucrose and lactose, respectively. The detection limits (LOD) of proposed method for determination of dextrose, sucrose and lactose were 0.5, 1.0 and 0.6 % w/v, respectively, while the quantitation limits (LOQ) were 1.6, 3.0 and 1.9 %w/v for anlysis of dextrose, sucrose and lactose, respectively. The interferences study for proposed method from sodium chloride was investigated. It had found that this salt shown none effect for the proposed method. The results acquired the proposed method were compared favorably with those acquired by the reference method at a 95% confidence level with no significant difference (n=10). The developed method had shown accurate, precise, and reproducible. Moreover, this method indicated the possibility of a modified polarimetric method for the quantification of sugars. The method is possible to apply both in simple quality control laboratory and local industrial that involved with pharmaceutical production.

Keywords: Sugars, Polarimetric method, UV-Visible quartz cuvette cell, Sodium chloride

Introduction

Scientifically, sugar refers to any monosaccharide or disaccharide. Monosaccharides called "simple sugars" as glucose or dextrose which stores chemical energy that biological cells convert to other type of energy. Glucose is also found in human blood plasma having the molecular formula $C_6H_{12}O_6$. While disaccharide, a crystalline solid, known as "table sugar" or "saccharose". Commonsugar (sucrose) is made from sugar beets or sugar cane, edible crystalline substances including sucrose, lactose and fructose. Biochemists regard sugars as relatively simple carbohydrates. Sugars monosaccharides, disaccharides, trisaccharides and the oligosaccharides; containing 1, 2, 3 and 4 or more monosaccharide units, respectively. Sugar is a building blocks of carbohydrates and it is naturally found in many foods such as fruit, milk, vegetables and grain, another kind of sugar is added sugar which can be founded in flavored yogurt, sweetened beverages, baked goods and cereals, and it is used widely in industry. There are several types of carbohydrates, including monosaccharide and polysaccharide, which have many properties, whether in terms of the food industry or nutritionally. In addition to main function of sugars as sweetness, they also have others roles in food industry such as preservation, antioxidants, enhance the color, flavor and texture [1]. Interestingly, dextrose is a typical constituent in some of pharmaceutical products such as 5% dextrose in 1/3 NSS, 5% dextrose in NSS, 5% dextrose in water and others related product.

Sugars are source of sweetener and energy for the body. Sugar is one of typical compound for life. Due to the functioning of the internal organs and body tissues, these all have to use energy from sugar. There are three main types of sugars including single, double and macromolecule sugar. But in this study, dextrose, sucrose and lactose were used for developing the simple determination method. The several methods were reported for analysis of the sugars in pharmaceutical products, fruit juices and food including spectrophotometric [2], cryoscopic [3], flow based analysis [4], anion-exchang chromatography [5], fourier transform infrared spectroscopy [6], high performance liquid chromatography [7, 8] and liquid chromatography incouple with mass spectrometry [9]. However, many previously method are consisting of sophisticated procedure and instrumentation. On the other hand, the proposed method is present as a simple method for analysis dextrose, sucrose and lactose with the high sample throughput rate.

This study was interested for determination of sugars level by using polarimetric method incouple with UV-Visible quartz cuvette with polarimeter. Simple polarimetric method for analysis dextrose, sucrose and lactose were launched and investigated. The simple UV-Visible quartz cuvette was used to replace the classical polarimeter sample cell for analysis of dextrose, sucrose and lactose. The classical polarimeter have some disadvantages including technique needed to get rid of the air bubble from sample solution before analysis sample, using specific cylindrical sample cell, needed high sample volume (5 or 10 ml) and leak problem may be found. In contrast, the UV-Visible quartz cuvette sample cell is very simple because it needed low sample volume, at least not more than 2 mL and was not designed for tapping the air bubble before anlysis sample. It was found that the proposed method was provided high sample throughput rate when compared with using classical polarimeter cell. This is very useful for quality control laboratory that needed to reduce for production time of some pharmaceutical products. Some pharmaceutical factory had some limitation which could be solved by using this method for reduce time consuming in quality control (QC) processes.

It was found that UV-Visible quartz cuvette has none effect from the sodium light source at wavelength of 589.0 nm and it possible to adapt for polarimetric technique. The proposed method has some advantages such as no need for air bubble trap and show good linearity range, accepted precision and robustness. The limit of detection (LOD) and limit of quantitation (LOQ) were studied. The method is possible to be an alternative method for analyze sugars in pharmaceutical formulation such as dextrose and sodium chloride injection solution. The method could be expected can be used for analysis others type of sugar sample [10, 11].

Materials and methods

Chemicals and apparatus

All chemicals were of analytical reagent grade and were used without further purifcations. All solutions were prepared with distilled deionized water. The dextrose ($C_6H_{12}O_6$), sucrose ($C_{12}H_{22}O_{11}$) and lactose standard ($C_{12}H_{22}O_{11}$) were purchased from Sigma-Aldrich, USA.

Conventional polarimeter (POLAX-2L), Atago, Tokyo, Japan, was used throughout the study. This polarimetric method was equipped with 0.1 dL polarimeter cell or 1 cm in length of UV-Visible quartz cuvette.

Procedure

The experiment was employed with polarimeter (Atago Co. Ltd, Tokyo, Japan) and read out the angle of rotation value. The proposed method was used UV-Visible quartz cuvette as the sample cell. The experimental conditions of method were validated with respect to linearity and range, precision, detection limits (LOD) and quantitation limits (LOQ). The angle of rotation for the sugar sample solutions was determined using the sodium light source at wavelength of 589.0 nm and 25.0 °C. All solution was prepared using deionized distillation water throughout the experiment.

Standard preparation

A stock standard solution of dextrose, sucrose or lactose were prepared by accurately weighing 2.5–15.0 g of standard and transferred into 100 mL volumetric flask. The standard of dextrose, sucrose or lactose was weighted and dissolved with deionize distilled water and made up to the marker of volumetric flask. The volume of assigned concentration was pipetted and subsequently adjusted to 50 mL which were gave the required standard concentration (2.5, 5.0, 7.5, 10.0 and 15.0 % w/v) of dextrose, sucrose or lactose solution.

Marketed product analysis

All sugar samples were collected from pharmaceutical products of the State Enterprise for Pharmaceutical Factory No.3, Vientiane Capital, Lao PDR. Dextrose, sucrose and lactose was taking 10 of each sugar using simple random sampling technique for collecting sample. Each sugar sample was diluted to the appropriate volume with deionized distillation water in order to obtained the appropriate concentration of sample for analysis.

Sugar quantification by modified and conventional method

The comparison between modified and conventional method was investigated. The angle of rotation of the sugars solutions were determined under sodium lamp at a wavelength of 589.0 nm and 25.0 °C using polarimeter with polarimetric sample tube and UV-Visible quartz cuvette. The resulting from both method was proved by using *pair t*-test at a 95% confidence level [12].

Results

The determination of sugars including dextrose, sucrose and lactose, were investigated. The concentration for each sugars standard was studied in range of 2.5–15% (w/v). The determination of sugars was used polarimetric method incouple with polarimeter sample tube and UV-Visible quartz cuvette. Then, each standard solution was prepared and measured the angle of rotation vales using polarimetric sample tube and compared the results when replaced the sample cell with UV-Visible quartz cuvette. The angle of rotation of the standard solutions was recorded using polarimeter with the sodium light source at wavelength of 589.0 nm and was kept temperature of sample cell at 25.0 °C.

The calibration curve of dextrose, sucrose and lactose standard solution were constructed by using polarimeter tube (Figure 1) and UV-Visible quartz cuvette (Figure 2). Linear regression equation from the angle of rotation vales with polarimeter tube was investigated. It was found that the relationship between the angle of rotation (y) and concentration of each sample (x) was expressed by the equation $y = 1.0941x - 0.1824$ (r^2= 0.9993), $y = 1.3515x - 0.1581$ (r^2= 0.9998) and $y = 1.0390x - 0.0162$ (r^2= 0.9997) for dextrose, sucrose and lactose, respectively. Interestingly, the calibration curve of sugars standard solution when using UV-Visible quartz cuvette as sample cell were shown $y = 0.1050x - 0.1038$ (r^2= 0.9990), $y = 0.1312x - 0.1458$ (r^2= 0.9989) and $y = 0.1072x - 0.0335$ (r^2= 0.9991) for dextrose, sucrose and lactose, respectively. It was found that all correlation coefficient (r^2) value are shown in acceptable value (Table 1).

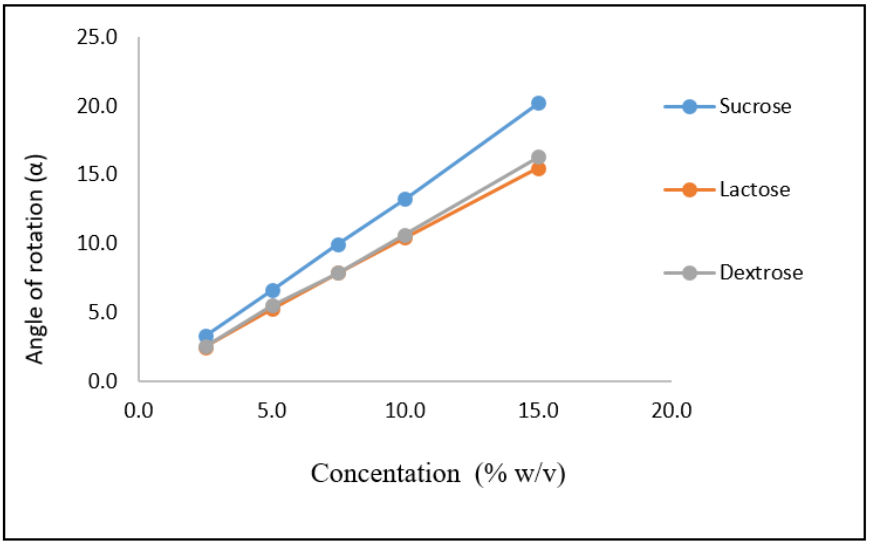


Figure 1 Clibration curve of sugars using polarimeter tube as sample container.

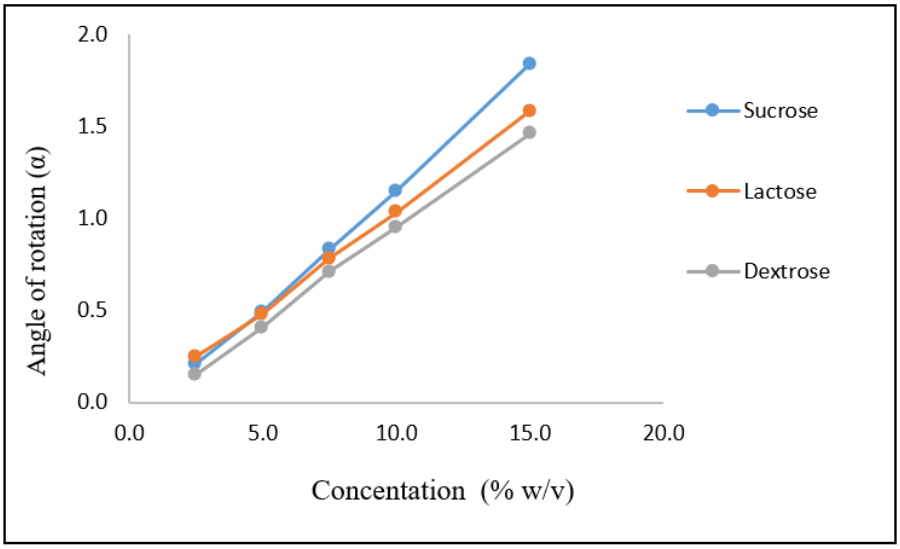


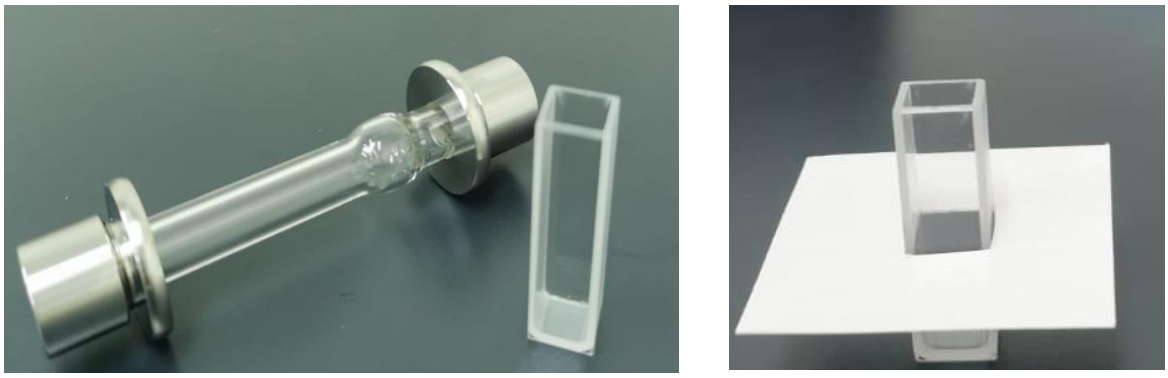
Figure 2 Clibration curve of sugars using UV-Visible quartz cuvette as sample container.

Table 1 Linear equation of sugars using polarimeter tube and UV- Visible quartz cuvette as sample container.

Sugars	Polarimeter tube using as sample container		UV-Visible quartz cuvette using as sample container	
	Regression equation	Correlation coefficient (r^2)	Regression equation	Correlation coefficient (r^2)
Dextrose	y = 1.0941x - 0.1824	0.9993	y = 0.1050x - 0.1038	0.9990
Sucrose	y = 1.3515x - 0.1581	0.9998	y = 0.1312x - 0.1458	0.9989
Lactose	y = 1.0390x - 0.0162	0.9997	y = 0.1072x - 0.0335	0.9991

Effect of UV-Visible quartz cuvette position on sugars determination

The effect of different placement region of modified sample UV-Visible quartz cuvette (Figure 3) use as sample container was investigated. The angle of rotation value was recorded for analysis dextrose, sucrose and lactose at five different concentrations (2.5, 5.0, 7.5, 10.0, and 15.0 %w/v) by placing the sample solution at three regions including extreme left, center and extreme right in the path of light (Figure 4). These were found that the different placement region of modified sample UV-Visible quartz cuvette had shown none affect on the angle of rotation value for analysis of dextrose, sucrose and lactose (Table 2).



(a) (b)

Figure 3 (a) Conventional polarimetry sample tube and UV-Visible quartz cuvette

(b) Modified sample UV-Visible quartz cuvette with paper holder

(a) (b) (c)

Figure 4 Studied effect of different placement region when using UV-Visible quartz cuvette as sample container.

(a) extreme left, (b) centre and (c) extreme righ

Table 2 Angle of rotation of sugars with different placement region.

Sugars Concentra-tion (%w/v)	Angle of rotation of sugars (*n*=3), (mean ± sd.)								
	Dextrose			Sucrose			Lactose		
	(a)*	(b)*	(c)*	(a)*	(b)*	(c)*	(a)*	(b)*	(c)*
2.5	0.15±0.00	0.15±0.00	0.15±0.00	0.21±0.03	0.21±0.03	0.21±0.03	0.18±0.03	0.17±0.03	0.17±0.03
5.0	0.41±0.03	0.40±0.00	0.40±0.00	0.55±0.00	0.52±0.03	0.53±0.03	0.43±0.03	0.42±0.03	0.42±0.03
7.5	0.71±0.03	0.70±0.00	0.70±0.00	0.92±0.03	0.88±0.03	0.91±0.03	0.70±0.00	0.68±0.00	0.67±0.00
10.0	0.95±0.00	0.95±0.00	0.93±0.03	1.23±0.03	1.23±0.03	1.22±0.03	0.98±0.03	0.97±0.03	0.95±0.00
15.0	1.47±0.03	1.47±0.03	1.47±0.03	1.90±0.00	1.91±0.00	1.93±0.03	1.43±0.03	1.43±0.03	1.42±0.03

(a)*; UV-Visible quartz cuvette placed extream left

(b)*; UV-Visible quartz cuvette placed centre

(c)*; UV-Visible quartz cuvette placed extream right

Validation of the method

The proposed method showed resonably analytical characteristic. The calibration curve of dextrose, sucrose and lactose standard solution using UV-Visible quartz cuvette was investigated and the correlation were 0.9990, 0.9989 and 0.9991, respectively.

The limit of detection (LOD) and the limit of quantitation (LOQ) were determined for sugars, based on the criteria that the signal to-noise ratio for LOD and LOQ are 3:1 and 10:1, respectively, for sample to blank. The detection limits for determination of dextrose, sucrose and lactose were 0.5, 1.0 and 0.6 % w/v, respectively, while the quantitation limits were 1.6, 3.0, 1.9 %w/v for anlysis of dextrose, sucrose and lactose, respectively.

The precision of the proposed method was studied through the repeatability by measuring twelve replicates of three standard sugars solutions (low, medium, and high: 3, 7 and 12 % w/v). Precision is expressed as standard deviation (sd.) of these three concentrations. It was found that the standard deviation was not higher than 0.02 for both of inter-day and intra-day studied (Table 3).

Accuracy was tested by means of recovery determinations. Seven replicated analyses of the same three standard sugars solutions (low, medium and high concentrations) led to mean recoveries values of 99.60±0.37–102.97±0.64, 98.44±0.49–103.01±1.05 and 99.29±1.20–103.06±1.12 for dextrose, sucrose and lactose, respectivelty. This shows that the proposed method provides accurate results (Table 4).

Table 3 Precision studies of sugars using UV-Visible quartz cuvette as sample cell.

Sugars	**Concentration**	**Angle of rotation, *n*=12 (mean±sd.)**	
	(%w/v)	**Inter-day**	**Intra-day**
Dextrose	3.0	0.19±0.02	0.19±0.02
	7.0	0.69±0.02	0.68±0.02
	12.0	1.18±0.02	1.19±0.02
Sucrose	3.0	0.26±0.02	0.26±0.02
	7.0	0.69±0.02	0.71±0.02
	12.0	1.41±0.02	1.42±0.02
Lactose	3.0	0.29±0.02	0.29±0.02
	7.0	0.71±0.02	0.70±0.00
	12.0	1.26±0.02	1.24±0.02

Table 4 Percentage of recoveries studies of sugars using UV-Visible quartz cuvette as sample cell.

Sugars	Concentration of tested (%w/v)	Percentage of recoveries, *n*=7 (mean±sd.)
Dextrose	3.0	102.05±1.01
	7.0	102.97±0.64
	12.0	99.60±0.37
	Mean	101.54±0.59
Sucrose	3.0	103.01±1.05
	7.0	98.44±0.49
	12.0	100.87±0.17
	Mean	100.77±0.57
Lactose	3.0	103.06±1.12
	7.0	99.29±1.20
	12.0	100.53±0.71
	Mean	100.96±1.01

Effect of sodium cation for dextrose determination

Synthetic standard of dextrose at concentration range 2.5–15.0 %w/v containing different concentrations of sodium cations were tested for interference using the proposed method. Sodium cation was added with the concentration in range of 0.0–4.0 %w/v to each of standard of dextrose solution (2.5–15.0 %w/v). The angle of rotation obtained were compared with the results recorded for the condition of without and with sodium cations. The results were found that the presence of sodium did not affect for dextrose determination (Table 5). This shows that the proposed method could be use for analysis dextrose in pharmaceutical formulation including dextrose and sodium chloride injection solution.

Table 5 Effect of sodium cation for dextrose determination.

Dextrose	Added Na (%w/v)	Angle of rotation, (n=3), (mean ± sd)	Relative of percentage (%)
2.5	0.0	0.25±0.00	100.0
	1.0	0.24±0.01	96.0
	2.0	0.24±0.01	96.0
	3.0	0.24±0.01	96.0
	4.0	0.24±0.01	96.0
5.0	0.0	0.48±0.00	100.0
	1.0	0.48±0.00	100.0
	2.0	0.50±0.02	104.0
	3.0	0.50±0.02	104.0
	4.0	0.50±0.02	104.0
7.5	0.0	0.78±0.00	100.0
	1.0	0.76±0.02	97.4
	2.0	0.76±0.02	97.4
	3.0	0.76±0.02	97.4
	4.0	0.76±0.02	97.4
10.0	0.0	1.05±0.00	100.0
	1.0	1.05±0.00	100.0
	2.0	1.06±0.01	100.9
	3.0	1.05±0.00	100.0
	4.0	1.05±0.00	100.0
15.0	0.0	1.61±0.00	100.0
	1.0	1.63±0.02	101.2
	2.0	1.63±0.02	101.2
	3.0	1.62±0.01	100.6
	4.0	1.63±0.02	101.2

Comparison between modified and conventional method

The method evaluation was proceeded by comparison between the classical polarimetric methods using the polarimetry sample cell and modified simple UV-Visible quartz cuvette. The commercial product (n= 10) were collected and determined dextrose, sucrose and lactose. The results acquired by the proposed method were compared favorably with those acquired by the reference method at a 95% confidence level with no significant difference by using pair t-test (Table 6).

Discussion

The calibration curve of sugars standard solution when using UV-Visible quartz cuvette as sample cell were shown $y = 0.1050x - 0.1038$ ($r^2 = 0.9990$), $y = 0.1312x - 0.1458$ ($r^2 = 0.9989$) and $y = 0.1072x - 0.0335$ ($r^2 = 0.9991$) for dextrose, sucrose and lactose, respectively. It was found that sucrose exhibited slightly higher sensitivity (defned as the slop of calibration graph) than dextrose and lactose. The detection limits for determination of dextrose, sucrose and lactose were 0.5, 1.0 and 0.6 % w/v, respectively, while the quantitation limits were 1.6, 3.0 and 1.9 %w/v for anlysis of dextrose, sucrose and lactose, respectively. Both of detection limits and quantitation limits are in acceptable values. Interestingly, the detection limits for determination of dextrose, sucrose and lactose were 0.4%, 0.2% and 0.2% w/v, respectively, when using the conventional method with polarimeter tube. The quantitation limits were 1.4%, 0.6% and 0.7% w/v for for dextrose, sucrose and lactose, respectively. It was found that the LOD and LOQ for dextrose determination were shown as same as for both of proposed and conventional method. The LOD and LOQ for sucrose or lactose determination from conventional method was slightly better than that received from the proposed method.

The precision of proposed method was not higher than 0.02 (sd.) for both of inter-day and intra-day studies. The average percentage recoveries of 3%, 7% and 12% w/v (n=7) of dextrose, sucrose and lactose were found to be 101.54±0.59, 100.77±0.57 and 100.96±1.01, respectively. It can be seen that the proposed method provided accurate results. The effect of sodium chloride was investigated with sodium ions which is known as dextrose in normal saline solution (NSS) products. The results were found that the presence of sodium did not affect for dextrose determination. The results acquired by the proposed method were compared favorably with those acquired by the classical polarimetric method at a 95% confidence level with no significant difference.

The advantages of this propose method may be summed up as follows: (1) It is faster in obtaining results, leading to tighter control during some pharmaceutical production; (2) it offers low-volume (1–2 mL) of sample for sugars analysis and ignores the angle of rotation value affect from air bubble of sugar solution sample; and (3) its simplicity compared to the classical polarimetric method. The disadvantage of this presented method could be not appropriate for determination of sugars which was found quantity lower than the LOQ of the methods but this study can prove that it can use UV-visible quartz cuvette replace polarimeter tube in general used for sugars determination.

Table 6 Statistical analysis of the proposed method compared with the official method of sugars determination.

No of commercial products	Dextrose (%w/v)		No of commercial products	Sucrose (%w/v)		No of commercial products	Lactose (%w/v)	
	Proposed method	Conventional method		Proposed method	Conventional method		Proposed method	Conventional method
1	4.43	5.25	1	4.80	4.84	1	4.59	5.02
2	3.83	5.23	2	4.80	4.84	2	4.91	5.02
3	4.43	5.25	3	4.93	4.82	3	4.59	5.02
4	5.04	5.27	4	4.54	4.86	4	4.91	5.10
5	4.73	4.23	5	4.80	4.85	5	4.91	5.10
6	5.04	5.27	6	4.80	4.86	6	5.23	5.09
7	9.87	9.92	7	9.20	9.33	7	9.02	9.06
8	9.27	9.82	8	9.20	9.33	8	9.02	10.01
9	10.01	9.78	9	9.20	9.33	9	9.97	10.06
10	9.87	9.80	10	9.46	9.32	10	10.28	10.10
t- test at 95 % confidence level:								
t-calculation	1.82			1.58			2.00	
t-critical value	2.26			2.26			2.26	

Conclusions

This study was method development for sugars determination by polarimeter couple with UV-Visible quartz cuvette. The calibration curve was linear (r^2 > 0.9989) for sugars determination in the range 2.5–15 % w/v. The proposed method is a simple and high sample throughput method for analysis of sugars. The developed method had shown accurate, precise, and reproducible. Moreover, this demonstrates method showed to be a modified polarimetric method for the quantification of sugars with low sample volume. From the result, this developed method is possible to apply for simple laboratory as same as local industrial including some pharmaceutical production or other kind of samples such as fruit juice, soft drink and others.

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