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Research Article

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A Standard Method Verification for Determination of Sugar Content of Commercial Fruit Juices by HPLC

Ticari Meyve Sularında HPLC ile Şeker Analizi için Standart Bir Metot Teyidi

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ABSTRACT

This study aimed to verify the standard test method TS EN 12630 and determine the sugar contents of commercially available fruit juices by high pressure liquid chromatography. A laboratory should verify the standard test method parameters in order to show its performance for the analysis, under consideration. For this purpose, Sucrose, Glucose and Fructose were analysed in orange juices obtained from the market. The principle of the method is based on the separation of sugars on a cation-exchange resin by isocratic elution with mobile phase, detection using a differential refractive index (RI) detector and external standard method. Accuracy and precision were performed via intraday and inter day studies to determine of accuracy and the precision (generally accepted as repeatability and reproducibility) for the standard test method. The recovery values of the sugars added into juice sample were found 92%, 99% and 96% for sucrose, glucose and fructose, respectively.

Key Words

Chromatography, sugar analysis, HPLC, packed fruit juice.

ÖZ

Bu çalışmada, TS EN 12630 standart metodunun doğrulanması ve piyasada bulunan meyve sularının şeker içeriklerinin yüksek basınçlı sıvı kromatografisi ile belirlenmesi amaçlanmıştır. Laboratuvar söz konusu standart test metodundaki performansını göstermek için, standart metodun parametrelerini teyit etmelidir. Bu amaçla piyasadan temin edilen portakal sularında Sükroz, Glikoz ve Fruktoz analizi yapılmıştır. Metodun prensibi, şekerlerin hareketli faz yardımı ile izokratik elüsyon ile bir katyon-değiştirici reçine üzerinde ayrılması, diferansiyel kırılma indisi (RI) dedektörü ve dış standart metodu kullanılarak saptanması esasına dayanmaktadır. Gün içi ve günler arası analizler ile yöntemin doğruluk ve kesinliği belirlenerek metot verifikasyonu gerçekleştirilmiştir. Meyve suyu örneğine eklenen şekerlerin geri kazanım değerleri sukroz, glikoz ve fruktoz için sırasıyla %92, %99 ve %96 bulunmuştur.

Anahtar Kelimeler

Kromatografi, şeker analizi, HPLC, paketli meyve suyu.

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INTRODUCTION

Fruit juice is considered to be one of the healthy foods in human diet. Industrialized fruit juices are the source of energy obtained via foods and beverages since they are considered healthy, practical, and nutritious. Fruit juice was recommended as the good source of vitamin and source of water for–infants and young children [1, 2, 3]. Even though there are some benefits of juice consumption, there are also some detrimental effects so that the high sugar content can result in increase in in taken calorie and dental problems. Additionally, the lack of protein and fiber in juice may result in inappropriate weight gain [1]. It is recommended that diabetic patients limit their sugar intake in line with studies showing that simple sugars may cause higher postprandial glycaemia than starch [4,5,6]. Hence, to avoid problems such as stimulating hyperglycemia, having recourse to insulin [7, 8], and causing possible cardiomyocyte dysfunction [9, 10] and/or enhanced loss of β-cells [11] diabetic patients have diets low in sugar [12].

The commercial juices are claimed to retain their nutritional effects. In the fruit juices production industry, there is a wide range of products that we can address according to the production, sourced and fruit itself and the properties we desire. Fruit juices differ from each other, especially natural and commercial juices. The different filtration, restoration, reconstitution and pasteurization techniques eventually give us a variety of features [13]. They have some components such as simple sugars and acids, originating from natural biochemical processes or some additional ones are also added. The flavor of fruits and juices are due to these constituents promoting a strong impact on the sensory quality and some chemical characteristics of this food, such as pH, total acidity, sweetness, microbial stability and general acceptability [14, 15].–The simple sugars which are glucose and fructose besides the disaccharide sucrose are found mainly in fruits and fruit juices [16]. They are present naturally or added externally to improve the sweetness and texture. Sucrose (table sugar) in commercial juices is probably added during production. Sugars are also used as preservatives [2].

In order to follow for the authenticity and purity of the fruit juices, the monitoring of these sugars is essential through quality control and process. Quantification of sugars can provide the estimation of the amount of artificial sugar present in the commercial products, allowing to detect the addition of a juice of different origin [17]. All these factors point to the reliable techniques to detect the quality of juices [2]. There are plenty of researches for the determination of various sugars in fruit juices via HPLC method. High performance liquid chromatography (HPLC) is a powerful technique for the analysis of sugars. Therefore, to evaluate the biochemical properties of commercial fruit juices, free sugars were measured by using the TS EN12630 (Fruit and Vegetable Juices-Glucose, Fructose, Sorbitol and Determination of Sucharose Content-High Performance Liquid Chromatography Method) Standard Method [18] with the refractive index detector. In the verification of a method, the laboratory has to confirm the parameters, assigned by the test method. Our laboratory verified a Turkish Standard Method, TS EN 12630. The standard method contains parameters for orange juice, apple juice, sour cherry juice, grape and orange juice such that the all experiment procedure is same for the each one except comparison parameters. We have used commercial orange juices for the verification studies but the standard method can be used for any commercial fruit juices.

MATERIALS and METHODS

The all sample preparations, calculations and verification were done as assigned in the standard method, TS EN 12630 (2001).

Materials

Reference standard materials for sucrose, glucose and fructose of high purity (99.5%) were obtained from Dr. Ehrenstorfer. EDTA tetrasodium salt was obtained from Merck. Deionized water was taken from a Millipore Simplicity. All solvents and reagents used in the study were HPLC or of analytical grade.

Standard Solutions

Standard solutions of glucose, fructose and sucrose were prepared in 10 g/L concentration in water, in volumetric flasks. After the confirmation of the retention times of the each one separately, standard solution was prepared in mixed form in 10 g/L concentration and used in the calculations.

Sample Preparation

The commercial orange juices, marked with “100 % and no sugar added”, bought from supermarket, were used in the experiments. Turbid samples were mixed thoroughly before dilution. Juices were diluted by using 1 part fruit juice and 4 parts water. These mixtures were centrifuged at 1400 g for 15 minutes. Then the samples were filtered

through a 0.45 µm mesh non-sterile hydrophilic syringe filter. The all samples were prepared as fresh and kept at 4°C (lab refrigerator) during the experiment day.

HPLC Instrument and Method Details

Analysis was carried out using Agilent 1260 Infinity Model HPLC equipped with a Refractive Index (RI) detector. TS EN 12630 standard method (Fruit and Vegetable Juices-Glucose, Fructose, Sorbitol and Determination of Sucharose Content-High Performance Liquid Chromatography Method) was used in the analysis. Chromatographic separation was performed on a Metacarb 67C (300 mm, 6.5 mm, A5235) model a cation-exchange column with a mobile phase of 0.1 mmol Calcium-disodium-EDTA solution in distilled water (HPLC grade). The flow rate was 0.5 ml/min and the temperature of the column oven was set at 90°C. The all sample injections were done two times from the same vial and the average value was used.

10 µl aliquots of the individual standards were injected onto the column and their retention times were determined. Identification of the peaks was done according to the response of the correspondence external standards. The mass concentration (ρ) of sugars and sorbitol were calculated using the following equation [18]:

$$\rho = \frac{P}{RF} \times F$$

ρ = Mass concentration of sugars and sorbitol in the sample (g/L)
P: The peak area or peak height of sugar or sorbitol
F: Dilution factor (This factor is 5 for fruit juices.)
RF: Appropriate response factor for sugar or sorbitol

$$RF = \frac{Ps}{\rho s}$$

Ps: Peak area or peak height obtained from the chromatogram of standard solutions of sugar
ρs : Mass concentration of sugar or sorbitol in standard solution (10 g/L)

A standard mixture of 10 g/L (glucose, fructose and sucrose) was prepared, and experiments were carried out, the obtained RF values were calculated separately for glucose, fructose and sucrose.

Method Verification

A verification study of the method was performed according to the "How to Meet ISO 17025 Requirements for Method Verification-2007 ALACC Guide" [19] description. For the repeatability of the method, fruit juice and sugar standards were analyzed 5 times on the same day. For the reproducibility of the method, two separate experiments were conducted consecutively in a day and the average was taken. It was studied for 2 non-consecutive days.

Standard deviation and relative standard deviation values, for both repeatability (Sr/RSDr) and reproducibility of within-laboratory tests (Si/RSDi) were calculated.

Recovery Studies

A standard addition technique was used in order to determine the percent recoveries of the sugars in juice samples for the accuracy of the method. The recovery experiments were carried out with all sugars standards for 6 consecutive days. The percent recovery was calculated as follows:
% Recovery = [C / (A + B)] × 100
where:

A – Amount of sugar found in the original sample (mg)
B – Amount of sugar added into the sample (mg)
C – Amount of sugar found in the standard added sample (mg)

RESULTS and DISCUSSION

Sucrose, fructose and glucose, which are the major sugars found in the orange juices were separated and analyzed with HPLC. A typical chromatogram of the sugar standards was given in Figure 1 and an example of the chromatogram of the commercial orange juice was given in Figure 2. Since the concentrations of glucose, fructose and sucrose in juices are major (>1%), the determination limit (LOD) has not been determined.

Quantification of sugars was based on TS EN 12630 standard method so sugar concentrations were determined from peak areas, by using the external standard method. During calculation, the dilution factor and the relationship between mass or volume values are taken into account. Retention times and average amounts of sucrose, glucose and fructose in orange juices are listed in Table 1. Standard deviation and relative standard deviation values, for both repeatability (Sr/RSDr) and reproducibility (Si/RSDi) are also given in the same

table. Intraday and interday analyses were performed according to the ALACC Guide description. The absolute difference, in between two single experiments for the identical samples, done by the same person, same method, by using the same equipment, can over the repeatability limit (r) one time in 20. For the repeatability, RSDr lab has to be lower than RSDr method and this requirement was verified by the laboratory. For the reproducibility, RSDi, coming from the same sample, same method, same person and the same laboratory but obtained in different consecutive times, has to be lower than the RSDRmethod, and this requirement was also confirmed by the laboratory (Table 1). Comparisons of the relative standard deviations of the lab and the standard method show that the all values of the laboratory for the glucose, sucrose and fructose are lower than the values of the method so that the laboratory confirmed the conditions assigned by the standard test method. The all actual values are given in Table 2.

The accuracy of the method was evaluated by means of recovery experiments. To show the accuracy of the method, a sample of fruit juice was analyzed before and after the addition of known amounts of the sugars. Recovery results between 80% and 110% are considered successful. The results of the recovery analysis were given in Table 3. The recovery for sucrose, glucose and fructose was found to be 92%, 99% and 96%, respectively so that it was shown that the method under consideration has the good accuracy.

In the case of the verification, which is a laboratory can adequately operate a standard method, the laboratory provide objective evidence for the performance parameters specified in the test method with the same matrices. Most often, the critical requirements are the accuracy and the precision (repeatability and reproducibility) which are important for the measurement uncertainty. They are assigned as the objective evidences for the actual lab data [19]. In the 100% juice category, orange is the favorite one representing 43.8% of the market, followed by apple with 16.9%, and multi fruit with 9.0%. Orange juice also occupies the first position with 26.2% of the market, with 16.0%, multi fruit is the second and with 8.5% mango is in the third position [20]. So, orange juice has a major role in the juice industry and the all practical and fast analyzing methods would find a place for the application in a way. Our laboratory verified the requirements for the accuracy and precision of TS EN 12630 (2001) standard test method which is a simple and fast sugar analysis method.

Table 1. Retention times of sugars and Repeatability and Reproducibility values of the study and standard method.

Sugars	Retention Time (min)	Average of amount (X) mg/mL	Sr	RSDr	Si	RSDi	RSDR Method	RSDr Method
Sucrose	7.72	32.14	0.13	0.004	0.18	0.006	0.024	0.013
Glucose	9.59	25.84	0.05	0.002	0.09	0.003	0.044	0.016
Fructose	11.47	27.53	0.03	0.001	0.11	0.004	0.040	0.014

Intraday (n=5) Inter day (n=2 for both 2 non-consecutive days)

X Arithmetic mean of measurements.

Sr Standard deviation of reproducibility.

RSDr Relative standard deviation of repeatability.

Si Standard deviation of within-laboratory relevance.

RSDi Relative standard deviation of within-laboratory relevance.

RSDR Relative standard deviation of within-laboratory relevance-given in standard method.

RSDr Relative standard deviation of repeatability-given in standard method.

Table 2. Comparisons of the relative standard deviation values.

Glucose
RSDr lab (0.002) <$RSDr_{method}$(0.016) RSDi lab (0.003) < $RSDR_{method}$ (0.044) RSDr < RSDi < RSDR 0.002 < 0.003 < 0.04
Fructose
RSDr lab (0.001) < $RSDr_{method}$ (0.014) RSDi lab (0.004) < $RSDR_{method}$ (0.040) RSDr < RSDi < RSDR 0.001 < 0.004 < 0.040
Sucrose
RSDr lab (0.004)< $RSDr_{method}$(0.013) RSDi lab (0.006) < $RSDR_{method}$ (0.024) RSDr < RSDi < RSDR 0.004 < 0.006 < 0.024

Table 3. Recovery values of sugars.

Sugars	Initial amount (g/L)	Added (g/L)	Found (g/L)	Recovery (%)
Sucrose	31.8 ± 1.9	5	36.5 ± 2.4	92 ± 0.2
Glucose	22.6 ± 2.5	5	27.6 ± 2.8	99 ± 0.1
Fructose	25.6 ± 2.1	5	30.4 ± 2.7	96 ± 0.2

Results are listed as mean ± SD (standard deviation) (n = 6).

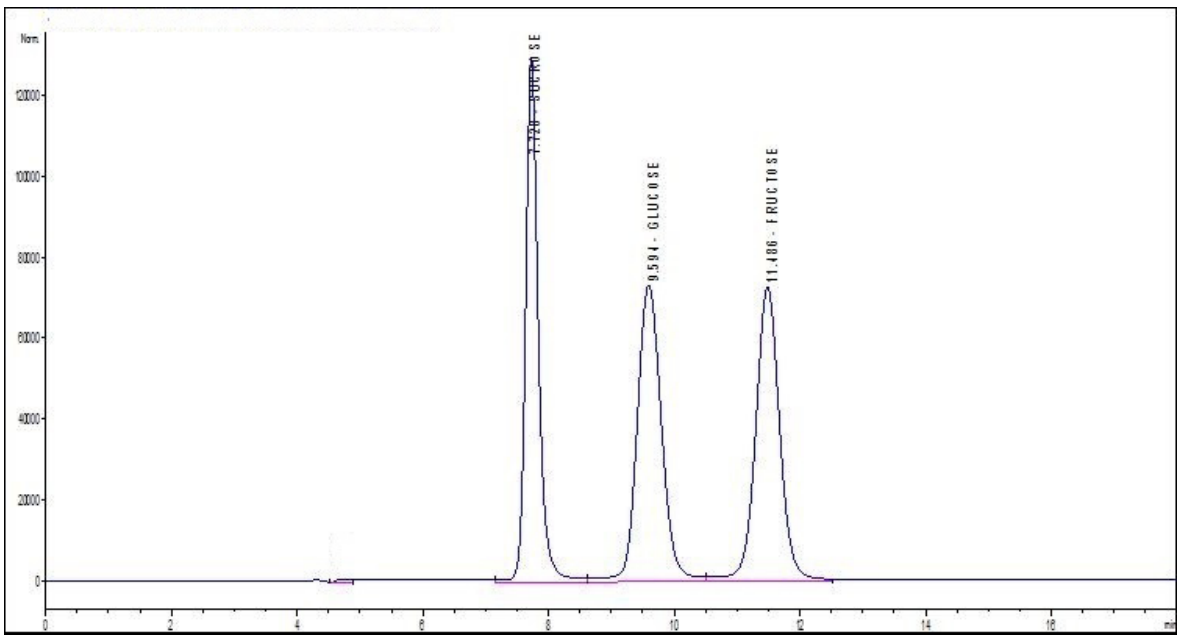

Figure 1. Chromatogram of the standards for sugars-10g/L.

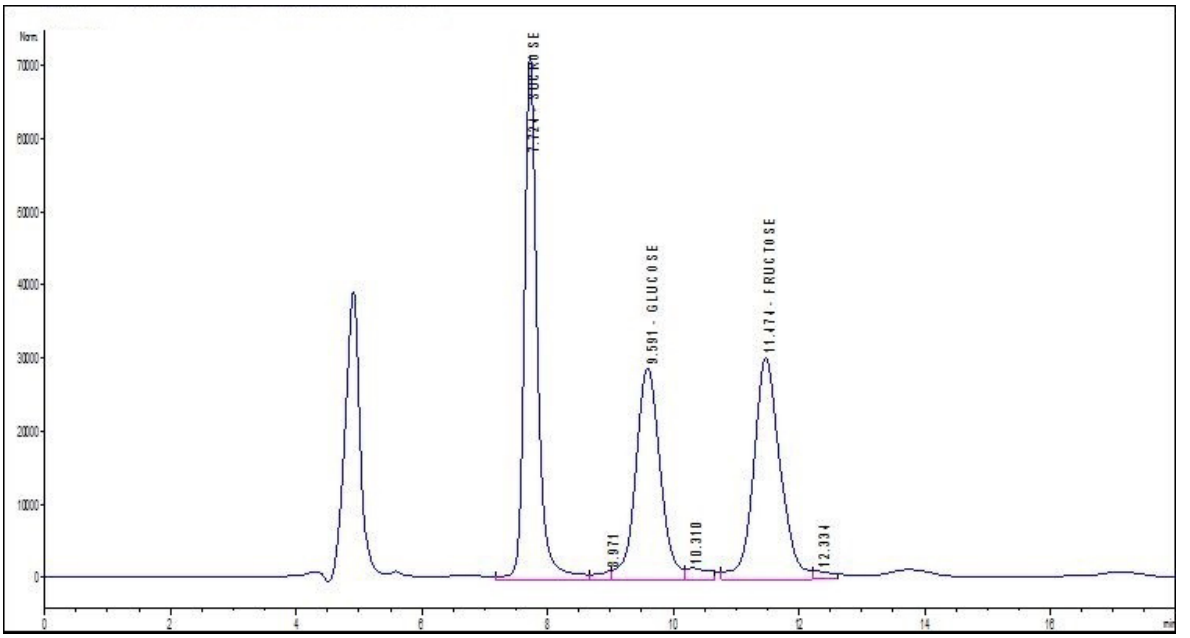

Figure 2. Chromatogram of a commercial orange juice.

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