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Evaluation of Different Sweetener Concentrations in Beverage Samples in Isfahan Market Using High Performance Liquid Chromatography

Abstract: Sweeteners were added in beverage formulations to provide sweetness with pleasant taste. In this paper, potent approach for quantification of acesulfame ok (ACE-ok), sodium saccharin (Na-SAC) and aspartame (ASP) contents in beverages had been applied with liquid chromatography (RP – HPLC) joint with diode array detection (DAD). Ninety-seven samples have been selected from Isfahan marketplace. Liquid chromatograph Agilent 1260 Infinity series was applied for analysis. Mobile phase was decided on as acetonitrile and phosphate buffer (pH= 3.5) with 15:85 extent ratio (V/V), and the float rate of the chromatographic device was Optimized at 1 ml min^{-1} using an isocratic elution system. All the beverages were saccharin-free and the mean concentrations of acesulfame and aspartame were lower than the general standards of the Iran National Standards Organization (600 mg L^{-1}). All the products were showed the acceptable levels of sweeteners, but, periodical and exact evaluation of these sweeteners in the beverages are still suggested to provide consumer health.
Key words: Saccharine, aspartame, acesulfame, beverages, HPLC.

Introduction

As the world's population increases, the request for beverages is increasing, so the investigation of the type and amount of these additives has become a global health challenge. Beverage companies use different additives such as preservatives and sweeteners to food products to maintain quality, taste, pH, increase shelf life, appearance, and consistency to make the product economical [1].

Artificial sweeteners are often included in drinks to improve taste and look [2]. These food additives are permitted for use according to the law, and in most countries, there are regulations that establish the highest allowable levels of these additives[3] or prohibit those that may lead to issues like asthma [4], allergies [5], cancer risk [6], hyperactivity [7] and Parkinson's disease [8]. It's crucial for people to understand the dangers linked to consuming these fake components and to carefully decide on their drink intake.

Among the artificial samples, acesulfame potassium, aspartame, saccharin and sucralose have the benefits such as cheaper rather than sweeteners and are more stable during food processing. Also, these sweeteners are not metabolized in the body and useful in patients with diabetes and overweight/obesity. However, in many countries, detecting the amount of sweeteners to avoid excessive addition or overuse has become important [9]. However, in most countries, detecting the content of artificial sweeteners to avoid extreme count or overuse has end up vital.

Evaluation and quantification of synthetic sweeteners is an important feature of quality control administration of meals stuff. To date, various analytical strategies had been detecting of sweeteners in beverage. Chromatographic technique such as high-performance liquid chromatography, (HPLC) [10, 11], gas chromatography (GC) [12], ion chromatography [13, 14] and spectroscopic techniques such as Infrared Spectroscopy (FTIR) [13-15] and UV/Vis Spectroscopy are in general used in quantification of sweeteners [16, 17].

Recently Cheng and co-workers developed a novel method by using column-switching UHPLC

single bond CAD for detecting 12 artificial and natural sweeteners [18]. In another work, Jankulovska developed a potent method for the determination of acesulfame (ACE-K), sodium saccharin (Na-SAC) and aspartame (ASP) in various drinks using reverse phase chromatographic system coupled with photodiode array detection technique [19].

Acesulfame (ACE-K), sodium saccharin (Na-SAC) and aspartame (ASP) are routine utilized in drinks as synthetic sweeteners. According to the Iran National Standards Organization, the acceptable range in carbonated soft drink is 600 mg kg^{-1} for ASP and ACE-K and all beverages should be saccharin-free [20]. Also, the suitable range in beverages are 80 mg L^{-1} for SAC, 350 mg L^{-1} for ACE-K and 600 mg L^{-1} for ASP based on European rules for food matrices additive [3].

In our study, HPLC-DAD method was utilized for the quantitation of ACE-K, SAC and ASP in different beverages available on the Isfahan markets. These sweeteners were chosen because they are mainly used in beverages, and HPLC method was used as an efficient technique because it is a fast, easy and obtainable method for many food quality centers.

Materials and methods

Instrumentation

HPLC analysis was performed with an Agilent 1260 Infinity II series contain a diode array detector (DAD) and quaternary pump, an autosampler part contain degasser, a thermostat and Adamas C18-Extreme (250*4.6 mm, 5 μm) column. The data was analyzed using Chem Station software. The pH of aqueous solutions was measured using a pH meter Metrohm (827, Switzerland). The solutions were sonicated with ultrasonic bath Elma. Both phosphate buffer and acetonitrile have been filtered through a 0.45 μm pore size filter (Whatman and MICROLAB).

Analytical standards and reagents

All the chemical substances have been used high purity grade or HPLC grade. Phosphoric acid (85.5%), methanol (>99.8%), acetonitrile (≥99.9 %), KH_2PO_4 (99%), and ultra-pure water have been purchased from Merck Chemical company, Germany. Analytical standards of Na-SAC (99.6%), ACE-K (99.4%), and ASP (99%) were obtained from Sigma-Aldrich chemical company.

Apparatus and chromatographic conditions

Analyst have been separated and quantified using a RP-HPLC system (Agilent Affinity 1260 II series) coupled with a degassing unit, pumps, autosampler, column oven, UV-Vis diode array detector (UV-DAD) with a reverse phase C18 column (Adamas C18-Extreme, 250*4.6 mm, 5 μm) at constant column temperature (25 °C). The drinks have been tested with DAD detector at 220 nm under isocratic system by utilizing acetonitrile/buffer phosphate (15:85) as mobile phase under optimal flow rate (1 ml/min).

Preparation of standard solutions

A stock standard solution was prepared by dissolving100 mg of each sweetener in a 100 ml volumetric flask with deionized water and brought up to volume. This solution contains 1000 ml/L of each sweetener and stored in brown glass for vessels. The working standard solutions were gained by diluting the sweeteners stock solutions in water to obtain aliquots of the following concentrations: 10, 20, 40, 60 and 80 mg L-1 for Na-SAC and ASP and 4, 8, 16, 24 and 32 mg L-1 for K-ACE. Working standard solutions were prepared daily.

Collection and preparation of samples

Commercially obtainable beverages (n = 97) were collected from the Isfahan market in Iran using a stratified random sampling method. All samples were collected in two different class, non-carbonated and carbonated drinks. In this work, samples were prepared easily. Carbonated drinks were placed in an ultrasound bath about 15 min at room temperature to eliminate the gasses. Then 10 ml of each sample transported into a 100 ml volumetric flask and up to a total volume with water. The non-carbonated semi solid samples were placed into a blender for 5 min until homogenized. The homogenized samples were vacuum filtered. After that, 10 ml of filtered was transported into a 100 ml volumetric flask and up to a total volume with water. The diluted solutions were then filtered with 0.45 μm syringe filter and were poured into a 2 mL sample vial for HPLC analysis. The filtered samples were injected replicate into the HPLC apparatus and the average values were calculated.

Preparation of buffer phosphate

0.1 g of potassium dihydrogen phosphate (KH_2PO_4) was poured into a 100 ml volumetric flask and up to a total volume with water. The buffer pH was set to 3.5 with diluted phosphoric acid (H_3PO_4). Filter under reduced pressure to remove insoluble substance before use with 0.45 μm membrane filter.

Statistical analysis

Statistical evaluation became finished by way of in Excel software program model 2019 in line with the descriptive exams. The attention of sweeteners becomes carried out in triplicate and final concentration were said as mean.

Results and discussion

Chromatographic settings were settled according to the European Standard EN-12856 [21]. In this manner, reverse phase C18 column was selected for the separation of ACE-K, Na-SAC and ASP because it could be used in different pH (2-9) of beverages [22]. UV-DAD detector was used for detection of artificial sweeteners because it shows suitable sensitivity in ppm levels for the quantities of artificial sweeteners. UV-DAD absorption was monitored at around 220. According to the literatures, the optimum separation for artificial sweeteners is in organic solvents by applying a phosphate buffer (pH 5-3.5) as a mobile phase [23]. Acetonitrile was selected as an organic solvent for quantification of sweeteners because of low back-pressure and shorter cutoff wavelength (190 nm) [24]. Mobile phase has been consisting of acetonitrile and phosphate buffer (V/V; 15:85, pH=3.5) at optimal flow rate of 1 ml min^{-1} under isocratic program. A typical chromatogram of a standard mixture of ACE-K (80 mg L^{-1}), Na-SAC (80 mg L^{-1}) and ASP (32 mg L^{-1}), at 220 nm was showed in Figure 1. As illustrated in Figure 1, the samples had been detected in the following retention times: 13.93, 4.45 and 13.5 minutes for ACE-K, Na-SAC and ASP respectively.

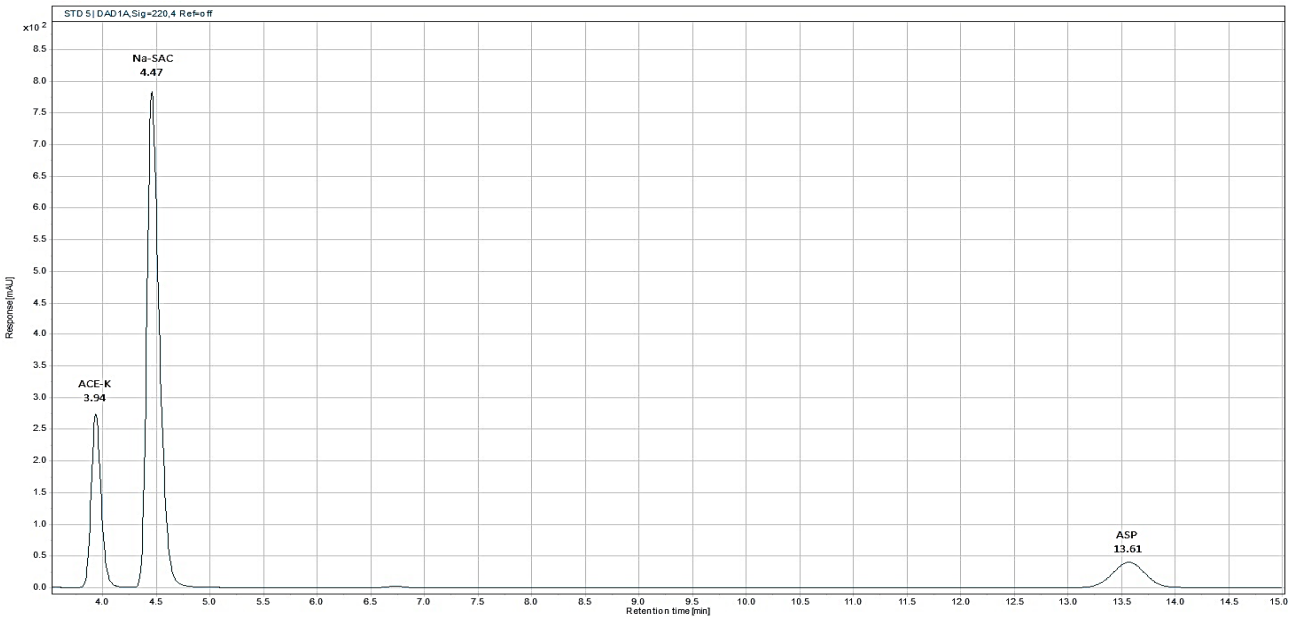

Figure 1 – Chromatograms of HPLC-DAD of a standard mixture of the examined sweeteners

For the linearity study, five concentration levels of synthetic sweeteners (10, 20, 40, 60 and 80 mg L^{-1} 1 for Na-SAC and ASP and 4, 8, 16, 24 and 32 mg L^{-1} for ACE-K) were selected and calibration curves were constructed (Figure 2). The calibration curve was made of three replications and fitted to a straight line using log10 scale values for both the x- and y-axes. Calibration equations and correlation coefficients (R^2) data of the standard solutions of sweeteners are illustrated in Table 1. According to the coefficient's values, the method exhibited good linearity of the analytical response in the examined concentration level. As can be seen in Table 1, the correlation coefficients for the standard curves of all sweeteners were more than 0.99, indicating that the method has suitable linearity.

The LOD is the lowest concentration of the sample that is detectable by of the analyte with certain level of certainty. The LOQ is the lowest concentration of the analyte that could be measured with appropriate precision and accuracy. The LOD and LOQ values are shown in Table 1. As shown in Table 1, the LOD values of sweeteners were 0.3, 0.2 and 4.2 ppm and the LOQ values were obtained 8, 8 and 13.8 ppm for ACE-K, Na-SAC and ASP respectively.

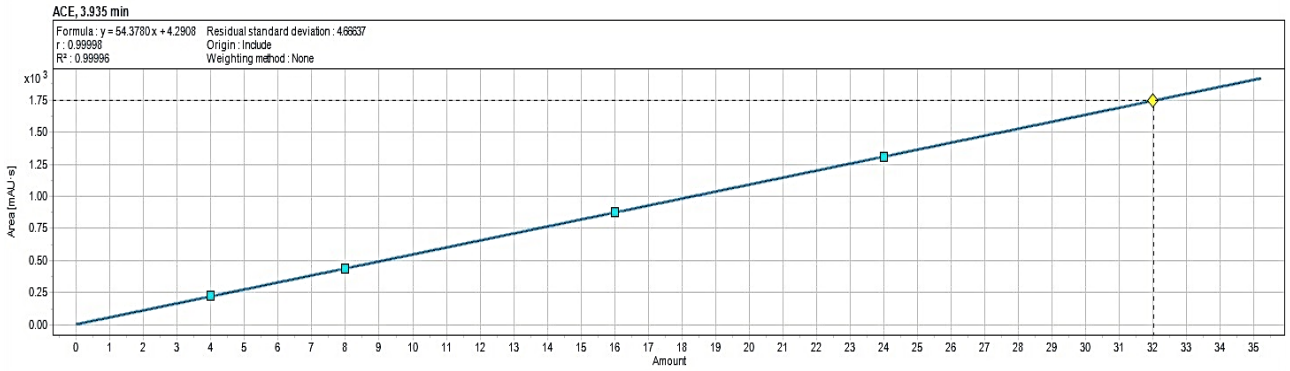

Figure 2 – The obtained calibration curve of ACE-K produce by HPLC–DAD method

Table 1 – Linearity and sensitivity obtained from the standard solutions of sweeteners (n=3)

Analyst	Calibration equations	R^2	LOD (mg L^{-1})	LOQ (mg L^{-1})
ACE-K	Y=54.241x+7.457	1	0.3	0.8
Na-SAC	Y=78.732x+14.405	1	0.2	0.8
Asp	Y=11.101x+15.657	0.9983	4.2	13.8

Repeatability is the precision under the same operational conditions over a short period of time [25]. In this study, the standard deviations (SD) of the pooled sample concentration were used to express the precision. The standard deviations were calculated for 12 and 8 samples of ACE-K and ASP respectively. The calculated standard deviations have been acquired 0.07 and 0.53 % for ACE-K and ASP respectively, since the results illustrated a proper intra-day precision.

Detected sweeteners (ACE-K, Na-SAC and ASP) by the optimized method were demonstrated in Table 2. According to the Table 2, between all the tested drinks, 12 samples contained acesulfame from 0.12 to 9.83 mg L^{-1} and showed in allowed concentrations according to the guideline. Also, none of the samples contained saccharin and aspartame were found in 8 samples ranging from18.19 mg L^{-1} to 40.42 mg L^{-1} that were lower than the standard range. Based on our results, 12.37%, 8.24%, 0% of all samples contained acesulfame, aspartame and saccharine respectively and were in acceptable ranges. The used method showed good linearity in the examined concentration levels and the correlation coefficients were more than 0.99 for all sweeteners and demonstrated that the method is linear. The method sensitivity of the method was acceptable because of the LOQs were less than the lowermost standard concentration except for ASP. Nevertheless, the LOQs for ASP was obtained near to the lowest minimum standard concentration. The data showed that the method is adequate for the detection and quantification of analyzed sweeteners.

Table 2 – The finding Concentrations (mg L^{-1}) of sweeteners (ACE-K, Na-SAC and ASP) in the collected beverages

Samples	Sample details	ACE-K, mg L^{-1}*	Na-SAC, mg L^{-1}	ASP, mg L^{-1}
S1	Carbonated with orange flavour	4.95	N.D [1]	40.42
S2	Black carbonated drink	9.83	N.D	18.19
S3	carbonated with mango flavour	5.07	N.D	26.60
S4	Carbonated with mojito flavour	3.76	N.D	27.57
S5	Carbonated with tropical flavour	4.52	N.D	23.45
S6	Landa, carbonated with peach flavour	3.69	N.D	26.50

Continuation of the table

Samples	Sample details	ACE-K, mg L^{-1}*	Na-SAC, mg L^{-1}	ASP, mg L^{-1}
S7	Sugar-free carbonated drink	7.73	N.D	39.05
S8	Non-carbonated with strawberry flavour	1.77	N.D	N.D
S9	Non-carbonated with mojito flavour	0.13	N.D	19.43
S10	Non-carbonated with malt flavour	0.27	N.D	N.D
S11	Non-carbonated with cherry flavour	0.12	N.D	N.D
S12	Non-carbonated with lemon flavour	6.61	N.D	N.D
Note: * indicates No detection				

Conclusion

In spite of the risk associated with the excessive use of sweeteners, the safe use of these additives in beverages can improve pleasant taste. For this reason, checking the usage of sweeteners in the meals enterprise and monitoring their quantity in excessive intake foods consisting of beverages is crucial for consumer fitness and economics. In this study, the amount of the three most common sweeteners namely saccharine, potassium acesulfame and aspartame were determined in different beverage samples in Isfahan, Iran. RP-HPLC method was used for determination of sweeteners. Chromatographic settings were applied according to the European Standard EN-12856. For this purpose, reverse phase C18 column and UV-DAD detector were used for detection of artificial sweeteners. According to the previous published, the best separation for artificial sweeteners is in organic solvents by applying a phosphate buffer (pH 5-3.5) as a mobile phase. Acetonitrile is a good selection as an organic solvent for quantification of sweeteners because of low backpressure and shorter cutoff wavelength (190 nm). In the optimal conditions, the mobile phase has been consisting of acetonitrile and phosphate buffer (V/V; 15:85, pH=3.5) at 1 ml min^{-1} flow rate under isocratic system. resulting retention times for samples were 13.93 min for ACE-K, 4.45 min for Na-SAC and 13.5 min for ASP under adjusted conditions. According to the results, all the beverages were saccharin-free and the mean concentrations of acesulfame and aspartame were lower than the general standards of the Iran National Standards Organization (600 mg L^{-1}). All the products were showed the acceptable levels of sweeteners, but, periodical and exact evaluation of these sweeteners in the beverages are still suggested to provide consumer health.

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Conflict of interest

The authors declare that they have no conflicts of interest.

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