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Original Research Article

Determination of Synthetic Food Colors, Caffeine, Sodium Benzoate and Potassium Sorbate in Sports Drinks

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Abstract

Purpose: *To employ high performance liquid chromatography (HPLC) techniques to determine edible additives in commercial sports drinks.*
Methods: *A total of 105 samples including 21 different brands of sports drinks were purchased from markets in Tehran, Iran. The process conditions included a C18 column, a mobile phase consisting of aqueous ammonium acetate buffer (pH = 4.2) and acetonitrile (80:20 v/v) at a flow rate of 0.8 ml min^{-1}. The injection volume was 20 µL and the UV detector was set at 225 nm.*
Results: *Mean recovery was between 95 and 106 %. The mean concentrations of sodium benzoate and potassium sorbate in Iranian brands (147.72 and 11.54 ppm, respectively) were significantly higher than in foreign brands (19.43 and 4.91 ppm, respectively, $p < 0.05$). The maximum amount of sodium benzoate and potassium sorbate was 251.50 ± 18.69 and 96.38 ± 38.56 ppm, respectively, while caffeine content was in the range of 293.48 ± 14.33 - 607.32 ± 135.33 ppm.*
Conclusion: *HPLC permits the detection of sodium benzoate and potassium sorbate at very low concentrations. Furthermore, caffeine was detected in all the sport drinks. Only Brilliant Blue and Allura Red were detected in the drinks.*

Keywords: *Edible additives, Sports drinks, Sodium benzoate, Potassium sorbate, Caffeine, Brilliant Blue, Allura Red*

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INTRODUCTION

Recently, sport drinks have gained popularity based on the attribution of energy-giving properties. Caffeine which is almost certainly the most widely consumed psychoactive substance in the world is a well-known stimulant effect over the central nervous system [1]. The US Food and drug Administration (FDA) limits the maximum amount in carbonated beverages to 6 mg/oz. [2]. On the other hand, preservatives are permitted as additives in energy and sport drinks [3-5]. Benzoic acid is sodium salt of benzene carboxylic acid and phenilcarboxylic acid, is 180 times more soluble than benzoic acid in water [6,7]. The additives may also cause allergic reactions in sensitive individuals as well as hyperactivity color. However, potassium solubility is more than 50 % in foods [6]. In Europe sorbates and benzoates are permitted in beverages [8].

For safety reasons, there have been recent reductions in the number of permitted food colors but because of their low price, effectiveness and stability they are still being used [9–11]. The lists of permitted food colors is definitely different between countries [12]. The beverage processing industry uses several types of food colors, but to minimize potential toxicity, the amounts of permitted synthetic colors used are strictly limited [11,13]. So they are permitted and frequently used in EU countries. However, they are not permitted in other countries, including Japan and USA [14].

The main objectives of this work were to evaluate the levels of mentioned additives compounds in 105 sport drinks and by comparing the levels of these compounds with the current available standards.

EXPERIMENTAL

A total number of 105 samples were purchased in accordance with the market availability, in Tehran - Iran. The samples were collected between December, 2013 and January. 2014. The studied commodities were 21 different brands of sports drinks. All stages of the experiment were done before expiry date of the drinks. The analyses were carried out in triplicate for each sample.

Standards and chemicals

All solvents/chemicals used were of analytical grade. In the study, high purity standards of potassium sorbate (> 99 %), sodium benzoate (> 99 %), sodium salicylate (> 99.5 %), Caffeine (> 99 %), ammonium acetate (98 %), acetic acid glacial (100 %) and HPLC - grade acetonitrile were purchased from Merck (Darmstadt, Germany). Deionised water was prepared by a Branstead Easypure II system (Thermo Fisher Scientific Inc., Waltham, MA, USA). Methanol (Merck) was HPLC quality. Acetic acid (Merck, d = 1.05) that was used for acetate buffer and NaOH (Merck, 5 mol/L) that was used for regulating pH were of analytical purity. Millipore Milli - Q water was used in all stages of the assay.

Chromatographic conditions

Sport drinks were filtered through 0.45 µm membrane filter and ultrasonicated, before HPLC analysis. Analytical separation of the caffeine, sodium benzoate and potassium sorbate was carried out by reverse phase liquid chromatography of an Agilent 1200 series liquid chromatograph equipped with a gradient pump capable of mixing four solvents, a vacuum membrane degasser, a 20 µL loop injector and a UV Detector (Agilent Technologies, Santa Clara, CA, USA). Analysis was performed on an Eclipse - XDB C18 column (150 × 4.6 mm, 5 µm). The mobile phase consisted of an aqueous ammonium acetate buffer (pH = 4.2) and acetonitrile (80:20 v/v) with a flow rate of 0.8 ml min – 1. The injection volume was 20 µL and the UV detector was set at 225 nm. The chromatographic system was initially conditioned by the mobile phase until a stable baseline signal was obtained, which needed at least 1 h.

To evaluate the synthetic colors, the mobile phase consisted of ammonium acetate solution (0.1 mol L-1, pH = 6.7), as solvent A and methanol - acetonitrile (50:50, v/v), as solvent B. A cellulose membrane with a pore diameter 0.45 µm was used to filter mobile phase A. In order to achieve a successful resolution, gradient elution programs were tested and the flow rate was always kept constant at 1 ml min-1. The final optimized gradient program was 3 % solvent B as the initial step; then it increased linearly to 60 % in 18 min and was held for 2 min. Analysis was performed on the UV detector with two optimized conditions. First, the fixed wavelength was set at 250 nm within the whole runtime to make it easy to use for amateur operators; next, it was programmed at 415 nm for tartrazine, 460 nm for Quinoline yellow, Ponceau 4R, and Sunset yellow, 500 nm for Allura red AC and Carmosine, and 600 nm for Indigo carmin and Brilliant blue for different colorants [11].

Data analysis

All measurements were replicated three times to improve the reliability of the results. Data were analysed using statistical program for social sciences (SPSS), version 21 (IBM SPSS Inc., Chicago, USA), Data are expressed as mean ± SD. One way analysis of variances (ANOVA - Dunnett T3) was used for determining significant difference which was set at $p < 0.05$.

Method validation

The LOD and the LOQ were calculated as LOD = 3.3 σ/S and LOQ = 10 σ/S, where σ is the standard deviation of the response and S is the slope of the calibration curve. The estimate of σ was carried out by analyzing blank samples seven times, measuring of the magnitude of the analytical background response and then calculating the standard deviation of the response. Recoveries were determined by spiking a sport drink known to be free of all

additives, in triplicate, with known amounts of studied additives at final concentrations between.

RESULTS

The analytical methodology revealed good linearity, sensitivity, exactitude and precision. Correlation coefficients (r^2) were comprised between 0.998 for caffeine acid and 0.999 for the other studied factors. Limits of qualification and quantification are presented in Table 1 respectively.

Recovery values were between 95 % and 106 % for studied additives at determined spiked levels. The developed method was successfully in the evaluation of the mentioned additives. Figure 1 shows the chromatograms of a standard solution containing sodium benzoate, potassium sorbate and also caffeine, while in Figure 2 the synthetic dyes resolution are illustrated.

The concentrations of preservatives quantified from sports- drink samples are shown in Table 2. Sodium benzoate was found in 14 brands at levels ranging between 12.50 and 251.50 ppm, (Table 2).

Moreover, 15 brands didn't have any potassium sorbate. The levels of sodium benzoate and potassium sorbate in Iranian brands (147.72 and 11.54 ppm, respectively) were significantly more than foreign studied brands (19.43 and 4.91 ppm respectively).

Also, methods to evaluate levels of coloring in foods for quality control are currently made by the TLC method. The mean concentration of colors in each sample group is presented in Table 3.

Table 1: Recovery data for the additives in sports drinks

Additive	Calibration Data	R^2	LOD (ppm)	LOQ (ppm)	Recovery (%)
Sodium benzoate	y=121.3x-56.82	0.999	0.6413	1.9435	99-102
Potassium sorbate	y=69.61x+36.64	0.999	1.1224	3.4013	98-100
Caffein	y=39.31x+9.87	0.998	1.8263	5.5344	97-101
Quinoline Yellow	y = 38.71x - 6.952	0.999	0.465	1.409	96-105
Sunset Yellow	y = 47.20x - 13.56	0.999	0.357	1.082	98-106
Carmoisine	y = 62.40x - 16.47	0.999	0.666	2.019	95-105
Ponceau 4R	y = 21.98x - 6.095	0.999	0.873	2.646	97-103
Allura Red	y = 70.08x - 10.09	0.999	0.510	1.547	95-102
Indigotine	y = 56.71x - 12.95	0.999	0.431	1.307	99-101
Brilliant Blue	y = 100.2x - 19.83	0.999	0.562	1.704	95-100

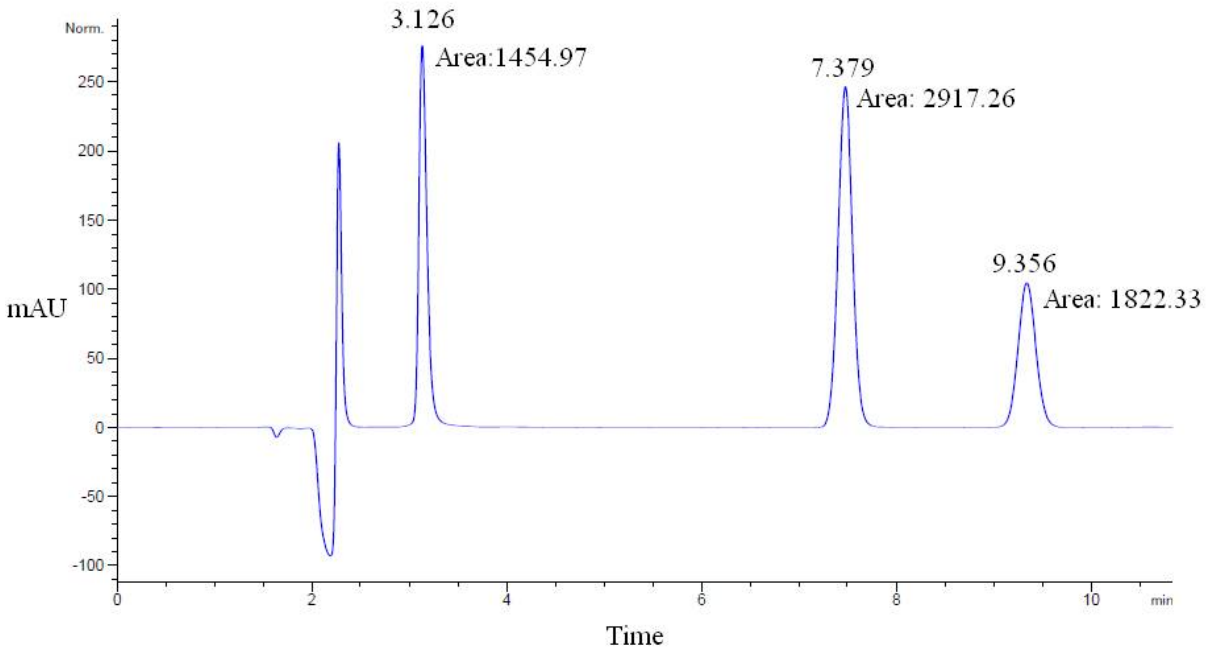

Fig 1: Chromatograms of a standard solution containing sodium benzoate, potassium sorbate and also caffeine.

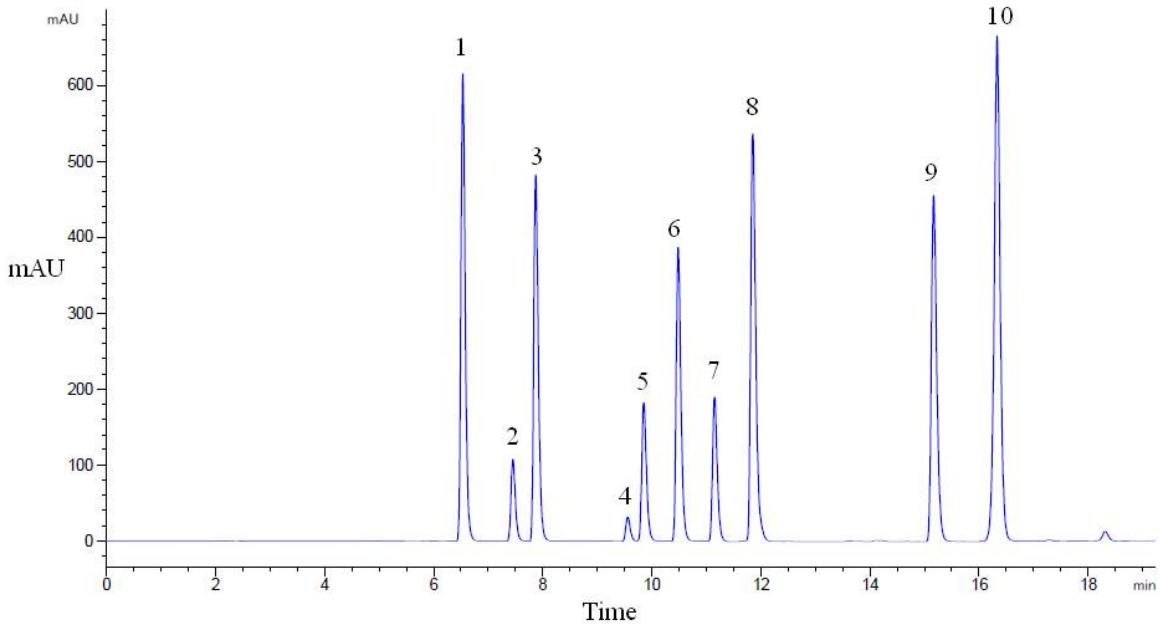

Fig 2: Resolution of chromatograms of synthetic dyes. 1 – Tartrazine; 2 - Quinoline yellow 1; 3 - Indigo carmine; 4 - Quinoline yellow 2; 5 - Ponceau 4R; 6 - Sunset yellow; 7 - Quinoline yellow 3; 8 - Allura red AC; 9 – Carmozine; 10 - Brilliant blue

DISCUSSION

Additives perform a variety of useful functions in foods that consumers often take for granted. Some additives could be eliminated if we were willing to grow our own food, harvest and grind it, spend many hours cooking and canning, or accept increased risks of food spoilage. Iranian National Standards [15] have some stringent rules for regulating the use of food additives. The level of sodium benzoate and potassium sorbate in Iranian standard (6693) [15] is 150 and 500 ppm, respectively. Therefore, based on the achieved results, 26.66 % of samples had higher sodium benzoate but all samples were in the range of sorbate.

Table 2: Sodium benzoate and potassium sorbate concentrations (ppm) in different brands of sports-drink

	Sodium benzoate		Potassium sorbate	
Brand	**Mean±SD**	**Range**	**Mean±SD**	**Range**
A	24.77±0.93	24.25-26.18	0	0
B	12.50±16.67	24.28-33.35	0	0
C	21.76±2.20	18.66-23.35	0	0
D	18.19±1.91	16.08-21.09	0	0
E	0	0	0	0
F	0	0	35.01±4.22	31.04-39.71
G	28.41±5.03	23.98-36.37	0	0
H	0	0	0	0
I	0	0	0	0
J	0	0	0	0
K	0	0	0	0
L	0	0	0	0
M	242.34±24.83	202.52-285.46	53.26±36.71	0-78.85
N	231.07±23.76	204.51-257.86	96.38±38.56	62.28-139.92
O	250.24±36.29	226.26-304.29	15.89±31.79	0-63.59
P	251.50±18.69	226.91-269.30	66.38±41.10	0-108.31
Q	144.99±77.02	17.75-218.55	28.80±29.51	0-66.61
R	138.43±17.74	123.24-150.93	0	0
S	163.14±18.40	131.99-178.51	0	0
T	105.18±6.62	94.30-111.86	0	0
U	95.66±109.51	18.23-173.10	0	0

Table 3: Caffeine and color concentrations (ppm) in different brands of sports-drink

Brand	Caffeine		Color Type	Color	
	Mean ±SD	Range		Mean ±SD	Range
A	345.21±114.24	174.45-389.60		0	0
B	443.87±36.75	403.21-496.36	Brilliant Blue	32.13±3.78	27.37-36.48
C	354.72±117.98	179.34-431	Allura Red	49.87±12.72	68.84-42.54
D	471.42±16.57	452.46-486.96		0	0
E	490.76±27.86	460-526.43		0	0
F	446.3±23.93	425.02-481.34		0	0
G	490.07±49.38	425.45-549.46		0	0
H	459.82±19.87	437.97-478.74		0	0
I	453.06±15.39	426.76-463.31		0	0
J	441.25±15.45	420.66-456.79		0	0
K	484.93±17.55	465.02-511.57		0	0
L	464.85±15	449.66-484.42		0	0
M	543.78±80.99	448.26-624.28	Allura Red	6.92±0.72	7.71-6.30
N	506.21±35.65	463.14-548.39		0	0
O	502.65±53.29	467.16-579.93		0	0
P	502±49.07	449.8-574.39		0	0
Q	607.32±135.33	503.61-836.99		0	0
R	514.02±36.16	499.39-572.56		0	0
S	293.48±14.33	284.77-314.68		0	0
T	547.72±223.68	235.66-776.31		0	0
U	531.28±37.83	504.53-558.04	Allura Red	14.54±0.52	14.92-14.17

For some foodstuff in the study by Mota *et al* [16], sorbic acid and/or benzoic acid were reported to range from not detected (n.d.) to 210 and n.d. to 153 mg/L, respectively. All sample concentrations of these compounds were below the stipulated limit of 300 mg/L for sorbic acid alone or 150 mg/L for benzoic acid alone [16]. In the three different samples of commercial wines, only sorbic acid was detected below 200 mg/L, which complied with the legislation for preservatives in wines [16]. In another related research which the benzoic and sorbic acids were in quince jam, all quince jams contained benzoic acid in concentrations ranging from 413.9.10.46 to 1501.4.2 mg of benzoic acid/kg [17]. In another study, it was shown that the amounts of benzoic and sorbic acid in jam were 639 mg/kg and 789 mg/kg, respectively. Furthermore, the benzoic acid content of five of jam samples in Ferreira *et al* was above the legal limits of 500 mg/kg in Portugal [17].

Although 300 mg/day of caffeine consumption is considered generally safe [18], there is no rapid method to determine caffeine content of sports-drinks. In this study, the caffeine determination in samples was in the range of 345.21 - 543.78 ppm in Iranian sports-drink samples. However, based on the results, other imported brands range was 293.40 - 607.33 ppm. Therefore, it must be asserted that caffeine amount in all samples was higher than the standard limitation of Iran (150 ppm) [15]. The lowest level of caffeine was detected in the soft drink, Coca Cola (43.71 ± 0.55), while the energy drinks showed the highest level of caffeine (58.31 ± 0.35 ppm). The range of caffeine content in the carbonated soft drinks group is between 43.71 and 45.83 ppm; while in it is from 47.56 to 58.31 ppm in the energy drink samples [19]. The mean caffeine content of carbonated soft drink was lower (44.52 ppm) than in the energy drinks (52.24 ppm. It was also observed that the caffeine level of the diet Cola (45.83 ± 1.05) was slightly higher compared to the regular cola drinks (Pepsi cola and Coca - cola). This is in agreement with previous work reported by Ali *et al* [20].

Food color additives are limited currently and only seven synthetic colors are permitted under these regulations. Color additives are used in foods for many reasons: to off-set color loss due to exposure to light, air, temperature extremes, moisture and storage conditions; to correct natural variations in colors; to enhance colors that occur naturally; and to provide color to colorless and “fun” foods. In the current study, all studied colors were not present in the sports drinks, but Brilliant blue and Allura Red were detected. In contrast to other countries, Ponceau 4R is prohibited in USA and Norway. Brilliant Blue is prohibited in many European countries. Tartrazine is also forbidden in the USA and Austria [11]. Based on the Iranian standard [15], there was no approved limits for sports drinks. Therefore, based on the achieved results, it seems that approved standards need to be revised both in terms of color type and related quantitative limitations made, regarding further risk assessments.

CONCLUSION

HPLC provides a suitable method for the detection of caffeine, sodium benzoate and potassium sorbate at very low concentrations in sports drinks. It can separate both preservatives at one wavelength in < 10 min and involves minimal sample preparation. The content of potassium sorbate was below legal limits. Non-compliance with legal limits was only encountered for sodium benzoate and synthetic colors in some samples.

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