CARPATHIAN JOURNAL OF FOOD SCIENCE AND TECHNOLOGY

journal homepage: http://chimie-biologie.ubm.ro/carpathian_journal/index.html

DETECTION OF SYNTHESIZED COLORANTS IN SPORTS DRINKS

Zhao Chen*

Track and Field Teaching and Research Office of Sports, Department of Physical Culture Instituteof Zhengzhou University, Zhengzhou, Henan, China; cdqdei@163.com

Article history:
Received:
13 January 2016
Accepted:
27 August 2016

Keywords:
Sports drink;
Colorants;
High performance liquid;
Detection method;
Artificial synthesis.

ABSTRACT

This study aimed at detecting synthesized colorants in sports drink to ensure the health of consumers. Synthesized colorants which are unbeneficial to human body in commonly seen sports drinks were taken as research subjects; detection and analysis method was used. First, the study discussed over the current research status using literature data method, expert interview method, interdisciplinarity method and experimental method. Then the pre-processed sports drink was detected using high pressure liquid chromatography (HPLC) and ultra-high pressure liquid chromatography (UPLC). Finally, the results obtained by detection based on HPLC and UPLC were compared and analyzed. Research results demonstrated that, the value of the linear correlation coefficient R for the detection of the pre-processed sports drink samples using HPLC was between 0.9906 and 0.9995, and the average recycling rate was between 80.5% and 97.1%. The linear correlation coefficient R for the detection of the pre-processed sports drink samples using UPLC was between 0.9909 and 0.9999, and the average recycling rate was between 71.3% and 113.5%. Compared to HPLC, UPLC was more sensitive. UPLC makes up the blank of multi-group detection of colorants and provides a technical support for the detection of colorants in sports drink.

1. Introduction

Food additives is a kind of material that can improve the color, favor and taste of food. The synthesized colorant, one kind of food additives, is able to control the color change of food and drink. Revision on the National food safety standard-standards for uses of food additives (GB 2760—2011) (Wang et al., 2011) includes 11 water-soluble artificially synthesized colorants (Liao et al., 2012; Cong et al., 2015) including quinoline yellow, brilliant blue, lemon yellow, sunset yellow, acid red, amaranth, new red, carmine, allura red, indigo blue and erythrosine. There are only 5 international standards for water-soluble artificially synthesized colorants in the current stage in China; besides, the components which require detection account for a small proportion and only cover a small part of foods in China.

Globally, many countries pay much attention to the application of colorants in food and drink. In 1960, American parliament passed Federal Food, Drug, and Cosmetic (FD&C) Act (Casadevall et al., 2014), emphasizing the importance of isolating colorants for management. The regulation claims that, colorants used in food, drugs and cosmetics must be approved by the Food and Drug Administration of America before entering into the market and samples of every batch produced by domestic or foreign producers should be strictly inspected for purification to confirm whether the colorants satisfy relevant limit standard. The European Union released 89/107/EEC which was related to food colorants (Türküler Isiksel, 2010). 94/36/EC which was proposed to normalize the use of food colorants gave a definition of colorants, and moreover the appendix exhibited the lists of colorants which were licensed to be used, forbidden to be used, limited to be used in usage and can be used in a moderate amount.

China also proposed the Edible Synthesized Dye Management Method (Wedge, 2013) in 1960 to temporarily monitor the application of food colorants in China. GBN 50-1977 The Applied Sanitary Standard of Food Additives (Sato, 2010) was formulated and put into trial use from 1973 to 1977. In 1980, Revision on the National Food Safety Standard-Standards for Uses of Food additives (GB 2760—2011) was released; afterwards, it was supplemented and amended for several times. Currently, GB 2760-2014 The Applied Sanitary Standards of Food Additives is taken as the national standard, and moreover the synthesized colorants allowed for use, the ones allowed for use within a limited quantity and those allowed for use in food according to the production needs.

Artificially synthesized coloring matters refer to organic coloring matters prepared using artificial chemical synthesis method. Most of the synthesized coloring matters that are allowed to be used globally are water-soluble coloring matters (Dupuy and Stéphanie, 2014). But the recent studies suggested that, nearly all the synthesized coloring matters could not provide human body with nutritional substances and some of them might even threaten the health of human body for its carcinogenicity (Verhagen et al., 2011; Boobis et al., 2013). In this study, synthesized colorants in sports drink were detected using high pressure liquid chromatography (HPLC) and ultra-high pressure liquid chromatography (UPLC). This work provides relevant departments with a reference and some suggestions in the aspect of the detection of synthesized colorants.

2. Study of detection methods for artificially synthesized colorants in sports drink

The intake of artificially synthesized colorants can block the absorption of vitamins, which can thereby damage nervous system and induce a series of symptoms such as the absence of mind. Moreover, artificially synthesized colorants are not easy to be oxidized and eliminated when accumulating in human body. Some colorants can affect liver functions as well and they will combine with target cells in human body to form tumor cells after decomposition. Therefore, the detection of colorants in sports drink is especially important.

2.1. Referable instrument method

With the development of science and technology, more and more novel instruments and methods have been developed. The detection methods of artificially synthesized coloring in sports drink include HPLC (Svec and Frechet, 2012), high-performance liquid chromatography-mass spectrometry (HPLC/ES-MS) (Vicente et al., 2015), electrochemical analysis method (Mattsson, 2015), capillary electrophoresis, fluorescence analytical method (Gostishchev et al., 2012), thin-layer chromatography and polyamide adsorption method. In this study, HPLC, HPLC-EC-MS, electrochemical analysis method and fluorescence analytical method were used.

HPLC adopts a novel infusion pump including a high-sensitive detector and a high efficiency particle stationary phase. It operates to elute and isolate solutes with different absorption capacity and molecular size through their multiple exchanges between stationary phase and moving phase. Combining the advantages of both HPLC and MS, HPLC/ES-MS not only has the high efficient isolation ability of liquid chromatography, but also can provide structural information like MS. Electrochemical analysis method is applied for analyzing the electrochemical properties of substances in solution which is also usually used for isolating and identifying substances such as protein, nucleic acid and amino acid. Fluorescence analytical method is established based on different absorption wavelengths of substances under electromagnetic radiation. It is featured by high sensitivity, less sampling volume and convenient use, but there are many interference factors.

2.2. Referable pre-processing method

Through looking up relevant literature data and interviewing experts, we found that, the preprocessing of sports drink before experiment was in certain relationship with the detection of some physiological indexes. In this study, the adopted methods included polyamide absorption method, liquid-liquid extraction method, direct sample introduction method, microwave assisted extraction method and solid phase extraction.

Polyamide absorption method completes the extraction of coloring matters through optimizing the dose of polyamide adsorbent, apparatus and eluent. Liquid-liquid extraction method purifies the samples by making use of the characteristic that the detected components and impurities are incompatible. Though it is easy to be operated, poor repeatability, low recycling rate and emulsification can frequently occur. Direct sample introduction method simplifies the complex preprocessing process, which can significantly improve working efficiency (Tokita et al., 2014) and save cost, and moreover, more microelements which are difficult to be detected can be detected using the method (Gomes, et al., 2015). Microwave assisted extraction method is featured by short extraction time, high extraction precision, high extraction efficacy and small dose of extractants. When solid phase extraction is used, substances in detected samples which have influence on target chemical are isolated effectively using non-liquid adsorbing agents at first and then the absorption of target chemical is relieved using eluent. The method can isolate target chemicals effectively (Behbahani et al., 2013).

2.3. Sample processing and condition optimization

Before HPLC and UPLC, the samples were pre-processed according to the national standard (Chang et al., 2013). The samples were scanned when the detection wavelength of HPLC was set as 500 nm and 20 mmol of ammonium acetate solution (A), acetonitrile (B) and methyl alcohol (C) were taken as flow phase and when the detection wavelength of UPLC was set as 260, 312, 300, 400, 427, 500, 540 and 634 nm respectively and 20 mmol of ammonium acetate solution (A) and methyl alcohol (B) were taken as flow phase. The set of elution procedures are shown in Table 1.

Table 1. Gradient elution procedures

		HPLC			UPLC	
Time	Flow speed mL/min	A Ammonium acetate (%)	B Acetonitrile (%)	C Methyl alcohol (%)	A Ammonium acetate (%)	B Methyl alcohol (%)
0	1	90	0	10	90	10
8	1	75	6	24	75	25
11	1	70	5	25	70	30
13	1	43	0	57	68	32
30	1	38	4	59	55	45
38	1	18	2	80	0	80
42	1	18	2	80	0	100
50	1	90	0	10	90	10

2.4. Two liquid chromatography methods

2.4.1 The detection of colorants using UPLC

The working principle of HPLC (Nugroho, 2011) is basically the same with that of general traditional detection methods; its characteristic lies on the application of infusion pump with relatively large pressure. In this study, the commonly seen sports drinks were taken as research subjects; five kinds of colorants, i.e., acid orange 10, acid red 1, acid red 14, acid black 1 and acid yellow 2 were detected using HPLC.

2.4.2 The detection of colorants using HPLC

Compared to HPLC, UPLC (Hung et al., 2011) can analyze compounds which are finite or exist in complex matrix, thus to achieve better isolation effect; the isolation of substances which is difficult to be realized using high performance liquid chromatograph in short time can be realized using ultra-high performance liquid chromatograph; besides, UPLC improves resolution and sensitivity, shortens time taken for analysis, reduces the dose of solvent and lowers analysis cost, which can effectively save cost and realize the optimization of time benefit.

3. Results and discussions

3.1. Chromatogram of HPLC and UPLC

The subjects of this study were all polar substances which are soluble in water or methyl alcohol. Therefore, reversed-phase chromatography was selected for separation and detection. A spectrogram was obtained based on the above chromatographic conditions, as shown in Figure 1.

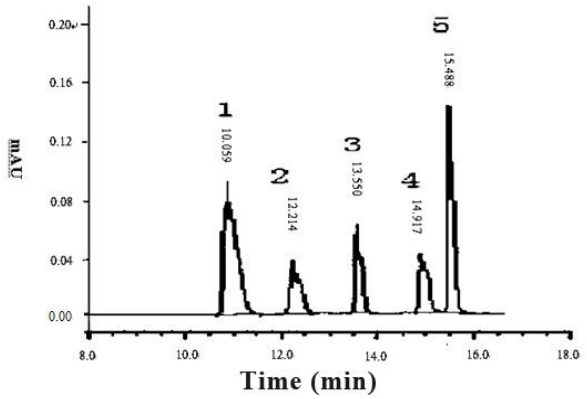

Figure 1. High performance liquid chromatograph of samples

As shown in Figure 1, 1 represents acid orange 10, 2 represents acid red 1, 3 represents acid red 14, 4 represents acid black 1, and 5 represents acid orange 2.

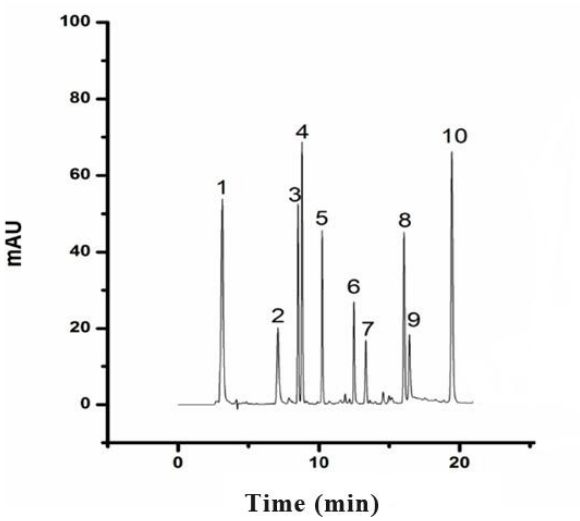

Figure 2. Ultra-high performance liquid chromatograph of samples

As shown in Figure 2, 1 represents acid yellow 23, 2 represents acid red 18, 3 represents acid purple 7, 4 represents acid orange 10, 5 represents acid red 35, 6 represents acid red 14, 7 represents acid green 5, 8 represents acid red 73, 9 represents acid black 1, and 10 represents acid orange 7. The experimental results demonstrated that, UPLC was applicable to the quantitative and qualitative detection and analysis of drink as it could detect multiple kinds of colorants.

3.2. Detection limit and linear range of colorants

HPLC: Five kinds of colorants were diluted into 5.0 mg/L, 10 mg/L and 100.0 mg/mL respectively using methanol solution. Then a coordinate was established, taking mass concentration (mg/ml) as the horizontal coordinate and peak area as the vertical coordinate. A standard curve was drawn after relevant data were substituted; then the linear regression equation was calculated. The correlation coefficients and detection items are shown in Table 2.

Table 2. Linear regression equations, correlation coefficients and detection limits of five kinds of colorants

Colorants	Linear range (mg/L)	Linear equation	Correlation coefficient	Detection limit (mg/kg)
Acid orange 10	5~100	y=19.7785x-3.6885	0.99995	0.016
Acid red 1	5~100	y=14.3568x-8.6271	0.99988	0.016
Acid red 14	5~100	y=18.8842x-12.1968	0.99995	0.061
Acid black 1	5~100	y=12.6795x-21.9655	0.99982	0.007
Acid yellow 2	5~100	y=41.5095x-9.8566	0.99996	0.092

UPLC: Ten kinds of colorants were diluted into 5.0 mg/L, 10 mg/L, 20 mg/L, 40 mg/L and 80 mg/L respectively using methanol solution. A coordinate was established, taking mass concentration (mg/mL) as the horizontal coordinate and peak area as the vertical coordinate. A standard curve was drawn after relevant data were substituted. The results are shown in Table 3.

Table 3. Linear regression equations, correlation coefficients and detection limits of 10 kinds of colorants

Colorants	Linear range (mg/L)	Linear equation	Correlation coefficient R
Acid yellow 23	5~50	y=25.0556x+33.2477	0.99096
Acid red 18	5~50	y=11.8875x-17.0491	0.99468
Acid purple 7	5~50	y=21.0102x-2.7975	0.99993
Acid orange 10	5~50	y=19.2741x-6.3333	0.99973
Acid red 35	5~50	y=5.51136x-2.0595	0.99952
Acid red 14	5~50	y=18.9478x-4.9321	0.99951
Acid green 5	5~50	y=68.1438x-2.4865	0.99968
Acid red 73	5~50	y=28.0635x+3.8665	0.99963
Acid black 1	5~50	y=20.3515x-32.1906	0.99536
Acid orange 7	5~50	y=39.2795x+2.4155	0.99983

3.3. Preciseness and recovery test

To test the preciseness of HPLC, mixed standard solutions made of five kinds of colorants (0.05, 0.1 and 0.2 mg/L) were added into blank drink samples respectively. The detection was performed according to the test method. After six times of parallel detection, the average value was calculated. The results are shown in Table 4. Results demonstrated that, the average recovery rates were larger than 83.5% and the relative standard deviations (RSD) were smaller than 1.55%, suggesting HPLC was accurate and reliable. Seven kinds of acid industrial dyes were extracted using alkalescence solvent. The experimental results demonstrated that, the recovery rate was high. Besides, a detection and analysis method which could be used for detecting seven kinds of acid industrial dyes in functional drink, i.e., HPLC, was set up. The method was simple, high-efficient and highly sensitive and moreover the RSD and recovery rate satisfied the requirements of relevant departments.

Table 4: The preciseness of adding standard recovery rate of HPLC

Component	Adding standard matter amount (mg/L)	Measured value (mg/L)	Relative standard deviation (%)	Recovery rate (%)
Acid orange 10	0.05	0.0416	0.33	83.6
	0.1	0.0806	1.58	80.4
	0.2	0.1733	0.26	86.6
Acid red 1	0.05	0.0405	0.45	81.3
	0.1	0.0829	0.27	82.6
	0.2	0.1765	0.99	88.1
Acid red 14	0.05	0.0415	0.32	82.6
	0.1	0.0918	0.58	91.5
	0.2	0.1964	0.40	98.3
Acid black 1	0.05	0.0407	0.52	81.1
	0.1	0.0901	1.25	89.8
	0.2	0.1914	0.33	95.7
Acid orange 2	0.05	0.0416	1.03	83.3
	0.1	0.0903	0.41	90.3
	0.2	0.1925	0.30	96.1

In the process of new product development, effects of the concentration of organic solvent, the category of buffer solution, the concentration of buffer salt, the acid value of flow phase, controlled temperature and the PH value of flow phase on products should be paid attention to.

In UPLC test, mixed standard solutions made of five kinds of colorants (5.0, 10.0 and 20.0 mg/L) were added into blank drink samples respectively. Parallel detection was performed for 6 times. The results are shown in Table 5.

Table 5. The preciseness of adding standard recovery rate of UPLC

Component	Adding standard matter amount (mg/L)	Measured value (mg/L)	Relative standard deviation (%)	Recovery rate (%)
Acid yellow 23	5	5.24	3.26	106.5
	10	10.19	3.42	101.9
	20	22.76	0.89	113.6
Acid red 18	5	4.88	1.86	102.5
	10	10.87	1.92	108.6
	20	19.75	1.51	98.8
Acid purple 7	5	5.026	1.72	100.5
	10	10.4	1.51	100.1
	20	19.72	1.45	98.6
Acid orange 10	5	4.88	2.24	97.5
	10	9.88	2.36	98.6
	20	19.16	4.01	95.8
Acid red 35	5	5.01	2.09	100.2
	10	10.68	2.55	106.8

	20	19.58	3.77	98.0
Acid red 14	5	4.73	3.38	94.3
	10	9.75	1.59	97.5
	20	17.51	5.18	87.6
Acid green 5	5	4.41	1.99	87.9
	10	9.43	1.02	94.3
	20	17.42	4.42	87.2
Acid red 73	5	4.88	1.71	97.5
	10	9.91	1.38	98.8
	20	19.56	3.62	97.8
Acid black 1	5	5.06	2.06	100.9
	10	10.55	7.51	105.2
	20	20.81	1.05	103.8
Acid orange 7	5	4.33	2.66	96.5
	10	9.39	0.82	93.9
	20	19.16	1.32	95.8

Results demonstrated that, the average recovery rates were larger than 89.8% and the RSD was between 1.50% and 3.58% (n = 4), suggesting UPLC was accurate and reliable. With the assistance of ultrasonic wave, 10 kinds of acid industrial colorants were extracted. Through optimization, the detection of sports drink became simpler. Compared to HPLC, UPLC is featured by simple operation, high efficacy, high sensitivity, high recovery rate and good repeatability, which provides relevant departments with a technical support in the aspect of the detection of sports drink.

4. Conclusions

In this study, HPLC and UPLC were applied to detect and analyze the artificially synthesized colorants in sports drink. The research results suggested that, the number of categories of synthesized colorants detected out by UPLC was more than that by HPLC; detecting colorants with UPLC was more sensitive than HPLC; moreover, the average recovery rate of samples detected by UPLC was larger. Thus, it can be concluded that, UPLC is more suitable for the detection and analysis of artificially synthesized colorants in sports drink. UPLC makes up the deficiency of colorant detection and provides relevant departments with a technical support in the aspect of colorants detection in sports drink, which can ensure the personal safety interest of consumers.

5. References

Behbahani, M., Salarian, M., Bagheri, A. et al. (2013). Synthesis, characterization and analytical application of Zn(II)-imprinted polymer as an efficient solid-phase extraction technique for trace determination of zinc ions in food samples. *Journal of Food Composition & Analysis*, 34(1), 81-89.

Boobis, A., Chiodini, A., Hoekstra, J. et al. (2013). Critical appraisal of the assessment of benefits and risks for foods, 'BRAFO Consensus Working Group'. Food & Chemical Toxicology An International *Journal Published for the British Industrial Biological Research Association*, 55, 659-675.

Casadevall, N., Felix, T., Strober, B.E. et al. (2014). Similar Names for Similar Biologics. *Biodrugs Clinical Immunotherapeutics Biopharmaceuticals & Gene Therapy*, 28(5), 439-44.

Chang, J., Xin, R., Ding, M. et al. (2013). Improvement of National Standard Method for the Determination of the Contents of Sorbic Acid, Benzoic Acid and Saccharin

Sodium in Wine and Mixed Wine. *Liquor-Making Science & Technology*, 16(3), 175-179.

Cong, K., Fodjo, E.K., Dan, L. et al. (2015). Chitosan-based adsorption and freeze deproteinization: Improved extraction and purification of synthetic colorants from protein-rich food samples. *Food Chemistry*, 188, 240-247.

Dupuy, S. (2014). Comparative Research of Standard and Management of Food Additives at Home and Aboard and Economic Benefit Analysis of Rational Use. *Advance Journal of Food Science & Technology*, 6(11).

Gomes, P.C.F.L., Tomita, I.N., Álvaro, J., Santos-Neto et al. (2015). Rapid determination of 12 antibiotics and caffeine in sewage and bioreactor effluent by online column-switching liquid chromatography /tandem mass spectrometry. *Analytical & Bioanalytical Chemistry*, 407(29), 8787-8801.

Gostishchev, V.K., Khomenko, V.A., Kuzmina, A.G. et al. (2012). Laser fluorescence analysis method use for express-diagnosis of antibiotic resistance among abdominal sepsis patients post-operation. *Journal of Innovative Optical Health Sciences*, 05(3):724-725.

Hung, P.V., Hatcher, D.W., Barker, W. (2011). Phenolic acid composition of sprouted wheats by ultra-performance liquid chromatography (UPLC) and their antioxidant activities. *Food Chemistry*, 126(4), 1896-1901.

Liao, Q.G., Wei, H.L., Lin, G.L. (2012). Applicability of accelerated solvent extraction for synthetic colorants analysis in meat products with ultrahigh performance liquid chromatography–photodiode array detection. *Analytica Chimica Acta*, 716(4), 128-132.

Mattsson, M.S. (2015). Li insertion into WO3: Introduction of a new electrochemical analysis method and comparison with impedance spectroscopy and the galvanostatic intermittent titration technique. *Solid State Ionics*, 131(3-4), 261-273.

Nugroho, A.S.U.W. (2011). Validation of High-Performance Liquid Chromatography Analysis on Phenolic Substances of Cirsium setidens and Sedative Effect of Pectolinarin as the Active Principle. *Natural Product Sciences*, 17(4), 342-349.

Sato, K. (2010). Trend in food additives administration of Japan. *Journal of the Food Hygienic Society of Japan*, 51(51), 331-335.

Svec, F., Frechet, J.M.J. (2012). Continuous rods of macroporous polymer as high-performance liquid chromatography separation media. *Journal of Japan Academy of Nursing Science*, 32(2), 279-281.

Tokita, K., Takazumi, K., Oshima, T. et al. (2014). A New Method for Analyzing the Characteristic Flavor of Beer Using Selectable One-Dimensional or Two-Dimensional Gas Chromatography-Olfactometry/Mass Spectrometry. *Journal of the American Society of Brewing Chemists*, 72(2), 154-161.

Türküler Isiksel, N. (2010). Fundamental rights in the EU after Kadi and Al Barakaat. *European Law Journal*, 16(5), 551–577.

Verhagen, H., Andersen, R., Antoine, J.M. et al. (2011). Application of the BRAFO tiered approach for benefit-risk assessment to case studies on dietary interventions. Food & Chemical Toxicology An International Journal Published for the British Industrial Biological Research Association, *50 Suppl*, 4(1), S710–S723.

Vicente, F.B., Vespa, G., Miller, A. et al. (2015). Quantification of Iohexol in Serum by High - Performance Liquid Chromatography - Tandem Mass Spectrometry (LC-MS/MS). *Methods in Molecular Biology*, 1383, 185-193.

Wang, H., Zhang, J., Zhang, J. (2011). Revision on the National food safety standard-standards for uses of food

additives (GB 2760—2011). *Chinese Journal of Food Hygiene*, 23(6), 571-575.

Wedge, N.E. (2013). Analysis of Interim Measures for the Administration Fixed Asset Loans. *Dummy Auxiliaries in First & Second Language Acquisition*, 49.