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UPLC Detection of Acid Dyes in Sports Drinks

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In recent years, sports drinks continue to be pursued and admired by sports groups, especially sports enthusiasts. It can be said that as long as there is a drinks area, it will exist. Because of the vigorous development of sports drinks market, some unscrupulous businessmen, in order to seize the market, reduce costs, and seek huge profits, illegally addsynthetic colorants to the products to replace the natural colorants. In contrast to the various illegally added substances suitable for drinks, it is found that acid dyes,this kind of synthetic pigment can effectively reduce the production cost in the production, and the production process relative to the natural edible pigment is short in cycle, high in efficiency, and low in price, thus easy to be used by unscrupulous businessmen. In this paper, the detection of the most common illegally added substances - synthetic colorants in sports drinks is studied. It establishes ultra performance liquid chromatography (UPLC) method for rapid detection of colour agent in the sports drink. The method is sensitive, efficient and good in separation effect, which can be used in routine testing for a variety of synthetic colorants - acidic industrial dyes in sports drinks.

1. Introduction

Because synthetic colorant is low in production cost, it gives people a good sense, and it is featured by easy to be dissolved in water and can be directly added. Some applications disable of adding pigment in food occurs, the acid dye synthetic pigment is taken as the object in this paper. For the commonly-used illegal addition of acid dyes contains the following materials: synthetic - acid yellow substances, synthetic - acid black substances, synthetic - acid orange substances, synthetic - acid red substance, synthetic - acid blue substance, synthetic - acid green substance, synthetic - acid brown substance, synthetic - acid violet substance more than 40 kinds of synthetic pigments acid dyes commonly used and commonly seen (Cao, et al., 2015). With the widely use of coloring purposes, related organizations and companies continue to develop and utilize new materials, and even substances have been banned are re-prohibited used. Adding and overuse of synthetic colorants in the drinks will cause certain harm to the health. The experimental results show that: if the human intakes a large number of synthetic pigments or industrial pigments, it will lightly appear allergies, breathing difficulties, throat swelling and redness, itching skin pain nerve, headache and other symptoms, while seriously it may lead to carcinogenesis or death. As a result, in the era with rapid development of sports drinks and sales surging, it is particularly important how to find the unsafe factors in sports drinks, to ensure sports drinks ingredients safety, and to establish a method of quickly detecting the adding pigment in sports drinks.

2. HPLC working principle

High performance liquid chromatography (HPLC) system consists of some major parts, including an equipment reserves liquid, a pressure pump, automatic sampling equipment, chromatographic column, automatic detection equipment, the recorder and so on. With the high-pressure of pump, the mobile phase in the equipment reserving liquid is introduced into the automatic sampling system. The sample solution and the mobile phase are combined through a certain proportion, and through the mobile phase, sample solution are brought into the chromatographic column, and separated by the column. The same material and different materials are grouped again, respectively through the instrument interface connector entering the mass spectrometer ion source. Different compounds components in the sample solution, through numerous

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repeated adsorption-desorption processes, make use of the difference of components in compound flow time to classify points into a single group, outflowing from the column according to the order (Chen, et al., 2016). When the detected compounds going through the detection channel, the quality concentration of the sample is converted into electronic signals form and transported to the electronic recording equipment. All kinds of data of compounds are displayed in the computer equipment in the form of wavelength.

At present, chromatographic analysis method often used and widely applied is high performance liquid chromatography HPLC. The whole system is composed of mobile phase transmission device, reserve liquid pressure pump, sample system, chromatographic column, detection device and recording display instrument. Generally speaking, its instrument device is similar to gas chromatography instrument, mainly making different changes for mobile phase liquid properties. High performance liquid has strict requirements on pump conditions. For example, the flow rate of fluid flow is supposed to be uniform; inlet system should be graded in the sample and good in sealing (Franquet-Griell, et al., 2016). Column is usually thick in the diameter and short in the length, for which mainly the liquid is easy to adsorb the column in the chromatographic column so as to make the column pressure not so high.

From the working principle of the instrument, there is no fundamental difference in high performance liquid chromatography with classical liquid chromatography. Its characteristic is also mainly adopting the infusion pump with higher pressure, the detector with sensitive response value, and high efficiency particulate stationary phase. For the organic compounds with different polarity, it can also make a good analysis of high boiling point, difficult to volatile, and high molecular weight ones.

3. Research objects and methods

Judge according to the national health standard, sports drinks can be divided into the following categories: sports drinks containing gas in a liquid, and that does not contain gas, solid sports drinks and solid inflatable sports drinks. The main object of this study is the detection method of synthetic pigment of two kinds of sports drinks, liquid sports drinks not containing gas and drinks containing gas. Data are processed and analyzed by using the method of experiment and with the help of HPLC instrument.

4. Materials and methods

4.1 Main instrument

Agilent - 1200 high performance liquid chromatography is equipped with DAD UV detector; AL204-IC electronic analytical balance (produced by Mettler Toledo Shanghai Instrument Co., Ltd.); PHS-3C electronic pH, Allegra X-22R centrifuge (produced by Chinese Beckman Kurt Trading Co., Ltd.); N-EVAP-112 nitrogen concentration instrument Organomation Assoeiate SIne (Gallo, et al., 2017); KQ-600 B ultrasonic cleaner (produced by Kunshan Ultrasonic Instrument Co., Ltd.); diaphragm vacuum pump (produced by Tianjin Auto Sainz Instruments Co. Ltd.); and 0.22 m organic membrane.

4.2 Materials and reagents

Acid orange 10, acid red 1, acid red 14, acid black 1, acid orange-yellow 2, acid violet 43, acid blue 62 (purity more than 98%, purchased from Dr Ehrenstorfer Corporation); acetonitrile and methanol (produced by Chromatography Pure Fisher Corporation); ammonia, pure analysis (produced by Tianjin Fuyu Fine Chemical Co. Ltd.), ammonium acetate, HPLC grade (produced by German Merck Corporation); formic acid, pure analysis (produced by Xi'an Chemical Reagent Factory), and test water for Milli-Q ultra-pure water.

4.3 Experiment method

4.3.1 Sample processing method

(1) For the optimum conditions of extraction effect, drinks containing gas are carried out with ultrasonic operation for 10 minutes by using ultrasonic instrument, and further heated for 30 minutes by using the heater to modulate temperature for 50 DEG C, to make the gas in the carbonated beverages further release cleanly.

(2) Take sample 10 g to add to 7 kinds of acid dye standard solution 10 g/g, fully mix, and place for 24 h.

(3) Take 28 branch of the centrifuge tubes and group the number.

(4) Accurately take 2 g beverage samples and place on the 50mL centrifugal tube, add 10 mL of methanol (containing 1% formic acid) -10 mmol/L ammonium acetate solution (volume ratio V:V=1:1) as extracted liquid, assisting extraction, ultrasound for 15 minutes (Li, et al., 2015); each sample extracts 2 times, combines the extraction liquid 2 times, volume set to 25 mL, take 2 mL of the sample extraction liquid, nitrogen blowing to nearly dry, use acetonitrile-water (volume ratio of 2: 8) dissolved 1 mL solution, passing over 0.22μm membrane, and finally determine by HPLC.

Table 1: Scalar quantity of all kinds of matters

Group	Name	Scalar quantity (mg/L)			
		Blank sample	No.1 sample tube	No.2 sample tube	No.3 sample tube
A	Acid orange 10	0	0.05	0.1	0.2
B	Acid red 1	0	0.05	0.1	0.2
C	Acid red 14	0	0.05	0.1	0.2
D	Acid black 1	0	0.05	0.1	0.2
E	Acid orange-yellow 2	0	0.05	0.1	0.2
F	Acid violet 43	0	0.05	0.1	0.2
G	Acid blue 62	0	0.05	0.1	0.2

4.3.2 Chromatographic condition

Column model: symmetry shield RP18 model, 250 mm x 4.6 mm, 5μm; column temperature: 30 degrees Celsius; detection wavelength: 500 nm. The mobile phase: acetonitrile and 10 mmoL ammonium acetate solution; flow rate: 1 mL/min; detector: UV detector; injection volume: 10μL. The gradient elution procedure was used in this experiment, and the elution conditions were shown in table 2.

Table 2: HPLC gradient elution condition

Time	Flow	%A	%B	%C	%D	Curve
0	0.8	90	0	10	0	6
10	0.8	75	0	25	0	6
18	0.8	0	0	100	0	6
22	0.8	0	0	100	0	6
26	0.8	90	0	10	0	6
29	0.8	90	0	10	0	6
30	0.8	90	0	10	0	6

5. Optimization of chromatographic conditions

5.1 Column selection

Using RP18 column symmetry shield 250 * 4.6 mm, 5 μm column can obtain a higher degree of separation, sample flux and sensitivity. Investigate the separation effect of different columns on seven kinds of acid dyes. C8(linear paraffin), HILIC (silica gel), and HSST3 (three C18 alkyl bonded) are compared respectively (Oh and Shin, 2015), and the results showed that the symmetry shield RP18 column has the best separation effect, so choose the column.

5.2 Mobile phase selection

Respectively select ultra-pure water, methanol and acetonitrile as the mobile phase to make different tests. The tests showed that taking acetonitrile -10mmoL ammonium acetate (Ph=4.5) as mobile phase, separation and chromatographic peak can reach ideal effect. Further compare the separation effect of acetonitrile -5mmoL ammonium acetate and acetonitrile -5mmoL ammonium acetate (Ph=4.5) 9Pan, et al., 2016). From mobile phase out of three groups, it is found that 10mmoL ammonium acetate is the optimal dose. By optimizing the mobile phase, effective separation of seven kinds of acid dyes in sports drinks is obtained, e.g. Figure 1, 2, and 3.

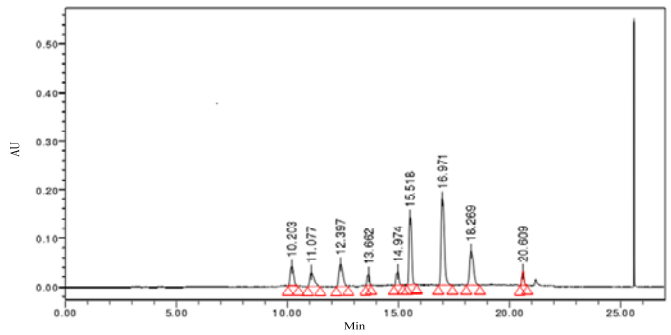

Figure 1: Acetonitrile-5mmoL ammonium acetate and acetonitrile

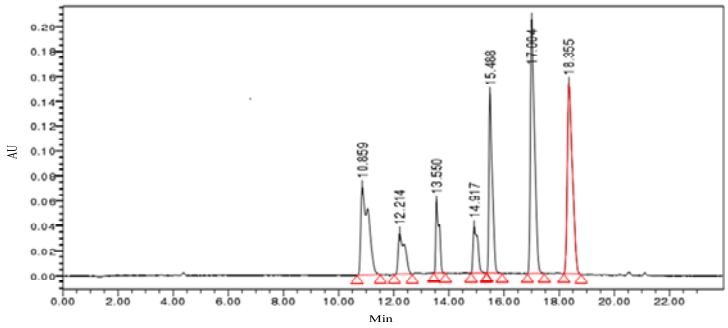

Figure 2: Acetonitrile-5mmoL ammonium acetate

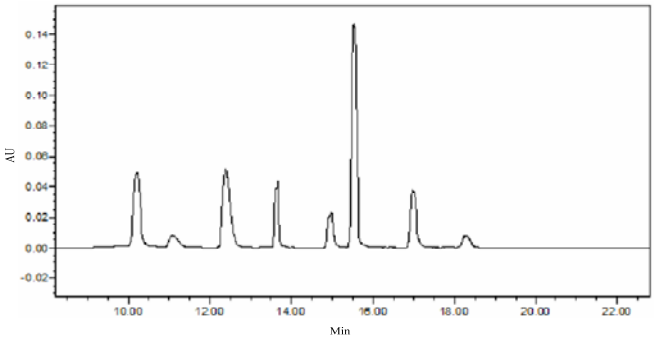

Figure 3: Acetonitrile-10 mmoL ammonium acetate (Ph=4.5)

5.3 Chromatographic behavior

According to the experimental method, test the spiked sample chromatogram in the blank sports drinks, e.g. Figure 4.

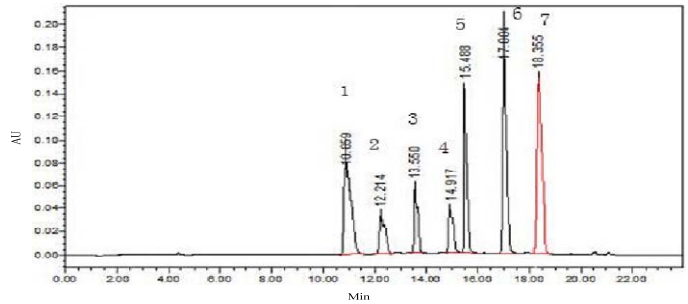

Figure 4: Sample UPLC chromatogram

Table 3: Compounds reserving time

No.	Compounds name	Reserving time (RT)	Wavelength (nm)
1	Acid orange 10	10.793	487
2	Acid red 1	11.925	517
3	Acid red 14	13.065	527
4	Acid black 1	15.249	334
5	Acid orange-yellow 2	15.748	467
6	Acid violet 43	17.248	238
7	Acid blue 62	17.894	282

5.4 Precision and recovery test method

The mixed standard solution of 0.05, 0.1, 0.2 mg/L of acid orange 10, acid red 1, acid red 14, acid black 1, acid orange-yellow 2, acid violet 43, and acid blue 62 was added to the beverage blank samples, and measured by the test method for 6 times, the results shown in table 4. The results show that the average recovery rate is more than 80.5% and the relative standard deviation (RSD) is less than 1.57%, which indicates that the method is accurate and reliable (Steiner, et al., 2016).

Table 4: Precision degree of scalar recovery rate (n=6)

Group	Scalar quantity (mg/L)	Tested value (mg/L)	RSD (%)	Recovery rate (%)
	0.05	0.0418	0.32	83.5
Acid orange 10	0.1	0.0805	1.57	80.5
	0.2	0.1731	0.27	86.5
	0.05	0.0406	0.46	81.2
Acid red 1	0.1	0.0828	0.26	82.8
	0.2	0.1762	0.98	88.0
	0.05	0.0413	0.30	82.5
Acid red 14	0.1	0.0916	0.57	91.6
	0.2	0.1967	0.39	98.4
	0.05	0.0406	0.51	81.2
Acid black 1	0.1	0.0902	1.24	90.0
	0.2	0.1912	0.30	95.6
	0.05	0.0415	1.02	83.2
Acid orange-yellow 2	0.1	0.0902	0.42	90.2
	0.2	0.1924	0.31	96.2
	0.05	0.0467	0.17	93.5
Acid violet 43	0.1	0.0946	0.97	94.6
	0.2	0.1931	0.35	96.5
	0.05	0.0425	0.49	85.1
Acid blue 62	0.1	0.0882	0.50	88.2
	0.2	0.1871	0.21	93.5

In the calculation of the recovery rate of the experiment, different concentrations of the standard samples were known (Togola, et al., 2015). And the average values of each sample were repeated for 6 times, then the recovery rate was calculated by the following formula (Wang, et al., 2015):

Recovery (%) = content of the sample to be measured (mg/g) - known content (mg/g) / sample added dose (mg/g)

6. Conclusion

In this experiment, on ultrasonic assisted conditions, alkaline solvent is used to extract seven acidic industrial dye in the samples. By rapid and convenient simple pre treatment, the high recovery rate was obtained. The

condition of high performance liquid chromatography was optimized, and the analytical method of seven kinds of acid industrial dyes in functional beverage was established. The method is simple, rapid, and highly sensitive, and the recovery rate and repeatability meet the detection requirements of Hygiene Inspection Department and Food Quality Inspection Department on acid orange 10, acid red 1, acid red 14, acid black 1, acid orange-yellow 2, acid violet 43, and acid blue 62 in sports drinks. The experimental results show that in the process of developing new method of sports drinks, it is necessary to pay special attention to the optimization of the following parameters: organic solvent concentration, buffer type, buffer salt concentration, mobile phase acidity value, control temperature, and mobile phase pH value. In addition, it is also of essence that different sports drink samples have a great impact on the development of method. What needs to pay attention to is that, in the process of the experiment, it is supposed to avoid the use of extreme PH, such as PH greater than 8 or less than 1, to ensure that the mobile phase pH value not too high nor too low. Since that working in extreme conditions will shorten the column time length, determine the optimal range of PH in the experiment as much as possible. Most isolates should be carried out between PH=2 ~ 8 (Yu, et al., 2015). The retention time of the analyte is very sensitive to PH, and significantly changes with the PH variation. In addition, the pH value of the mobile phase is tested under different conditions, and it is more favourable for the development of test methods.

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