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Determination of eight synthetic food colorants in drinks by high-performance ion chromatography

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Abstract

Eight synthetic food colorants (Amaranth, Brilliant Blue, Indigo Carmine, New Red, Ponceau 4R, Sunset Yellow, Tartrazine, Allura Red) were determined by high-performance ion chromatography on an anion-exchange analytical column with very low hydrophobicity and visible absorbance detection. Gradient elution with hydrochloric acid–acetonitrile effected both the chromatographic separation of these colorants and the on-line clean-up of the analytical column, which was very advantageous for routine analysis. High-performance ion chromatography may be a solution to the chromatographic analysis for some water-soluble, organic analytes with strong hydrophobicity. The method has been applied to the determination of colorants in drinks and in instant drink powder. No time-consuming pretreatment, as used in conventional liquid chromatography, was needed.

Keywords: Food analysis; Colorants; Fruit juices; Soft drinks

1. Introduction

Color is often the first sensory quality by which foods are judged. Food colorants have been used to make food more attractive and appetizing for centuries, and some synthetic dyes have been added legally into foods since the 1880s [1]. Although the number of permitted food colorants was reduced for food safety reasons in recent years, many kinds of synthetic food colorants are still widely used all over the world because of their low price, effectiveness and stability.

At present, nine synthetic colorants are permitted for use in foods in China: Amaranth (C.I. Food Red 9, AMA), Ponceau 4R (C.I. Food Red 7, PON), Erythrosine (C.I. Food Red 14), Allura Red (C.I. Food Red 17, ALL), Sunset Yellow (C.I. Food Yellow 3, SUN), Tartrazine (C.I. Food Yellow 4, TAR), Indigo Carmine (C.I. Food Blue 1, IND), Brilliant Blue (C.I. Food Blue 2, BRI) and New Red (NEW). All these colorants are permitted for use in most developed countries except New Red, which was synthesized by Chinese scholars [2] and permitted for use only in China. These colorants may be used separately or in combination with other colorants, and the latter is more common because it can cover all different shades and hues. Because many synthetic colorants are potentially toxic, the usage of colorants is limited very strictly. As a result, accurate and reliable methods for the determination of synthetic food colorants are required for the assurance of food safety.

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Many analytical techniques have been used for the simultaneous determination of various synthetic food colorants such as thin-layer chromatography [3,4], derivative spectrometry [5–7], and adsorptive voltammetry [8], but they all require time-consuming pretreatment or cannot be used for complex colorant mixtures. Capillary electrophoresis has also been used, but the proposed methods [9–12] have not been applied to the analysis of real samples. Reversed-phase liquid chromatography (RPLC) [13–15] and ion-pair RPLC [16–18] are still the most widely used methods. Both isocratic and gradient systems are used, and the latter are preferred for the separation of the more complex mixtures. In addition, several anion-exchange chromatographic assays have been developed for the separation of intermediates in single colorants by using buffered eluents such as borate and perchlorate mixtures with gradient elution [19–22]. To the best of our knowledge, no ion chromatographic separation of synthetic food colorant mixtures has been reported hitherto. In this paper, novel high-performance ion chromatography (HPIC) for the simultaneous determination of eight synthetic food colorants is presented; all the synthetic food colorants permitted in China except Erythrosine can be separated in a single run. The retention mechanisms of these colorants on an anion-exchange separation column with very low hydrophobicity are discussed. This method has been applied to the analysis of real samples.

2. Experimental

2.1. Apparatus

A Dionex Model DX-500 ion chromatograph (Sunnyvale, CA, USA) equipped with a 50-μl sample loop was employed along with a Dionex PEAKNET chromatography workstation for instrument control as well as data acquisition and processing. The separation was achieved by a Dionex IonPac AG11 guard column (50×4 mm I.D., 13 μm) and a Dionex IonPac AS11 analytical column (250×4 mm I.D., 13 μm), the detection by a Dionex AD20 absorbance detector. The flow-rate of eluent was 1.5 ml/min. All separations were carried out at room temperature.

The spectrophotometric experiments were carried out by using a Shimadzu UV-120-02 spectrophotometer (Kyoto, Japan) with 1-cm quartz cells.

2.2. Reagents

All solutions were prepared with distilled deionized water and all chemicals were of analytical reagent grade unless otherwise stated.

Indigo Carmine (purity: 90%) was obtained from Sigma (St. Louis, MO, USA), New Red (purity: 85%) and Allura Red (purity: 90%) from Shanghai Dye Institute (Shanghai, China). The stock solutions (0.5 mg/ml) of these three colorants were prepared separately by dissolving appropriate amounts of these compounds in water. The stock solutions (1.0 mg/ml) of the other five colorants were obtained from the National Research Center for Certified Reference Materials (Beijing, China). The working solutions were prepared by serial dilution of the stock solutions with water.

2.3. Preparation of real samples

The samples, three carbonated drinks (samples A, B and C), one fruit juice drink (sample D), one health drink (sample E) and one fruit flavored instant drink powder (sample F), were purchased from local market. A portion (1.0 g) of powder (sample F) was accurately weighed and dissolved in 25 ml of water. A 5-ml sample of fruit juice drink, health drink or degassed ultrasonically carbonated drink was diluted with the same volume of water. All sample solutions (50 μl) were injected after filtering through a 0.45-μm filter.

2.4. Analytical conditions

The chromatographic separation was effected by a gradient program (shown in Table 1) which consisted of three eluents: 2.0 mol/l HCl (E1), degassed ultrasonically acetonitrile (E2) and water (E3) [as the Dionex equipment was made of polyether ketone (PEEK), it was not damaged by the HCl eluent]. A wavelength-switching technique [23] was employed for the visible absorbance detection. When the detection wavelength was switched from one wavelength to another, the absorbance offset was set to 'on' to maintain a flat and low baseline. The gradient

Table 1
Gradient and wavelength program

Time (min)	E1 (%)	E2 (%)	E3 (%)	Wavelength (nm)	Absorbance offset
0.0	10.0	50.0	40.0	625	On
6.0	10.0	50.0	40.0	430	On
9.5	10.0	50.0	40.0	430	
9.6	2.5	95.0	2.5	430	
12.0	2.5	95.0	2.5	480	On
20.0	2.5	95.0	2.5	480	
20.1	10.0	90.0	0	480	
23.0	10.0	90.0	0	525	On
35.0	10.0	90.0	0	525	
35.1	10.0	50.0	40.0	625	

needs 5–7 min for equilibration prior to another injection. In this study, the peak area was used for quantification.

2.5. Identification

The colorants were identified by comparison of the retention times in real samples with those in standard solutions, and by the absorbance ratios between the two wavelengths.

3. Results and discussion

3.1. Choice of separation system

A simple method for the determination of all permitted synthetic food colorants is advantageous to practical application. The present National Standard Method of Determination in China [15] is RPLC with gradient elution for the simultaneous determination of all permitted synthetic food colorants except Allura Red. Because Erythrosine would precipitate in acid solution [2] which we chose finally as eluents in this study, the separation conditions of the eight other colorants (for structures, see Fig. 1) were optimized as described below.

In theory, ion-exchange chromatography is not suitable for separating organic multivalent ions with strong hydrophobicity because the very strong interactions between ions and stationary phase result in very long elution times. Apart from pure ion-exchange processes, additional non-ionic adsorption interaction between the highly polarizable ions and the stationary phase contributes to the retention [24]. Each colorant in this study exists as an acidic multivalent anion with at least two $-SO_3$ groups in aqueous solution. Our preliminary studies showed that all the colorants could not be eluted within an acceptable time from the commonly used anion-exchange separation columns such as Dionex IonPac AS4A-SC (medium hydrophobicity) using conventional eluents, including mixtures of carbonate or iodide and acetonitrile. Therefore, an anion-exchange separation column with very low hydrophobicity should be chosen to reduce the adsorption, and a strong acid solution should be selected as the eluent to suppress ionization. A high concentration of organic solvent should also be added to the eluent to improve the selectivity. In this study, a Dionex IonPac AS11 analytical column with very low hydrophobicity was employed. It remains stable between pH 0 and 14 and compatible with eluents containing 0–100% organic solvents, which facilitates eluent selection [25]. In this study, acetonitrile was preferred to methanol because of its relatively higher elution strength as well as the lower column back pressure it creates. In principle, many inorganic acids such as HCl, H_2SO_4, HNO_3 and $HClO_4$ can be used as the eluent. Because most colorants are susceptible to oxidation [1,2] and the solubility of HCl in high concentration of acetonitrile is better than that of H_2SO_4, the former was chosen. The Cl^- ions can elute the colorants retained by anion-exchange interaction. In order to separate all eight colorants in a single run, a gradient elution program was employed, and 2.0 mol/l HCl, acetonitrile and water were chosen as E1, E2 and E3, respectively. It

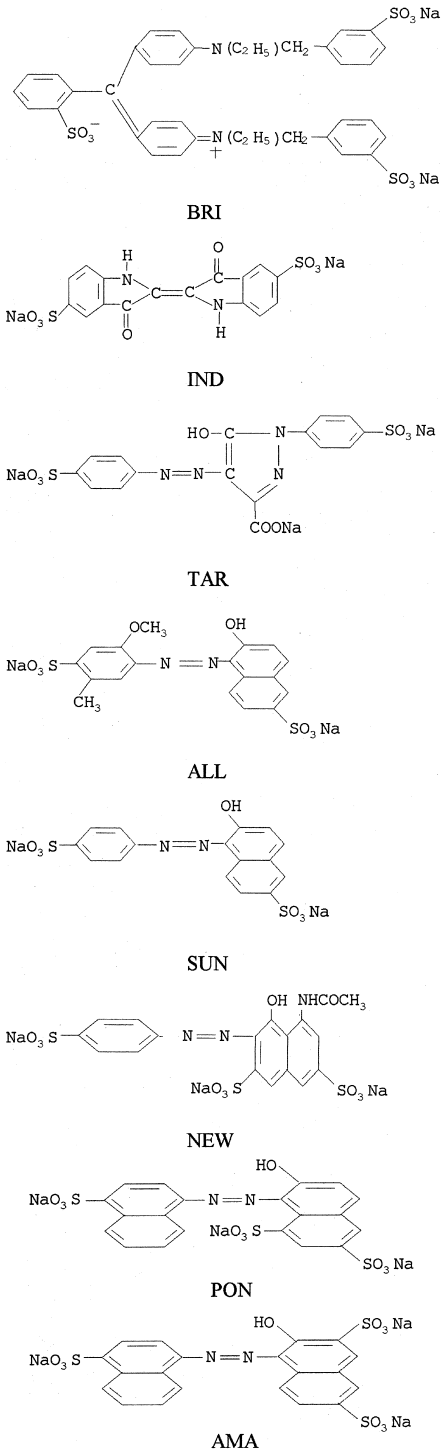

Fig. 1. Structures of eight synthetic food colorants.

was noteworthy that the two non-azo colorants (Brilliant Blue and Indigo Carmine, which chemical structure types are triarylmethane and indigoid [26], respectively) eluted first, followed by azopyrazolone colorant (Tartrazine) and then monoazo colorants (the other five colorants). The elution sequence may be explained by the molecular structures. The hydrophobicities of non-azo colorants were weaker than those of azo colorants. Among azo colorants, the hydrophobicities of the colorants with a naphthalene ring were stronger than that of the colorants with benzene rings, and the hydrophobicities increased with the increase of the naphthalene rings. New Red was eluted after Allura Red and Sunset Yellow because it possessed more $-SO_3$ groups. Due to steric effect, one of $-SO_3$ groups of Ponceau 4R could not interact effectively with the anion-exchange sites on stationary phase, so it eluted before Amaranth with similar structures and the same charges. According to the elution sequence of all the colorants, three groups were discerned, i.e., gradient program should consist of three steps, and the elution condition for each step was studied separately.

Brilliant Blue, Indigo Carmine and Tartrazine eluted first. Fig. 2a illustrates the effect of the HCl concentration on the retention times of these three analytes when the concentration of acetonitrile is 50%. Because the maximum absorption wavelength in visible region of Tartrazine was very different from those of Brilliant Blue and Indigo Carmine, the wavelength-switching technique should be employed. The peak of Tartrazine should then not be very near to that of Indigo Carmine. Therefore, 0.20 mol/l was selected as the concentration of HCl. Fig. 2b shows that the retention times of all three colorants decrease with the increase in the acetonitrile concentration. Taking the factors of separation and detection into account, the concentration of acetonitrile was set at 50%. So, the eluent composition during the first step of the gradient program from 0 to 9.5 min of run time was chosen as 0.2 mol/l HCl–acetonitrile (50:50, v/v).

Following the first group of colorants, Allura Red and Sunset Yellow eluted next. It was very difficult to achieve the baseline separation of these two colorants because of their similar molecular structures and charges. When the acetonitrile concentration was 80%, the effect of the HCl concentration

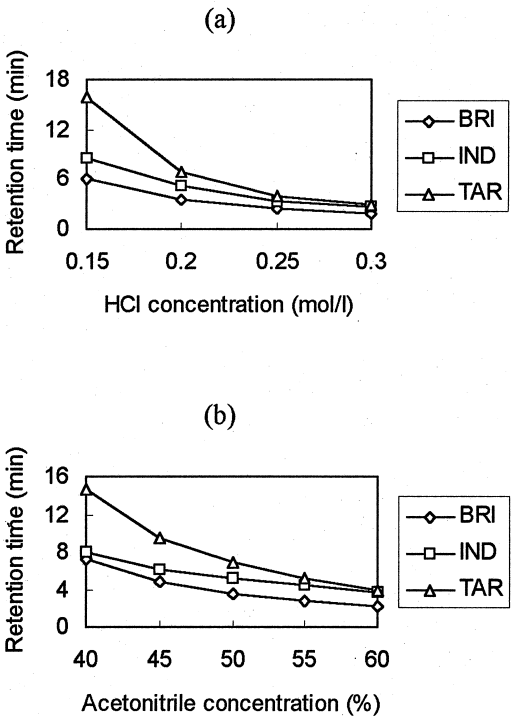

Fig. 2. Effects of the HCl (a) and acetonitrile (b) concentrations on retention times of Brilliant Blue, Indigo Carmine and Tartrazine.

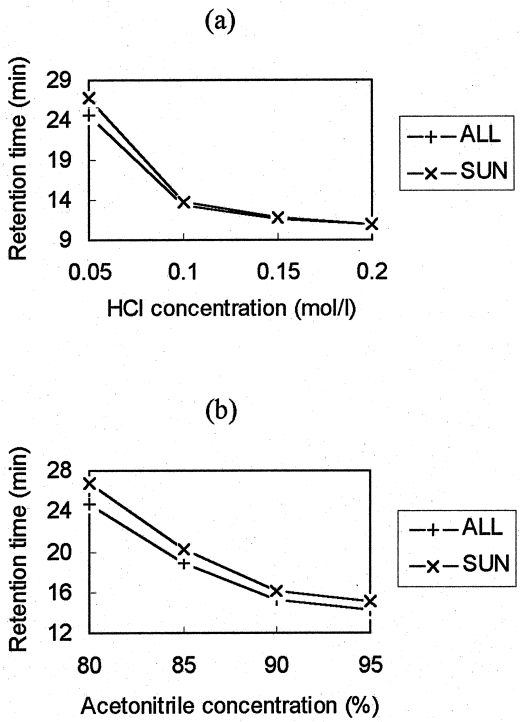

Fig. 3. Effects of the HCl (a) and acetonitrile (b) concentrations on retention times of Allura Red and Sunset Yellow.

on the retention times of the two colorants is shown in Fig. 3a. If the acid concentration was too high, they would be coeluted. So, 0.05 mol/l was chosen as the HCl concentration. Fig. 3b demonstrates the effect of the acetonitrile concentration on the retention times of the two colorants. An increase of the acetonitrile concentration causes the decrease of the retention times of the two analytes as well as the improvement of the peak shapes and the resolution of the two peaks. When the acetonitrile concentration was 95%, the resolution of Allura Red and Sunset Yellow was more than 0.8. Finally, 0.05 mol/l HCl–acetonitrile (5:95, v/v) was selected as the eluent for the second step of the gradient program, that ranged from 9.6 to 20.0 min. The retention times of Allura Red and Sunset Yellow differ just enough to allow for identification. If both colorants are present in the same sample, however, the lack of resolution excludes quantification. As the spectra of the two colorants are very similar, selective wavelengths cannot be found to quantitate both colorants separately. Fortunately, the possibility of Allura Red and Sunset Yellow mixtures for practical application is very small because of their similar color profiles. In the following sections, all quantitative data of Allura Red and Sunset Yellow were obtained under the conditions that only one of them occurred in the sample.

New Red, Ponceau 4R and Amaranth were the strongest retained group of colorants. The chromatographic conditions could be optimized simply by varying the percentages of E1 and E2 in eluent, and the results are shown in Table 2. Finally, the eluent

Table 2
Effect of the percentages of E1 and E2 in eluent on the retention times of New Red, Ponceau 4R and Amaranth

E1 (%)	E2 (%)	Retention time (min)		
		NEW	PON	AMA
20	80	22.66	24.41	25.23
15	85	23.28	25.26	26.78
10	90	25.60	28.08	31.70
5	95	50.98	>60	>60

during the third step of gradient program from 20.1 to 35.0 min was chosen as 0.2 mol/l HCl–acetonitrile (10:90, v/v). From here, we can find that the decrease of the retention times caused by increasing the acetonitrile concentration was offset completely by the increase of the retention times caused by decreasing the HCl concentration. So, the HCl concentration in eluent was possibly the most important experimental parameter that determined the retention behaviors of the three colorants.

3.2. Choice of detection wavelength

Generally, the absorbance detection was the first choice in the LC analysis of synthetic food colorants owing to its simplicity and reliability. The colorants themselves were often detected in the visible wavelength range, and the intermediates as well as other organic impurities in the ultraviolet range [26]. In this study, the experimental results obtained from a spectrophotometer showed that the absorbances of all colorants in the ultraviolet range were not higher than those in visible range, and the background absorbance of the eluent in ultraviolet range was very high. Table 3 shows the maximum absorption wavelength (λ_{max}) and the optimal detection wavelength (λ_{opt}) of each colorant in the visible range. Because of the significant differences between the maximum absorption wavelengths, a wavelength-switching technique should be a better solution for improving the detection limits of all colorants. For the third group of colorants, 525 nm was a compromise because the maximum absorption wavelengths of these three colorants were very near. For the second group, 480 nm was preferred because the application sphere of Sunset Yellow was much wider than that of Allura Red [27]. For the first group, 430 nm was selected for the detection of Tartrazine and 625 nm for the other two analytes. The wavelength of 610 nm was not chosen because Indigo Carmine had seldom been used in recent years [2]. Under the adopted experimental conditions, the chromatogram of a standard solution is shown in Fig. 4a, where the concentrations of all colorants are 40 μg/ml except that of Brilliant Blue (20 μg/ml).

Table 3
λ_{max}, λ_{opt} and percentage less in sensitivity at λ_{opt} for each colorant

Analyte	λ_{max} (nm)	λ_{opt} (nm)	Less in sensitivity at λ_{opt} (%)
BRI	625	625	0
IND	610	625	19
TAR	430	430	0
ALL	505	480	21
SUN	480	480	0
NEW	530	525	0.6
PON	510	525	9.6
AMA	520	525	0.8

3.3. Interference study

In general, a variety of additives such as preservatives, acidulants and artificial sweeteners were frequently added into many kinds of foods. Under the specified experimental conditions, the commonly used additives including saccharin, citrate, malate, benzoate, and sorbic acid did not interfere with the detection of the colorants.

3.4. Analytical data

Under the optimized experimental conditions, all the colorants showed good linearities between the concentrations and peak area responses. The detection limits, defined as the signals three times the noise levels, were also calculated. All the results are listed in Table 4. The precisions were evaluated by performing seven replicate analyses of a standard solution where the concentration of Brilliant Blue was 10 μg/ml and the concentrations of the other seven colorants were all 20 μg/ml. The relative standard deviations were 2.15, 2.26, 2.34, 2.45, 2.01, 1.53, 1.53 and 2.36% for Brilliant Blue, Indigo Carmine, Tartrazine, Allura Red, Sunset Yellow, New Red, Ponceau 4R and Amaranth, respectively.

3.5. Real samples

For most LC methods, synthetic food colorants must be extracted firstly from the food matrices and purified as well as concentrated prior to chromatographic analysis, although some liquid samples can be injected directly without any pretreatment in

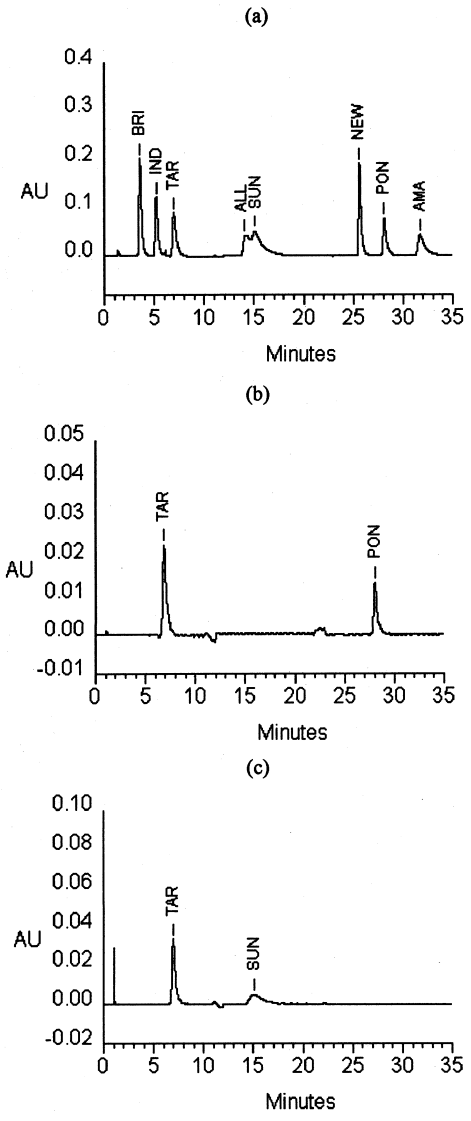

Fig. 4. Chromatograms of standard solution where the concentrations of all analytes were 40 μg/ml except the Brilliant Blue concentration which was 20 μg/ml (a) and the final solutions of real samples: (b) sample D; (c) sample F. For analytical conditions see text.

individual assays [13]. Sample pretreatment techniques include: (1) adsorption by using cellulose, alumina and polyamide, among other materials [15,16,26], (2) extraction with organic solvents [15] or ion-pairing reagent as well as organic solvents and then back-extraction with aqueous solutions, and (3) solid-phase extraction with cartridges [18]. All the methods are time-consuming, and sometimes so expensive that they were not suitable for routine analyses of large number of samples. For an anion-exchange separation column with very low hydrophobicity, the elution program containing strong acid and high concentrated organic solvent was essentially an on-line clean-up program for the column [25]. Under this condition, cations will not be retained and the majority of anions will be converted to neutral molecules or cations, which will elute from the columns easily. The proposed gradient program was advantageous not only for the analysis of real samples but also for the protection of the separation column. In our laboratory, any evident change in the separation performance of the columns was not observed after more than eighty analyses for real samples. In all samples, Indigo Carmine, Allura Red, New Red and Amaranth were not detected. The results are shown in Table 5, and the chromatograms of samples D and F shown in Fig. 4b and c. Spike studies were performed by using sample D. Two concentration levels of each analyte were added (3 and 6 μg/ml for Brilliant Blue, 10 and 20 μg/ml for Allura Red and Sunset Yellow, 6 and 12 μg/ml for the other colorants), the average recoveries (n=4) ranged from 94.7 to 109.0%.

4. Conclusions

A HPIC method for the simultaneous determination of eight permitted synthetic food colorants in a single run was developed. Both the chromatographic separation of colorants and the on-line clean-up of analytical columns were achieved by using the same gradient elution program. The applicability was verified by the determination of colorants present in liquid and instant drinks. This proposed method has shown good operational stability over 1 month for at least 80 assays, and gave reliable and reproducible results with simple sample pretreatment operation. In addition, HPIC proved to be a promising method for the separation of water-soluble, multivalent organic ions with strong hydrophobicity by both ion exchange and ion suppression.

Table 4
Linearities and detection limits for colorants

Analyte	Concentration range (μg/ml)	Regression equation[a]	Correlation coefficient (n=5)	Detection limit (μg/ml)
BRI	0.2–20	$A=(2.14c-0.37)\cdot10^5$	0.9991	0.03
IND	0.4–40	$A=(6.35c-0.86)\cdot10^4$	0.9993	0.1
TAR	1.0–40	$A=(6.69c-2.03)\cdot10^4$	0.9989	0.5
ALL	2.0–40	$A=(5.13c+1.87)\cdot10^4$	0.9990	2
SUN	2.0–40	$A=(7.80c-0.29)\cdot10^4$	0.9978	2
NEW	0.4–40	$A=(9.80c-0.56)\cdot10^4$	0.9994	0.2
PON	1.0–40	$A=(5.16c-1.29)\cdot10^4$	0.9998	0.5
AMA	2.0–40	$A=(5.57c-1.12)\cdot10^4$	0.9992	1

[a] A=Peak area response (arbitrary unit), c=concentration of analytes (μg/ml).

Table 5
Analysis of real samples

Sample	Content[a] (μg/ml or μg/g)			
	BRI	TAR	SUN	PON
A			42.5±1.0	
B			67.0±1.4	
C	0.82±0.04	15.6±0.7		
D (wild jujube)		21.1±0.3		13.6±0.5
E		4.0±0.2		11.6±0.5
F		325.0±4	176.0±4	

[a] Mean±standard deviation (n=4), μg/g for solid sample (sample F) and μg/ml for the other samples.

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