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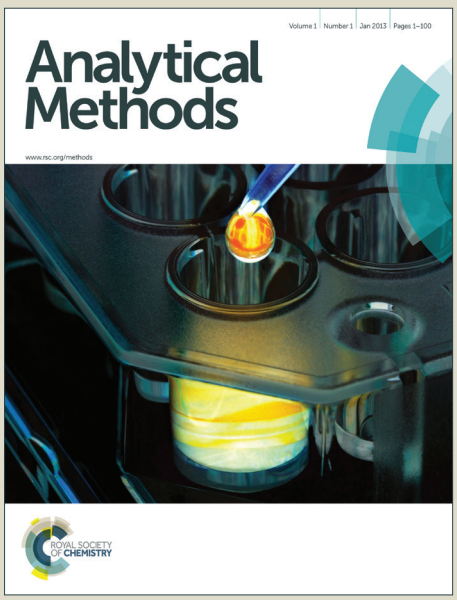



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# Quantitative Analysis of Fourteen Synthetic Dyes in Jelly and Gummy Candy by Ultra Performance Liquid Chromatography

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**Abstract**

An anlytical method was developed for simultaneous determination of fourteen dyes (tartrazine, sunset yellow, poncean4R, allura red, brilliant blue, carmoisune, amaranth, indigo carmine, erythosine, new red, acid green, patent blue, acidrose red B and rose bengale) in jelly and gummy candy using ultra performance liquid chromatography (UPLC) with a photodiode array detector. Analytes were extracted with hot water (55±5 °C) and ammonium sulfate was added for selectively precipitating protein and polisacchsride. Then the supernatant was purified by an Oasis WAX solid-phase extraction cartridge. Chromatographic separation was performed on an ACQUITY UPLC® BEH shield RP18 column with the gradient elution using acetonitrile and water containing 10 mmol $L^{-1}$ ammonium acetate as mobile phase. Analytes were quantified by external calibration curves over ranges of 0.25-50 mg $L^{-1}$, with correlation coefficients >0.9999. Recoveries of the target compounds (spiked at levels of 1, 5, 25 mg $kg^{-1}$) ranged from 81.4±6.34% to 97.5±3.05%, while intraday and interday relative standard deviations were from 0.90% to 8.74% and from 3.36% to 9.62%, respectively. Limits of quantifications for fourteen dyes were 0.25 mg $kg^{-1}$.

**Keywords:** Dyes, jelly, gummy candy, UPLC.

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## Introduction

Coloring agents were widely used in food manufacture to increase the attraction for consumers, since lively color of food always associates with the first sensory quality and freshness even the good taste value. Both natural and artificial colors were permitted to add in processed food, while the natural food colors were increasingly used for the consumer preference in recent years. However, many natural dyes are relatively unstable and easily undergo degradation during the food processing. Synthetic food dyes are still applied in many foods, such as candy, sweet, and jelly.

The synthetic dyes might exert adverse effects on human health after excessively consuming. It was demonstrated that ponceau SX was toxic and carcinogenic *in vitro* and *in vivo*.[1] Kornbrust and Barfkencht suggested that brilliant blue was genotoxic according to the results of rat hepatocyte primary culture/DAN repair assays.[2] In addition, allergy and asthmatic reactions were the potential side effect for some synthetic dyes.[3-6] Therefore, the use of food dyes was strictly controlled in many countries and regions. Ten synthetic dyes were permitted using in food according to Chinese government, including tartrazine, sunset yellow, poncean 4R, allura red, brilliant blue, carmoisune, amaranth, indigo carmine, erythosine and new red which was only permitted using in China.[7-8] The other four synthetic dyes, including acid green, patent blue, acidrose red B and rose bengale, were not permitted using in food in China. But patent blue and acid green can be used in food in European and Russia, and acidrose red B and rose bengnale were permitted as food additives in Japan.[8] The maximum allowable levels of these fourteen dyes in jelly and candy for China, EU and USA were listed in Table 1.

Jelly and gummy candy were the popular confectioneries, especially for children. The main components of jelly and gummy candy were agar, gelatin and carrageenen, which were not dissolved in organic solution or cold water. Hence, routine analytical methods for analysis of dyes in sweets usually weren't suitable for the direct analysis of jelly-based candy because of



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the tendency of gelatin to block the column. Some studies have demonstrated the possibility to use polyamide SPE to avoid this problem. Polyamide powder adsorption methods were developed in previous papers.[9,10] However, these methods were not suitable for routine analysis because of complicated and un-controlled procedures. Therefore, specific pretreatment methods were urgent need for the surveillance of synthetic dyes in jelly and gummy candy. As for the detection assay, HPLC[11-13] was the most commonly-used method because of its high sensitivity and specificity, compared to other method such as TLC,[14-16] CE[17-19] and ion chromatography.[21,22] In consideration of above adverse effect of synthetic dyes, strict control of synthetic dyes concentration in jelly and gummy candy was necessary, which should be supported by reliable and relative simple analytical methods. Therefore, it was important to develop a suitable method for synthetic dyes in jelly and gummy candy. In this study, a rapid and simple method for the simultaneous determination of fourteen dyes in jelly and gummy candy was developed based on solid-phase extraction purification following an ultra performance liquid chromatography (UPLC) coupled with a photodiode array (PDA) detector.

## Experimental

### Reagents and chemicals

Tartrazine, sunset yellow, carmoisine, amaranth, ponceau 4R, erythrosine, allura red, patent blue, new red and brilliant blue were purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany). Indigotine, acid green, rose bengale and acidrose red B were obtained by Tokyo Chemical Industry Co. (Tokyo, Japan). Ultra-pure water (18.2 MΩ $cm^{-1}$) used was acquired by a Milli-Q apparatus (Millipore, Bedford, MA). HPLC grade of methanol and acetonitrile were purchased from Fisher Scientific (Fair Lawn, NJ). Formic acid (98%) was from Acros organics (New Jersey, USA). Ammonium acetate used as the mobile phase additive was from Fluka (Buchs, Switzerland) with purity≥99%. Ammonium sulfate was supplied by Beijing Chemical (Beijing,



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China). Oasis WAX SPE cartridges (60 mg, 3 ml) were supplied by Waters Corporation (Milford, MA).

**Ultra Performance Liquid Chromatography analysis**

A Waters Acquity UPLC® system (Milford, MA) coupled with a Waters Acquity eλ PDA detector was used in this study. UPLC analysis was performed on an Acquity UPLC® BEH shield RP18 column (50 mm×2.1 mm, 1.7 μm). The mobile phase was consisted of (A) pure water containing 10 mmol $L^{-1}$ ammonium acetate and (B) acetonitrile. With a total flow rate of 0.4 ml $min^{-1}$, the gradient schedule of the mobile phase was listed in Table 2. The column oven was set at 40°C and the injection volume was 10 μL. The PDA detector was operated under absorption spectrum scanning mode (200-650 nm). Identities of the chromatographic peaks were confirmed by comparing their UV-visible spectral characteristics and retention times to that of standards. Quantitative analysis was accomplished using the external calibration.

**Sample preparation**

One gram of crushed jelly or gummy candy sample was placed in a 15 ml centrifuge tube containing 10 ml of water. Then the sample was ultrasonically extracted at 55±5 °C for 30 min to completely dissolve. Immediately, 0.5 ml saturated ammonium sulfate solution was added. After vortex mixed for 10 seconds, the extract was stayed at room temperature for 10 min to precipitate, and then centrifuged at 9000 rpm for 10 min.

The supernatant obtained above was introduced to an Oasis WAX cartridge preconditioned sequentially with 1 ml methanol and 1 ml water at a flow rate of approximate 3 ml $min^{-1}$. The procedure of SPE was referred to our previous study.[23] After sample loading, the cartridge was rinsed with 1 ml water containing 2% formic acid and 1 ml methanol, and the dyes were eluted with 2 ml methanol containing 5% ammonia. Elute was dried under a gentle nitrogen stream,



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finally reconstituted with 1 ml water for UPLC analysis.

**Method validation**

The principle of method development and validation abided by CITAC/ EURACHEM GUIDE: Guide to Quality in Analytical Chemistry (2002).[24] The linearity of method was evaluated with working standard mixtures of fourteen dyes over ranges of 0.25 - 50 mg $L^{-1}$ (e.g. 0.25, 0.5, 1, 2.5, 5, 10, 25 and 50 mg $L^{-1}$). Integrated peak areas of the selected absorbing wavelength for each dye were used to construct eight-point calibration curves, which were applied for quantification. The limit detection (LOD) and limit of quantification (LOQ) were estimated by analyzing 10 control samples, and calculated as 3 and 10 times the standard deviation of the signals from the blank matrix, respectively.

Recoveries and precision of the method were evaluated by assaying the fortified jelly samples at three concentration levels. In this study, the precision of method was determined by calculating the relative standard deviation (RSD) of repeatability (intraday) and intermediate precision (interday). Intraday precision was evaluated using six replicate injections with the same sample in a day. Interday precision was determined from six injections for six consecutive days.

**Real sample detection**

Different fifteen real candy samples, including 6 jelly and 9 gummy candy samples with different brands were purchased from local supermarkets or grocery stores in Beijing. These samples were crashed and homogenized for analysis using the new developed method.

## Results and Discussion

**Optimization of liquid chromatography conditions**



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Nowadays, ultra-fast or ultra-rapid HPLC method has been adopted in variety of analytical fields due to its significantly high performance and efficiency, which can essentially improve separation compared to the conventional HPLC. An UPLC column (Acquity UPLC® BEH shield RP18, 2.1 mm×50 mm, 1.7 μm) was applied to separate the fourteen target dyes. As shown as figure 1, all target dyes except for rose bengale and erythrosine were present sulfonic acid groups. Therefore it was necessary to add buffer in mobile phase for satisfactory result on reverse phase chromatography. In this study, water with four different concentrations (5, 10, 20 and 50 mmol $L^{-1}$) of ammonium acetate and acetonitrile as mobile phase were tested, respectively, for the sake of achieving well-shaped peaks and good resolutions. As a result, 10 mmol $L^{-1}$ ammonium acetate was selected. Using the optimized mobile phase with flow rate of 0.4 ml $min^{-1}$, a total analysis was done in 8 min (Fig.2).

Since each synthetic dye presents different wavelength of maximum absorption, the separated analytes were monitored at the wavelengths nearest to their own maximum absorption by a photodiode array detector in order to increase the sensitivity of this method. For achieving higher sensitivity, the detection wavelengths of tartrazine and sunset yellow were set at 420 nm and 480nm, respectively. The detection wavelengths of new red, amaranth, ponceau 4R, allura red, carmoisine and erythrosine were set at 500nm, while acidrose red B and rose bengale were monitored at 550 nm. The 630 nm were selected for the detection of indigotine, acid green, brilliant blue and patent blue.

**Optimization of sample preparation**

Jelly and gummy candy were composed of thickener and food additives, such as dyes, essences and sweeteners. Commonly, the thickeners used into jelly were agar, carrageenan and gelatin, which were also the main components of gummy candy. Gelatin was a kind of water soluble protein derived from collagen protein, while agar and carrageenan were polysaccharide



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extracted from marine plants. These components were not dissolved either in cold water or organic solvent. Considering that agar, carrageenan and gelatin can be solved in hot water, we put the jelly and gummy samples into water and then heated it with an ultrasonic water-bath to make it dissolved completely.

The solubility of gelatin, agar and carrageenan (the thickener of jelly and gummy candy) is highly temperature-dependent. These components only dissolved in hot water. When the temperature decreased, the sample extracts will be jellied or semi-solidified, which isn't suitable for the subsequent SPE processing. Therefore, the thickener needs to be removed from hot extracts. A precipitant reagent, which can selectively precipitate protein and polisacchsride, was added immediately in extracts to precipitate the gelatin, agar and carrageenan. Here, we used a precipitation procedure by salting out, which is the most commonly applied for ploisacchsride and protein precipitation[25]. Three conventional inorganic salts, sodium chloride, saturated ammonium sulfate and sodium sulfate were compared. One gram NaCl, 1 g $NH_4SO_4$ and 1 g $Na_2SO_4$ were added into the hot extract, respectively. Obvious floccus precipitate was occurred immediately using $NH_4SO_4$; while no evident precipitation was observed for the adding of NaCl or $Na_2SO_4$. $NH_4SO_4$ was consequently selected as the precipitation reagent. 0.5 ml saturated $NH_4SO_4$ was added into extract after ultra-sonic extracting immediately, and then the extract was set alone for 10 min at room temperature to avoid co-precipitation.

In order to increasing the sensitivity of the whole method, SPE technology was used to enrich and purify target dyes. C18 cartridge[26] and polyamide colunm[9,10,27] were applied in previous study. But the selectivity and specificity of method using C18 cartridge was not satisfactory, e.g. the poor recovery of tartrazine. As for the ployamide-based SPE, both polyamide power and commercial ployamide cartridge were reported. Recently, weak anion-exchange (WAX) SPE technology was frequently used for enriching and purifying dyes due to its convenience, comprehensiveness as well as the favorable selectivity and specificity for compounds with



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strong polarity and sulfonic acid groups.[23,28,29] In this study, an Oasis WAX SPE cartridge was selected and the procedure referred to our previous study.[23] To investigate the performance of SPE for all fourteen dyes, 10 ml 1 mg $L^{-1}$ mixed standard solution was introduced to WAX cartridge, and processed as above procedure. The recoveries of fourteen target dyes were from 85.9% to 98.7%, which were satisfied with routine analysis requirements.

**Robustness study**

The robustness was determined by analyzing the same samples under variety of conditions. Extraction temperatures (45°C, 50°C，55°C and 60°C) and volume of precipitant reagent (0.2ml, 0.5ml and 1ml) were compared. As a result, extraction temperature at 50±5°C, volume of precipitant reagent between 0.5ml and 1ml did not have significant effects on the method.

**Validation of the method**

Good linearity of the method was achieved for all target dyes over the range of 0.25-50 mg $L^{-1}$. The correlation coefficients ($r^2$) of the calibration curves were greater than 0.9999. For each dye, the LOD and LOQ calculated in jelly sample were 0.10 and 0.25 mg $kg^{-1}$, respectively, as shown in Table3.

As presented in Table 4, average recoveries of each dye ranged from 81.4±6.34% to 97.5±3.05% at three spiked levels. The precision of this method at each fortification level, represented by the intraday and interday RSD% were 0.90%-8.74% and 3.36%-9.62%, respectively. These results demonstrated that the recoveries and precisions of this method were suitable for routine monitoring purpose.

Additionally, inter-lab validation experiment of the new method was performed in five different laboratories. The results of linear range, LOD, LOQ, recovery and precision were quite similar to that of our laboratory.



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**Application to real samples**

This new developed method was used to analyze six jelly and nine gummy candy samples of Chinese local brands from local markets. In all fifteen samples, only five out of fourteen dyes were detected, including tartrazine, sunset yellow, poncean 4R, allura red and brilliant blue. The concentrations of these five dyes detected in samples are shown in Table 5.

Tartrazine was not permitted using in jelly in EU,[30] as well as poncean 4R was not permitted using in candy in USA.[31] Thus, jelly samples (No.12 and 14) containing tartrazine did not meet requirements of EU, while 3 gummy candies and 1 jelly with poncean detected were not up to the standard of USA. Nevertheless, these candy samples were all Chinese local brands and only sold in China mainland. The maximum levels permitted in China of tartrazine, sunset yellow, poncean 4R, allura red and brilliant blue in candy were 100, 100, 50, 50 and 25 mg $kg^{-1}$, respectively.[7] Tartrazine was found in 9 samples, including 7 gummy candy and 2 jelly samples, at a concentration range of 1.7-80.4 mg $kg^{-1}$. Sunset yellow was detected in 4 samples at levels less than 2 mg $kg^{-1}$. Poncean 4R was occurred in 3 gummy samples and 1 jelly at level of 1.6-33.0 mg $kg^{-1}$. Allura red and brilliant blue were only in gummy candy at level of 1.0-47.8 mg $kg^{-1}$ and 0.7-9.6 mg $kg^{-1}$, respectively. Concentrations of all of these five dyes in fifteen real samples were lower than the maximum level permitted in China.

## Conclusions

A rapid and reliable method has been established and validated for simultaneous determination of fourteen dyes in jelly and gummy candy samples. Acceptable accuracy and precision were obtained for all fourteen dyes. This method is applicable for the routine surveillance of dyes in jelly and gummy candy.



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## Figure Captions

Fig. 1 Chemical structures of the fourteen target dyes.

Fig. 2 LC chromatograms (420 nm) of fourteen dyes using the mobile phase consisted of acetonitrile and water containing 10 mM of ammonium acetate: a, tartrazine; b, new red; c, amaranth; d, indigotine; e, ponceau 4R; f, sunset yellow; g, allura Red; h, carmoisine; i, acid green; j, brilliant blue; k, erythrosine; l, acidrose red B; m, patent blue V; n, rose bengale.

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Table 1 The maximum allowable levels of 14 dyes in jelly and candy for China, USA and EU (mg $kg^{-1}$)

| Dyes | China[a] | | USA[b] | | EU[c] | |
|---|---|---|---|---|---|---|
| | jelly | candy | jelly | candy | jelly | candy |
| Tartrazine | 50 | 100 | GMP[d] | GMP | NP[e] | 300 |
| New red | NP | 50 | GMP | GMP | NP | NP |
| Amaranth | 50 | 50 | GMP | GMP | NP | NP |
| Indigotine | NP | 100 | GMP | GMP | NP | 300 |
| Poncean 4R | 25 | 50 | NP | NP | 100 | 50 |
| Sunset yellow | 25 | 100 | GMP | GMP | 100 | 50 |
| Allura red | 25 | 50 | GMP | GMP | NP | 300 |
| Carmoisine | NP | 50 | NP | NP | NP | 50 |
| Acid green | NP | NP | NP | NP | 100 | 300 |
| Brilliant blue | 25 | 25 | GMP | GMP | NP | 300 |
| Erythrosine | NP | 50 | GMP | GMP | NP | NP |
| Acidrose red B | NP | NP | NP | NP | NP | NP |
| Patent blue V | NP | NP | NP | NP | NP | 300 |
| Rose bengale | NP | NP | NP | NP | NP | NP |

a. Ministry of Health of the People's republic of China, GB2760-2011, 2011.

b. U.S. Food and Drug Administration, Code of Federal Regulation, Title 21, Parts 70-74 and 80-82.

c. European Parliament and Council, Directive 94/36//EC, 1994.

d. No maximum level is specified, but the usage must meet the requirement of Good Manufacturing Practice (GMP).

e. NP=Not Permitted

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Table 2 Gradient elution program of chromatography

| Time (min) | A (%) | B (%) |
|---|---|---|
| 0 | 98 | 2 |
| 1 | 94 | 6 |
| 3.3 | 82 | 18 |
| 4.5 | 65 | 35 |
| 6 | 40 | 60 |
| 8 | 40 | 60 |
| 8.1 | 98 | 2 |
| 10 | 98 | 2 |

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Table 3 linearity, limits of detection (LOD) and limits of quantification (LOQ) for 14 dyes

| Dyes | RT (min) | Linear range ( mg $L^{-1}$ ) | Slope ( $\times 10^4$ ) | Intercept ( $\times 10^3$ ) | correlation coefficients ( $r^2$ ) | LOD (mg $kg^{-1}$) | LOQ (mg $kg^{-1}$) |
|---|---|---|---|---|---|---|---|
| Tartrazine | 1.20 | 0.25－50 | 7.02 | 6.92 | 0.999942 | 0.10 | 0.25 |
| New red | 1.80 | 0.25－50 | 5.36 | 4.78 | 0.999907 | 0.10 | 0.25 |
| Amaranth | 1.91 | 0.25－50 | 6.88 | 7.31 | 0.999919 | 0.10 | 0.25 |
| Indigotine | 2.21 | 0.25－50 | 3.84 | 1.28 | 0.999965 | 0.10 | 0.25 |
| Poncean 4R | 3.09 | 0.25－50 | 7.15 | 11.8 | 0.999913 | 0.10 | 0.25 |
| Sunset yellow | 3.36 | 0.25－50 | 5.03 | -0.27 | 0.999995 | 0.10 | 0.25 |
| Allura red | 3.84 | 0.25－50 | 6.68 | 1.50 | 0.999977 | 0.10 | 0.25 |
| Carmoisine | 4.81 | 0.25－50 | 4.16 | 1.89 | 0.999994 | 0.10 | 0.25 |
| Acid green | 4.92 | 0.25－50 | 2.65 | 3.07 | 0.999968 | 0.10 | 0.25 |
| Brilliant blue | 5.09 | 0.25－50 | 2.23 | -4.07 | 0.999996 | 0.10 | 0.25 |
| Erythrosine | 5.36 | 0.25－50 | 1.88 | 2.05 | 0.999994 | 0.10 | 0.25 |
| Acidrose red B | 5.68 | 0.25－50 | 5.72 | 6.08 | 0.999973 | 0.10 | 0.25 |
| Patent blue V | 5.78 | 0.25－50 | 4.59 | 2.62 | 0.999991 | 0.10 | 0.25 |
| Rose bengale | 6.02 | 0.25－50 | 2.05 | 3.16 | 0.999986 | 0.10 | 0.25 |

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Table 4 Recoveries of 14 dyes from jelly samples at three spiked concentrations ($n$=6)

| Dyes | Spiking level (mg kg$^{-1}$) | Mean recovery ±SD (%) | RSD (%) | |
|---|---|---|---|---|
| | | | Intraday | Interday |
| Tartrazine | 1 | 81.4±6.34 | 7.94 | 8.67 |
| | 5 | 84.5±3.77 | 3.61 | 6.03 |
| | 25 | 92.5±3.05 | 3.45 | 5.76 |
| New red | 1 | 90.7±4.96 | 5.81 | 6.88 |
| | 5 | 93.8±5.13 | 6.05 | 3.36 |
| | 25 | 92.4±3.85 | 4.72 | 4.09 |
| Amaranth | 1 | 89.6±5.06 | 6.31 | 6.58 |
| | 5 | 94.7±3.90 | 3.96 | 5.34 |
| | 25 | 95.9±3.55 | 3.53 | 5.06 |
| Indigotine | 1 | 91.9±4.69 | 5.35 | 5.04 |
| | 5 | 93.6±3.58 | 3.06 | 4.11 |
| | 25 | 93.8±1.94 | 2.78 | 3.52 |
| Ponceau 4R | 1 | 83.8±3.75 | 2.97 | 5.42 |
| | 5 | 87.0±3.82 | 2.52 | 8.27 |
| | 25 | 91.3±5.62 | 4.06 | 7.04 |
| Sunset yellow | 1 | 82.2±4.55 | 2.73 | 4.98 |
| | 5 | 84.6±3.41 | 2.99 | 6.55 |
| | 25 | 88.5±4.05 | 5.33 | 7.09 |
| Allura Red | 1 | 84.6±1.63 | 0.90 | 5.66 |
| | 5 | 84.7±3.05 | 2.58 | 7.83 |
| | 25 | 96.5±8.01 | 8.74 | 9.62 |

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| | | | | |
|---|---|---|---|---|
| Carmoisine | 1 | 95.4±7.55 | 7.04 | 6.77 |
| | 5 | 93.6±5.88 | 3.11 | 5.43 |
| | 25 | 97.5±3.05 | 2.95 | 4.08 |
| Acid green | 1 | 93.8±3.12 | 4.67 | 4.15 |
| | 5 | 96.6±4.68 | 5.32 | 4.62 |
| | 25 | 95.0±2.05 | 3.58 | 3.05 |
| Brilliant blue | 1 | 81.9±5.15 | 4.97 | 5.38 |
| | 5 | 87.5±3.06 | 4.63 | 7.11 |
| | 25 | 84.9±4.77 | 5.81 | 6.50 |
| Erythrosine | 1 | 94.1±3.85 | 5.66 | 7.15 |
| | 5 | 95.5±2.96 | 2.18 | 4.88 |
| | 25 | 95.1±3.06 | 3.65 | 3.92 |
| Acidrose red B | 1 | 96.5±4.15 | 6.09 | 6.55 |
| | 5 | 94.8±3.59 | 5.57 | 5.98 |
| | 25 | 98.6±4.36 | 4.88 | 5.06 |
| Patent blue V | 1 | 85.5±7.90 | 8.10 | 8.59 |
| | 5 | 89.3±5.06 | 6.45 | 7.35 |
| | 25 | 87.1±4.28 | 5.59 | 5.99 |
| Rose bengale | 1 | 90.6±4.55 | 5.66 | 6.05 |
| | 5 | 95.8±1.85 | 3.82 | 3.56 |
| | 25 | 94.2±2.60 | 4.05 | 4.11 |

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Table 5 Dye concentrations in the real jelly and gummy candy samples

| Sample | | | Dyes (mg $kg^{-1}$) | | | | |
|---|---|---|---|---|---|---|---|
| | | | Tartrazine | Sunset yellow | Poncean 4R | Allura red | Brilliant blue |
| 1 | Brand 1 | Gummy candy | 80.4 | ND[a] | ND | ND | 9.6 |
| 2 | Brand 2 | Gummy candy | 54.3 | ND | ND | 22.3 | 0.7 |
| 3 | Brand 2 | Gummy candy | 13.7 | ND | ND | ND | ND |
| 4 | Brand 2 | Gummy candy | 71.6 | ND | 9.6 | ND | ND |
| 5 | Brand 2 | Gummy candy | ND | 0.8 | 33.0 | 1.0 | ND |
| 6 | Brand 2 | Gummy candy | ND | ND | ND | 41.6 | 7.9 |
| 7 | Brand 2 | Gummy candy | ND | ND | ND | ND | ND |
| 8 | Brand 2 | Gummy candy | 51.9 | ND | 8.3 | 2.0 | ND |
| 9 | Brand 3 | Gummy candy | 1.7 | ND | ND | 47.8 | ND |
| 10 | Brand 4 | Jelly | 20.2 | 0.6 | ND | ND | ND |
| 11 | Brand 5 | Jelly | ND | ND | ND | ND | ND |
| 12 | Brand 6 | Jelly | 2.2 | ND | ND | ND | ND |
| 13 | Brand 7 | Jelly | ND | 1.8 | ND | ND | ND |
| 14 | Brand 8 | Jelly | 13.6 | 1.4 | ND | ND | ND |
| 15 | Brand 9 | Jelly | ND | ND | 1.6 | ND | ND |

a. ND= not detected

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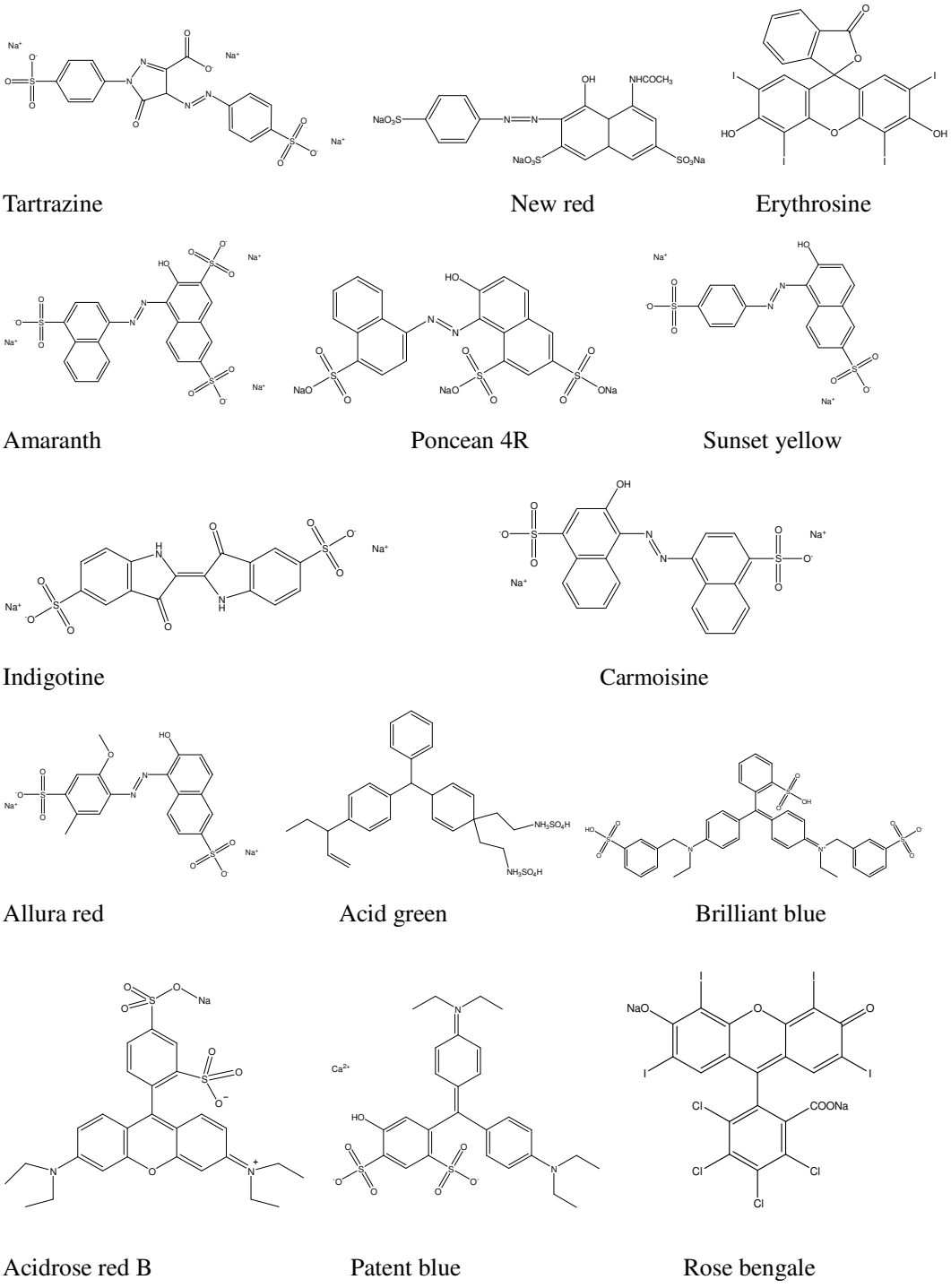


Fig. 1 Chemical structures of the fourteen target dyes

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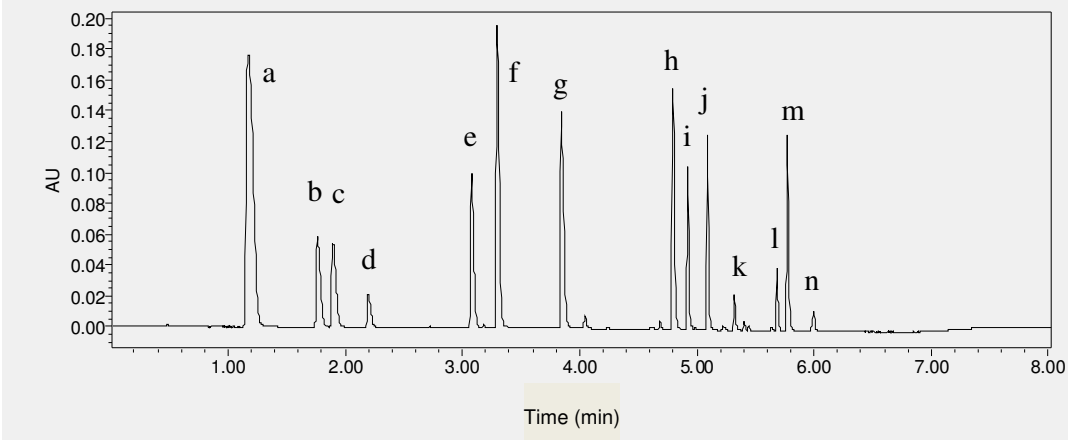


Fig. 2 LC chromatograms (420 nm) of fourteen dyes using the mobile phase consisted of acetonitrile and water containing 10 mM of ammonium acetate: a, tartrazine; b, new red; c, amaranth; d, indigotine; e, ponceau 4R; f, sunset yellow; g, allura Red; h, carmoisine; i, acid green; j, brilliant blue; k, erythrosine; l, acidrose red B; m, patent blue V; n, rose bengale.

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