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Analytical Methods

Fast and simultaneous determination of eleven synthetic color additives in flour and meat products by liquid chromatography coupled with diode-array detector and tandem mass spectrometry

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ABSTRACT

In this study, an efficient, fast and sensitive method for the simultaneous determination of eleven synthetic color additives (Allura red, Amaranth, Azo rubine, Brilliant blue, Erythrosine, Indigotine, Ponceau 4R, New red, Sunset yellow, Quinoline yellow and Tartrazine) in flour and meat foodstuffs is developed and validated using HPLC coupled with DAD and MS/MS. The color additives were extracted with ammonia–methanol and was further purified with SPE procedure using Strata-AW column in order to reduce matrix interference. This HPLC–DAD method is intended for a comprehensive survey of color additives in foods. HPLC–MS/MS method was used as the further confirmation and identification. Validation data showed the good recoveries in the range of 75.2–113.8%, with relative standard deviations less than 15%. These methods are suitable for the routine monitoring analysis of eleven synthetic color additives due to its sensitivity, reasonable time and cost.

1. Introduction

Color additives have been widely used as coloring agents in the food industry for many years. They are usually classified as natural (or identical natural) and synthetic. Natural color additives generally have a lower tinctorial strength than synthetic color additives, which are generally more sensitive to light, temperature, pH, and redox agents. At present, it is more frequent that single or mixtures of several synthetic color additives are used as food colorants in foodstuff to obtain attractive colors of a product. However, the use of these synthetic color additives must be permitted and controlled because they can occasionally produce allergy, asthma and other health disorders in sensitized individuals (Amate, Unterluggauer, Fischer, Fernández-Alba, & Masselter, 2010; Boeniger, 1980).

The Food Safety Law of the People's Republic of China requires the application of synthetic color additives to be kept under surveillance by the China Food and Drug Administration (CFDA) and listed in Direct GB 2760-2011 of the Ministry of Health, in order to be legally used in food markets in China. According to the Direct GB 2760-2011, eleven synthetic color additives are listed as certifiable food color additives that can be added to food products. Permitted synthetic food color additives are: Allura red, Amaranth, Azo rubine, Brilliant blue, Erythrosine, Indigotine, Ponceau 4R, New red, Sunset yellow, Quinoline yellow and Tartrazine. Based on their chemical structure, they can be divided into the azo (Sunset yellow), triarylmethane (Brilliant blue), xanthene (Erythrosine), and indigo (Indigotine) colorant classes. These synthetic food color additives are usually used as the water-soluble sodium salts. Their name, structure and properties were summarized in Table S1. Moreover, the Direct GB 2760-2011 also regulates the fields of application of the synthetic food color additives and the permitted maximum quantities allowed for coloring foodstuffs. In China, the maximum amount allowed for most synthetic food color additives is no more than 100 mg/kg. Even it is non-permitted that these synthetic color additives are used in several kinds of foods, such as stewed meat, roast meat and stream born products. When they are consumed in excessive amounts, these substances and their metabolites also pose potential health risk to human beings and may even be carcinogenic (Price et al., 1978; Robens et al., 1980). Therefore, CFDA usually makes the plan to monitor and investigate the levels of the certified synthetic color

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additives in high consumption and risk products such as meat and flour products every year. In response to the CFDA's plan, a new, fast, accurate and robust method for the quantitative determination of the certified synthetic color additives should be developed in food products, particularly complex solid-matrix foods.

Based on HPLC, several types of methods have been reported for the determination of 3–40 colorants in food products (Alves, Brum, Branco de Andrade, & Pereira Netto, 2008; Dossi, Toniolo, Susmel, Pizzariello, & Bontempelli, 2006; Garcia-Falcón & Simal-Gandara, 2005; Ma, Luo, Chen, Su, & Yao, 2006; Minioti, Sakellariou, & Thomaidis, 2007; Yoshioka & Ichihashi, 2008; Yuet-Wan Lok, Chung, Benzie, & Woo, 2010). However, most of them only focused on liquid samples, like soft drinks and juice drinks, or water-soluble foods such as fruit jelly, jam and confectionery because the colorants can be analyzed directly with little sample preparation. Obviously, these methods are not suitable for the complex solid-matrix foods. The other methods (González, Gallego, & Valcárcel, 2003; Sun, Sun, Li, Zhang, & Yang, 2013; Tao et al., 2011; Tavakoli, Shemirani, & Hajimahmoodi, 2014) reported for more complex foods use procedures for the determination of the color additives that are not suitable for CFDA's use, because they are either very time-consuming or only applicable to the chili foods (chili powder, chili paste) or they require the use of special cleanup materials and instruments. In addition, most researchers pay much more attentions to the determination of illegal dyes (Alesso, Bondioli, Talío, Luconi, & Fernández, 2012; Chang et al., 2011; Enríquez-Gabeiras, Gallego, Garcinuño, Fernández-Hernando, & Durand, 2012; Zhu et al., 2014; Zou, He, Yasen, & Li, 2013), such as Sudan dyes, Rhodamine B, Para red in foods. Not many researches were reported for the detection of permitted synthetic color additives. However, the usage of permitted synthetic color additives sometimes was above the authorized levels or beyond the scope of application in foods. Until now, to the best of our knowledge, there are no reports in detail on the simultaneous determination of all the certified synthetic color additives in solid-matrix foods, especially in animal origin foods. Animal origin foods have very complex matrices. They typically contain high concentrations of fats, proteins and other additives, which often caused the interference in the confirmation of color additives.

Thus, in our study, a new method was developed and validated for the simultaneous determination of eleven permitted synthetic color additives in high protein and fat content food products. Such method will be employable for routine applications where high sample throughput is required without affecting the accurateness and the sensitivity of the determinations. The analysis was mainly performed with high performance liquid chromatography (HPLC), coupled diode array detector (DAD) or tandem mass sepctrometry operated in negative electro-spray mode (ESI-MS/MS). This HPLC–DAD method was intended for a comprehensive survey of color additives in foods. However, it was not sufficient for the identification by the retention time and spectrum because of the interference of food matrix. The high-performance liquid chromatography–tandem mass spectrometry (HPLC–MS/MS) can distinguish and identify targets from background matrix ions, which can increase sensitivity and specificity. Therefore, HPLC–ESI-MS/MS method was chosen and developed for the further confirmation purposes to assure accuracy of the results. The influences of extract preparation condition, mobile phase, SPE condition, and MS parameters were investigated and optimized. The ionization behavior and the MS/MS fragmentation behavior of dyes were researched. The proposed method can realize fast separation of the 11 color additives in a 10-min gradient elution. The method was validated by evaluating recovery, selectivity, linearity, accuracy and repeatability according to the China FDA guideline GB/T 27404.

2. Materials and methods

2.1. Reagents and materials

Certificated reference materials of Azo rubine, New red, Erythrosine and Quinoline yellow were obtained from Dr. Ehrenstorfer GmbH (Augsburg, Germany). Appropriate amounts of powder of these color additives were dissolved in methanol/water (1:1 v/v) to give a concentration of 1.00 mg/mL. The other color additives (1.00 mg/mL) used as standards were purchased from Chemical Metrology & Analytical Science Division (Beijing, China). Matrix-matched mixed working standard solutions were prepared by adding desired volume of individual stock standard solutions into the blank matrix. These solutions were stored at 4 °C in the dark. All working solutions for the calibration were prepared fresh before use.

All the water used was purified by Sartorius Arium 611 system with a resistance of 18.2 MΩ/cm. HPLC grade methanol and ammonium acetate were purchased from Sigma–Aldrich (St. Louis, MO, USA). Analytic grade ammonium hydroxide, ethanol and n-hexane were purchased from Guangzhou Chemical Reagent Company (Guangzhou, China). SPE Strata-X-AW, Strata-X-C, Strata-X and Strata-X-CW cartridges (200 mg, 6 mL) were obtained from Phenomenex (Torrance, CA, USA), which were used in the purification step. Teflon membrane syringe filters (0.22 μm) were bought from Anpel Company (Shanghai, China).

2.2. Sample collection

All of the food samples such as corn steamed bun, barbecued pork and roasted duck were purchased from local markets. The manufacturer or distributor declared that these products didn't contain any synthetic color additives. Prior to analysis, the products were mixed homogeneously and stored in 50 mL PTFE centrifuge tubes at −20 °C. Spiked samples were prepared in a 50 mL centrifuge tube by mixing 2.0 g of homogenized samples with a series of the 11 color additives standard solutions at various concentrations.

2.3. Sample preparation

First, 2.0 g of homogenized sample and 10 mL of n-hexane were shaken by vortex mixer for 5 min, and then the n-hexane layer was discarded to eliminate fat. Second, the sample was extracted with 10 mL of methanol–ammonia–water (80:2:18, V/V/V) for 10 min in an ultrasonic bath at 40 °C. The super-extracts were collected. The above procedure was repeated 1 more time. Finally, the pooled super-extracts were collected and evaporated to dryness by rotary evaporator at 35 °C. The evaporation residues of pooled extracts were redissolved in 20 mL of deionized water as the loading solution of SPE. Third, 10 mL of redissolved solution was loaded onto the Strata-X-AW cartridge that was preconditioned with 6 mL of methanol and 6 mL of water. After washing with 6 mL of water/methanol (1:1, v/v), the retained constituents were eluted with 20 mL of ethanol that contained 10% (v/v) ammonia–water, followed by the evaporation to dryness by rotary evaporator. Finally, the evaporated residue was reconstitute in 5 mL of methanol–water (1:9, v/v), and then it was filtered through a 0.22 μm Teflon syringe filter for HPLC or HPLC–MS/MS analysis.

2.4. Apparatus

The HPLC–DAD method was developed using an Agilent 1260 HPLC system with binary-pump, auto-sampler, temperature controlled column oven and DAD detector (Agilent Technologies,