Simultaneous Determination of Seven Synthetic Colorants in Wine by Dispersive Micro-Solid-Phase Extraction Coupled with Reversed-Phase High-Performance Liquid Chromatography

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A novel and effective dispersive micro-solid-phase extraction (d-µ-SPE) using ethanediamine-functionalized magnetic Fe₃O₄ polymer (EDA-MP) as an efficient adsorbent in wine sample was developed. Based on this, a simple and time-saving analytical method for the simultaneous determination of seven synthetic colorants (i.e., tartrazine, amaranth, carmine, sunset yellow, allura red, brilliant blue and erythrosine) in wine by reversed-phase high-performance liquid chromatography with an ultraviolet detector was established. The experimental parameters, including the chromatographic retention behavior of studied synthetic colorants, the effect of the usage amount of cross-linking monomer, the effect of the usage amount of EDA-MP on the recovery and the recyclability of the adsorbents, were studied in detail. The results showed that the EDA-MP could be reused efficiently at least six times. Under optimized conditions, the recoveries for all analytes were in the range of 88.6–105.2%, with the intraday relative standard deviations (RSDs) ranging from 2.1 to 8.2% and the interday RSDs ranging from 3.4 to 8.7%, and all the analytes had good linearity in the tested ranges with correlation coefficients \((r^2)\) >0.9995. The limits of quantification for seven synthetic colorants were between 0.12 and 0.45 mg L\(^{-1}\). The developed method was successfully applied to wine samples, and it was confirmed that the EDA-MP particles were highly effective d-µ-SPE materials.

Introduction

Food colorants, including natural colorants and synthetic colorants, are an important kind of food additives, which are widely used in drinks and food products to restore the natural color and provide the desired colored appearance (1). Natural colorants are preferred by consumers; however, synthetic colorants are widely used because of their low price, high effectiveness and excellent stability (2). In recent years, more and more evidences indicate that some synthetic colorants and their metabolites pose a potential risk to human health and may even be carcinogenic, especially when they are consumed excessively (3, 4). Therefore, to protect public health, the use of synthetic colorants is strictly controlled by different national legislations, while acceptable daily intakes are determined and evaluated by the World Health Organization and the United Nations Food and Agricultural Organization (5). In China, the maximum amount allowed for most colorants is no >100 mg kg\(^{-1}\) (6). Consequently, accurate and reliable methods for the determination of synthetic colorants in drinks and food products are required for the public health.

Some analytical methods for synthetic colorants have been proposed, such as capillary electrophoresis (7, 8), spectrophotometry (9), ion chromatography (10, 11), thin-layer chromatography (12), liquid chromatography–mass spectrometry (2, 13, 14) and high-performance liquid chromatography (HPLC) with ultraviolet/visible or diode-array detectors (5, 15–18). HPLC-based methods are the most commonly used technique with excellent precision, accuracy and relatively lower cost (19). Owing to the complexities of matrices and the low levels of colorants in real samples, it is difficult to determine the synthetic colorants so the sample pretreatment is crucial for simultaneous determination. Zhang et al. (20) used polyamide adsorption method for the determination of five synthetic colorants, such as tartrazine, amaranth, carmine, sunset yellow and brilliant blue in beverage. Unfortunately, the polar analyte, i.e., erythrosine, could not be eluted from polyamide adsorbents, and the phenomenon is also found in literature (21).

Therefore, in our work, hydrophilic–lipophilic balanced (HLB) solid-phase extraction (SPE) cartridges were used in the cleanup procedure of synthetic colorants in wine. The reason for choosing the HLB SPE cartridges was that its copolymer contained two monomers: the hydrophilic N-vinylpyrrolidone and the lipophilic divinylbenzene (DVB). The N-vinylpyrrolidone could provide a special polar hook for enhanced capture of polar analytes, and the DVB could provide a better reservation for weak polar analytes. Although HLB SPE has been verified to be effective for cleaning the wine samples in our study, compare with the dispersive micro-solid-phase extraction (d-µ-SPE), it is relatively expensive, complicated, time-consuming and requires large volumes of solvents. Based on the HLB SPE method, we inspired that amino-functionalized superparamagnetic polymers with magnetic separation would probably become a powerful adsorbent to carry out d-µ-SPE procedure, which is considered as one of the most powerful cleanup and extraction technologies.

In this study, an effective ethanediamine-functionalized magnetic Fe₃O₄ polymer (EDA-MP) has been synthesized, which is expected to have large adsorption capacity for synthetic colorants and possesses superparamagnetic features for more convenient separation under a magnetic field. The object of this study was to prepare a novel amino-functionalized magnetic polymer (EDA-MP) and substantiated its d-µ-SPE ability for the analysis of synthetic colorants including tartrazine, amaranth, carmine, sunset yellow, allura red, brilliant blue and erythrosine in wine samples. This paper also aimed to develop a rapid and efficient analytical method for the simultaneous determination of seven
synthetic colorants mentioned above by reversed-phase high performance liquid chromatography (RP-HPLC). The comparison of the polyamide adsorption method, HLB SPE and EDA-MP d-μ-SPE were also described in detail.

**Material and Methods**

**Chemicals and reagents**

Ferrous sulfate (FeCl₂·6H₂O, 99%), ferric chloride tetrahydrate (FeCl₃·4H₂O, 99%), oleic acid (OA, 90%), aqueous ammonia (NH₃·H₂O, 25% in H₂O), ethanediamine (EDA, 99.5%) and absolute ethanol (99.7%) were analytical grade and used without any further purification. Glycidyl methacrylate (GMA, 97%), styrene (St, 99.5%) and DVB (80% mixture of isomers) were analytical grade (Sigma-Aldrich), which were distilled under reduced pressure to remove inhibitors. Polyvinyl alcohol (217, degree of polymerization 1,700, degree of hydrolysis 88%) and benzoyl peroxide (99.9%) were analytical grade, which were purchased from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). The standard solutions (1,000 mg L⁻¹ from Merck (Darmstadt, Germany). Water for all applications in our study was obtained from an in-house Milli-Q ultra-pure water system (Millipore, Bedford, MA, USA) with resistivity equal to or >18.2 MΩ cm. The wine samples including dry red wine, semi-dry wine and sweet wine were acquired in local markets (Zhejiang, China).

Working standard mixture solution at a concentration of 100.0 mg L⁻¹ was prepared by appropriate dilution of the stock solutions with 20 mmol L⁻¹ AmAc in water : ethanol (A), and methanol as eluent (B). The linear gradient was: 0–5.0 min, 92–65% A (8–35% B); 5.0–10.0 min, 65–20% A (35–80% B) and 10.0–14.0 min, 20% A (80% B). Finally, the mobile phase concentration was returned to 92% A (8% B), and held for 2 min for column equilibration. The detection wavelength of the colorants was carried out according to the following procedure: 0–10.20 min, 254 nm; 10.21–12.00 min, 600 nm; 12.10–15.00 min, 254 nm. Chromatographic separation of the synthetic colorants was accomplished on the Kromasil C18 column at a constant flow of 1.0 mL min⁻¹ and the injection volume was 10.0 μL. The column temperature was maintained at 30°C.

**Equipment**

The characterization of EDA-MP were conducted by using transmission electron microscopy (TEM) H-7650 (Hitachi, Japan) and vibrating sample magnetometer (VSM) Lake Shore 7410 (Westerville, OH, USA) for the magnetic separation. A 1.5-T NdFeB permanent magnet (MCE Products, Torrance, CA, USA) was used for magnetic separation. A vortex mixer Hualida WH-866 (Taicang, China) was used during extraction. HPLC analysis was performed with an Agilent 1100 system equipped with a G1311A pump, a G1314A UV detector and a G1313B autosampler (Palo Alto, CA, USA). Separation of synthetic colorants was made on a Kromasil C18 chromatographic column (250 × 4.6 mm × 5 μm). SPE cartridges (Oasis® HLB; 6 mL, 200 mg) were purchased from Waters (AGB, Belfast, USA). The polyamide adsorbent was purchased from Nankai University Chemical Co. (Tianjin, China).

**Synthesis of EDA-MP**

EDA-MPs with varied amounts of cross-linking monomer (DVB) were prepared via suspension polymerization according to our previously reported procedures after minor modification (22). The paramagnetic Fe₃O₄ was firstly coated with OA. GMA, St and DVB were then copolymerized via the suspension polymerization procedure over the magnetic core to obtain epoxide-functionalized magnetic polymer. Finally, EDA was grafted onto the surface of the polymer via ring-opening reaction. Thus, the target EDA-functionalized magnetic polymer was obtained. The preparation procedure was illustrated in Figure 1. The series of EDA-MPs with different amount of DVB (0.5, 1.0, 1.5 and 2.0 mL) were synthesized in a similar way, and named as EDA-MP-0.5, EDA-MP-1.0, EDA-MP-1.5 and EDA-MP-2.0.

**Characterization of EDA-MP**

The morphology and dimensions of the synthesized EDA-MP were examined by TEM at 80 kV. Each sample was prepared by placing a very dilute particle suspension onto 400 mesh carbon grids coated with copper film. The nitrogen contents of EDA-MP were determined by elemental analysis (EA). Magnetic behavior was analyzed by a VSM. XPS spectra were obtained with XPS, and a monochromatic Al-Kα X-ray source (1486.6 eV) was used.

**Chromatographic conditions**

Analytes were separated by RP-HPLC using 20 mmol L⁻¹ AmAc in water as eluent (A), and methanol as eluent (B). The linear gradient was: 0–5.0 min, 92–65% A (8–35% B); 5.0–10.0 min, 65–20% A (35–80% B) and 10.0–14.0 min, 20% A (80% B). Finally, the mobile phase concentration was returned to 92% A (8% B), and held for 2 min for column equilibration. The detection wavelength of the colorants was carried out according to the following procedure: 0–10.20 min, 254 nm; 10.21–12.00 min, 600 nm; 12.10–15.00 min, 254 nm. Chromatographic separation of the synthetic colorants was accomplished on the Kromasil C18 column at a constant flow of 1.0 mL min⁻¹ and the injection volume was 10.0 μL. The column temperature was maintained at 30°C.

**Sample pretreatment**

The extraction procedure, EDA-MP d-μ-SPE, was performed and compared with the polyamide adsorption method and HLB SPE, with the other variables unchanged. An overview of three different extraction procedures was shown in Figure 2.

**The EDA-MP d-μ-SPE procedure**

An exactly 1.0 mL of wine (adjusted to pH 3.0 with HCl 0.5 mol L⁻¹) was transferred to a glass centrifuge tube containing 20 mg of EDA-MP. The mixture was immediately vortexed for 1.0 min, then separation under a magnetic field for 1 min. Subsequently, the adsorbed EDA-MP was eluted by 5.0 mL of 2% ammonia (v/v) in methanol. The supernatant concentrated to dryness with a nitrogen stream and was re-dissolved with 1.0 mL of 20 mmol L⁻¹ AmAc in water : methanol (92:8, v/v) and filtered using a 0.45 μm membrane prior to its injection into the RP-HPLC system. The used EDA-MP was recycled by washing with methanol for 30 min.

**The HLB SPE procedure**

For the HLB SPE method, 1.0 mL of wine sample was placed into an open evaporating dish and evaporated to dryness in a water
bath at 80°C. Afterwards, the residue was redissolved with pure water and transferred onto the HLB SPE cartridge, which was conditioned sequentially with methanol (5.0 mL) and 0.1% methanoic acid : water (5.0 mL, v/v). The extraction cartridge was washed by another 0.1% methanoic acid : water (5.0 mL, v/v). The absorbed colorants were then eluted with 2.0% ammonia solution : methanol (5.0 mL, v/v). The final eluate was collected and concentrated to dryness with a nitrogen stream and was redissolved with 1.0 mL of 20 mmol L\textsuperscript{-1} AmAc in water : methanol (92 : 8, v/v) and filtered using a 0.45 μm membrane prior to its injection into the RP-HPLC system.

Spiked samples
Spiked recoveries were performed at concentrations of 1.0, 5.0 and 20.0 mg L\textsuperscript{-1} for seven synthetic colorants in the samples. For each spiked sample, the stock mixture solution of the standards was added to 1.0 mL wine, which was free from the target compounds. The spiked samples were stored at 4°C for ~12 h to let the synthetic colorants permeate uniformly into the samples. All recoveries at each level were run along with both a reagent and a sample blank.

**Results**

**Characterization of EDA-MP**

*TEM, EA and VSM analysis of EDA-MP*

The TEM images of EDA-MPs were shown in Figure 3. It revealed that the average diameters of EDA-MPs increased gradually (∼0.5, ∼0.6, ∼0.9 and ∼1.0 μm) with an increased amount of DVB during the copolymerization procedure. However, there are more and more small spherical growths on the surface microspheres in the particles in Figure 3a–d, which can be attributed to the residual polymers in the copolymerization procedure. With the increasing amount of cross-linking monomer (DVB) (0.5, 1.0, 1.5 and 2.0 mL), the vinyl groups on the polymer microspheres increased correspondingly, caused excessive self-cross-linking and subsequently leaded to the highly cross-linked copolymers. So there are small spherical growths on the surface microspheres in some of the particles.

The nitrogen contents of these series of EDA-MPs obtained from elemental analysis decreased (7.89, 6.60, 6.33 and 5.60%), indicating that with the increasing of the amount of DVB, the copolymers were highly cross-linked, which subsequently resulted in the decreasing of the epoxy groups on the surfaces, and eventually leading decreasing of amino groups via ring-opening reaction. The
paramagnetic properties of the EDA-MP-0.5 was verified by VSM and the saturation intensities of magnetization obtained from the hysteresis loop was 10.59 emu g$^{-1}$ (Supplementary data, Figure S1), which was expected to respond well to magnetic fields for easy separation.

**XPS analysis of EDA-MP**

The chemical composition of the EDA-MP was investigated by XPS. As shown in Figure 4a, characteristic signals for carbon (C 1s) and oxygen (O 1s) were clearly detected at 282.6 and 529.6 eV. The appearance of a signal at 396.4 eV was assigned to nitrogen (N 1s), which was introduced by the functional monomer EDA. Furthermore, the C 1s high-resolution scan could be fitted into four peaks (Figure 4b) with binding energies of 282.6, 283.7, 284.3 and 286.5 eV, which were attributed to aliphatic carbon (C−C/C−H), ether (C−O), C−N and an ester carbon (O−C=O), respectively. The successful functionalization was also affirmed by the appearance of a strong N 1s signal between 390 and 410 eV in a high-resolution XPS spectrum (Supplementary data, Figure S2). No characteristic signal for iron (Fe 2p) between 710 and 740 eV in the high-resolution XPS spectrum was observed, confirming that no leakage of Fe$_3$O$_4$ on the surface of EDA-MP.

**Method validation**

The linearity of the calibration curves obtained by peak area (y) vs. concentration (x, mg L$^{-1}$) were studied using calibration standards in the solvent at seven concentrations of 0.5, 1.0, 2.0, 5.0, 10.0, 20.0 and 50.0 mg L$^{-1}$. The response function of the seven synthetic colorants was found to be linear with correlation coefficients ($r^2$) $>0.9995$ in the tested range, as listed in Table I. The method accuracies and precisions were obtained by seven synthetic colorants spiked at concentrations of 1.0, 5.0 and 20.0 mg L$^{-1}$ in blank red wine. The method accuracies were expressed as the recoveries, and the method precisions were expressed as the intra- and interday relative standard deviations (RSDs). The intraday RSDs were obtained by repeating the three levels of spiked samples five times within a day, and the interday RSDs were obtained by repeating the three levels of spiked samples in triplicate on 9 separate days within a 2-week period. The results are summarized in Table I. It shows that the majority of mean recoveries are in the range of 88.6–105.2% with
the intraday RSDs ranging from 2.1 to 8.2% and interday RSDs ranging from 3.4 to 8.7%.

The limits of detection (LOD) and limits of quantification (LOQ) were calculated in blank extracts as the lowest analyte concentration that yielded a signal-to-noise ratio of 3 and 10, respectively. The LODs and LOQs were in the range of 0.04–0.11 mg L$^{-1}$ and 0.12–0.45 mg L$^{-1}$, respectively, as shown in Table I.

Sample analysis

Three kinds of wine (six samples for each kind), including dry red wine, semi-dry wine and sweet wine, were analyzed by the developed EDA-MP d-$\mu$-SPE-RP-HPLC method. Each batch of samples was processed together with a matrix blank (synthetic colorants free sample). The matrix blank eliminated the false positive as result of contamination in the extraction process, chemicals or instrument. A reagent blank was obtained by performing the whole process without a sample. This sample eliminated the possible false positives produced by contamination in the instrument or solvent used. A blank extract spiked at concentrations of 1.0 mg L$^{-1}$ permitted was to control the extraction efficiency (Figure 5). The findings indicated that the synthetic colorants, including tartrazine, amaranth, carmine, sunset yellow, allura red brilliant blue and erythrosine, were not found above LOQ in the wine.

Discussion

Effect of AmAc on the chromatographic separation

In order to achieve an optimal chromatographic separation, the effects of AmAc as ion suppressor on the retention behavior of the studied synthetic colorants were investigated. A series of aqueous mobile phases were composed of different concentrations of AmAc prepared at 1.0, 2.0, 5.0, 10.0, 20.0 and 25.0 mmol L$^{-1}$ under the gradient elution condition described above. The results indicated that with the increasing of the concentration of AmAc, the retention times for the seven colorants decreased significantly. The phenomenon could attribute to the fact that AmAc not only has an ion suppression effect but also has an organic modification.
Effect on the synthetic colorants in the mobile phase system. The baseline separation of seven synthetic colorants can be achieved by using AmAc solution (20.0 mmol L\(^{-1}\)) and methanol as the mobile phase system, as shown in Figure 6.

**Effect of the usage amount of DVB on the extraction properties**

The effects of the usage amount of DVB on extraction efficiency of EDA-MP have been investigated. The series of EDA-MP-0.5, EDA-MP-1.0, EDA-MP-1.5 and EDA-MP-2.0 were applied to the d-μ-SPE extraction procedure for seven synthetic colorants at concentrations of 1.0, 5.0 and 10.0 mg L\(^{-1}\). The results, as shown in Table II, indicated that with the increasing of DVB, the average recoveries at 5.0 mg L\(^{-1}\) for the series of EDA-MPs decreased correspondingly. This can be explained as follows: on the one hand, with the increasing of DVB, the particle diameters increased and the surface areas decreased correspondingly, resulting in the decline of extraction efficiency; on the other hand, the amount of amino groups on the adsorbent surfaces decreased attributed to the highly cross-linked copolymers, which also led to the decreasing of extraction efficiency. The results from the elemental analysis of nitrogen contents of the series of EDA-MPs further confirmed the above conclusion. This phenomenon was also observed at the 1.0 and 10.0 mg L\(^{-1}\) levels.

Therefore, the EDA-MP, synthesized with 0.5 mL of DVB, was chosen as the adsorbents in the following d-μ-SPE procedure.

**EDA-MP d-μ-SPE procedure and comparison of the method with others**

In order to minimize the influence and achieve the qualitative and quantitative analysis, three different pretreatment procedures, the polyamide adsorption method, the HLB SPE and the EDA-MP d-μ-SPE were all investigated and compared with obtain reasonable experimental results and satisfactory efficiency, with the other variables unchanged. Wine samples spiked with seven synthetic colorants at a concentration of 5.0 mg L\(^{-1}\) was used to compare the pretreatment procedures via the three different approaches. The average recoveries of the analytes studied were shown in Figure 7. It can be seen that with the use of polyamide adsorption method, though the recoveries of tartrazine, amaranth, carmine, sunset yellow, allura red and brilliant blue were >80%, the recovery of erythrosine was close to 0, which meant it was hardly eluted from the polyamide adsorbents. With the use of HLB SPE method, the recoveries of all the seven synthetic colorants were in the range of 91.5-111%, with the RSDs ranging from 1.5 to 6.3%. The average recoveries obtained by EDA-MP d-μ-SPE procedure were 96, 97, 94.3, 100.3, 101.5, 94 and 102.5% for tartrazine, amaranth, carmine, sunset yellow, allura red, brilliant blue and erythrosine, respectively, with the RSDs ranging from 1.4 to 4.9%. Though both of the EDA-MP d-μ-SPE and HLB SPE methods had obtained satisfactory recoveries, the EDA-MP d-μ-SPE method was faster and more convenient than the HLB SPE method and consumed less solvent.

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**Figure 4**. (a) XPS analysis of the EDA-MP and (b) the fit of C 1s XPS spectrum.

**Table I**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Retention time (min)</th>
<th>Linear equation (y = ax + b)</th>
<th>(r^2)</th>
<th>Average recovery, % (RSD, %)</th>
<th>LOD (mg L(^{-1}))</th>
<th>LOQ (mg L(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1.0 mg L(^{-1})</td>
<td>5.0 mg L(^{-1})</td>
<td>10.0 mg L(^{-1})</td>
</tr>
<tr>
<td>Tartrazine</td>
<td>5.526</td>
<td>(y = 31.70x + 6.588)</td>
<td>0.9999</td>
<td>98.2 (7.0, 5.1)</td>
<td>96.8 (7.2, 7.9)</td>
<td>93.3 (7.2, 8.7)</td>
</tr>
<tr>
<td>Amaranth</td>
<td>6.123</td>
<td>(y = 27.31x + 5.044)</td>
<td>1.0000</td>
<td>93.6 (2.7, 4.6)</td>
<td>92.2 (4.8, 6.2)</td>
<td>96.2 (2.6, 4.1)</td>
</tr>
<tr>
<td>Carmine</td>
<td>8.054</td>
<td>(y = 25.76x + 1.449)</td>
<td>1.0000</td>
<td>96.2 (5.6, 4.4)</td>
<td>90.6 (5.8, 5.7)</td>
<td>95.2 (3.6, 5.2)</td>
</tr>
<tr>
<td>Sunset yellow</td>
<td>9.053</td>
<td>(y = 23.39x + 1.320)</td>
<td>0.9998</td>
<td>90.2 (6.2, 7.3)</td>
<td>92.3 (2.1, 3.4)</td>
<td>96.8 (5.6, 5.5)</td>
</tr>
<tr>
<td>Allura red</td>
<td>9.831</td>
<td>(y = 7.732x - 0.720)</td>
<td>0.9996</td>
<td>99.6 (6.8, 8.2)</td>
<td>105.2 (3.7, 6.2)</td>
<td>96.2 (4.8, 6.6)</td>
</tr>
<tr>
<td>Brilliant blue</td>
<td>10.93</td>
<td>(y = 33.33x + 3.309)</td>
<td>0.9997</td>
<td>90.2 (2.6, 5.6)</td>
<td>88.6 (5.8, 8.7)</td>
<td>91.9 (3.9, 5.4)</td>
</tr>
<tr>
<td>Erythrosine</td>
<td>13.09</td>
<td>(y = 14.96x + 1.048)</td>
<td>0.9995</td>
<td>92.9 (6.8, 4.3)</td>
<td>90.8 (6.2, 6.9)</td>
<td>89.8 (8.2, 8.5)</td>
</tr>
</tbody>
</table>

\(y\), peak area; \(x\), the concentration of target compound, mg L\(^{-1}\).

\(\text{Intraday, } n = 5 \text{ replicates.}\)

\(\text{Interday, } n = 3 \text{ replicates } \times 7 \text{ days within a 2-week period.}\)
Therefore, the EDA-MP \(d_\mu\)-SPE method was used in all following extraction experiments.

**Effect of the amount of EDA-MP on the extraction properties**

According to the discussion above, the EDA-MP particles were very effective to extract the target analytes. When designing the EDA-MP \(d_\mu\)-SPE pretreatment optimized experiments, it was the primary consideration to employ a suitable amount of the EDA-MP without affecting the synthetic colorants recoveries. For this purpose, the effectiveness of various amounts of adsorbents on extraction efficiency was carried out by red wine samples spiked with each of the seven synthetic colorants at \(10.0 \text{ mg} \text{ L}^{-1}\). The spiked samples were purified by using different amounts of the EDA-MP particles, and the results were shown in Figure 8. The dispersive EDA-MP adsorbents had an impact on recoveries of the studied synthetic colorants. With the increasing of the amount of EDA-MP adsorbents from 5 to 20 mg, the recoveries of the seven synthetic colorants were all gradually increased. They ranged from 64.4 to 96.4% for tartrazine, from 63.8 to 95% for amaranth, from 66 to 95% for carmine, from 64.2 to 94.4% for sunset yellow, from 65 to 98.2% for allura red, from 61 to 96.1% for brilliant blue and from 64.4 to 95.6% for erythrosine. However, with the increasing from 20 to 35 mg, the recoveries of seven synthetic colorants seemed to be unobvious. Based on the obtained results, it could be concluded that

![Figure 5. Chromatograms of seven synthetic colorants spiked at 1.0 mg L\(^{-1}\) level with peak numbering: tartrazine (1), amaranth (2), carmine (3), sunset yellow (4), allura red (5), brilliant blue (6) and erythrosine (7).](image)

![Figure 6. Chromatograms of seven synthetic colorants (5.0 mg L\(^{-1}\)) in the mobile phase system of 20.0 mmol L\(^{-1}\) AmAc and methanol with peak numbering: tartrazine (1), amaranth (2), carmine (3), sunset yellow (4), allura red (5), brilliant blue (6) and erythrosine (7).](image)

![Figure 7. The comparison of three cleanup procedures on the recoveries of seven synthetic colorants.](image)

![Figure 8. Effect of the amount of EDA-MP on the extraction properties.](image)

### Table II

The Average Recoveries of Seven Synthetic Colorants at 5.0 mg L\(^{-1}\) in Wine by Using EDA-MPs with Different Usage Amount of DVB

<table>
<thead>
<tr>
<th>Compound</th>
<th>Average recovery (%)</th>
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<tbody>
<tr>
<td></td>
<td>EDA-MP-0.5</td>
</tr>
<tr>
<td>Tartrazine</td>
<td>96.8</td>
</tr>
<tr>
<td>Amaranth</td>
<td>92.2</td>
</tr>
<tr>
<td>Carmine</td>
<td>90.6</td>
</tr>
<tr>
<td>Sunset yellow</td>
<td>92.3</td>
</tr>
<tr>
<td>Allura red</td>
<td>105.2</td>
</tr>
<tr>
<td>Brilliant blue</td>
<td>88.6</td>
</tr>
<tr>
<td>Erythrosine</td>
<td>90.8</td>
</tr>
</tbody>
</table>
the combination of the amount of EDA-MP adsorbents in the proportion of 20 mg per 1.0 mL wine sample would ensure the extraction efficiency while maintaining quantitative recovery of the target synthetic colorants.

**Reusability of the adsorbents**

In order to investigate the recycling of the EDA-MP, all the EDA-MP used for d-μ-SPE procedure was collected and soaked in sodium hydroxide solution at a concentration of 1.0 mol·L⁻¹ for at least 30 min and then was washed with water and methanol to remove the redundant sodium hydroxide until the eluent pH value was ≏7.0. Finally, the EDA-MP was separated and dried under a vacuum at 60°C for 12 h and reused for d-μ-SPE. The results showed that the EDA-MP can be reused for at least six times without much sacrifice of the recoveries of the analytes, as shown in Figure 9.

**Conclusions**

In this work, an efficient EDA-MP was synthesized and a simple and reproducible analytical EDA-MP d-μ-SPE-RP-HPLC method for the simultaneous determination of seven synthetic colorants in wine was developed. The results showed that the extraction efficiency using EDA-MP was more effectively than that of the polyamide adsorption and HLB SPE method. It also showed that the synthesized EDA-MP had potential applications in sample extraction and cleanup procedures.

**Supplementary data**

Supplementary data are available at *Journal of Chromatographic Science* online.

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