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EXPLORING DEEP EUTECTIC SOLVENTS AS A POTENTIAL SOLVENT FOR THE EXTRACTION OF FOOD ADDITIVE ALLURA RED IN FOOD TECHNOLOGY APPLICATION

(Mengeksplorasi Pelarut Eutektik Mendalam sebagai Pelarut Berpotensi untuk Pengekstrakan Pewarna Makanan Merah Allura dalam Aplikasi Teknologi Makanan)

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Abstract

In recent years, synthetic food dyes have gained significant popularity as additives, replacing natural dyes, with azo dyes comprising approximately 65% of the commercial dye market. However, concerns regarding the toxic effects of azo dyes used in food colouring have prompted the development of advanced analytical methods to detect them in various food matrices. These methods typically involve intricate pre-concentration processes, time-consuming steps, and the use of expensive instruments, thus emphasising the need for highly sensitive and selective approaches. Hence, this study aims to explore the application of deep eutectic solvent (DES) as a potential solvent for the extraction of Allura Red AC (ARAC). First, the synthesised magnetic nanoparticles functionalised with DES (silicone surfactant: dodecanoic acid) (SS: DoAc@Fe_3O_4) adsorbent was successfully characterised using attenuated total reflectance-Fourier transform infrared spectroscopy (ATR-FTIR) for the identification of functional bonds. This adsorbent was subsequently applied in the magnetic solid phase extraction (MSPE) technique to extract ARAC in soft drink samples. In contrast to pure Fe_3O_4 particles, the targeted ARAC can be easily adsorbed onto the SS: DoAc@Fe_3O_4 surface due to the formation of strong hydrophobic interaction between ARAC and the adsorbents, thus amplifying the extraction efficiency. Further optimisation was done via one-variable-at-a-time (OVAT) and Taguchi experimental design to determine the optimised condition for ARAC extraction. The OVAT analysis identified that using methanol: acetonitrile (1:1) as the desorption solvent, incorporating Na_2SO_4 to boost extraction efficiency, employing salt volume of 200 µL, and utilising a sample volume of 20 mL produced the most favourable outcomes. Meanwhile, the Taguchi experiments pinpointed the optimum conditions as pH 2, an adsorbent dosage of 25 mg, an extraction duration of 2 minutes, a desorption period of 3 minutes, and a desorption solvent volume of 400 µL. Under the optimised conditions, the R^2 value obtained was 0.999 while the limit of detection (LOD) and limit of quantification (LOQ) values were 0.1 mgL^{-1} and 0.4 mgL^{-1}, respectively. The studied ARAC also obtained relative recovery values between 80% to 111%. Therefore, it is safe to conclude that the newly proposed SS: DoAc@Fe_3O_4-based MSPE is an environmentally friendly, simple, affordable, and effective technique for the extraction of ARAC in drink samples.

Keywords: Allura red AC, deep eutectic solvents, dodecanoic acid, magnetic solid phase extraction, silicone surfactant

Abstrak

Kebelakangan ini, pewarna makanan sintetik semakin popular dan kerap digunakan sebagai bahan tambahan menggantikan pewarna semula jadi, dengan pewarna azo merangkumi kira-kira 65% daripada pasaran pewarna komersial. Walau bagaimanapun, kebimbangan mengenai kesan toksik pewarna azo yang digunakan dalam pewarna makanan telah mendorong pembangunan kaedah analisis lanjutan untuk mengesannya dalam pelbagai matriks makanan. Kaedah tersebut biasanya melibatkan proses pra-kepekatan yang rumit, prosedur yang memakan masa, dan penggunaan instrumen yang mahal, sekaligus menekankan keperluan untuk pendekatan yang sensitif dan terpilih. Oleh itu, kajian ini bertujuan untuk meneroka aplikasi pelarut eutektik mendalam (DES) untuk mengekstrak pewarna merah allura AC (ARAC). Pertama, penjerap zarah nano magnetik difungsikan dengan DES (surfaktan silikon:asid dodekanoik) (SS:DoAc@Fe_3O_4) telah berjaya disintesis dan dianalisis menggunakan spektroskopi transformasian Fourier inframerah dengan pantulan keseluruhan dikecilkan (ATR-FTIR) untuk mengenal pasti ikatan berfungsi. Bahan penjerap ini kemudiannya digunakan dalam teknik pengekstrakan fasa pepejal magnetik (MSPE) untuk mengekstrak ARAC dalam sampel minuman ringan. Berbeza dengan zarah Fe_3O_4 tulen, ARAC lebih mudah diserap ke permukaan SS:DoAc@Fe_3O_4 disebabkan oleh pembentukan interaksi hidrofobik yang kuat antara ARAC dan penjerap, sekaligus menguatkan kecekapan pengekstrakan. Pengoptimuman lanjut kemudiannya dilakukan melalui satu-pembolehubah-pada-satu-masa (OVAT) dan reka bentuk eksperimen Taguchi bagi menentukan keadaan optimum untuk pengekstrakan ARAC. Analisis OVAT mendapati bahawa penggunaan metanol:acetonitrile (1:1) sebagai pelarut penyahjerapan, penambahan garam untuk meningkatkan kecekapan pengekstrakan, penggunaan isipadu garam sebanyak 200 µL, dan penggunaan isipadu sampel 20 mL memberikan hasil yang paling baik. Sementara itu, eksperimen Taguchi menunjukkan keadaan optimum sebagai pH 2, dos penjerap sebanyak 25 mg, tempoh pengekstrakan selama 2 minit, tempoh penyahjerapan selama 3 minit, dan isipadu pelarut penyahjerapan sebanyak 400 µL. Dibawah keadaan yang dioptimumkan, nilai R^2 yang diperolehi adalah 0.999 manakala nilai had pengesanan (LOD) dan had kuantifikasi (LOQ) masing-masing adalah 0.1 mgL^{-1} dan 0.4 mgL^{-1}. ARAC yang dikaji juga memperoleh nilai pemulihan relatif antara 80% hingga 111%. Oleh itu, dapat disimpulkan bahawa MSPE berasaskan SS:DoAc@Fe_3O_4 yang dicadangkan adalah satu teknik mesra alam, mudah, berpatutan, dan berkesan untuk pengekstrakan ARAC dalam sampel minuman.

Kata kunci: Pewarna merah allura AC, pelarut eutektik mendalam, asid dodekanoik, pengekstrakan fasa pepejal magnetik, surfaktan silikon

Introduction

Food colourants, also known as pigments, encompass dyes or substances that impart colour to food and beverages. They are broadly utilised both in commercial food production and home cooking settings [1]. These colourants consist of diverse chemical substances added to food matrices, serving several purposes. They are employed to enrich or safeguard the sensory attributes of food items, which could otherwise be compromised or destroyed throughout the processes of manufacturing or storage [2]. Additionally, food colourants are employed in food technology applications to achieve the desired appearance and colour of the final product. The visual aspects are considered a vital role in the modern consumer's food choices, with colour being an integral part of food and beverages [3]. Over the past few years, a multitude of artificial food dyes have been introduced as an alternative to natural colourants. They are predominantly utilised to attain distinct attributes, such as enhanced visual appeal, intensified hues, increased colour stability, and uniformity [4, 5].

Synthetic food colourants offer several commercial benefits, such as affordability, resilience to light and pH changes, and excellent colour stability in comparison to natural colours [6]. These colourants are chemical compounds derived from coal tar derivatives, with many of them containing azo-group [7]. However, there are concerns in the market as some foods and beverages may contain unapproved synthetic colourants or excessive amounts of approved synthetic colourants [8]. Extensive research has revealed synthetic colourants as a significant contributor to food poisoning and can lead to severe health issues. It is also not safe to use approved synthetic colourants for the long term [9]. The use of unapproved colourants or the indiscriminate use of approved colourants is known to have adverse effects on animal and human health. There is a growing demand for the development of precise, accurate, sensitive, and selective analytical methods for the qualification and quantification of food colourants, owing to their extensive use in food manufacturing processes [10]. Among the synthetic dyes utilised in the food industry, azo dyes dominate the commercial dye market, accounting for about 65% of the share [11].

Azo dyes are synthetic organic colourants known for their vibrant and intense colours, making them popular additives in various food products. However, these dyes possess certain characteristics that warrant attention. They are typically resistant to aerobic conditions but can be metabolised by intestinal flora, leading to the formation of aromatic amines [12]. Such compounds have been associated with adverse effects on health, including headaches in adults, neurotoxicity, genotoxicity, and carcinogenic properties [13]. Most countries worldwide have taken measures to prohibit the usage of a significant number of azo dyes in food products as a response to the associated health concerns. Over the past few decades, strict regulations have been put in place to govern the use of azo dyes in both domestic food supplies and exports of various nations [14]. However, despite the presence of diverse regulatory frameworks, the overall approach is grounded in similar stages that demand well-established protocols for risk assessment.

Magnetic solid phase extraction (MSPE) has been developed based on traditional SPE [15]. In recent years, nano-magnetic materials have attracted much interest due to their unique size and physical properties. They possess a high surface area, resulting in greater adsorption capacity for analytes [16]. Because of their super-paramagnetic property, nanoparticles can be attracted by a magnet, but do not retain magnetism after the field is removed. Additionally, they do not agglomerate after the magnetic field is removed, thus not affecting sorption ability [17]. Meanwhile, the magnetic sorbent can be used repeatedly to enrich and separate the target analyte [18]. However, the naked Fe_3O_4 adsorbent is limited for selective analyte adsorption. Therefore, surface modification is necessary to design highly selective adsorbents to improve selectivity or affinity towards analytes [19]. Compared with traditional sample pre-treatment, MSPE offers many advantages, such as simplicity, low cost, and environmental friendliness, making it highly suitable for isolating and enriching target analytes from food samples [20]. The demand for environmentally friendly solvents has led to the adoption of ionic liquids (ILs) as replacements for traditional organic solvents, which are volatile and toxic. However, the green aspects of ILs have come under scrutiny due to their poor biocompatibility and biodegradability [21].

As a solution to this concern, deep eutectic solvents (DESs) have been introduced as a green alternative to ILs since 2004. DESs can be tailored to exhibit specific properties, such as hydrophobicity or hydrophilicity, providing the advantage of selectively extracting target compounds. This selectivity leads to higher purity and concentration of the emerging contaminants (ECs) obtained from the extraction process [22]. DESs can be easily modified by adjusting the composition of their components, making them adaptable to meet the specific demands of the extraction process. This versatility makes DESs well-suited as adsorbents for extracting or removing ECs in various matrices [23]. They offer numerous benefits, including high solvency, low toxicity, low environmental impact, biodegradability, and cost-effectiveness, as they are derived from renewable resources. Furthermore, DESs are easy to recycle without imposing negative effects on the environment.

Food analysis has become an exceptionally crucial procedure in ensuring the safety and quality of food products [24]. Furthermore, the adverse toxic effects linked to azo dyes employed for food colouring have spurred the advancement of exceptionally sensitive and selective analytical methods for their detection in various food matrices [25]. Despite the existence of many analytical methods previously studied, the majority of conventional methods, such as liquid-liquid extraction (LLE) and solid phase extraction (SPE), regrettably entail intricate pre-concentration, lengthy procedures, and the use of expensive equipment [26, 27]. An appropriate approach for preparing samples is imperative before conducting instrumental analysis, aiming to eliminate interferences arising from intricate food matrices that could potentially obstruct the chromatographic system. Hence, the development of green solvents has attracted significant interest in the demand for sustainable and green processes [28].

Based on our previous laboratory findings, a newly synthesised magnetic adsorbent named SS:DoAc@Fe_3O_4 using non-ionic silicone surfactant and dodecanoic acid as a DES in magnetic solid-phase extraction (MSPE) was reported as a reliable approach for the extraction of organophosphorus in honey and vegetable samples. In this study, similar magnetic adsorbent is employed to explore the extraction performance towards Allura Red AC (ARAC) using the MSPE technique. The focus on ARAC is prompted by its extensive application as food colorant in the food industry. ARAC (E129), also known as Red 40, is an azo dye originally derived from petroleum with a dark red colour and water solubility. It serves as a food colouring substitute for amaranth and is commonly added to sodas, baby medicines, and cotton candy. In the United States, ARAC is the most frequently used food colouring in beverages, candy, popsicles, deli meats, cheeses, salmon, over-the-counter (OTC) medications, and liquid suspensions. This study explored the potential application of DES-based magnetic adsorbent, a novel adsorbent synthesised by Elencovan et al. in solid phase extraction methods for the determination of ARAC in food matrices [29]. Attenuated total reflectance-Fourier transform infrared spectroscopy (ATR-FTIR) analysis was conducted to confirm the successfully re-synthesised material. Then, the newly synthesised SS:DoAc@Fe_3O_4 adsorbent was applied in the MSPE technique. All the methods were optimised and validated for the analysis of ARAC in soft drink samples.

Materials and Methods

Chemicals and reagents

The food dye (ARAC) was purchased from R&M Chemicals (Essex, UK). Ultrapure water (18.2 MΩ/cm) supplied by the Sartorius Milli-Q system (Aubagne, Germany) was used to prepare the standard stock solution for ARAC at the concentration of 1000 mgL^{-1}. Daily preparation of the standard working solutions involved dilution of a sufficient quantity of the stock solutions with ultrapure water for the extraction analysis. Additionally, the stock solution for salt was created by dissolving the requisite amount of salt in ultrapure water to achieve a concentration of 25% (w/v). Miracle S240, a non-ionic silicone surfactant, was obtained from G-Planter (Johor, Malaysia) with the IUPAC designation of 3-[methyl-bis(trimethylsilyloxy) silyl]propan-1-ol;2-methyloxirane; oxirane. Sodium sulphate (Na_2SO_4) was purchased from Qrec (Chonburi, Thailand) while methanol and acetonitrile of HPLC grade were obtained from Fisher Scientific (Seoul, Korea). R&M Chemicals (Essex, UK), Friedemann Schmidt Chemicals (Parkwood, Australia), Sigma-Aldrich (Darmstadt, Germany), and Chemiz (Shah Alam, Malaysia) supplied the iron (II) chloride tetrahydrate ($FeCl_2.4H_2O$), iron (III) chloride hexahydrate ($FeCl_3.6H_2O$), ammonium hydroxide (25%), sodium hydroxide (NaOH), and hydrochloric acid (HCl), respectively. Absolute ethanol was purchased from Fisher Scientific (Seoul, Korea).

Instrumentation

Attenuated total reflectance-Fourier transform infrared spectroscopy (ATR-FTIR) was employed to identify the functional bonds present in the newly synthesised SS:DoAc-based DES. The spectra were examined using Fortier from Perkin Elmer USA in the 4000 to 600 cm^{-1} range. The Perkin Elmer Lambda 25 UV-Vis spectrophotometer was used to analyse the analytical response of ARAC. Meanwhile, the Minitab 2022 (v21.3.1) software was used to investigate the signal-to-noise ratios of the experimental parameters that were optimised for the MSPE technique via the Taguchi design of experiments.

Synthesis of SS:DoAc-based DES

The SS:DoAc-based DES used in this study was synthesised according to the procedure mentioned in Elencovan et al. [29]. The co-precipitation method was used to synthesise the SS:DoAc@Fe_3O_4 adsorbent. The DES was made by combining silicone surfactant (SS) as a hydrogen bond acceptor (HBA) and dodecanoic acid (DoAc) as a hydrogen bond donor (HBD) at a molar ratio composition of 1:5, respectively. A clear, homogeneous liquid of SS:DoAc-based DES was produced by heating the mixture at 70 °C for 15 min with continuous stirring. Then, 150 µL of DES was added to the mixture of 1.4576 g of $FeCl_2.4H_2O$ and 3.7306 g of $FeCl_3.6H_2O$. Subsequently, 75 mL of ultrapure water was incorporated into the mixture. The mixture underwent vigorous stirring using a magnetic stirrer for 30 minutes at 85 °C under nitrogen gas stream protection. Next, 10 mL of ammonium hydroxide (25%) was added dropwise to the mixture and agitated constantly for 60 minutes. The synthesised SS:DoAc@Fe_3O_4 adsorbent was later collected using an external magnet and rinsed repeatedly, alternating between ethanol and ultrapure water to ensure complete removal of any residual unreacted chemicals. Lastly, the isolated SS:DoAc@Fe_3O_4 adsorbent was placed to dry in an oven for 24 hours at 80 °C.

Proposed SS:DoAc-based DES in MSPE technique

The magnetic solid-phase extraction (MSPE) method was conducted as described by Elencovan et al. with some modifications [29]. Firstly, 25 mg of SS:DoAc@Fe_3O_4 adsorbent was weighed precisely and incorporated into 10 ml of sample solution that was spiked with targeted analyte, followed by the addition of 800 µL of 25% (w/v) sodium sulfate (Na_2SO_4) to increase the ionic strength. The sample vial was vortexed for 2 minutes at 2500 rpm using a vortex mixer to promote the adsorption of the targeted analyte onto the SS:DoAc@Fe_3O_4 adsorbent. An external magnet was later used to isolate the SS:DoAc@Fe_3O_4 adsorbent and supernatant was discarded. Next, 700 µL of methanol:acetonitrile (1:1) acting as a desorption solvent was added to the isolated SS:DoAc@Fe_3O_4 adsorbent. The mixture underwent sonication for 2 minutes to facilitate the elution of the target dye analyte from the SS:DoAc@Fe_3O_4 adsorbent into the desorption solvent. Subsequently, the SS:DoAc@Fe_3O_4 adsorbent was separated using an external magnet and the desorption solvent was carefully retrieved with the aid of a micropipette tip and filtered using a 13 mm nylon syringe filter with a pore size of 45 µm. The final solution was then transferred into a 20 mL vial. Lastly, the desorption solvent containing the targeted analyte was subjected to UV-Vis spectrophotometry analysis for further identification and quantification.

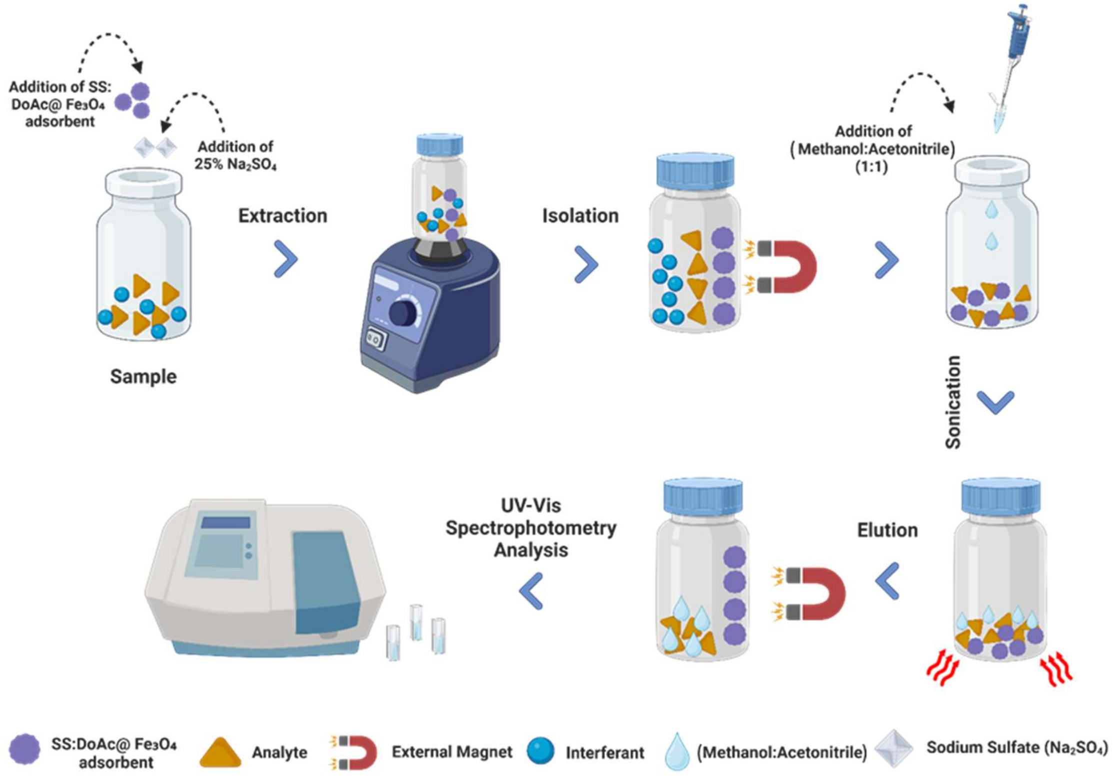

Figure 1. Schematic diagram of MSPE technique

Preliminary screening

A preliminary study was conducted via the MSPE approach to compare the performance of newly synthesised SS:DoAc@Fe_3O_4 adsorbent with control (Fe_3O_4 particles). Other extraction parameters included 25 mg of adsorbent, 10 mL of sample solution, 2 minutes of extraction time, 800 µL of 25% (w/v) Na_2SO_4, 700 mL of methanol:acetonitrile as desorption solvent, and 2 minutes of sonication. The adsorbent's performance in extracting ARAC was analysed using UV-Vis spectrophotometry at λ_{max}=501 nm.

Optimisation of MSPE technique via One-Variable-at-A-Time (OVAT) design of experiments

The OVAT (one-variable-at-a-time) design of experiments is a simple and straightforward approach in experimental design where one independent variable is varied while keeping all other variables constant. This method involves changing one factor at a time to observe its individual effect on the dependent variable. It is often used in the preliminary stages of research and allows researchers to quickly evaluate the impact of individual factors on a system without the complexity of considering interactions between multiple variables. In this study, in-depth analysis was conducted on the types of desorption solvent, addition of salt, volume of salt, volume of sample solution, and point zero charge as well as how they affect the extraction performance of the SS:DoAc-MSPE method. All experiments were conducted in triplicates (n=3). Four different types of desorption solvents, including methanol, acetonitrile, a 1:1 mixture of methanol and acetonitrile, and isopropanol, were evaluated in this study. To assess the effectiveness of target analyte extraction and recovery with and without the addition of salt, 25% (w/v) Na_2SO_4 was used to study the effect of salt. The effect of salt volume was investigated using volumes of 25% (w/v) Na_2SO_4 in the range of 200 to 800 µL. Different volumes of sample solution were investigated in this procedure ranging between 10 to 25 mL.

Point of zero charge

The points of zero charges (pzc) of the adsorbent were determined using this approach [18] where 25 mg of newly synthesised SS:DoAc@Fe_3O_4 adsorbent and control (Fe_3O_4 particles) were added to 10 mL solutions with pH levels of pH 2, 4, 6, 8, and 10, respectively. An orbital shaker was used to agitate the mixture for 24 hours. Subsequently, the final pH of the mixtures was determined using a pH meter and the graph of ΔpH versus the initial pH was plotted.

Optimisation of MSPE technique via Taguchi design of experiments

A process can be optimised using the Taguchi design of experiments method by identifying the crucial parameters that influence the output and determining the ideal levels of these parameters. This strategy uses the multi-variable at a time method, which is comparable to the conventional approach of OVAT. Five different parameters (pH of sample solution, mass of adsorbent (mg), extraction time (min), desorption time (min), and volume of desorption solvent (µL)) were analysed simultaneously where only one parameter was manipulated at a time whereas the rest were held constant. The extraction efficiency was assessed using the average of three replicate measurements (n=3), which were conducted for each parameter. Deionised water with 10 mgL^{-1} of ARAC was used to conduct the optimisation analysis. Spectrophotometric peak regions corresponding to the target dye wavelength were employed to analyse the effects of various factors on the effectiveness of the targeted dye's extraction. The effect of sample pH was studied at the range of pH 2, 4, 6, and 8. The SS:DoAc@Fe_3O_4 adsorbent ranging from 5 to 25 mg was also investigated. Additionally, the effect of extraction time was analysed for durations between 0.5 to 3 mins; the effect of desorption time was analysed for durations between 1 to 5 mins; and the effect of desorption solvent volume was analysed at the range of 400 to 700 µL. In this study, the extraction efficiency is the target property and should be maximised; thus, the favourable optimisation criterion is the larger-the-better (LTB) type of signal of noise.

Method validation

The linearity range, precision, limit of detection (LOD), limit of quantification (LOQ), recovery values, and matrix effect of the proposed SS:DoAc@Fe_3O_4-based MSPE approach was evaluated by conducting a range of tests on real matrix samples according to the International Council for Harmonisation of Technical Requirements of Pharmaceuticals for Human Use (ICH) guidelines [31].

Linearity

The linearity of the proposed DES in the MSPE method was examined by employing the optimised parameter conditions. Samples of soft drinks were spiked with various amounts of the standard dye analyte, ranging from 0.5 to 10 mgL^{-1}. A peak area following sample pre-treatment was applied to create a linear calibration curve with a coefficient of determination (R^2) close to 1.

Limit of detection (LOD) and limit of quantification (LOQ)

To assess the sensitivity of the proposed technique, the LOD and LOQ were calculated using the formulas in Eq. 1 and Eq. 2, respectively, where s is the standard deviation of ten replicate readings of the blank sample and b is the gradient of the calibration line [32].

$$\text{LOD} = \frac{3.3\text{s}}{\text{b}} \qquad (1)$$

$$\text{LOQ} = \frac{10\text{s}}{\text{b}} \qquad (2)$$

Precision analysis

The intra-day and inter-day precision at dye concentrations of 10 mgL^{-1} were measured to determine the precision of the proposed method. Both intra-day and inter-day precision analyses were conducted using three replicates (n=3). Prior to establishing the precision value, the standard deviation (SD) of the data was determined and provided in the form of percentage of relative standard deviation (%RSD). Subsequently, the SD and %RSD values were calculated using Eq. 3 and Eq. 4, respectively.

$$\text{SD} = \sqrt{\frac{\sum(\text{x} - \bar{\text{x}})^2}{\text{n} - 1}} \qquad (3)$$

$$\text{RSD} = \frac{\text{SD}}{\bar{\text{x}}} \times 100 \qquad (4)$$

where x is the outcomes of each run, n is the number of measurements, and $\bar{x}$ is the mean.

Recovery study

Three different dye concentrations were incorporated into the sample matrix to evaluate the accuracy of the suggested technique. The relative recovery (RR%) of dye was determined for each concentration level using three replicates (n=3) via Eq. (5).

$$\text{RR (\%)} = \frac{\text{Cs-Cu}}{\text{Co}} \text{ x } 100 \qquad (5)$$

Where C_s is the dye concentration that has been spiked into the sample matrix, C_u is the dye concentration that has not been spiked into the sample matrix, and C_0 is the initial standard solution spiked into the sample matrix. In this study, the standard dye solution was spiked with dye at low, medium, and high concentrations (1, 3, and 10 mgL^{-1}) to analyse the drink samples.

Matrix effect

The process of co-extracting and co-eluting chemicals from actual matrix samples can have a significant impact on the spectrophotometry signals of dye. Therefore, Eq. (6) was used to study the matrix effect [33].

$$\text{Matrix effect (\%)} = \frac{(A_{M+S} - A_M)}{A_O} \text{ -1 x } 100 \qquad (6)$$

Where A_{M+S} denotes the spectrophotometric response of the dye in a sample matrix that has been spiked, A_M denotes the spectrophotometric response of the dye in a sample matrix that has not been spiked, and A_O denotes the spectrophotometric response of the dye in a standard solution. A dye concentration of 10 mgL^{-1} was used to assess the matrix effect.

Real sample analysis

Commercial soft drinks were purchased from the local supermarket. First, an adequate amount of liquid drink sample was degassed for 10 minutes via sonication [34]. The liquid sample was later filtered through 0.45-µm nylon syringe filters, and 1.0 mL of the sample solution was taken and transferred into a 100-mL volumetric flask and diluted with ultrapure water [35]. Afterwards, the pH of the liquid samples was adjusted to 2 using 1 M hydrochloric acid. The MSPE technique was then applied to analyse the liquid samples.

Results and Discussion

Characterisation of DES

Figure 2 displays the FT-IR spectra of Fe_3O_4, SS:DoAc-based DES, and SS:DoAc@Fe_3O_4. The existence of the Fe-O stretching vibration at a frequency between 580 and 620 cm^{-1} serves as evidence for the successful formation of the Fe_3O_4 particles in spectra (a) and (b). The large absorption peak of approximately 3000 cm^{-1} in the Fe_3O_4 and SS:DoAc@Fe_3O_4 adsorbents indicated the existence of hydroxyl group (OH stretching). The Si−O−Si functional group of silicone surfactant (SS) is responsible for the stretching vibration in SS:DoAc@Fe_3O_4 materials at a wavenumber of around

1402 cm^{-1}. When Fe_3O_4 was coated with SS:DoAc-based DES, the C=O stretching band associated with the dodecanoic acid (DoAc) of SS:DoAc DES produced a wavenumber of 1622 cm^{-1}. These results are in line with the findings reported by previous studies [36, 37]. When compared to pure Fe_3O_4, the distinctive peaks of Si−O−Si stretch at 1402 cm^{-1} and C=O stretch at 1622 cm^{-1} established the success of the coating of SS:DoAc on Fe_3O_4. The attribution of the FT-IR spectra is summarised in Table 1. The FT-IR results denote the successful synthesis of the unique SS:DoAc@Fe_3O_4 material.

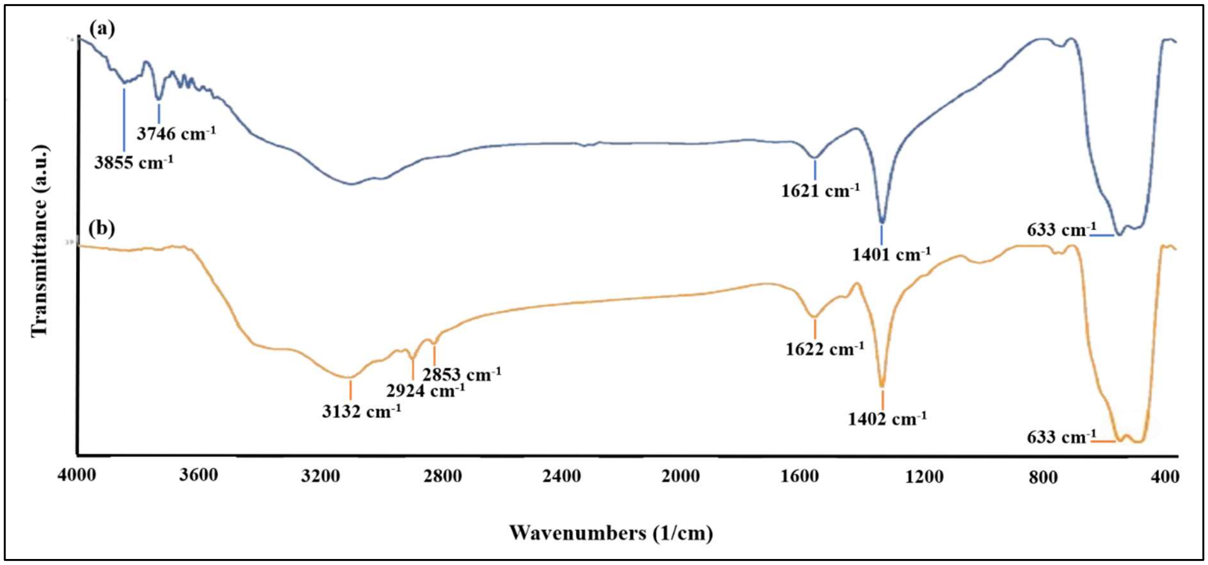

Figure 2. FT-IR spectra of (a) pure Fe_3O_4, (b) SS:DoAc@Fe_3O_4

Table 1. Main IR frequencies with assignments

	Wavenumber (cm^{-1})	
Assignment	**Fe_3O_4**	**SS:DoAc@Fe_3O_4**
OH stretching	3855	3132
Fe−O stretching vibration	633	633
Si−O−Si stretching	-	1402
C=O stretching	-	1622
C−H	3746	2924

Preliminary screening

An initial study was conducted using the MSPE technique with pure Fe_3O_4 particles and SS:DoAc@Fe_3O_4 adsorbent. As illustrated in Figure 3, the SS:DoAc@Fe_3O_4 adsorbent demonstrated a greater analytical response to dye extraction compared to pure Fe_3O_4 particles. This demonstrates that the coating of DES-based SS:DoAc on Fe_3O_4 particles enhanced the adsorbent's capacity for extraction. The extraction of the non-polar dye investigated in this study is facilitated by the hydrophobic SS:DoAc-based DES. Due to the strong hydrophobic interaction that developed between the target dye and the adsorbents, the target dye could be readily adsorbed onto the SS:DoAc@Fe_3O_4 surface. In the MSPE extraction technique, hydrophobic deep eutectic solvents (HDES) have been reported as an alternative to traditional extraction medium. HDES has been proven to be efficient absorbents with excellent CO_2 affinity and selectivity [38]. They are also efficient in removing hydrophobic compounds from aqueous samples [39]. Moreover, HDES has been utilised as an effective modification of inexpensive adsorbent for the extraction of pharmaceuticals and dyes [40]. Hence, the SS:DoAc@Fe_3O_4 adsorbent was preferred for further optimisation because the SS:DoAc-based DES coating could significantly enhance the extraction performance.

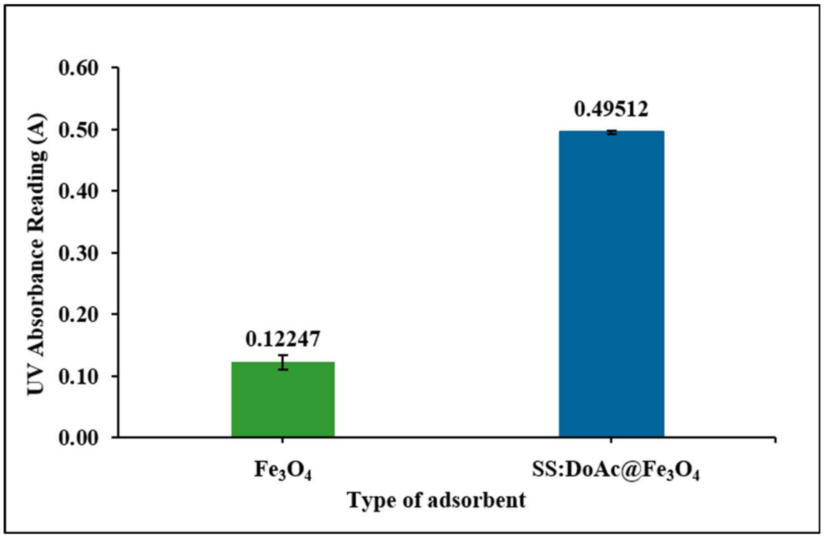

Figure 3. Type of adsorbent. (Condition: 10 mL of sample solution; 25 mg of adsorbent; 2 mins of extraction time; 800 µL of 25% (w/v) Na_2SO_4; 700 µL of methanol and 2 mins for desorption time), n=3

Optimisation of MSPE via OVAT design of experiments: Type of desorption solvent

The three frequently used organic solvents (methanol, acetonitrile, and methanol:acetonitrile (1:1) and isopropanol) with varied polarity were evaluated to determine their ability to elute the target dye to ensure the maximum recovery feasible. The results in Figure 4 show that the application of methanol:acetonitrile (1:1) as eluent resulted in the highest analytical signal, which was subsequently followed by methanol, acetonitrile, and isopropanol. The polarity and solvent characteristics of methanol and acetonitrile contribute to their efficacy as desorption solvents. Methanol is a polar-protic solvent, whereas acetonitrile is a polar-aprotic solvent and has a greater dipole moment. These two solvents are compatible with standard mobile phase additives and buffers and are completely miscible with water [41]. Methanol and acetonitrile, however, have been discovered to complement each other in terms of extraction efficiency. Both solvents can interact with non-polar compounds like ARAC due to their mid-polarity, making it easier to extract these compounds from food samples [42]. As a result, the target ARAC is more easily eluted from the SS:DoAc@Fe_3O_4 adsorbent into methanol:acetonitrile. Therefore, methanol: acetonitrile was chosen as a potential candidate for the desorption solvent in the forthcoming optimisation study.

Salt addition

The addition of salt was found beneficial in the extraction of ARAC because it enhances extraction efficiency by effectively separating dye molecules from the aqueous phase [43]. The recovery of the target analyte was favoured when salt was added to the sample solution due to the salting-out effect where it spiked the ionic strength of the aqueous matrix without causing additional reactions or chemical changes. This condition selectively reduced the analyte's solubility in the aqueous phase and increased the amount of analyte that could be adsorbed onto the SS:DoAc@Fe_3O_4 adsorbent [44]. Sodium sulfate (Na_2SO_4) is often used for salting out processes due to its properties where it is highly soluble in water and does not react with most compounds, making it an inert substance in this type of extraction. The effect of salt addition on the extraction performance was studied where sample solution added with salt was compared to sample solution without the addition of salt. The type of salt employed in this procedure is Na_2SO_4 with a concentration of 25% (w/v). In contrast to the standard sample solution, adding salt drastically increased the extraction yield, as shown in Figure 5. Therefore, 25% (w/v) of Na_2SO_4 was chosen for further investigation.

Volume of salt

The effect of salt volume was investigated by adding 25% (w/v) Na_2SO_4 at the range of 200 to 800 µL. As depicted in Figure 6, the highest analytical response was the extraction yield obtained in 200 µL of 25% (w/v) Na_2SO_4. However, the extraction efficiency decreased with the addition of 800 µL of 25% (w/v) Na_2SO_4. This is due to the possibility of a salt-rich layer forming at the interface between the aqueous and organic phases, which potentially reduces the efficacy of the extraction method [45]. Thus, 200 µL of 25% (w/v) Na_2SO_4 was preferred for further investigation.

Volume of sample solution

The effect of sample solution volume was investigated with the range of 10 to 25 mL. As depicted in Figure 7, the highest analytical response was the extraction yield obtained in 20 mL of sample solution. A considerable amount of sample solution is necessary to obtain a higher pre-concentration factor [46]. The adsorption rate exhibited little impact for sample volumes between 10 and 20 mL but it drastically dropped between 20 and 25 mL (see Figure 7). This resulted from the use of high volume of sample solution, which may dilute the target analyte and minimise the efficacy of extraction and the analytical response of the method.

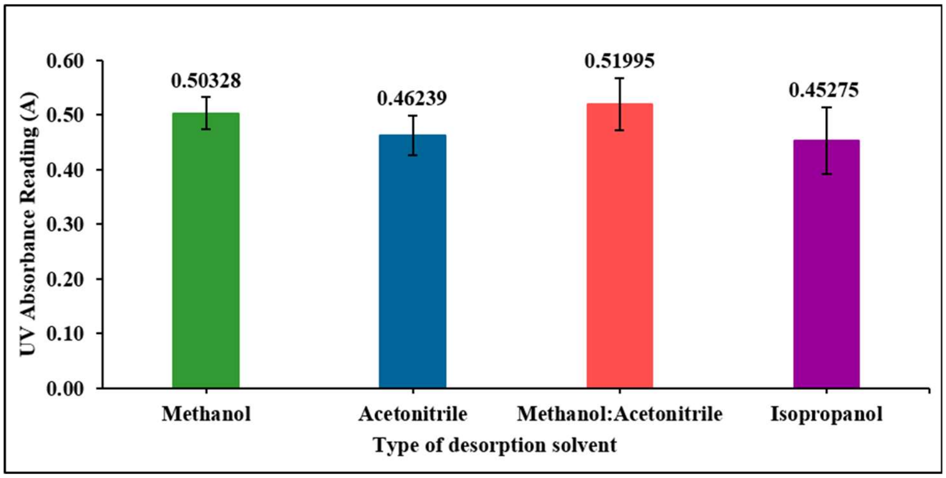

Figure 4. Effect of desorption solvent. (Condition: 10 mL of sample solution; 25 mg of adsorbent; 2 mins of extraction time; 800 µL of 25% (w/v) Na_2SO_4; and 2 mins for desorption time), n=3

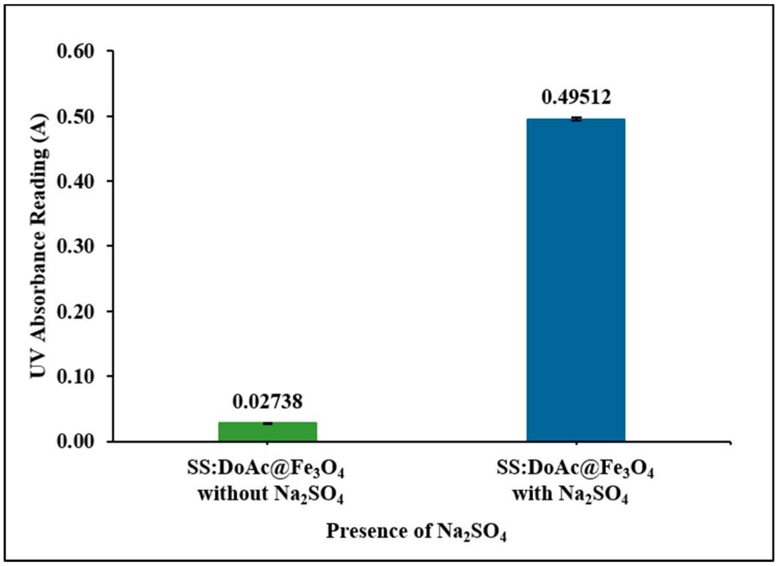

Figure 1. Effect of presence of Na_2SO_4. (Condition: 10 mL of sample solution, 25 mg of adsorbent; 2 mins of extraction time; 800 µL of 25% (w/v) Na_2SO_4; and 2 mins for desorption time), n=3

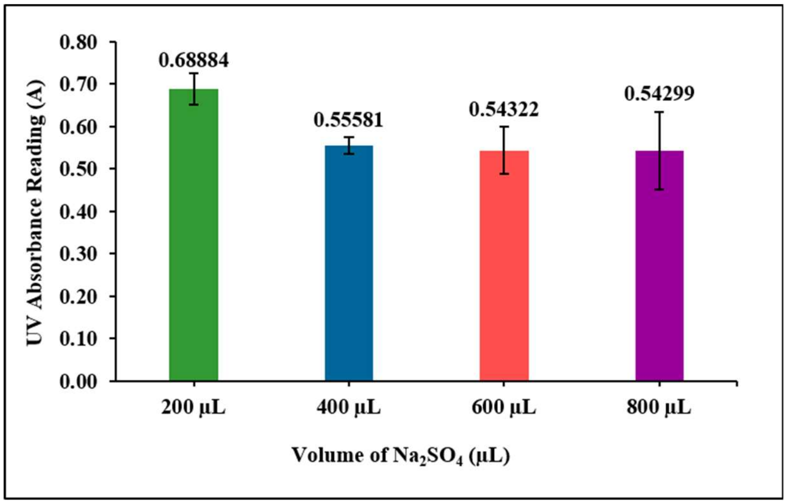

Figure 2. Effect of volume of Na$_2$SO$_4$. (Condition: 10 mL of sample solution, 25 mg of adsorbent; 2 mins of extraction time; and 2 mins for desorption time), n=3

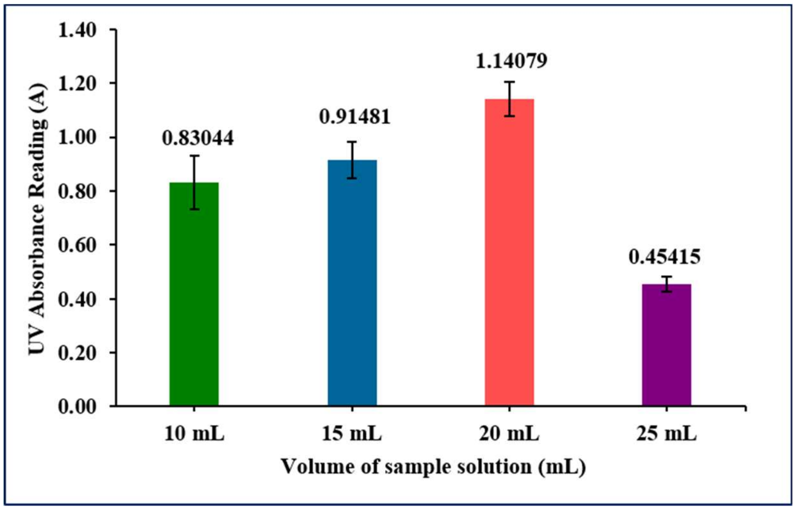

Figure 3. Effect of volume of sample solution. (Condition: 800 µL of 25% (w/v) Na$_2$SO$_4$, 25 mg of adsorbent; 2 mins of extraction time; and 2 mins for desorption time), n=3

Point of zero charge

The pH at which the net surface charge of the particles reaches zero is known as the point zero charge (pzc) of an adsorbent [47]. Determining the point of zero charge (pzc) of an adsorbent is crucial in adsorption studies. The surface of the adsorbent is positively charged whenever the solution's pH is lower than pzc. The surface may attract anions in this condition. However, if the pH of the solution is greater than pzc, the surface is negatively charged and may attract cations [48]. Figure 8 shows the Δ pH plotted against the initial pH graph. The pH value for zero-point charge of the SS:DoAc@Fe$_3$O$_4$ adsorbent was found to be at pH 2. Whereas the surface of SS:DoAc@Fe$_3$O$_4$ adsorbent became positively charged at pH level less than pH 2, making it feasible for anionic species like Allura Red AC to be adsorbed.

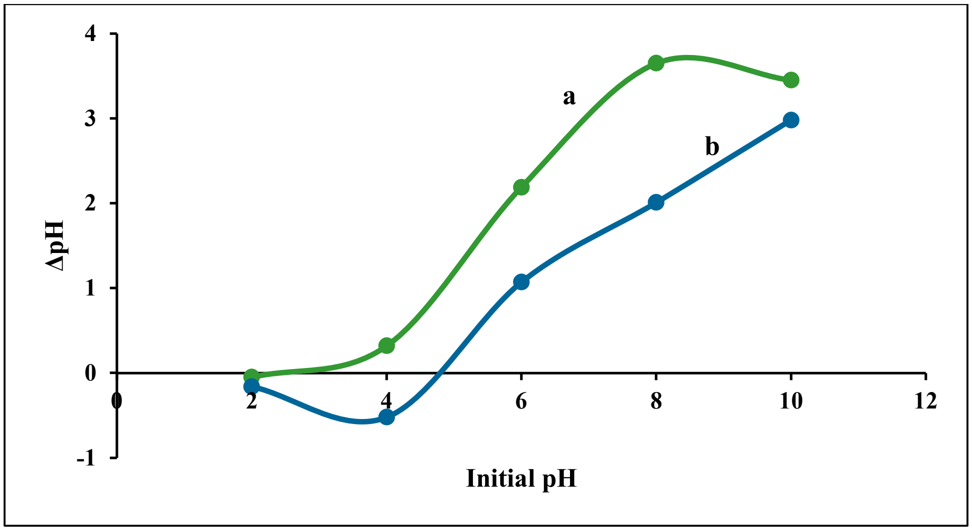

Figure 8. Point of zero charge of a) Fe_3O_4 and b) SS:DoAc@Fe_3O_4, (n=1)

Optimisation of MSPE technique via Taguchi design of experiments: pH of sample solution

The efficacy of the extraction is significantly affected by the pH of the sample solution by establishing the ionic state of any possible adsorbents, adsorbates, and other species in the sample [49]. The effect of sample pH was investigated with pH values ranging from 2 to 8. As shown in Figure 9, the extraction recovery of ARAC significantly increased in the pH range of 2 to 4 and decreased from 6 to 8. Given that the pKa of the target analyte, ARAC, is 11.4 and that 50% of this dye is protonated at the pH value of 11.4, the partial deprotonation of the phenol group for ARAC may be the cause of the decreased extraction of the analyte [50]. The results align with those reported by Yu and Fan who found that the adsorption rate of ARAC was higher at pH 2 or 3 but decreased as the pH increased from 3 to 7 [46]. Another study also mentioned that in an acidic solution (pH 2), the ARAC dye functions as an anionic dye molecule and two of its sulfonate (SO^{3-}) groups may be electrostatically attracted by protonated amino (NH^{2+}) groups. The maximum dye adsorption was seen at solution pH 2, which may be due to the electrostatic interaction between dye molecules and adsorption sites [51]. The target ARAC could be present as a stable acidic molecule with a greater affinity for adsorbents at pH 2, which resulted in the maximum extraction yield. As a result, pH 2 was selected as the appropriate pH for subsequent tests.

Mass of adsorbent

The quantity of adsorbent was optimised to achieve the highest extraction yield where different amounts of SS:DoAc@Fe_3O_4 in the range of 5 to 25 mg were investigated. As shown in Figure 9, the extraction recovery for the investigated ARAC rose exponentially with adsorbent mass up to 25 mg. The dosage of the adsorbent determines the amount of target dyes being adsorbed since it offers the sites needed to load the dyes. In this regard, more accessible sites are deemed advantageous for maximising extraction [49]. This agrees with Saha et al. who stated that surface area and the number of accessible adsorption sites increased as the mass of adsorbents increased in the extraction of Allura Red [51]. Therefore, 25 mg of the SS:DoAc@Fe_3O_4 adsorbent was established for the subsequent experiments.

Extraction time

According to Yu and Fan, the extraction method used in MSPE is based on a time-dependent equilibrium in which target molecule mass transfer rises with time until adsorption equilibrium is attained [46]. The period of time needed to establish extraction equilibrium is crucial for improving both the mass transfer and the interaction between the sample solution and the solvent [50]. The extraction time was examined between 0.5 and 3 mins in order to maximise the extraction yield. As seen in Figure 9, the analytical response elevated significantly in the first 2 mins. This could be prompted by the powerful attraction forces between the dye molecules and the adsorbents. To achieve equilibrium, dye molecules quickly adsorb onto the adsorbents' exterior surfaces, followed by pore diffusion into their matrix [51]. Further prolongation of the extraction process notably after 2 mins demonstrates that the adsorption of ARAC dye onto the adsorbent did not show a significant progress, which could be related to back extraction [52]. Hence, 2 mins of extraction time was selected for forthcoming investigations.

Desorption time

The impact of desorption time was investigated between 1 and 5 mins. The results in Figure 9 show that 3 mins of sonication was sufficient to elute all the target ARAC from the adsorbent into methanol:acetonitrile (1:1). The analytical response increased gradually and declined after 3 mins of elution. According to Naing et al., prolonged sonication may have caused the target analytes to re-adsorb onto the adsorbent, thus decreasing the extraction yield [52]. Conversely, the adsorbents' magnetic properties allowed them to be quickly isolated from the sample solution in less than 2 mins using a powerful magnet as opposed to filtration or centrifugation [46]. Therefore, the optimal desorption time was established at 3 mins of sonication.

Desorption solvent volume

The impact of desorption solvent volume on the elution of target ARAC was also investigated to obtain the highest possible extraction yield. As shown in Figure 9, the analytical signal quickly decreased following the increasing volume of elution solvent. Methanol:acetonitrile (1:1) beyond 400 µL caused the target ARAC to be diluted in the solvent, which gradually reduced the spectrophotometric response. Desorption solvent volumes below 400 µL were not employed in this study because the remaining solvent obtained after the desorption process was insufficient to be subjected to UV-Vis spectrophotometry analysis. Subsequently, 400 µL of methanol:acetonitrile (1:1) was fixed to prepare for future optimisation.

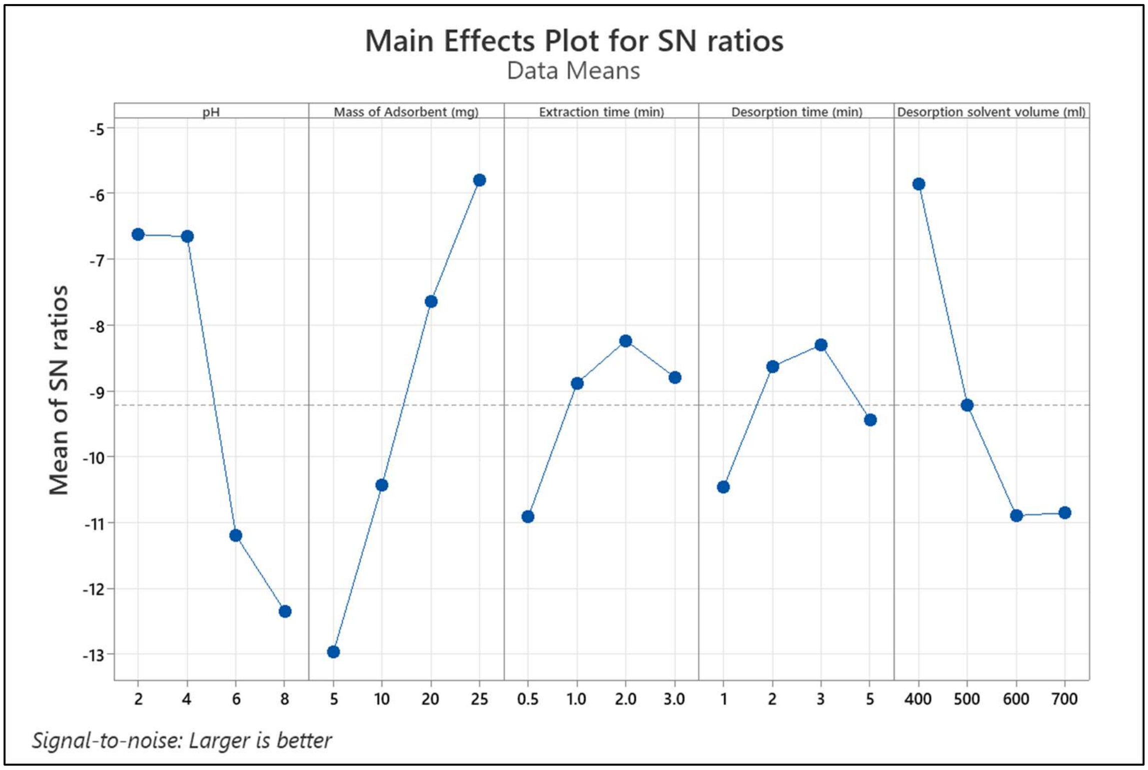

Figure 9. Signal-to-noise ratios involving sample pH; mass of adsorbent, extraction time, desorption time, desorption solvent volume

Method Validation

The proposed approach was implemented to monitor ARAC in soft drink samples to evaluate its feasibility. The calibration curve was plotted within the range of 0.3 to 10 mgL^{-1} for soft drinks samples under optimised experimental parameters. The coefficient of determination (R^2) derived from the linearity curve was 0.999. The LOD obtained was 0.1 mgL-1 and the LOQ was 0.4 mgL-1, and both values are lower compared to the maximum permitted level of ARAC in soft drinks. In accordance with Directive 94/36/EC from the European Parliament and Council, the highest acceptable concentration of ARAC in soft drinks is 100 mgL^{-1} [53]. The intra- and inter-precision values for the developed technique ranged between 5.05% and 5.37%, respectively. The matrix effect obtained is 4% across all investigated ARAC, indicating that the analysis of the soft drink samples had minimal effect on the recovery of ARAC. Table 2 provides a summary of the figure of merits.

Table 2. Analytical performance data of MSPE technique using SS:DoAc@Fe_3O_4 adsorbent

Assay	**Allura Red AC**
Linearity (mgL^{-1})	0.3 - 10
Coefficients of determination (R^2)	0.999
Intraprecision (%) (n=3)	5.05
Interprecision (%) (n=3)	5.37
LOD (mgL^{-1})	0.1
LOQ (mgL^{-1})	0.4
Matrix effect (%)	4

Real sample analysis

Samples of commercial soft drinks were subjected to the validated MSPE method using the SS:DoAc@Fe_3O_4 adsorbent. The samples were spiked with 1, 3, and 10 mgL^{-1} of the target ARAC to examine how the sample matrix affected the results. The recoveries obtained from soft drinks samples ranged from 80% to 111%. Table 3 lists the recovery values for the real sample.

Table 3. Relative recovery study of SS:DoAc@Fe_3O_4 based MSPE of ARAC in soft drink samples.

Analyte	**Spiked (mgL^{-1})**	**% Recovery (% RSD, n = 3)**		
		Sample A	**Sample B**	**Sample C**
Allura Red AC	0	nd	nd	nd
	1	111 (11.6)	101 (7.4)	93 (4.6)
	3	88 (9.2)	84 (6.2)	85 (1.1)
	10	80 (10.7)	83 (2.6)	90 (3.9)

Linear range: 0.3-10 mgL^{-1}

[a] nd-not detected.

[b] Mean of triplicate analysis.

[c] RSD-Relative standard deviation (n = 3)

Comparison with previous studies

As shown in Table 4, the developed method obtained lower LOD compared to those reported by dos Reis et al. [54]. Compared to other methods, the LOD obtained was slightly lower due to the instrument used in this study. UV-Vis has lower sensitivity compared to other instruments, such as HPLC. However, the result is comparable to other studies as the extraction time needed in this study was only one minute.

Table 4. Comparison of performance of ARAC extraction using various extraction techniques

Extraction Technique	Matrix	Instrument	LOD (mg L^{-1})	LOQ (mg L^{-1})	Extraction Time (min)	Reference
VA-SS-LPME	Confectioneries, beverages, drugs and syrups	UV-Vis spectrometer	0.051	0.172	2	[55]
LLE	Milk, meat and fish products, confections, wines, juices and beverages	HPLC-DAD	0.029	0.088	2 – 5 (based on samples)	[56]
MSPE	Paediatric syrup, colored sugar-coated chickpeas, and mineral water	HPLC-UV	0.05	0.1	5	[48]
LLE	Guava pulp	HPLC-DAD	15	30	0.5	[54]
MSPE	Soft drink	UV-Vis spectrometer	0.1	0.4	2	This study

Abbreviation: VA-SS-LPME: vortex assisted sequential-simultaneous liquid phase micro-extraction; LLE: liquid-liquid extraction; MSPE: magnetic solid phase extraction; HPLC-DAD: high performance liquid chromatography with diode array detector; HPLC-UV: high performance liquid chromatography with ultraviolet detector.

Conclusion

This study aimed to explore the application of SS:DoAc-based DES functionalised Fe_3O_4 (SS:DoAc@Fe_3O_4) adsorbents, which are inexpensive and environmentally benign solvents, to increase the effectiveness and sustainability of the extraction technique. The work was successful in demonstrating the feasibility and efficacy of SS:DoAc@Fe_3O_4 for the extraction of ARAC through an extensive experimental method and analysis. The results showed that the proposed adsorbent has higher extraction efficiency, high selectivity, and great reusability. Compared to unmodified Fe_3O_4 particles, the SS:DoAc-based DES functionalised Fe_3O_4 allowed for simple and quick separation of the adsorbent from the sample matrix. This was made possible by the strong hydrophobic interaction between ARAC and the SS:DoAc@Fe_3O_4 adsorbent.

Through a combination of OVAT and Taguchi design methodologies, we successfully optimised the extraction conditions for ARAC using magnetic solid phase extraction. The OVAT analysis revealed that having a methanol:acetonitrile (1:1) mixture as the desorption solvent, salt addition for enhanced extraction performance, salt volume of 200 µL, and a sample volume of 20 mL yielded optimal results. Meanwhile, Taguchi experiments determined the ideal conditions as pH 2, 25 mg of adsorbent dosage, 2 minutes of extraction time, 3 minutes of desorption time, and a desorption solvent volume of 400 µL. These findings enhance the current understanding of the extraction process and offer practical guidelines for maximising ARAC extraction efficiency. Under optimised experimental parameters, the studied ARAC derived the R^2 value of 0.999 for linearity curve while the LOD and LOQ were 0.1 mgL^{-1} and 0.4 mgL^{-1}, respectively. This infers that the developed MSPE approach capitalising on the SS:DoAc@Fe_3O_4 adsorbent is indeed an excellent tool for the analytical separation and identification of ARAC. The findings of this work significantly support the notion that DES-based MSPE is an extraction technique with high potential for the extraction of azo food dyes, hence providing new opportunities for efficient and sustainable dye analysis in the food industry. This research represents a significant advancement in the direction of more environmentally friendly and effective extraction techniques, making a substantial contribution to the overall development of green techniques for the analysis of food dyes.

Several suggestions for further research can be made based on the findings and conclusion deduced from this study. First, it is important to work on optimising the DES composition to find the best solvent system for a variety of azo food dyes. This can be accomplished by experimenting with various molar ratios and DES component combinations. Moreover, examining the applicability of the suggested approach using other analytical tools, such as high-performance liquid chromatography (HPLC) and gas chromatography-mass spectrometry (GC-MS), can expand our knowledge in this specific field of study and improve its credibility. Investigating the application of DES-based MSPE to complex food matrices, like processed foods and different types of beverages, will also offer beneficial practical insights. For industrial implementation, it is essential to evaluate the scalability, viability, and compatibility of the process. Furthermore, it would be desirable to conduct an environmental impact assessment (EIA) to compare the sustainability and energy efficiency of DES-based MSPE to traditional approaches. These recommendations for future research must be taken into consideration to ensure consumer safety and environmental protection. This will help to promote effective and sustainable methods for food dye analysis in food technology applications.

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