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Organic solvent-free air-assisted liquid–liquid microextraction for optimized extraction of illegal azo-based dyes and their main metabolite from spices, cosmetics and human bio-fluid samples in one step

Behruz Barfi, Alireza Asghari*, Maryam Rajabi, Sedigheh Sabzalian

Department of Chemistry, Semnan University, Semnan 35195-363, Iran

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ABSTRACT

Air-assisted liquid–liquid microextraction (AALLME) has unique capabilities to develop as an organic solvent-free and one-step microextraction method, applying ionic–liquids as extraction solvent and avoiding centrifugation step. Herein, a novel and simple eco-friendly method, termed one-step air-assisted liquid–liquid microextraction (OS-AALLME), was developed to extract some illegal azo-based dyes (including Sudan I to IV, and Orange G) from food and cosmetic products. A series of experiments were investigated to achieve the most favorable conditions (including extraction solvent: 77 μL of 1-Hexyl-3-methylimidazolium hexafluorophosphate; sample pH 6.3, without salt addition; and extraction cycles: 25 during 100 s of sonication) using a central composite design strategy. Under these conditions, limits of detection, linear dynamic ranges, enrichment factors and consumptive indices were in the range of 3.9–84.8 ng mL^{-1}, 0.013–3.1 μg mL^{-1}, 33–39, and 0.13–0.15, respectively. The results showed that –as well as its simplicity, fastness, and use of no hazardous disperser and extraction solvents- OS-AALLME is an enough sensitive and efficient method for the extraction of these dyes from complex matrices. After optimization and validation, OS-AALLME was applied to estimate the concentration of *1-amino-2-naphthol* in human bio-fluids as a main reductive metabolite of selected dyes. Levels of *1-amino-2-naphthol* in plasma and urinary excretion suggested that this compound may be used as a new potential biomarker of these dyes in human body.

1. Introduction

One of the most important new aspects of chemistry is the development of “Green Chemistry”. To date, most of the efforts at greening analytical chemistry have focused on either replacement of existing solvents with greener alternatives or on overall reduction in the amount of used solvent and generated waste [1]. Sample preparation is the stage of the analytical process where greenness-related issues can likely play the most important role. With the exception of direct methods for solid sample analysis, for most analytical methods it is necessary to carry out a certain number of operations to make the sample amenable to the instrument. These operations, which may include digestion, extraction, dissolution, preconcentration and clean-up, typically require the use of large amounts of acids, organic solvents, and in general, chemicals that can often be persistent, bioaccumulative and toxic as well as operating conditions that can become unsafe and energy-consuming. Therefore, sample preparation should be targeted as a priority when green chemistry principles are to be adapted to analytical activities [2]. Of special significance to environmental friendly sample preparation are extraction methods in which liquid solvents are used in reduced amounts, replaced with green solvents or even completely eliminated from the analytical procedure. Furthermore, the number of operations and processes involved in the extraction methods should be kept to a minimum.

Air-assisted liquid–liquid microextraction (AALLME) is one of the most recently used dispersive solvent-free LPME methods, which has been reported by Farajzadeh in 2012 [3]. In this method, a few microliters of the organic solvent (denser or lighter than water) is transferred into the aqueous sample solution in a conical centrifuge tube, and the mixture is then repeatedly withdrawn into a glass syringe and pushed out into the tube. By this action, fine organic droplets are formed, and the extraction solvent is entirely dispersed in the sample solution. After centrifugation of the cloudy

* Corresponding author. Fax: +98 23 33654110.
E-mail address: aasghari@semnan.ac.ir (A. Asghari).

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solution formed, the extractant is settled down at the bottom of the centrifuge tube or gathered on the surface of sample and used for further analysis [4–8]. AALLME has a great potential to consider as a greener microextraction method, if organic solvents used can be replaced with other green solvents.

Ionic liquids (ILs) have gained widespread recognition as novel solvents in chemistry. They are considered to be "environmental friendly solvents" due to their negligible vapor pressure, good thermal stability, and wide liquid phase range [9,10]. The immiscibility of ILs in water and their capability to solubilize organic species has made them particularly suited to isolation and preconcentration of compounds from aqueous solutions, and so they have been a valuable alternative to the volatile organic solvents used in conventional liquid-based extraction processes [11–13].

However, ILs have not yet been employed as an extractant in AALLME, maybe due to their high viscosity and incomplete dispersion in aqueous solutions. To overcome this limitation, sonication can be a good assistant choice. Simultaneous application of ultrasound irradiations and common AALLME can lead to the rapid formation of sub-micron droplet size of the IL in the aqueous solution, and the contact surface between both immiscible liquids is significantly enlarged. Smaller fine droplets of the IL and enlarged interfaces between the extractant and aqueous sample lead to a significant increase in the analyte mass transfer into the extractant. Consequently, high extraction efficiency could be achieved in a short period of time. If this action can be performed in a single device like a syringe, with no need to centrifuge, a simple and one-step ionic liquid-based AALLME method is developed which is simple and environmentally friendly, too.

In recent years, identification and quantitation of illegal additives in food products is of great importance to human health. Sudan dyes (Sudan I–IV), and Orange G, as instances of 1-amino-2-naphthol-based azo dyes, have been illegally used as additives in a variety of food products to maintain their intense red–orange color and thus stimulate sales of these products on the market [14,15]. When the foods are ingested, these azo dyes are initially contacted with the gastrointestinal tract, where the dyes can be reduced to corresponding aromatic amines (mainly, 1-amino-2-naphthol), which are toxic, water-soluble, and easily absorbed by the human intestine [16,17]. Due to this fact their use in foodstuffs has been prohibited by the European Commission. However, in some Eastern countries, these dyes are still used in order to intensify the red-orange color in spices. From the toxicological and nutritional viewpoint, several studies have been carried out in order to check the noxious effects on humans; thus, there is a pressing need to develop simple and sensitive methods for their determination in contaminated foods.

Furthermore, these compounds have long been used in industries of plastics, inks, waxes, leather, fabrics, shoe and floor polishing, cosmetics, paper, textile and pharmaceutical industries [18–20]. Therefore, they can enter to human body not only by ingestion but also using inhalation or skin contact in different ways. As an instance, most of the cosmetic products are directly applied to the human skin. Although the skin provides a great protective barrier, some of the ingredients in cosmetic products are still able to penetrate the skin and reach vital internal organs via the systemic circulation [21]. Cosmetic products that are applied to mucous membranes, such as lipsticks, are even more hazardous. In addition, lipsticks also have a higher risk of direct oral ingestion, which can aggravate the negative effects of the chemicals contained in them [22]. Basically, providing the pigment in colored lipsticks is quite worrisome especially in the longer lasting red lipsticks. In recent years, the Sudan dyes in food products and lipsticks have been detected in some countries, which exports many cosmetic products to different countries including Iran [23]. Since there are not enough ability and time to control and detect all suspected contaminated sources, development of comprehensive and reliable methods to track residues or metabolites of these dyes (as biomarkers) in human biological fluids can be a desirable solution, as well as their detection in food and cosmetic products. Biomarkers of nutrient intake (or exposure) are able to objectively assess dietary intake/status without the bias of self-reported dietary intake errors, and also overcome the problem of intra-individual diet variability. In order to select an analyte as a potential biomarker, the analyte has to meet some requirements [24], such as bioavailability in human fluids, sensitive to the changes in intake of the dietary component of interest, and quantifiably using a suitable technique accurately.

In this way, 1-amino-2-naphthol (as a main reductive metabolite of Sudan dyes) could be a good choice to consider as a potential biomarker to indicate the entrance of Sudan dyes to human body.

The objectives of the present work were followed as, (i) to introduce a simple and eco-friendly method based on use of ionic–liquid as extraction solvent, termed one-step air-assisted liquid–liquid microextraction (OS-AALLME), which able to simultaneously extract the sudan dyes in the complex matrices such as foods and cosmetics. (ii) to evaluate and optimize the OS-AALLME method with the aid of response surface methodology. (iii) to investigate the possibility of being 1-amino-2-naphthol as a new potential biomarker of Sudan I–IV dyes, and Orange G, which are the most illegal azo-based dyes added to daily consumed edible and cosmetic products.

2. Experimental

2.1. Reagents and solutions

Standards of Sudan dyes (Sudan I–IV), Orange G and *1-amino-2-naphthol* were purchased from Sigma–Aldrich (St. Louis, MO, USA). HPLC grade methanol and acetonitrile were from Fluka (Buches, Switzerland). Acetone, sodium chloride (NaCl), and ultra-pure water were all from Merck (Darmstadt, Germany). 1-hexyl-3-methylimidazolium hexafluorophosphate [C_6MIM][PF_6] (purity >98%), 1-butyl-3-methylimidazolium hexafluorophosphate [C_4MIM][PF_6] (purity >98%), and 1-Hexyl-3-methylimidazolium bis(trifluoromethylsulfonylimide) [C_6MIM][$N(SO_2CF3)_2$] (purity >98%) were purchased from IoLiTec Company (Heilbronn, Germany). Syringe filters (0.2 μm) were purchased from Sigma–Aldrich. Sodium hydroxide and concentrated hydrochloric acid, were bought from Merck used to adjust the pH of the samples. The lipstick samples were purchased from a cosmetics store (Mashhad, Iran). Chilli powder and its sauce were purchased from local markets (Mashhad, Iran).

A mixture of stock solution containing azo dyes at 1000 μg mL^{-1} was prepared in HPLC grade methanol. A series of standard solutions were prepared by mixing an appropriate amount of the stock solution with ultra-pure water in a 5 mL volumetric flask. The aqueous solutions were prepared daily by diluting the standards mixture with ultra-pure water. All the standard solutions were stored at 4 °C.

The optimum separation condition was achieved using an isocratic elution with a binary mobile phase (methanol:acetonitrile, 20:80 (v/v)). In order to achieve the maximum peak areas and higher sensitivity for the analytes, two different wavelengths at 250 and 480 nm were used for 1-amino-2-naphthol and Sudan dyes (Sudan I–IV) and Orange G, respectively. The injection volume was 20 μL.