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Determination of diphenylamine residue in fruit samples by supercritical fluid extraction followed by vesicular based-supramolecular solvent microextraction

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ABSTRACT

The combination of supercritical fluid extraction (SFE) with supramolecular solvents-based microextraction (SSME) has been developed for extraction and determination of diphenylamine (DPA) in the peel of fruit samples. High performance liquid chromatography (HPLC) coupled with ultraviolet (UV) detection was applied for determination of the DPA. The supramolecular solvent was produced from coacervation of decanoic acid aqueous vesicles in the presence of tetrabutylammonium (Bu_4N^+). In SFE–SSME procedure, the dehydrated samples were loaded into SFE extraction vessel and extraction was performed in a prescribed time. The extracted analyte was collected in 5 mL aqueous solution (pH = 3) and introduced to SSME. The effective parameters on the SSME efficiency were studied and optimized. The Taguchi orthogonal array (OAD) experimental design with an OA_{16} (4^5) matrix was employed to optimize the SFE conditions. The calibration plot was linear in the range of 0.5–7.0 mg kg^{-1} and the limit of detection (LOD), based on *S*/*N* of 3 was 0.3 mg kg^{-1}. Interday RSDs% lower than 10.3%, and intraday RSDs% lower than 7.1% were obtained. Analysis of DPA in different fruit peel showed that the improved technique has great potential for extraction and determination of DPA in fruit samples.

1. Introduction

The use of pesticides in agriculture is necessary to combat a variety of pests that could destroy crops, and to improve the quality of the food produced. Agricultural use of pesticides plays a beneficial role in providing a plentiful, low-cost supply of high-quality fruits and vegetables. On the other hand, as a consequence of this use, the presence of residues in food that was critical elements of overall population health is unavoidable and pesticide residues in food is of great importance in the evaluation of food quality [1]. Diphenylamine (DPA) is one of the most used pesticides worldwide. It is used as a pre- or postharvest scald inhibitor for some fruits include apples and pears. Its anti-scald activity is the result of its antioxidant properties, which protect the fruit skin from the oxidation products of alpha-farnesene during storage [2]. Therefore, residues of DPA are often found in agricultural crops. However, the presence of pesticide residues in foods can be considered as a hazard to human health [3,4].

According to EU regulations in foodstuffs, the maximum residue levels (MRLs) for DPA are 5 and 10 mg kg^{-1} for apples and pears, respectively [5]. Therefore, qualitative and quantitative determination of DPA in these materials is of biological and environmental importance. Several methods have been described in the literature for the determination of DPA using different analytical techniques such as GC [6], HPLC [7] and spectrophotometery [8]. Spectrophotometric methods are more useful for the determination of diphenylamine at low concentration level, but these methods suffer from poor linear dynamic ranges and some of methods require expensive instruments.

Different analytical procedures have been used for extraction of pesticides from solid samples. Conventional methods, such as liquid–liquid extraction (LLE) [9] solid-phase extraction [10] and Soxhlet [11] have been used for extraction of organic compounds from the soil and sediment samples. However, these techniques are tedious and time or/and solvent consuming. In the last decades, new extraction methods such as supercritical fluid extraction (SFE) [12], pressurized liquid extraction (PLE) [13] and microwave assisted extraction (MAE) [14] were introduced. The unique properties exhibited by supercritical fluids have already been applied for the analysis of pesticide residues in solid

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samples [15]. SFE is selective and less-solvent-consuming, thus it is environmental friendly. The most serious problem of off-line SFE methods is evaporation of collecting solvent at the end of extraction to acquire high preconcentration factor. However, this procedure is a time-consuming step and contaminates the environment and collected analytes may be lost or degradated in this step.

Modern trends in analytical chemistry now lean towards the simplification and miniaturization of sample preparation, as well as the minimization of the organic solvents used. In 1996, Jeannot and Cantwell [16] developed a liquid-phase microextraction (LPME) technique, which is based on analyte partitioning between a drop of organic solvent (extraction phase) and a bulk aqueous sample. Several different types of LPME have been developed, including single drop microextraction (SDME) [17], hollow fiber LPME [18], and homogeneous liquid-liquid extraction (HLLE) [19]. Microextraction techniques are fast, simple, inexpensive, environmentally friendly, and compatible with many analytical instruments. Nevertheless, some drawbacks, such as instability of the droplet and relatively low precision are often reported [20]. Not long ago, a new LPME method namely solidified floating organic drop microextraction (SFODME), which is a modified solvent extraction method, was proposed for extraction and determination of organic analytes [21]. In this method, no specific holder, such as the needle tip of microsyringe and the hollow fiber, is required for supporting the organic microdrop due to the using of organic solvent with low density and proper melting point. Moreover, the extractant droplet can be collected easily by solidifying it in the lower temperature.

The surfactant-rich phase is a nano-structured liquid, recently named as supramolecular solvent (SUPRAS), which is generated from the amphiphiles through a sequential self-assembly process occurring on the molecular and nano-scales [22]. In 2006, Pérez-Bendito et al. have investigated the potential of the tetrabutylammonium-induced liquid–liquid phase separation in alkyl carboxylic acid vesicular solutions for the extraction of organic compounds prior to HPLC for the first time [23]. SUPRASs are water-immiscible liquids made up of supramolecular assemblies dispersed in a continuous phase [24]. Two characteristics make the alkyl carboxylic acid-based Coacervates to have a high potential for analytical extraction processes. First, the polar region of molecular aggregates consists of protonated and deprotonated carboxylic groups and ammonium groups, so a number of interactions (e.g. electrostatics, cation-π, hydrogen bonds, formation of mixed aggregates, etc.) can be established with analytes, in addition to hydrophobic interactions in the hydrocarbon region. Second, vesicles have a number of available solubilization sites so, high concentrations of polar and apolar molecules can be solubilized in each aggregate. The formation of vesicles in aqueous solution before adding Bu_4N^+ ions was not essential to achieve liquid–liquid phase separation [25–27]. Recently, our research group firstly described the potential of SUPRAS for solidification of floating drop method [28]. In this method, a small volume of a vesicular coacervate (melting point ≈10 °C) is floated on the surface of aqueous solution. After the extraction, the floated extractant droplet can be collected easily through solidification at low temperature. The solidified solvent can be melted quickly at room temperature, which is then introduced to HPLC.

Although SUPRAS-based microextraction (SSME) has a lot of advantages such as low cost, low consumption of organic solvent and high enrichment factor, it is not suitable for extraction of compounds from solid samples. There are only a few papers reporting the use of SSME in solid samples [25–28]. In the present study, SFE coupled to SSME was applied for extraction and determination of DPA in fruit samples.

2. Experimental

2.1. Chemicals and reagents

All of the reagents used were of analytical grade. Decanoic acid was purchased from Fluka (Buchs, Switzerland). Tetrabutylammonium hydroxide (Bu_4NOH, 40% w/v in water) was obtained from Sigma-Aldrich (Milwaukee, WI, USA). The ultra-pure water was prepared by a model Aqua Max-Ultra Youngling ultra-pure water purification system (Dongan-gu, South Korea). HPLC grade methanol and acetonitrile were purchased from Caledon (Ontario, Canada). Standard of diphenylamine (DPA) was purchased from Merck (Darmstadt, Germany). Carbon dioxide with a minimum 99.99% purity was obtained from Sabalan (Tehran, Iran) and used in all of the extraction experiments.

Stock standard solutions of 1000 μg mL^{-1} DPA was prepared by dissolving appropriate amount of the compound in methanol and stored at 4 °C. Working standard solutions were prepared daily by diluting the stock standard solution with ultra-pure water to the required concentrations.

2.2. Apparatus

A Suprex (Pittsburgh, PA) MPS/225 system in SFE mode was utilized for all extractions. Extractions were accomplished using a 1 mL volume stainless steel extraction vessel. An adjustable restrictor (ISCO, USA) was used in the SFE system to collect the extracted analytes. In order to prevent sample plugging, the restrictor point was warmed electrically. Chromatographic separations were carried out on a Varian HPLC equipped with a 9012 HPLC pump (Mulgrave, Victoria, Australia), a six-port Valco HPLC valve (Houston, USA) equipped with a 20 μL sample loop and a Varian 9050 UV–vis detector. Chromatographic data were recorded and analyzed using Chromana software (version 3.6.4). The separation were carried out on an ODS-3 column (150 mm × 4.6 mm, with 5 μm particle size) from MZ-Analysentechnik (Mainz, Germany). A mixture of ultra-pure water and methanol (30:70) at a flow rate 1.0 mL min^{-1} was used as a mobile phase and the DPA was detected at 280 nm.

2.3. SFE–SSME procedure

The fruit samples were peeled and then peel of fruit placed into a freeze-dry machine that removes water from them. Dehydrated peels were homogenized and sieved (for particle sizes in the range of 0.02–0.05 mm). Samples were stored in glass bottles with Al-foil cover. The homogenized sample was placed in the SFE extraction vessel and spiked with 50 μL of DPA standard (100 mg L^{-1}). The sample was mixed completely and another filter was placed on top of the vessel and the filter was then closed. Finally, SFE was carried out using a combination of static extractions to enhance the sample-solvent contact, and thus a better penetration of the fluid in the matrix, followed by dynamic extraction steps (in which the supercritical fluid passed continuously through the extraction chamber). Extractions were conducted under the following conditions: 15 min static extraction, 30 min dynamic extraction at 240 bar and 75 °C, a CO_2 flow-rate of 0.5 mL min^{-1} and temperatures of restrictor body and tip equal to 90 °Cand 95 °C, respectively. The extracted analyte was collected in 5 mL aqueous solution (pH = 3) which was located in a 10.0 mL volumetric flask and diluted to 25 mL with deionized water. Then, pH of the solution was adjusted at 7.0 μL volume of vesicular coacervative droplet was delivered to the surface of the aqueous sample, and the sample was stirred for a desired time. The sample vial was cooled by immersing it into an ice bath for 3 min. The solidified solvent was