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In-syringe reversed dispersive liquid–liquid microextraction for the evaluation of three important bioactive compounds of basil, tarragon and fennel in human plasma and urine samples

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ABSTRACT

In the present study, an efficient and environmental friendly method (called in-syringe reversed dispersive liquid–liquid microextraction (IS-R-DLLME)) was developed to extract three important components (i.e. *para-anisaldehyde*, *trans-anethole* and its isomer *estragole*) simultaneously in different plant extracts (*basil*, *fennel* and *tarragon*), human plasma and urine samples prior their determination using high-performance liquid chromatography. The importance of choosing these plant extracts as samples is emanating from the dual roles of their bioactive compounds (*trans*-anethole and estragole), which can alter positively or negatively different cellular processes, and necessity to a simple and efficient method for extraction and sensitive determination of these compounds in the mentioned samples. Under the optimum conditions (including extraction solvent: 120 μL of *n*-octanol; dispersive solvent: 600 μL of acetone; collecting solvent: 1000 μL of acetone, sample pH 3; with no salt), limits of detection (LODs), linear dynamic ranges (LDRs) and recoveries (R) were 79–81 ng mL^{-1}, 0.26–6.9 μg mL^{-1} and 94.1–99.9%, respectively. The obtained results showed that the IS-R-DLLME was a simple, fast and sensitive method with low level consumption of extraction solvent which provides high recovery under the optimum conditions. The present method was applied to investigate the absorption amounts of the mentioned analytes through the determination of the analytes before (in the plant extracts) and after (in the human plasma and urine samples) the consumption which can determine the toxicity levels of the analytes (on the basis of their dosages) in the extracts.

1. Introduction

Nowadays, plant extracts are considered as potential bioactive agents that can interfere and positively or negatively alter different cellular processes. Basil, tarragon, and fennel are three edible plants which have frequently been used as cooking spices, popular aromatic, and medicinal plants. For medicinal purposes, they are used to treat dyspeptic complaints and catarrh of the respiratory tract and as a mild expectorant [1,2]. The major components of these plants are *para-anisaldehyde*, *trans-anethole* and *estragole*. There are close relations between the functionality and mechanisms of these compoundsin the human body [3,4]. It seems they are responsible for the effective health benefits of these plants.

However, recent studies conducted on mice have shown that the existence of high levels of estragole could have genotoxic and carcinogenic properties [5]. Consequently, the addition of high levels of estragole as a flavoring substance in different types of foods has been banned in the recently adopted EU Regulation on flavoring substances [6].

In addition, because of the widespread use of large amounts of these compounds as flavoring additives for foods, medicine, and in the perfumery industry, as well as dose-dependent properties of estragole as a suspected carcinogenic agent [7], their accu-

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rate measurement in plant extracts and human fluids plays an important role in many areas of science. However, due to low concentrations of these compounds and complicated matrices of real samples, effective extraction and preconcentration of these analytes is needed before their determination to achieve good results.

Dispersive liquid–liquid microextraction (DLLME) [8] is an attractive pretreatment technique that allows reduction of sample and solvents consumption, high preconcentration factors and simplicity of operation [9–11], simultaneously. It also provides the analytes in a small organic droplet, as extraction solvent, which is more suitable to be injected in a chromatographic system. Up to now, based on the density of extraction solvents, DLLME has two modes. One mode, which called *Normal-DLLME* (N-DLLME), usually uses chlorinated solvents whose densities are higher than that of water. However, the number of organic solvents used in N-DLLME are relatively few and they are hazardous, too [12,13].

One of the principal driving forces for the broad investigations in the area of new sample preparation methods is the need to find replacements for hazardous volatile organic solvents. Hence, low density solvents were applied and inverted or reversed-DLLME (R-DLLME) was introduced, as a latter mode [14]. Although this method is more environmental friendly, there needs additional modifications of the extraction solvent collection which leads to the necessity of new device development [15].

However, the main drawback of both modes of DLLME is the necessity to centrifugation which is usually needed more expensive equipment and wasted time. In this way, a new DLLME method without centrifugation was introduced by Chen et al. and Navarro et al. [16–18]. However, the collection of the micro-volume of the floating organic solvents is still problematic.

In the present study, a novel and simple DLLME method (termed *in-syringe reversed dispersive liquid–liquid microextraction* (IS-R-DLLME)) was proposed using a syringe as an alternative for the flask to carry out the extraction, dispersion and phase separation steps in a single syringe. In order to repeatable collection of the separated floating organic solvent, a capillary tube was fitted at the top of the syringe. By pressing the syringe plunger, the floating organic solvent was moved and collected in the capillary tube. The performance of the IS-R-DLLME was demonstrated for the extraction, preconcentration, separation and determination of three bioactive compounds (i.e. *para*-anisaldehyde, *trans*-anethole and its isomer estragole) in different plant extracts (basil, fennel and tarragon), human plasma and urine samples at trace concentration levels using high performance liquid chromatography (HPLC). The IS-DLLME offers a simpler and more convenient method from a handling point of view without losing sensitivity and efficiency. The importance of choosing basil, fennel and tarragon as the samples for analysis is emanating from the dual roles of their bioactive compounds (*trans*-anethole and estragole), which can alter positively or negatively different cellular processes, and necessity to a simple and efficient method for extraction and sensitive determination of these compounds in the mentioned samples. In the end, determination of the compounds levels in the plant extracts and comparison with their amounts in human bio-fluids was used to determine the extent of their degradation or absorption in the human body.

2. Experimental

2.1. Reagents and solutions

para-Anisaldehyde, estragole and *trans*-anethole, HPLC grade acetonitrile and methanol were from Merck (Darmstadt, Germany, www.merck.de). 1-octanol, toluene, *n*-heptane, cyclohexane, 2-dodecanol, 1-undecanol, *n*-hexane, acetone, methanol, ethanol, sodium chloride, and ultra-pure water were from Merck. Trichloroacetic acid (TCAA) was obtained from Sigma–Aldrich (St. Louis, MO, USA, www.sigmaaldrich.com). Sodium hydroxide and concentrated hydrochloric acid were bought from Merck, used to adjust the pH of the samples. Other reagents were of analytical grade and purchased from Merck.

Stock standard solutions of each analyte were prepared separately by dissolving proper amounts of each drug in methanol at 1000 mg mL^{-1} and stored at 4 °C. Mixtures of standard working solutions for extraction at different concentrations were prepared by dilution with ultra-pure water for optimization of parameters. The working solutions were freshly prepared by diluting the mixed standard solutions in ultra-pure water for the required concentrations. All the standard solutions were stored at 4 °C.

The optimum separation was achieved by gradient elution with a binary mobile phase (A, water; B, acetonitrile) and flow programming. The gradient elution program was: 0–4 min 60% B (1 mL min^{-1}); 4–7 min 70% B (1 mL min^{-1}); 7–10 min 75% B (0.9 mL min^{-1}); 10–15 min 80% B(0.7 mL min^{-1}); 15–16 min 75% B (0.9 mL min^{-1}); 16–20 min 90% B (1 mL min^{-1}). The injection volume and detection wavelength were 20 μL and 280 nm, respectively.

2.2. Apparatus

Chromatographic measurements were carried out using a Knauer HPLC system (Berlin, Germany, www.knauer.net) equipped with a K-1001HPLC pump and a UV detector K-2800. The other HPLC equipment included a Knauer K-1500 solvent organizer and a Knauer K-500 degasser (Berlin, Germany, www.knauer.net). Chromgate software (version 3.1) for HPLC system was employed to acquire and process chromatographic data. The chromatographic determinations were performed using an ODS III column (250 mm × ID 4.6 mm, 5 μm) from Capital (Broxburn, UK, www.capitalhplc.com). The pH values for the solutions were measured using a PHS-3BW model pH-meter (Bell, Italy, www.belltechnology.co.nz).

2.3. Sample preparation

2.3.1. Volunteers

Volunteers were all apparently healthy with no history of disease of the gastrointestinal tract. These volunteers followed a vegetable-free diet for one week before the study and during the study day. They were given oral instructions on their diet and a list of prohibited foods, which included all foods and beverages known or suspected to contain the analytes studied. The volunteers were also asked to restrain from using dietary supplements during this period. Blank urine (analyte-free) was provided by healthy volunteers who had not consumed any prohibited vegetables or other foods [19]. It should be mentioned that all ethical and human rights guidelines in the sampling procedure were obeyed.

2.3.2. Plasma

Blood samples were collected into Plasma Separation Tubes (PSTs) and centrifuged at 10,000 rpm. Separated plasma was withdrawn into a Pyrex centrifuge tube and stored at −20 °C until analysis.

Plasma sample (2.5 mL) was spiked with particular level of the drug and sonicated for 5 min. The mixture was acidified with 200 μL hydrochloric acid (37%) to disturb the drug protein binding [20]. Then, 250 μL TCAA (100%, w/v) was added to denature the proteins. These processes eventually led to the precipitation of proteins. Subsequently, the sample was centrifuged at 10,000 rpm for 5 min. A volume of 2 mL of the supernatant was transferred to the sample vial and diluted with doubly distilled water to 5 mL [21]. The result-