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Two-phase hollow fiber-liquid microextraction based on reverse micelle for the determination of quercetin in human plasma and vegetables samples

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ABSTRACT

A new two-phase hollow fiber-liquid microextraction based on reverse micelle as sensitive and efficient method for the extraction of quercetin in human plasma, onion and tomato samples was developed for the first time. The extraction is based on the solubilization of analyte in the aqueous core of the reverse micelle of cationic cethyltrimethyl ammonium bromide surfactant, CTAB, which are present in the organic phase and are used as acceptor phase in hollow fiber-liquid phase microextraction. Experimental results show that the electrostatic interaction between the oppositely charged surfactant head group present in the reverse micelles and the analyte molecule has a key role in the extraction. Extraction parameters including the organic phase, pH, surfactant concentrations, stirring rate, extraction time, temperature and ionic strength were optimized. The optimum condition is obtained when the variables are set to: pH= 7.5, organic solvent= 1-octanol, CTAB concentration= 7 mmol L^{-1}, stirring rate= 900 rpm at room temperature and extraction time= 30 min. Under the mentioned condition, the calibration range was found to be linear over 0.5–1000 ng mL^{-1} with the limit of detection of 0.1 ng mL^{-1}. The extraction recovery of 97.4%, with relative standard deviation of 3.5%, was obtained via tree replicated measurements on a 15 ng L^{-1} quercetin standard solution. Finally, the newly developed method was successfully used for the preconcentration and determination of quercetin in plasma, onion and tomato samples.

1. Introduction

Flavonoids are a various group of polyphenolic compounds with antioxidant activity present in vegetables, fruits, wine and tea. Because of its health-related properties, the determination of flavonoids has attracted much attention of researchers. Quercetin is the most active compound in flavonoid family and the most common in nature [1]. Epidemiological studies relating to the biological properties of quercetin have indicated its important role in the prevention of a wide range of diseases human, such as cancer, cardiovascular and inflammatory disorders, diabetes, ulcer, cataract and allergies [2–6]. It has also been claimed that quercetin can reduce blood pressure in hypertensive subjects [7]. Quercetin has free-radical scavenger properties and shows high tendency for electron transfers and are so considered as dietary antioxidants to be helpful for human health [8].

In recent years, the interest in natural antioxidants, especially in those of plant origin (berries, vegetables, fruits, tea), has increased considerably. Among vegetables, Onion (Allium cepa L.) and tomato (Solanum lycopersicum) are very important for its high content of healthy constituents in human diet. They are rich in phenolic compounds (flavonoids and phenolic acids), especially quercetin. Onion ranked highest in quercetin content in a survey of 28 vegetables and 9 fruits [9]. Quercetin-3-rutinoside (rutin) and quercetin-4′-glucoside are the predominant forms of quercetin in onion and tomato [10,11].

Analytical methods for the determination of quercetin concentrations in the vegetables samples have mostly been based on high performance liquid chromatography (HPLC) with ultra violet (UV) detection [12–15], capillary electrophoresis with UV [12], HPLC with diode-array detection [16], HPLC with electrochemical detection (ED) [17] and photodiode array spectrophotometer [18].

Quercetin is mostly present as glycosides, such as quercetin-4-glucoside, quercetin-3-rutinoside (rutin) and quercetin-3-galactoside [19]. Studies demonstrated that some quercetin glycosides able to be absorbed in humans and the bioavailability of various quercetin glycosides is affected by their sugar moiety [20], so, in order to the pharmacokinetic and bioavailability investigations of flavonoid glycosides possessing quercetin, the measurement of quercetin in biological samples is imperative. Numerous analytical methods appropriate for the measurement of quercetin in biological samples are included gas chromatography (GC) with mass spectrometry (MS) detection [21],

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liquid chromatography (LC) with MS [22,23], LC with MS/MS [24], HPLC with UV [25,26], fluorimetry [27] and ED [28] detection.

The monitoring of target compounds present in representative samples at trace/ultra-trace concentration level generally needs a primary step of isolation and/or preconcentration of analytes, especially in samples characterized by complex matrices composition. Traditional preconcentration techniques are based on liquid–liquid extraction (LLE) and solid phase extraction (SPE) but disadvantages such as tedious and time consuming, high consumption of organic solvents, expensive and labor intensive are associated with these methods. So to overcome LLE and SPE disadvantages, several new miniaturized extraction techniques inclusive solid phase microextraction (SPME) [29], stir-bar sorptive extraction (SBSE) [30], single drop microextraction (SDME) [31], cloud point extraction (CPE) [32], dispersive liquid–liquid microextraction (DLLME) [33] and hollow fiber–liquid phase microextraction (HF-LPME) [34] have been introduced and extensively applied to different type of samples.

HF-LPME has been developed based on the principle of a supported liquid membrane in 1999 [34]. According to the HF-LPME method, the porous polypropylene hollow fiber acts as an interface between the donor phase and micro-volumes of the acceptor phase and causes efficient sample microfiltration through the pores of the HF. HF-LPME can be performed both two phase and three phase mode. In two-phase mode which is mainly utilized for hydrophobic compounds the target analytes are extracted from the aqueous sample solution into the organic solvent located both inside the lumen of the hollow fiber and in the pores of the wall based on classical partition. In another mode, the acceptor phase can be an aqueous solution, providing a three-phase extraction system which is preferable for the extraction of compounds with ionizable functionalities. The distinguished advantages of HF-LPME include simplicity, negligible consumption of organic solvent, inexpensiveness and high ability to cleanup, no memory effect due to the single-used and disposable nature of HF, tolerance of wide pH range by HF and high enrichment factor because of high ratio of surface area to volume of the hollow fiber column like configuration.

Our investigations showed that there is not any report about preconcentration and extraction of compounds with ionizable functionalities by two phase HF-LPME technique. From this perspective, surfactants are excellent candidates to achievement to this purpose. Surfactants include both hydrophilic and hydrophobic groups in their structure whereby caused advantages such as high solubility in different solvents and high efficiency to solubilize different compounds. The most outstanding feature of the surfactants is theirs trend to form the micelles, the large aggregates, in critical micelle concentration. The tendency of many water soluble solutes or polar substances to partition into the aqueous inner core of reverse micelles present in the organic phase provides using such systems in preconcentration techniques as potential extracting media.

In this work we present for the first time a hollow fiber-liquid microextraction based on reverse micelle (HF-LLME-RM) of cethyltrimethyl ammonium bromide (CTAB) following by HPLC-UV for extraction of quercetin, one of the most important flavonoids, in onion and human plasma samples.

2. Experimental

2.1. Chemicals and materials

Quercetin and CTAB were purchased from Sigma–Aldrich (Steinheim, Germany). Hydrochloric acid, phosphoric acid, sodium hydroxide, sodium chloride, 1-octanol, 5-nonanol, 2-ethyl 1-hexanol, 1-undecanol, HPLC-grade methanol and HPLC-grade acetone were obtained from Merck (Darmstadt, Germany). The Accurel Q3/2 polypropylene hollow fiber (600 μm internal diameter, 200 μm wall thickness and 0.2 μm average pore size) bought from Membrana GmbH (Wuppertal, Germany), was applied for all experiments. The water used throughout the work was double distilled deionized. Water filtration was accomplished through a 0.45 μm filter (Millipore membranes, Bedford MA, USA). A stock standard solution of quercetin (100 μg mL^{-1}) was prepared by dissolving a certain amount of the compound in methanol and stored at 4 °C in the dark. The working solutions were prepared daily by a proper dilution of stock solution with double distilled deionized water.

2.2. Instrumentation and operating condition

The chromatographic analysis was carried out by a Waters HPLC system equipped with model 1525 Binary HPLC Pump and model 7125i manual Rheodine injector fitted with a 20 μL loop and a model 2487 Waters dual λ absorbance UV detector. Separation was performed at flow rate of 1.0 mL min^{-1} by an isocratic elution on a Waters C18 column (150 mm × 4.6 mm I.D., 5 μm particle size). The mobile phase was consisted of a mixture of 58% methanol and 42% aqueous phase containing 0.3% phosphoric acid and detection was performed at 370 nm. All of analyzes were done at ambient temperature. For adjustment of solution pH, a 3030 Jenway pH meter was used and also a magnetic stirrer plate bought from Heidolph was utilized to stir the sample solution and accelerate the extraction.

2.3. Real sample preparation

Quercetin mostly exists as glycosides. In order to simplifying chromatographic data specifically in instances where appropriate standards of most individual flavonoid glycosides are commercially unavailable and to minimize interferences in subsequent chromatography, preliminary hydrolysis of samples is necessary. Hence, in this work, the flavone glycosides were hydrolyzed before the analysis and free quercetin was determined. The elimination of the protein from plasma was carried out by addition 4 mL methanol to 2 mL of the plasma. Then the mixture was extremely vortexed for 5 min and was kept in the frozen at −20 °C for 10 min. After thawing frozen plasma at ambient temperature, it was centrifuged at 5000 rpm for 10 min then supernatant was decanted into another clean tube and filtered through a 0.45 μm filter.

For hydrolysis of quercetin glycosides in deprotonated plasma sample, 1 mL of 25% HCl was added into 4.0 mL of plasma sample and was mixed well at 80 °C water bath for 30 min [35]. Afterward, the sample solution was transferred into a 100 mL volumetric flask and diluted with double distilled deionized water. Finally, the diluted and hydrolyzed sample was applied to HF-LLME-RM as mentioned in Section 2.4.

Onion bulbs, outer dry skins and any inedible outer sections were removed. The tomato sample was washed with water after removing manually inedible parts. 1 g each of crushed and dried (in oven at 60 °C for 6 h) tomato and onion was thoroughly extracted by 10 mL of 25% HCl at 80 °C water for 30 min, separately. Then, the sample extracts were filtered using a Whatman filter paper No. 41 (Macherey-Nagel, Germany) and diluted to 100 mL with double distilled deionized water prior to HF-LLME-RM procedure.

2.4. HF-LLME-RM procedure

The hollow fiber membrane was manually and carefully cut into segments with a length of 9 cm (the approximate internal volume of 25 μL) and ultrasonically cleaned in HPLC-grade acetone for 5 min to remove any contaminants in the fiber and then dried completely in air before use.

The detailed extraction and preconcentration procedure for target analyte was according to the following procedure. A 10 mL of the sample solution (pH adjusted with 0.5 M HCl to 7.5) was transferred to the vial, and a magnetic bar was placed in the solution to ensure