Journal of Chromatography B 1072 (2018) 17–24

Contents lists available at ScienceDirect

Journal of Chromatography B

journal homepage: www.elsevier.com/locate/jchromb

Fibroin/dodecanol floating solidification microextraction for the preconcentration of trace levels of flavonoids in complex matrix samples[1]

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ARTICLE INFO

Keywords:
Fibroin
Floating solidification microextraction
Flavonoids
Complex sample
High performance liquid chromatography

ABSTRACT

A new fibroin/dodecanol floating solidification microextraction, coupled with high performance liquid chromatography, was developed and applied for enrichment and quantification of the trace flavonoids in traditional Chinese medicine and biological samples. Also, fibroin sensibilization mechanism was described, and influence of sample matrix to enrichment factor was investigated. In this method, a homogeneous fibroin/dodecanol of dispersed solution was employed as microextraction phase to flavonoids (myricetin, quercetin, isorhamnetin, chrysin, kaempferide), the several critical parameters affecting the performance, such as organic extractant, amount of fibroin in organic extractant, volume of extraction phase, dispersant, salt concentration, pH of sample phase, stirring rate, extraction time, and volume of sample phase were tested and optimized. Under the optimized conditions, enrichment factor of flavonoids ranged from 42.4 to 238.1 in different samples, excellent linearities with $r^2 \geq 0.9968$ for all analytes were achieved, limits of detection were less than or equal to 5.0 ng/mL, average recoveries were 92.5% to 115.0% in different samples. The new procedure is simple, fast, low cost, environmentally friendly and high *EF*, it can also be applied to the concentration and enrichment of the trace flavonoids in other complex matrixes.

1. Introduction

Flavonoids, secondary metabolites of plants [1], widely exist in traditional Chinese medicines (TCMs). A large number of experiments [2,3] have illustrated that flavonoids can be used for the treatment of many diseases, such as antitumor [4,5], antioxidant [6], anti-inflammatory, prevention of cardiovascular disease [7], and reducing blood lipid [8]. However, flavonoids have a wide variety of structure types and lower content in TCMs, and it can generate multiple metabolites come from the metabolism of human body, the disease mechanism and pharmacodynamic material basis are still unclear. So, it is very meaningful to research and establish the analytical method for separation, enrichment and determination of the trace flavonoids in traditional Chinese medicine and biological samples so as to improve their sensibilities in subsequent analysis and detection.

Liquid phase extraction (LPE) [9,10] and solid phase extraction (SPE) [11,12] are common sample pretreatment technologies, which possess defects of time-consuming, complex operation, more organic solvent, higher cost and much pollution [13]. Therefore, miniaturized sample preparation technology, for example liquid phase microextraction (LPME) [14–17] and solid phase microextraction (SPME) [18–20], have been widely used for enrichment and concentration of the trace compounds in various complex samples. LPME and SPME, are more applicable to modern drug analysis, because of simplicity, less solvent consumption, lower cost, environmental protection and higher enrichment factor (*EF*).

In 2007, Khalili-Zanjani et al. [21] proposed a new LPME method named solidification of floating organic droplet LPME (SFODLPME), which was used for preconcentration of polycyclic aromatic hydrocarbons in well water samples. In SFODLPME, it is essential that the melting point (MP) of extractant must be close to or below to room temperature and its density should be lower than water, therefore, *n*-undecane (MP −26 °C, density 0.74 g/cm^3), *n*-dodecane (MP −9.6 °C, density 0.75 g/cm^3), 1-undecanol (MP 19 °C, density 0.83 g/cm^3), and 1-dodecanol (MP 24 °C, 0.83 g/cm^3) are usually used as extraction solvent in the conventional SFODLPME. When the extraction solvent is rapidly added into the sample solution at normal agitation, the cloudy suspension forms instantly and a large sum of the organic droplets are uniformly dispersed in the solution. These little droplets, with large surface areas, contact with sample solution to accelerate extraction

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[1] Abbreviations: F/D-FSME, fibroin/dodecanol floating solidification microextraction; HPLC, high performance liquid chromatography; UV, ultraviolet detection; *EF*, enrichment factor; TCM, traditional Chinese medicine; LPE, liquid phase extraction; SPE, solid phase extraction; LPME, liquid phase microextraction; SPME, solid phase microextraction; SFODLPME, solidification of floating organic droplet liquid phase microextraction; D-FSME, dodecanol floating solidification microextraction.

https://doi.org/10.1016/j.jchromb.2017.09.023
Received 1 April 2017; Received in revised form 26 July 2017; Accepted 6 September 2017
Available online 18 September 2017
1570-0232/

equilibrium and to improve enrichment factor of the trace target analytes. SFODLPME has been applied to the preconcentration and preenrichment of the trace lignans [22], flavonoids [23] in TCMs; heavy metals [24–27] in water samples; phthalate esters [28] in lotions; molybdenum [29] in beverages and food samples; polybrominated diphenyl ethers [30] in water and urine samples; phenobarbital, phenytoin, and lamotrigine [31] in plasma and urine samples.

Fibroin, is a kind of natural macromolecule protein consisting of 18 kinds of amino acids, has been used in medical field, such as artificial skin [32], drug release material [33], cell culture media [34], and immobilized enzyme carrier [35] because of its good biocompatibility, mild physical and chemical properties. In addition, fibroin is a good adsorbent due to the active centres of nitrogen and oxygen in its molecular structure [36]. Weitao Zhou [37] et al. used fibroin to adsorb Cu^{2+} from waste water in order to reduce the toxicity to human. Altiok E. et al. [38] isolated polyphenols from the extracts of olive leaves by using fibroin. Sun Y.Y. et al. [39] made a study about the adsorption capacity of silk fibroin to three heavy metal ions.

In our study, a new fibroin/dodecanol floating solidification microextraction (F/D-FSME) combined with high performance liquid chromatography and ultraviolet detection (HPLC/UV) was developed for enrichment and determination of the trace flavonoids including myricetin, quercetin, isorhamnetin, chrysin, kaempferide (Fig. 1) from TCMs and biological samples. By comparing to traditional dodecanol floating solidification microextraction (D-FSME), the sensibilization mechanism of the F/D-FSME caused by fibroin was expounded, and that the influence of sample matrix on *EFs* of flavonoids were analysed and described. The variables affecting extraction behavior were optimized, and the methodology of F/D-FSME was evaluated and validated. This study aims to establish a novel F/D-FSME coupled with HPLC/UV for concentration and quantitation of the trace flavonoids in TCMs and biological samples.

2. Experimental

2.1. Instruments

The following apparatus were used in the present experiment: An Agilent 1260 series of high performance liquid chromatograph (Agilent, USA) with G1311C infusion pump, G1329 B autosampler, G1316A column oven, G1314 B UV detector, and C18 column (250 mm × 4.6 mm, 5 mm; Elite analytical instruments Co., Ltd., Dalian, China); High-speed freezing centrifuge (TGL–16 M Changsha, China); DragonLab BlueSpin magnetic stirrer (MS-H280-Pro, Beijing, China).

2.2. Reference substances, materials, and reagents

All reference substances of flavonoids: myricetin (batch number: 16012504, purity: 98.31%), quercetin (batch number: 16031804, purity: 99.35%), isorhamnetin (batch number: 16032910, purity: 99.99%), chrysin (batch number: 16042821, purity: 98.47%), kaempferide (batch number: 16041502, purity: 99.74%), were provided by chengdu MUST Bio-Technology CO., Ltd. (Chengdu, China). *Tribulus terrester, Ginkgobiloba,* and *Platycladus orientalis* were obtained from Beijing Tongrentang drugstore (Taiyuan, Shanxi, China). 1-Dodecanol was purchased from Tianjin Guangfu Fine Chemical Research Institute (Tianjin, China). HPLC-grade methanol, acetonitrile, and acetone were purchased from Tianjin Siyou Chemical Co., Ltd. (Tianjin, China). Double-distilled water was used throughout the whole test. Fibroin powder was provided by Execute Brigitte Biotechnology Co., Ltd. (Shanghai, China). Fresh blank human plasma was purchased from Taiyuan Red Cross Blood Center (Taiyuan, Shanxi, China). Blank human urine was obtained from healthy volunteer.

2.3. Preparation of standard and sample solutions

Individual reference stock solutions of myricetin, quercetin, chrysin, kaempferide (1 mg/mL) and isorhamnetin (0.2 mg/mL) were prepared in methanol. The mixed working standard solution containing 125 μg/mL of myricetin, quercetin, chrysin, kaempferide and 100 μg/mL of isorhamnetin was prepared by mixing and diluting all of stock solutions with methanol.

1.0 g of herbal powder was soaked in 40 mL of methanol for 10 min, weighed, and treated by ultrasonic for 30 min. After supplying the weight loss of methanol at room temperature, the herbal extract was added into a flask with 5 mL of 25% HCl solution, which was refluxed for 30 min, centrifuged for 10 min at 3500 rpm, and then the volume of the supernatant was adjusted to 50 mL. The above herbal sample was diluted 20 times by double-distilled water before use.

The simulated biological sample was prepared by mixing the blank human plasma or urine and the mixed working standards, which was diluted 50 times by double-distilled water before use.

All of standard solutions were stored in darkness and kept at −4 °C. The blank human plasma and urine were stored at −20 °C.

2.4. Chromatographic condition

The five flavonoids from different samples were analysed by HPLC with UV at 340 nm. The column temperature was maintained at 35 °C

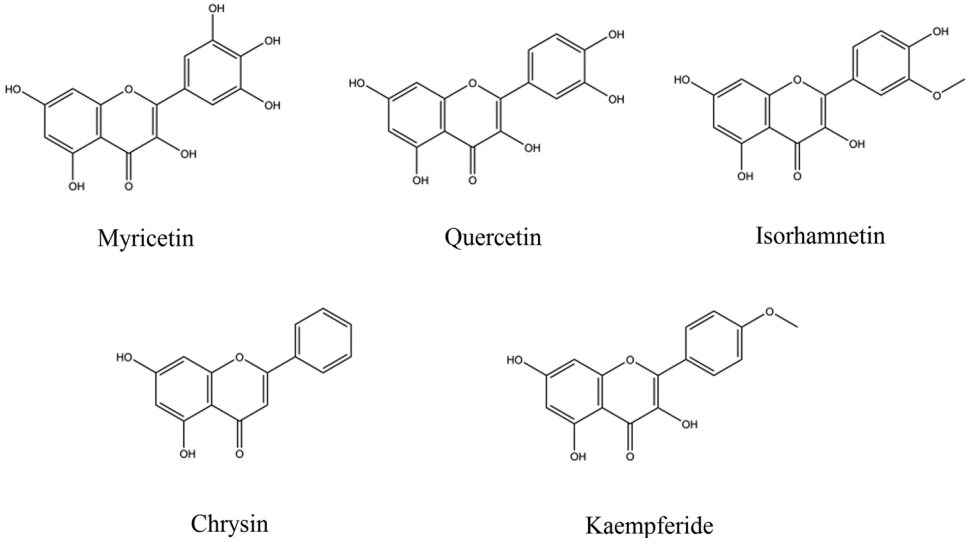

Fig. 1. The structure of five flavonoids.