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Comparison of dual solvent-stir bars microextraction and U-shaped hollow fiber–liquid phase microextraction for the analysis of Sudan dyes in food samples by high-performance liquid chromatography–ultraviolet/mass spectrometry

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Abstract

Two sample preparation methods, dual solvent-stir bars microextraction (DSSBME) and U-shaped hollow fiber–liquid phase microextraction (U-shaped HF–LPME), are proposed and critically compared for high-performance liquid chromatography (HPLC)–ultraviolet (UV)/mass spectrometry (MS) analysis of Sudan dyes in this paper. In DSSBME, the organic solvent was confined to a pair of hollow fiber membrane fixed on a stir bar, which can stir by itself, while the hollow fiber in U-shaped HF–LPME was fixed by two microsyringes. The significant factors affecting the microextraction of Sudan dyes in both microextraction techniques have been examined and no obvious difference in the effect of extraction solvent, pH and salt concentration on the extraction efficiency of Sudan dyes was observed except extraction time and stirring speed. Both microextraction techniques were similar in terms of analytical performance from aqueous solutions (LODs ranged from 0.09 to 0.95 $\mu g L^{-1}$ by HPLC–UV and 2.5–6.2 $\mu g L^{-1}$ by HPLC–MS; the absolute LODs ranged from 0.9 to 11.25 pg by HPLC–UV and 5–21.2 pg by HPLC–MS), however, DSSBME was more stable (lower stirring speed required), less sample consuming and much shorter time required to reach extraction equilibrium; while U-shaped HF–LPME was easier to operate and no more special device required. The two microextraction techniques combined with HPLC–UV/MS were successfully applied to the analysis of real samples including strawberry sauce, capsicum oil, salted egg, and two kinds of chilli sauce. Although the LODs of HPLC–UV are lower than that of HPLC–MS by a factor of 10 in this work, the absolute LODs for both HPLC–UV and HPLC–MS are comparable. HPLC–UV cannot identify the suspicious peaks at the same retention time as that of Sudan II and III in salted egg, while HPLC–MS can give exact information of Sudan I–IV in real sample analysis and is more reliable. The sensitivity of HPLC–MS is enough for real sample analysis.

Keywords: Dual sovlent-stir bars microextraction (DSSBME); U-shaped hollow fiber–liquid phase microextraction (U-shaped HF–LPME); Sudan dyes; HPLC–UV/MS; Food

1. Introduction

Sudan dyes are a group of azocompounds which have been classified as a possible human carcinogen and thus, the presence of Sudan dyes in foodstuff is forbidden in any national and international food regulation act. However, they are still used as food additives in some eastern countries due to their colorfastness and low price. Since the occurrence of Sudan I in chilli products and Sudan IV in salted eggs was determined in food market, there is a surge in the detection of Sudan dyes in China and these incidents cause panic in some customers. In view of the above incidents, suitable analytical methods for identification and quantification of such compounds in foodstuff are necessary.

Considering the complexity of matrix and the very low concentration of Sudan dyes in real samples analysis, a sample preparation step is required for isolation or enrichment of the analytes. Conventional sample preparation method for Sudan dyes analysis is liquid–solid extraction [1]. This method requires large amounts of organic solvent, it is often time-consuming and potentially toxic. Other sample preparation techniques, such as ultrasonic-assisted extraction [2–4] and centrifugal sedimentation [5–8] were also reported for Sudan dyes analysis. In recent

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years, solid phase extraction (SPE) packed with Alumina N was used for clean-up of the food samples prior to the analysis [9,10]. Although this procedure is easy to operate and inexpensive, it requires long time of extraction and large amounts of organic solvent for elution. In addition, activation of Alumina N has great effect on the recoveries of the target analytes. Therefore, the development of simple, rapid and high efficient sample preparation techniques is significant for the trace analysis of Sudan dyes in foodstuff.

Liquid phase microextraction (LPME) is a simple, fast, efficient and inexpensive sample preparation technique which was developed in 1996 [11], it integrates extraction, concentration and sample-introduction into one step. Hollow fiber protected LPME (HF–LPME) was considered to be more robust, because the organic solvent is contained within the lumen of a porous hollow fiber, so the organic solvent is not in direct contact with the aqueous solution. To increase the interface area of the extractant, the fiber is presented in a U shape with each end connected to a microsyringe needle. As protected by the hollow fiber, HF–LPME can be applied to complex samples such as plasma [12], urine [13] or slurry [14] without any pretreatment, and endure high-speed agitation. Another alternative microextraction method derived from HF–LPME was solvent bar microextraction (SBME) which was proposed by Jiang and Lee [15] to improve the extraction efficiency. Since the organic phase in the solvent bar was stirred as well as the aqueous phase, faster extraction equilibrium can be achieved compared with HF–LPME. However, large amounts of aqueous solution would be needed if the fiber was lengthened [16], which restricted the application of SBME in some real sample analysis.

In this work, a novel solvent microextraction method called dual solvent-stir bars microextraction (DSSBME) was proposed and compared with U-shaped hollow fiber–liquid phase microextraction (U-shaped HF–LPME) for HPLC–UV/MS analysis of Sudan dyes in food samples. The developed methods have been successfully applied to the analysis of Sudan dyes in strawberry sauce, capsicum oil, salted egg and two kinds of chilli sauce.

2. Experimental

2.1. Materials and reagents

Sudan I (90% purity), Sudan II (90% purity), Sudan III (96% purity) and Sudan IV (91% purity) were purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany). Stock solutions (0.5 mg mL^{-1}) of Sudan I and II were prepared in methanol, whereas stock solutions (0.5 mg mL^{-1}) of Sudan III and IV were prepared in ethyl acetate, and all stock solutions were preserved in the dark.

The Q3/2 Accurel KM polypropylene hollow fiber was bought from Membrana GmbH (Wuppertal, Germany). The inner diameter of the hollow fiber was 600 μm, the thickness of the wall was 200 μm, and the wall pore size was 0.2 μm.

Water was purified with a Milli-Q water purification system (Millipore, Bedford, MA, USA). Methanol and acetonitrile (HPLC grade) were purchased from Fisher (Fisher, Scientific, Pittsburgh, PA, USA). 1-Octanol, tributyl phosphate (TBP), ethyl acetate, acetone, formic acid (FA) and ethanol were analytical grade (China National Medicine Corporation Ltd., Shanghai, China).

2.2. Apparatus

For HPLC–UV analysis, an Agilent 1100 series HPLC–UV system (Agilent Technologies, Waldbronn, Germany) consisting of vacuum degasser, a quaternary pump and a variable wavelength detector was used for identification and quantification of Sudan dyes. The separation was performed on a reversed phase C_{18} HPLC column (Lichrospher ODS, 5 μm, 4.6 mm × 200 mm, Hanbon, Jiangsu, China). Methanol–tetrahydrofuran–water (40:30:10, v/v/v) was used as an isocratic eluent at a flow rate of 1 mL min^{-1} and the UV detector was set at 478 nm for Sudan I and II, and 520 nm for Sudan III and IV. The retention time for Sudan I–IV was 3.37, 4.46, 4.93, and 6.85 min, respectively.

For liquid chromatography–electrospray ionization-quadrupole-time of flight-mass spectrometry (LC–ESI-Q-TOF MS) analysis, HPLC was performed using an Agilent-1200 liquid chromatographic system (Agilent Technologies, Waldbronn, Germany) equipped with vacuum degasser, a quaternary pump, a column oven and a photodiode array detection. A reversed phase C_{18} HPLC column (Zorbax ODS, 3.5 μm, 2.1 mm × 150 mm, Agilent, Santa Clara, USA), heated at 30 °C, was eluted isocratically by a solvent mixture (1%, v/v, FA in water/acetonitrile 5/95, v/v) at a flow rate of 0.2 mL min^{-1}. The LC system was coupled to a microTOF-Q mass spectrometer (Bruker Daltonics GmbH, Rheinstetten, Germany) equipped with an electrospray ionization source. The optimum conditions for operating the mass spectrometer in positive ion (ESI+) mode and the interface were electrospray voltage 4.5 kV, electrospray pressure 1.6 bar, desolvation gas 8.0 L min^{-1}, desolvation temperature 180 °C, scan range 70–400 *m/z*. The calibrant was 0.1% FA solution (isopropanol:water = 1:1) containing 10 mM NaOH. Fig. 1 shows the product-ion spectra and the fragmentation pathway for Sudan I–IV. The retention time for Sudan I–IV was 3.83, 5.35, 6.29, 9.62 min, respectively.

The Delta 320 pH meter was from Mettler-Toledo Instruments Co. Ltd. (Shanghai, China). The ultrasonic equipment was from Shengyuan Ultrasonic Equipment Co. Ltd. (Shanghai, China).

2.3. DSSBME procedure

The technical set-up of DSSBME is shown in Fig. 2a. The device consisting of a stainless-steel stir bar with four sites for fixing each end of the two hollow fibers, was sequentially cleaned by high purity deionized water and ethanol. The hollow fiber was cut into 3.5-cm length, and a pair of pliers was used to seal one side at 2 mm from the end of the fiber. These segments were immersed in acetone for 30 s to get rid of the organic contamination and dried in the air prior to use. The sealed end of the fiber was set onto one fixed site of the stirrer by a forceps carefully. Eighteen microlitres of 1-octanol was