

Rapid Qualitative Detection of Rhodamine B in Chili Oil by Deep Eutectic Solvent Extraction and a Microplate Reader

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Abstract

Rhodamine B, an alkaline industrial dye, was forbidden in food due to its potential carcinogenicity to humans. However, some unscrupulous merchants often used it as a food colouring agent to dope chili oil. In this research, a microplate reader was utilized for rapid qualitative detection of rhodamine B in chili oil with the deep eutectic solvent which was comprised of choline chloride and ethylene glycol (mole ratio 1:3) as an extraction medium.

Keywords: Rapid qualitative; Rhodamine B; Deep eutectic solvent

Introduction

Rhodamine B ($pK_a=3.7$) [1] which was an artificial synthetic xanthene dye was harmful if swallowed by human beings and animals [2]. As a result of its potential carcinogenic properties, Rhodamine B was forbidden to use in food by the European Food Safety Authority [3]. However, some counterfeiters stained chili oil with rhodamine B to enhance its natural colour in disregard of the law. Although many methods for testing rhodamine B had been studied such as HPLC, LC-MS and ELISA, these methods were time-consuming and couldn't be applied for screening multiple batches of chili oil. Hence, there is a great need for developing a method in rapid qualitative determination of rhodamine B (Figure 1).

Recently, deep eutectic solvents (DESs) as a novel class of green solvents attracted considerable attention in separation technique. We also found that the deep eutectic solvent (DES-1) comprised of choline chloride and ethylene glycol (mole ratio 1:3) could selectively extract rhodamine B from chili oil in our previous report [4]. In consideration of the fluorescence nature of rhodamine B, the idea that we can realize rapid qualitative analysis of rhodamine B by directly observing fluorescence signal from the extract of chili oil appeared. In order to put this idea into practice, a microplate reader was employed to collect the fluorescence data in this research. As we expected, the fluorescence could be detected in chili oil spiked with rhodamine B. Importantly, the process in reading fluorescence signal only took several seconds. As far as we know, this was the first-time deep eutectic solvent extraction assisted with a microplate reader was applied to rapid qualitative analysis of rhodamine B.

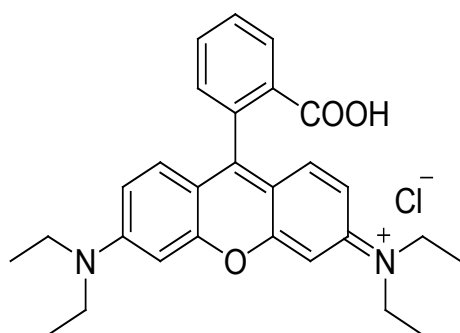


Figure 1: Molecular structure of rhodamine B.

Materials and Methods

Materials

Choline chloride and ethylene glycol were purchased from Aladdin Industrial Corporation and Lingfeng Chemical Co. Ltd., respectively. DES-1 was prepared according to the reported literature [5]. Chili oil was provided by Jikuaifu Food Co. Ltd. from China. Methanol (HPLC grade) and n-hexane (HPLC grade) were obtained from Merck (Darmstadt, Germany). Rhodamine B standard material (purity 95.0%) was supplied by Dr. Ehrenstorfer GmbH (Augsburg, Germany). Rhodamine B was dissolved in methanol to prepare the stock solution (1.00 mg/L).

Instruments

Fluorescence measurement was accomplished by a multi-mode microplate reader (BioTek Synergy HTX, USA). A vortex device (IKA Vortex Genius 3, Germany) was utilized for the extraction procedure and a high-speed centrifuge (SIGMA 2-16P, Germany) was used for phase separation process.

Analytical procedures

DES-1 (1.0 mL) and n-hexane (5 mL) were added to a 15 mL centrifuge tube which was charged with chili oil (1.0 g). The mixture above was vortexed for 1 min and then centrifuged at 9000 rpm for 2 min. The upper phase was removed with a straw and the residue was diluted to a total volume of 5 mL with methanol. The final solution was filtered through a 0.22 μ m membrane for the following test experiment. 200 μ L of chili oil extract was placed into a 96-pore ELISA plate. The excitation wavelength and detection wavelength were set at 530/25 nm and 590/35 nm respectively.

Results and Conclusion

As depicted in Figure 2, fluorescence intensity of spiked samples increased upon addition of rhodamine B. It was notable that the test for

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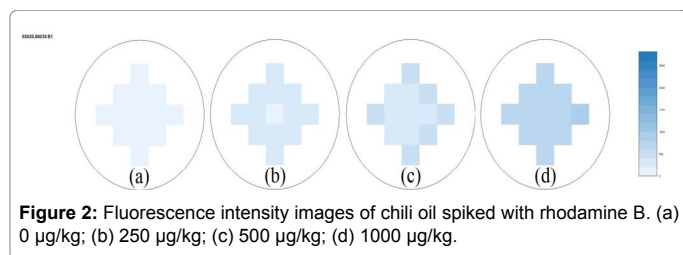


Figure 2: Fluorescence intensity images of chili oil spiked with rhodamine B. (a) 0 $\mu\text{g}/\text{kg}$; (b) 250 $\mu\text{g}/\text{kg}$; (c) 500 $\mu\text{g}/\text{kg}$; (d) 1000 $\mu\text{g}/\text{kg}$.

a sample could be finished in several seconds. The results indicated that the proposed method could meet the requirement for rapid screening multiple batches of chili oil from markets. In summary, this method based on deep eutectic solvent extraction assisted with a microplate reader had potential application in rapid qualitative detection of rhodamine B in chili oil.

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