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# A rapid and simple pretreatment method for benzoylurea insecticides in honey samples using in-syringe dispersive liquid–liquid microextraction based on the direct solidification of ionic liquids

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ABSTRACT

A pretreatment method using in-syringe dispersive liquid–liquid microextraction based on the direct solidification of ionic liquids before high performance liquid chromatography analysis was developed for the determination of benzoylurea insecticides (BUs) in honey samples. The hydrophobic ionic liquid [$N_{4444}$][$PF_6$], formed in situ by the hydrophilic ionic liquid [$N_{4444}$]Cl and the ion exchange reagent $KPF_6$, was used to extract the target analytes. The entire extraction procedure was performed in a syringe. The extractant was solidified at room temperature and collected using a nylon membrane filter. This technique did not require a dispersive solvent, vortex mixer, ultrasound bath, or centrifugation. The parameters affecting the extraction efficiency were investigated through an experimental design. Under the optimal conditions, the limits of detection for the four BUs varied from 0.21 to 0.42 μg $L^{-1}$ in solution (2.1–4.2 μg $kg^{-1}$ in honey). Good linearities were obtained in the range of 2–300 μg $L^{-1}$, with coefficients of determination greater than 0.999. The recoveries of the four BUs ranged from 80.94% to 84.59%. The intra-day ($n$ = 3) and inter-day ($n$ = 3) relative standard deviations were less than 5.08%. Finally, the proposed method was applied to the determination of BUs in commercial honey samples with satisfactory results.



## 1. Introduction

Honey is a highly valuable natural food product due to its characteristic flavor, nutritional value and therapeutic applications [1,2]. The chemical safety of honey is important because it affects consumer health. Therefore, honey should be free from contaminants. However, due to the widespread use of pesticides and their persistence in the environment, they may be introduced into honey. Acaricides, fungicides, antibiotics and other chemical agents that are used to control bee diseases in the hive have the potential to directly contaminate honey [3,4]. There are also indirect sources of contamination. As beehives are frequently pastured on plants or crops treated with pesticides, these chemicals can contaminate honey through pollen, nectar or the bees' bodies [5,6]. Furthermore, the level of pesticides in honey can provide information about the use of pesticides in agricultural practice [7]. Thus, accurate and reliable analytical methods are needed for the determination of pesticide residues in honey.

In recent years, advanced chromatographic techniques, such as gas chromatography (GC) [8–10] and liquid chromatography (LC) [11–14], have been widely used for the analysis of pesticides in honey. Despite the high selectivity and sensitivity of these techniques, sample preparation is essential in the analysis of pesticide residues in honey because of its complex composition [15]. The main objectives of this critical step are to promote the extraction, enrichment, and purification of the analytes. Recently, due to the use of highly toxic organic solvents and multiple sample handling steps [10,16], traditional liquid–liquid extraction (LLE) and solid-phase extraction (SPE) have been replaced by simple, efficient, and miniaturized methods such as solid-phase microextraction (SPME) and liquid-phase microextraction (LPME) [17,18]. LPME is a miniaturized version of LLE that requires minimal amounts of organic solvent. It has the advantages of low cost, simple operation, environmental friendliness, and high enrichment factors [19]. Different modes of LPME have been developed, including single drop microextraction (SDME), hollow fiber-supported LPME

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(HF-LPME) and dispersive liquid–liquid microextraction (DLLME) [20–22]. DLLME has been widely applied to the extraction of different analytes from various matrices [23,24].

Based on a ternary component solvent system, DLLME was first reported by Rezaee and collaborators in 2006 [25] and has been widely used in the determination of pesticides in honey [26,27]. In this method, a mixture of extraction and dispersive solvents is rapidly injected into an aqueous sample, which immediately forms an emulsion. The large contact area between the phases in DLLME establishes extraction equilibrium more quickly than in traditional LLE [28,29]. However, the use of dispersive solvents (e.g., methanol, acetone, and acetonitrile) leads to lower extraction efficiencies due to the increased solubilities of the analytes in the solution and makes the technique less environmentally friendly [30,31]. Another major obstacle in DLLME is the separation and recovery of the extraction solvents. Centrifugation and a micro-syringe, respectively, are commonly used to separate the phases and to collect the extraction solvents [26,29]. To overcome these limitations, ultrasonic-assisted DLLME (UA-DLLME), vortex-assisted DLLME (VA-DLLME), temperature-controlled DLLME (TC-DLLME), and DLLME based on a solidified floating organic drop (DLLME-SFOD) have been developed [32–35]. In UA-DLLME, VA-DLLME, and TC-DLLME, the extraction solvents are dispersed by ultrasound radiation, vortex energy, and high temperature, respectively, without using dispersive solvents. However, these methods usually require longer extraction times than traditional DLLME [29]. In DLLME-SFOD, an extractant with a lower density than water, a low toxicity and an appropriate melting point (10–30 °C), such as 1-undecanol, 1-dodecanol, or 2-dodecanol, is used. The extractant is solidified in an ice bath and floats on the top of the solution, facilitating easy collection. However, the number of useful organic solvents for this technique is greatly restricted by the melting point requirement, and this method does not avoid the need for centrifugation [36]. Therefore, DLLME still requires a number of modifications.

Recently, ionic liquids (ILs), which are eco-friendly solvents, have received great interest in analytical fields because of their unique physicochemical properties, such as a low vapor pressure, good extractability for various organic compounds and metal ions, and miscibility with water and organic solvents [37,38]. In situ solvent formation for microextraction based on ILs (in situ IL-DLLME) is a simple, fast, and efficient DLLME technique that eliminates the need for a dispersive solvent. In this method, the hydrophilic IL is transformed into a hydrophobic IL through reaction with an anion-exchange reagent, and the extraction process is completed simultaneously. Thus, this method has the advantages of simple operation and high extraction efficiency [39,40]. In the present study, $[N_{4444}]Cl$ (tetrabutylammonium chloride) and $KPF_6$ were selected as the starting extraction solvent and anion-exchange reagent, respectively.

Benzoylurea insecticides (BUs) are a class of powerful insect growth regulators that inhibit or block the synthesis of chitin, a vital part of the insect exoskeleton [33]. Their high selectivity with respect to non-target insects, rapid degradation in soil and water, and low acute toxicity to animals make them suitable for integrated pest control programs [41]. However, due to their widespread use, BU residues in foods and the environment can have negative effects on human health through chronic exposure and long-term toxicity [42,43]. According to the European Union (EU) Directive, the BU content in honey must be less than 0.05 mg $kg^{-1}$ [16]. Thus, it is important to develop a simple, fast and sensitive analytical technique for the determination of BUs in honey.

In this study, a rapid in-syringe dispersive liquid–liquid microextraction based on the direct solidification of ionic liquids (in-syringe DLLME-DSIL) technique combined with high performance liquid chromatography analysis was developed for the determination of BUs in honey samples. The entire extraction process was simple and fast because the formation of the hydrophobic IL $[N_{4444}][PF_6]$ and the extraction of the analytes occurred simultaneously. The high melting point (244–246 °C) extractant $[N_{4444}][PF_6]$ could be solidified and separated from the aqueous sample at room temperature, eliminating the need for an ice bath and centrifugation. Additionally, polar solvents as dispersive agents and specific instruments, such as a vortex mixer, magnetic stirrer or ultrasonic bath, were not required. The current method was considerably simplified. Thus, it has great potential for the rapid detection of BUs. The parameters that affect the extraction efficiencies of the target analytes were screened and optimized using the Plackett-Burman (P-B) design and the central composite face-centered design (CCF). Finally, the developed method was used to extract and detect BUs in honey samples.

## 2. Experimental

### 2.1. Chemicals and reagents

The pesticide standards (triflumuron, hexaflumuron, lufenuron and chlorfluazuron) were purchased from Aladdin Reagent Corporation (Shanghai, China). Tetrabutylammonium chloride ($[N_{4444}]Cl$) was supplied by the Center for Green Chemistry and Catalysis, Lanzhou Institute of Chemical Physics, Chinese Academy of Sciences. Potassium hexafluorophosphate ($KPF_6$, analytical grade) was acquired from Aladdin Reagent Corporation (Shanghai, China). Sodium chloride (NaCl, analytical grade) was purchased from Beijing Chemical Reagent Factory (Beijing, China). Acetonitrile (HPLC grade) and methanol (HPLC grade) were obtained from Dikma Ltd. (Beijing, China). Pure water was prepared using a Milli-Q water purification system (Millipore, Bedford, MA, USA).

Individual stock solutions of BUs (1 g $L^{-1}$) and a mixed stock solution (100 mg $L^{-1}$ of each analyte) were prepared in acetonitrile and stored in a refrigerator. Working solutions were prepared weekly by diluting the stock solutions with acetonitrile.

### 2.2. Instruments

Chromatography analysis was performed on an Agilent 1260 HPLC system equipped with an ultraviolet detector (UVD) and an autosampler. Sample separations were conducted on a Dikma Spursil C18 column (250 × 4.6 mm i.d., 5 μm) with a Spursil C18 guard column (10 × 2.1 mm i.d., 5 μm). A mixture of methanol (A) and water (B) was used as the mobile phase at a flow rate of 0.8 mL $min^{-1}$. The gradient conditions were 83% A and 17% B for 2 min, then increased from 83% A to 93% A over 21 min, decreased from 93% A to 83% A over 2 min and finally maintained at 83% A for 2 min. The column temperature was maintained at 25 °C, the injection volume was 10 μL, and the detection wavelength was 265 nm. The sample pH was measured using a pHS-3C pH meter (Shanghai, China). Ten-milliliter medical plastic syringes were purchased from Nanquan Polymer Products Co., Ltd. (Jiangyin, China).

### 2.3. Preparation of honey samples

Four honey samples of different floral origin were purchased from the local supermarket. To reduce the viscosity and facilitate handling, 10 g of homogenized honey was diluted to 100 mL with ultrapure water, and the solution pH was adjusted to 7 with 0.1 M NaOH. The samples were then spiked with the BU standards. A blank honey sample collected from an apiary with no history of BU contamination was prepared in the same manner and was used for optimization and validation.