Original Research Article

Ion-pair dispersive liquid–liquid microextraction solidification of floating organic droplets method for the rapid and sensitive detection of phenolic acids in wine samples using liquid chromatography combined with a core–shell particle column

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ABSTRACT

This study describes a novel sample preparation method for extraction of phenolic acids from wine using ion pair dispersive liquid–liquid microextraction based on the solidification of a floating organic droplet (IP-DLLME-SFO). The ion-pairing technique combined with DLLME-SFO dramatically enhanced the extraction efficiency for very polar phenolic acids, such as gallic acid and protocatechuic acid, which could not be extracted by DLLME-SFO in the absence of an ion-pairing reagent. The effects of the parameters that can affect the extraction efficiency were systematically investigated, including the type and concentration of ion-pairing reagent, type and volume of extraction and dispersive solvents, extraction time, sample pH, and ion strength. The method linearity was constructed in the range of 0.01–15 μg/mL, and the sensitivity expressed as limit of detection was as low as 10 ng/mL. The method that we developed was applied for the analysis of commercial wine samples, revealing different levels of phenolic acids among these products.

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1. Introduction

A new liquid phase microextraction technique, dispersive liquid–liquid microextraction (DLLME), has been extensively explored in recent years because of its simplicity, rapidity, convenience, and low cost (Rezaee et al., 2010). However, the DLLME technique is limited in its application to the extraction of polar compounds or organic acids because their polar ionizable groups restrict transfer to water-immiscible solvents. To solve this problem, some successful methods for extraction of polar compounds have been developed. For example, in liquid-liquid extraction (LLE), extraction of ionizable compounds into an organic phase was made possible using an ion-pairing agent, resulting in improved recovery and selective isolation from complex matrices (Fernandes and Ferreira, 2000; Xu et al., 2009). Similarly, the ion-pairing technique was applied to LPME, as exemplified by ion pair-based surfactant-assisted microextraction (IP-SAME) of fluoroquinolones (Ebrahimpour et al., 2012) and ion

pair-based surfactant assisted DLLME (IP-SA-DLLME) of heavy metals (Yousefi and Shemirani, 2013). In view of those studies, we employed a modified version of DLLME based on the solidification of floating organic droplets (DLLME-SFO) in the current study and first proposed to apply IP-DLLME-SFO coupled to LC using a core–shell particle column for the analysis of polar compounds in a liquid sample.

Among numerous polar compounds, phenolic acids are one of the most important classes of organic acids because they are common constituent in honey, fruits, vegetables and plants. Additionally, phenolic compounds are usually responsible for wine colour and contribute to the bitter flavour of wine (Porgalı and Büyüktuncel, 2012; Vaquero et al., 2007). Their antioxidant properties are beneficial for overall human health, due to their scavenging of reactive free radicals that are associated with the pathophysiology of various diseases, such as inflammatory and degenerative diseases (Liu et al., 2012). The potential of phenolic compounds as preservatives in winemaking has been confirmed (Garcia-Ruiz et al., 2012). Phenolic acids are a predominant subclass of phenolic compounds comprising almost a third of phenolics in plants (Robbins, 2003). They are naturally present in red and white wine as free acids, glycosylated derivatives, or esters of tartaric, quinic, and shikimic acids (Mudnic et al., 2010).

Different numbers and positions of hydroxyl groups on the aromatic ring of phenolic acids produce a variety of similar chemical structures, contributing to the complexity of phenolic acid analysis (Stalikas, 2007). Among numerous methods developed for the analysis of phenolic acids in wine samples (Dong et al., 2013; Irakli et al., 2012; Porgalı and Büyüktuncel, 2012), liquid chromatography (LC) has predominantly been used, due to its high accuracy and sensitivity. Before chromatographic analysis, pre-treatment steps such as extraction and concentration are usually required because wine matrices are very complex and the levels of phenolic acids in wines are low. The most frequently used sample preparation techniques are liquid–liquid extraction (LLE) (del Álamo et al., 2004; Garciafalcon et al., 2007; Malovana et al., 2001; Porgalı and Büyüktuncel, 2012) and solid-phase extraction (SPE) (Alarcon Flores et al., 2012; Irakli et al., 2012; Michalkiewicz et al., 2008). However, LLE usually requires a large volume of organic solvents and is very time-consuming. Although SPE consumes much less solvent and time than LLE, it still involves the consumption of expensive SPE cartridges. Alternatively, a number of microextraction methods such as liquid-phase microextraction (LPME) and solid-phase microextraction (SPME) have been developed for more sensitive and environmentally-friendly analysis of phenolic acids (Becerra-Herrera et al., 2014; Irakli et al., 2012; Michalkiewicz et al., 2008).

The goal of this study was to develop a new, eco-friendly analytical method to rapidly and efficiently determine the levels of various phenolic acids in wine. We employed DLLME based on the solidification of floating organic droplets (DLLME-SFO) in the current study because it can reduce matrix effects and facilitate the selective collection of the organic phase free from matrix interferences, as we found in our previous studies (Jia et al., 2013a, 2013c). In the method that we developed, 10 phenolic acids were effectively extracted and concentrated by IP-DLLME-SFO, followed by a rapid LC analysis using a column packed with sub-3 μm core–shell particles. To the best of our knowledge, this study is the first application of IP-DLLME-SFO coupled to LC using a core–shell particle column for the analysis of phenolic acids in wine.

2. Materials and methods

2.1. Chemicals and reagents

Phenolic acids (gallic acid, *p*-hydroxybenzoic acid, vanillic acid, caffeic acid, *p*-coumaric acid, ferulic acid, sinapic acid, cinnamic

acid) were purchased from Sigma–Aldrich (St. Louis, MO). Tetraethylammonium bromide (TEAB), tetrapropylammonium bromide (TPAB), tetrabutylammonium bromide (TBAB), tetraheptylammonium bromide (THPAB), tetrahexylammonium bromide (THAB), protocatechuic acid, *trans-m*-coumaric acid, 1-undecanol, and 2-dodecanol were obtained from TCI (Tokyo, Japan). All other regents were purchased from Sigma–Aldrich unless otherwise noted. LC-grade acetone, water, methanol, and acetonitrile were obtained from Duksan Chemical Co. Ltd. (Ansan, Korea).

2.2. Instrumentation

Chromatographic analysis was performed using a Flexar FX-10 UHPLC system (PerkinElmer, Shelton, CT) with a Flexar FX PDA (PerkinElmer, Shelton, CT). The detection wavelength was 280 nm. An Agilent Poroshell EC-C18 column (2.1 × 150 mm) packed with 2.7 μm core–shell particles was used for chromatographic separation of the 10 phenolic acids. A gradient elution was carried out using a binary mobile phase composed of eluent **A** (water with 0.1% formic acid) and eluent **B** (acetonitrile with 0.1% formic acid). The linear gradient program was as follows: 0–1 min, 10% **B**; 1–25 min, 10–15% **B**; 25–35 min, 15–50% **B**; 35–36 min, 50–100% **B**; 36–46 min, 100% **B**. After each run, the gradient was held at 100% **B** for 12 min for column washing and then returned to 10% **B** for 10 min for column equilibration. The column temperature was kept at 30 °C. The flow rate was 0.2 mL/min and the injection volume was 3 μL.

2.3. Preparation of standard solutions and wine sample

Stock solutions of phenolic acids were prepared in methanol and working solutions were freshly made by appropriate dilution of the stock solutions in methanol. All the wine samples were purchased from local markets in Korea. The wine samples were filtered through a 0.2 μm hydrophobic filter (Toyo Roshi Kaisha, Ltd., Tokyo, Japan) and stored at 4 °C until use. Optimization studies for extraction conditions were performed using pure water spiked with standards. Spiked wine samples used for method application and method validation were prepared as follows: a fresh standard solution was evaporated in a glass test tube under a stream of high-purity nitrogen, and blank wine was added to the tube to produce a wine sample at the desired concentration of phenolic acids. To reduce sample matrix effects, red and white wine samples were diluted with water by ten-fold and five-fold, respectively, before processing using the microextraction procedure described below.

2.4. Optimized procedure for IP-DLLME-SFO

A total of 1.5 mL diluted wine (1.0 mL of wine + 0.5 mL of water) or water was mixed with 1.0 mL of 50 mM sodium phosphate buffer (pH = 6.0) and 2.5 mL of 10 mM THAB to produce a final 5.0 mL solution containing 5 mM THAB. Five hundred microlitres of a mixture of 1-dodecanol (extraction solvent) and methanol (dispersive solvent) mixed at 1:9 (v/v) were rapidly injected into the solution using a 1.0-mL gastight Hamilton syringe, forming a stable, cloudy solution. Fine droplets of the organic phase containing the ion pairs of phenolic acids and THAB accumulated at the surface of the sample solution after centrifugation at 3500 *g* for 5 min. The glass tube was placed on ice for 10 min, and then the solidified droplet was quickly transferred to a 200-μL Eppendorf tube. After thawing, the droplet was centrifuged (10,000 *g*, 3 min), and 30 μL of the upper layer were diluted to 90 μL with methanol. Three microlitres of the diluted solution were directly injected onto the LC.