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Determination of chloramphenicol, thiamphenicol and florfenicol in milk and honey using modified QuEChERS extraction coupled with polymeric monolith-based capillary liquid chromatography tandem mass spectrometry

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ABSTRACT

A poly(lauryl methacrylate-*co*-methacrylic acid-*co*-ethylene glycol dimethacrylate) [LMA-MAA-EDMA] monolithic column was used to simultaneously determine amphenicol antibiotics (chloramphenicol/CAP, thiamphenicol/TAP, and florfenicol/FF) in milk and honey samples by capillary liquid chromatography tandem mass spectrometry (LC-MS/MS). QuEChERS (quick, easy, cheap, effective, rugged, and safe) method was optimized for sample pretreatment. Good linearity ($0.1-15$ ng g^{-1}) and extraction recoveries ($95.8-100.2\%$ and $95.6-99.3\%$ for milk and honey samples, respectively; $n=3$) with minor matrix effect ($\leq$5% ion suppression) were obtained. Limits of detection were estimated at $0.02-0.045$ ng g^{-1}. Good intra-day/inter-day precision ($0.2-9.1\%/0.3-8.7\%$) and accuracy ($90.5-110.0\%/93.4-109.3\%$) were achieved. With more than 200 analyses of real samples, no noticeable carry-over and deterioration of separation efficiency were observed using the monolithic column. The applicability of the developed QuEChERS-capillary LC-MS/MS method was demonstrated by determining the occurrence of CAP, TAP, and FF in various milk and honey samples.

1. Introduction

Amphenicol antibiotics including chloramphenicol (CAP), thiamphenicol (TAP), and florfenicol (FF) were used to treat various infections in animals. Since the association of CAP with several health side-effects such as bone marrow suppression and carcinogenic properties was reported in human [1,2], the use of CAP has been banned in many countries including Canada, USA, and European Union. Due to the health concerns, European Union has set a MRPL (minimum required performance level) at 0.3 ng g^{-1} for CAP, and MRLs (maximum residue levels) at 50 ng g^{-1} for TAP as well as $100-2500$ ng g^{-1} (depending on the food matrix) for the sum of FF and its major metabolite in foodstuffs of animal origin [3]. Therefore, developing a reliable and sensitive analytical method became an important issue to accurately determine trace antibiotics in food samples.

In addition to immunoassay [4] for rapid screening of the occurrence of amphenicol antibiotics in food samples, liquid chromatography (LC) techniques [5–7] have been extensively utilized to quantitatively determine CAP, TAP, and/or FF in various food matrices by coupling with a variety of detection methods including UV [8], fluorescence detection (FLD) [9], and mass spectrometry (MS) [3,10–14]. For example, Alechaga et al. [3] applied solvent extraction followed by SPE (HLB cartridge with hydrophilic–lipophilic balanced reversed-phase sorbent for honey samples; MCX cartridge with mixed-mode anion exchange sorbent for chicken, pork, fish, and prawn samples) to isolate amphenicol antibiotics from complex sample matrix. The extracted analytes were further separated and analyzed by ultra-high-performance liquid chromatography (UHPLC)-tandem mass spectrometry (MS/MS) for simultaneous determination. The authors reported the instrument LOD values of target antibiotics (CAP, TAP, and FF) ranging from 0.02 to 0.07 ng g^{-1} for quantitative analyses in various food samples.

In order to reduce the matrix effect for trace analysis, sample pretreatment was usually involved in quantitative determination of target analytes in complex samples. Liquid–liquid extraction (LLE) [15,16], solid-phase extraction (SPE) [17,18], matrix solid-phase dispersion (MSPD) [19], and molecular imprinted polymer (MIP) extraction [10,20] have been utilized for sample

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pretreatment [21] prior to quantitative determination of CAP, TAP, and/or FF in various food matrices. In early 2000s, a new extraction method [22], QuEChERS (quick, easy, cheap, effective, rugged, and safe), was first developed to determine pesticide residues in fruits and vegetables. QuEChERS method includes solvent extraction combining with salting out and dispersive SPE (dSPE) to remove water and unwanted matrix components such as proteins from samples. Since 2003, QuEChERS has been widely utilized as a simple and effective sample pretreatment process in food safety and environmental applications [23–28]. For example, Abdallah et al. [26] employed QuEChERS extraction for analyzing 22 sulfonamides and their metabolites in various edible animal tissues by HPLC-high resolution MS. Bruzzoniti et al. [28] recently published a critical review discussing the use of QuEChERS sample preparation method for determining pesticides and other organic residues in various environmental matrices such as soil and water samples. QuEChERS was also applied to determine residual amphenicol antibiotics in various food samples [29]. However, poor extraction recoveries were obtained when employed to milk and honey samples.

For trace analysis, reduced dimensions on separation channels such as chip-based nano LC have been proved to offer enhanced sensitivity of target analytes in food samples [30]. Liu et al. utilized chip-based nano LC-MS/MS, which offered LOD values as low as $0.004-0.008\ ng\ g^{-1}$, for quantitative determination of five aflatoxins in various peanut products. In this study, capillary LC-MS/MS was used for sensitive determination of amphenicol antibiotics (CAP, TAP, and FF) in milk and honey samples using a polymer-based monolithic column. The polymer-based monoliths have served as stationary phases in different types of chromatographic separation modes including reversed-phase capillary LC to successfully separate large biomolecules such as proteins [31,32] and/or small molecules [33,34]. For example, the poly(lauryl methacrylate-*co*-methacrylic acid-*co*-ethylene glycol dimethacrylate) [LMA-MAA-EDMA] monolithic column was recently developed by Lin et al. [34] to successfully separate three amphenicol antibiotics for potential food safety applications. As reported by the authors, good stability and repeatability of the monolithic column indicated the potential of utilizing the LMA-MAA-EDMA column for routine capillary LC analysis. However, these home-made monolithic columns haven't been used for routine capillary LC analysis in real samples.

In this study, we developed a modified QuEChERS method to effectively reduce matrix effect in milk and honey samples prior to capillary LC-MS/MS and explored the utilization of polymeric monolith-based LMA-MAA-EDMA column for routine food safety analysis. Different dSPE sorbents were evaluated and optimized for diminishing interfering components and enhancing extraction recovery. The experimental conditions for MS detection, capillary LC analysis, and QuEChERS extraction were optimized prior to quantitative determination of amphenicol antibiotics. Method validation was performed by investigating linearity, LOD, as well as method precision and accuracy under the optimal conditions using the proposed QuEChERS-capillary LC-MS/MS method. The determination of amphenicol antibiotics (CAP, TAP, and FF) in various milk samples and honey samples was also performed to demonstrate the capability of the developed QuEChERS-capillary LC-MS/MS method for routine analysis in food safety applications.

2. Experimental

2.1. Materials and reagents

Sodium hydroxide (NaOH), hydrochloric acid (HCl), TPM (3-[trimethoxysilyl]propyl methacrylate), AIBN (2,2-Azobis[2-methylproprionitrile]), lauryl methacrylate (LMA), ethylene dimethacrylate (EDMA), methacrylic acid (MAA), 1,4-butanediol, thiamphenicol (TAP), and florfenicol (FF) were obtained from Sigma-Aldrich (Steinhelm, Germany). Acetic acid, chloramphenicol (CAP), and the internal standard (ISTD): CAP-D_5, were purchased from Fluka (Steinheim, Germany). Methanol (MeOH), acetonitrile (ACN), and 1-propanol were purchased from Mallinckrodt Baker, Inc. (Phillipsburg, NJ, USA). Solvents and reagents used in this study were all HPLC or analytical grade. Deionized water was obtained from a Milli-Q Integral 5 water purification system (Millipore, Bedford, MA, USA). All stock solutions ($1000\ \mu g\ mL^{-1}$) were prepared individually in ACN and then stored at $-20\ ^{\circ}C$. A mixture of FF, TAP, and CAP ($1000\ ng\ mL^{-1}$ each) was also prepared in ACN as the working solution. Each calibration standard containing FF, TAP, and CAP was prepared by spiking the working solution into milk or honey extract at a desired concentration including $0.30\ ng\ g^{-1}$ of ISTD. Anhydrous sodium sulfate (Na_2SO_4), sodium chloride (NaCl), and C18 powders in the extraction kit as well as the clean-up kit were from Getech (Kaohsiung, Taiwan). Agilent C18 endcapped sorbents (Lake Forest, CA, USA) and Supelco QuE Z-Sep$^+$ powders (Bellefonte, PA, USA) were also applied to remove unwanted matrix components from milk and honey samples. Fused silica capillaries (250-μm i.d., 360-μm o.d.) were purchased from Polymicro Technologies (Phoenix, AZ, USA). Various brands of full-fat milk and honey samples (including longan honey, litchi honey, and herbs honey) were purchased from local markets in Taipei city, Taiwan.

2.2. Preparation of LMA-MAA-EDMA monolithic column for capillary LC

As described in the previous study [34], a fused silica capillary was treated prior to use and a polymerization solution containing monomers (LMA: 7.5%, w/w; MAA: 4.5%, w/w), cross-linker (EDMA: 18%, w/w), porogenic solvents (1-propanol and 1,4-butanediol: 35% each, w/w), and initiator (1% AIBN, w/w; relative to the sum of LMA, MAA, and EDMA) was applied to prepare 30% LMA-MAA-EDMA monolithic column. The monolithic polymerization was then carried out at 60 °C for 2 h in a GC oven. After polymerization, the monolithic capillary column was rinsed sequentially by ACN (250 μL) and 50% ACN-water solution (250 μL) to remove remaining chemicals and porogenic solvents.

2.3. Instrumentation and conditions

An Agilent 6410 series Triple Quad LC/MS mass spectrometer (Agilent, Waldbronn, Germany) was utilized for MS detection under the negative mode with electrospray ionization source at a spray voltage of -3800 V. A heated N_2 gas ($5\ L\ min^{-1}$ at 325 °C) was introduced for solvent evaporation in the ionization chamber. Agilent Mass Hunter Workstation (version B.03.01) was used for system control, data acquisition, and data processing. Multiple reaction monitoring (MRM) was performed for quantitative determination. A funnel shaker with centrifuge tube holder (Shin-Kwang precision industry, Taipei, Taiwan) and Hitachi CF15RXII high-speed micro-centrifuge equipped with a T15A42 rotor (Tokyo, Japan) were also used in the study.

An Agilent 1100 Series capillary LC system (Agilent, Waldbronn, Germany), including an online degasser, a binary capillary-pump, and a micro well-plate auto-sampler, was applied to perform chromatographic separation using the LMA-MAA-EDMA monolithic column (15-cm × 250-μm i.d.). Solvent A (Milli-Q water) and solvent B (MeOH:ACN/10:90) were delivered by the capillary pump for chromatographic separation. For gradient separation of analytes, the initial mobile phase (2% B) was increased to 40% B in 3 min and then gradually increased to 50% B in another 3 min