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Method validation and measurement uncertainty for the simultaneous determination of synthetic phenolic antioxidants in edible oils commonly consumed in Korea

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ABSTRACT

This study investigated a method for the validation and determination of measurement uncertainty for the simultaneous determination of synthetic phenolic antioxidants (SPAs) such as propyl gallate (PG), octyl gallate (OG), dodecyl gallate (DG), 2,4,5-trihydroxy butyrophenone (THBP), *tert*-butylhydroquinone (TBHQ), butylated hydroxyanisole (BHA), and butylated hydroxytoluene (BHT) in edible oils commonly consumed in Korea. The validated method was able to extract SPA residues under the optimized HPLC-UV and LC–MS/MS conditions. Furthermore, the measurement of uncertainty was evaluated based on the precision study. For HPLC-UV analysis, the recoveries of SPAs ranged from 91.4% to 115.9% with relative standard deviations between 0.3% and 11.4%. In addition, the expanded uncertainties of the SPAs ranged from 0.15 to 5.91. These results indicate that the validated method is appropriate for the extraction and determination of SPAs and can be used to verify the safety of edible oil products containing SPAs residues.

1. Introduction

Synthetic phenolic antioxidants (SPAs) such as propyl gallate (PG), octyl gallate (OG), dodecyl gallate (DG), 2,4,5-trihydroxybutyrophenone (THBP), *tert*-butylhydroquinone (TBHQ), butylated hydroxyanisole (BHA), and butylated hydroxyltoluene (BHT) have been used as food additives to retard or prevent lipid oxidation. Because SPAs are low-cost, widely available, and high-performance compared to natural antioxidants, food manufacturers prefer to use SPAs. Although they are effective at prolonging the shelf life and preventing the oxidative rancidity of food, the addition of excess antioxidants to food might produce toxicities or mutagenicities (Xiu-Qin, Chao, Yan-Yan, Min-Li, & Xiao-Gang, 2009). In the USA, EU, and other countries, these antioxidants are authorized as food additives and can be added alone or in combination up to a final concentration of 0.01% or 0.02% (European Commission, 2011, FDA., 2015; USDA Foreign Agricultural Service, 2011). However, in Korea, PG, TBHQ, BHA, and BHT are authorized as food additives, whereas OG, DG, and THBP are not. Consequently, a validated method for the simultaneous analysis of unauthorized SPAs in edible oils commonly consumed in Korea is necessary.

Various analytical methods have been reported for the determination of SPAs in edible oils, including high-performance liquid chromatography (HPLC) with UV, photodiode array detector (PDA) (Andrikopoulos, Brueschweiler, Felber, & Taeschler, 1991; Wang et al., 2012), liquid chromatography time-of-flight mass spectrometry (LC-TOF/MS) (Xiu-Qin et al., 2009) gas chromatography (GC) (González, Gallego, & Valcárcel, 1999; Yang, Lin, & Choong, 2002), gas chromatograph-mass spectrometry (GC–MS) (Guo, Xie, Yan, Wan, & Wu, 2006), capillary electrophoresis (CE) (Jaworska, Szulińska, & Wilk, 2005), and thin-layer chromatography (TLC) (Ragazzi & Veronese, 1973). The Association of Official Analytical Chemists (AOAC) and American Oil Chemists' Society (AOCS) official methods are based on the HPLC-UV determination of SPAs (AOAC official method 983.15, 1995; AOCS official method Ce 6-86, 1997). However, the limit of detection (LOD), limit of quantification (LOQ), and measurement uncertainty of these methods have not been provided.

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In analytical chemistry, method validation is conducted to ensure reliable results. The validation approach relies on the evaluation of specificity, linearity, LOD, LOQ, accuracy and precision (International Conference on Harmonization (ICH)., 2005). However, these validation factors do not account for the measurement uncertainty associated with factors like the reference material, balances, volumetric measuring devices, and calibration curves. Measurement uncertainty represents the state of dispersion for a reasonable estimate of the quantitative indicators of the reliability of the measurement results. The precision of analytical validation only indirectly represents the reliability of the analysis results; it cannot quantitatively determine the measurement uncertainty of the exact concentration of the SPAs in the analysis result.

Therefore, the aim of this study was to develop and validate an HPLC-UV method for the simultaneous determination of SPAs in edible oils commonly consumed in Korea. We further investigated an LC–MS/MS analytical method to simultaneously confirm the identities of the SPAs. The measurement uncertainty was also evaluated for validation. Additionally, to demonstrate the effective application of the established method on real samples, various edible oils and fats were collected from grocery markets in Korea and other countries and monitored for their SPA contents.

2. Materials and methods

2.1. Reagents

Pure standards such as PG, OG, DG, THBP, TBHQ, BHA, BHT were obtained from Sigma-Aldrich (St Louis, MO, USA). All solvents were suitable for HPLC analysis and were purchased from J.T. Baker (Phillipsburg, NJ, USA).

2.2. Food materials

One hundred and eight food samples were analyzed, including 98-vegetable oil (olive, soybean, corn, grape seed, sunflower, canola, blended oil, etc.) and 10-bread spread (nine-types of butters and one-type of margarine) samples. Commonly consumed edible oils and their products were purchased from grocery markets in Korea. Several edible oils (12 samples; six types of olives, two types of canola, and one of each of soybean, sunflower, coconut and seasame) were purchased as control samples from grocery markets in the USA. Each shelf life of the samples was enough to be investigated. To validate the procedure, vegetable oil that was found to be free of synthetic antioxidants was selected. All samples with the exceptions of cooking oils were kept refrigerated before use.

2.3. Optimization of HPLC instrument conditions

SPAs were tested using HPLC. Analytical conditions were chosen for SPA analysis as this achieved the best results. Firstly, we compared different official methods including AOAC, AOCS, and Korean Food Standards Codex for standardization. All of the official methods were conducted with C_{18} column and detected with UV (280 nm). But there are differences in mobile phases and eluent conditions (AOAC official method, 1995; AOCS official method, 1997; Korean Food Standards Codex., 2015). Then, we evaluated HPLC analytical parameters such as columns and oven temperature until the optimum conditions for the separation of SPAs from edible oils were obtained. The sensitivity of the analytical method was based on the maximum admissible levels of SPAs in foreign countries (EU, USA, and China). The basis of evaluation was conducted with LOD and LOQ. The LOD and LOQ were calculated for the analysis according to the following equations: LOD = 3.3 σ/S and LOQ = 10 σ/S, where σ is the mean standard deviation and S is the slope of the same equation.

The used HPLC apparatus was an iLC3300 HPLC system (Labogene, Eresing, Germany) equipped with a binary pump, an autosampler, a column heater, and a UV detector. Chromatographic separation was performed by a Shiseido Capcell Pak C_{18} UG120 (Shiseido, 4.6 × 250 mm, 5.0 μm, Tokyo, Japan) column maintained at 30 °C. The mobile phase was composed of A (5% acetic acid in acetonitrile) and B (5% acetic acid in water) with gradient elution as follows: initially (<2 min) 40% A followed by 50% A (2–7 min); 50% A followed by 70% A (10–15 min); 70% A followed by 82% A (20–23 min); 82% A followed by 40% A (28–31 min); and hold for 4 min. The solution was then filtered through a 0.45 μm membrane filter (Whatman, Amersham, UK) and degassed under vacuum. The sample injection volume was 20 μL, and the flow-rate was set at 1.0 mL/min. Peaks were detected at 280 nm and applied for the method validation and analysis. Data acquisition and the remote control of the HPLC system were performed using DataApex Clarity™ software (DataApex, Praha, Czech Republic).

2.4. Optimizations of extract method and sample preparation

Using the optimum HPLC-UV analysis conditions, the preparation methods for SPAs in vegetable oil were optimized. The recovery was used to evaluate each sample preparation method. The recovery test was evaluated by preparing a sample solution spiked with PG at 50 ppm; OG and DG at 100 ppm; THBP at 25 ppm; TBHQ, BHA and BHT at 200 ppm, respectively. An edible oil matrix was prepared using the previously reported method (AOAC official method, 1995; AOCS official method, 1997; Korean Food Standards Codex, 2015; Saad et al., 2007) and optimized by the followed sample preparation method for SPAs analysis.

The samples were prepared according to the method of the Korean Food Standards Codex (2015). The edible oil (liquefied in a 60 °C water bath) samples were accurately weighed to 5 g into a beaker, quantitatively transferred to a separatory funnel rinsed with 20 mL *n*-hexane, and extracted with 50 mL portions of acetonitrile saturated with hexane. The acetonitrile phase was collected, and the extraction procedure was repeated twice. Subsequently, the extracted phases were evaporated to 3–4 mL using a rotary evaporator (EYELA, N-1200A, Tokyo, Japan) with a ⩽40 °C water bath (EYELA, SB-1200, Tokyo, Japan) within 10 min. The flask was rinsed with small portions of non-saturated acetonitrile and transferred to a 10 mL volumetric flask. The flask was again rinsed with small portions of 2-propanol, and all rinsings were transferred to the volumetric flask until exactly 10 mL was collected. All samples were filtered through a 0.45 μm syringe filter (Millex-HV, Millipore, Bedford, MA, USA).

2.5. Method validation

2.5.1. LC–MS/MS

LC–MS/MS was performed using a Finnigan Surveyor HPLC system and TSQ Quantum mass spectrometer (Thermo Finnigan, San Jose, CA, USA) with electrospray ionization (ESI) capabilities. Liquid chromatography separation was performed on an Accucore RP-MS Column (Thermo Scientific, 2.1 × 100 mm, 2.6 μm, Waltham, MA, USA) maintained at 30 °C using a gradient program consisting of mobile phase A (10 mM ammonium acetate in 10% acetonitrile) and mobile phase B (10 mM ammonium acetate in 90% acetonitrile) at a flow rate of 0.25 mL/min. The initial conditions (<1 min) were 10% A followed by 95% A (1–7 min), 10% A (8–9 min), and finally holding for 3 min. The total run time was 12 min. The injection volume was 10 μL. The MS/MS data for SPAs