Short Communication

Quantification of propionic acid from Scutellaria baicalensis roots

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ABSTRACT

Background: Propionic acid is a widely used preservative and has been mainly formed by artificial synthesis or fermentation. In the case of natural products, the presence of propionic acid is viewed as a sign that an additive has been introduced for antimicrobial effects.
Methods: In this work, the propionic acid that occurs in Scutellaria baicalensis roots was studied. A quantification method was developed, validated, and showed good linearity, low limit of detection, and limit of quantification values, as well as fine precision and recovery rate. The developed method was applied to the analysis of S. baicalensis roots collected in South Korea and China.
Results: The detection rate for all samples was 94.0%. The average concentration was 0.41 ± 0.24 mg/g from the China sample and 0.76 ± 0.28 mg/g from the Korea sample.
Conclusion: This study is the first to report that propionic acid exists in S. baicalensis roots and also provides a useful ultra performance liquid chromatography analysis method for its quantification.

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1. Introduction

Scutellariae Radix is the dried root of Scutellaria baicalensis, which is widespread in East Asia, North America, and Europe, and it is widely used as a botanical medicine and dietary supplement. In East Asia, Scutellariae radix has been used in traditional oriental medicine for thousands of years and has been listed in the Pharmacopoeia of Korea, China, and Japan as a treatment for allergic and inflammatory diseases. Modern research has elucidated the effects of Scutellariae radix and its chemical components. To date, more than 295 compounds have been identified from 35 Scutellaria species since the first isolation of scutellarein in Vietnam. The major identified phytochemicals were phenolics and terpenoids, and small amounts of alkaloids, phytosterols, and polysaccharides were also found. Regarding the organic acids obtained from S. baicalensis, tartaric, citric, malic, malonic, succinic, and fumic acids were reported, but so far propionic acid has not yet been reported.

Short-chain volatile organic acids with carbon numbers ranging from two to 12 significantly affect the flavor and quality of foods. In particular, propionic acid was reported to reduce Salmonella typhimurium in food and feed. In 1938, propionic acid was suggested for use as a bread dough preservative, and currently, the sodium propionate and calcium propionate salt forms of propionic acid are widely used for bakery and cheese products.

Propionic acid is generated from dairy products as a result of the metabolism and breakdown of milk, protein, fat, lactose, and citrate, and is listed as “Generally Recognized as Safe.” However, some countries including Korea, China, and Japan, have strictly restricted herbal materials for import clearance if any artificial preservative including propionic acid is added.

In our preliminary study, we discovered the presence of propionic acid in Scutellaria roots, and, therefore, in this study, we seek to analyze many different Scutellaria root samples to study the differences in which propionic acid exists and to investigate its content variation, followed by the development of a quantitative analysis using ultra performance liquid chromatography (UPLC).

2. Methods

2.1. Sample and standard preparation

S. baicalensis roots were collected in 2014 from different areas of South Korea and China. The Scutellaria roots from China were purchased from the Hebei Anguo and Anhui Bozhou Medicine Market in unprocessed form and identified by Professor Liu Xiangqian (Hunan University of Chinese Medicine, Yuelushan, Changsha, Hunan, P. R. China). The Scutellaria roots from Korea were purchased from nine medicinal herbs market in Korea, and all samples were identified by Professor KiHwan Bae (Chungnam National University, South Korea).

Each 1-g sample was extracted with 10 mL of 70% aqueous ethanol for 2 days at room temperature, then filtered and injected into UPLC. For the preparation of the standard solution, 10.07 μL of propionic acid standard material (density, 0.993 g/mL at 25 °C, purity 99.5%) was carefully measured and then dissolved in 1 mL of water for the preparation of the 10 mg/mL stock solution, and six working standard solutions were prepared by serial dilution from the stock solution with final concentrations of 10 μg/mL, 100 μg/mL, 200 μg/mL, 500 μg/mL, 1000 μg/mL, and 2000 μg/mL.

2.2. Instrument conditions for quantitative analysis

The analysis system consists of a Waters Acquity Ultra Performance LC equipped with a binary solvent manager and a TUV Detector (Waters, Milford, MA, USA). The stationary phase was an Acquity UPLC BEH C18 column, 1.7 μm, 2.1 mm × 50 mm (Waters). The mobile phase consisted of a 15 mM aqueous phosphate buffer at pH 2.1 (A) and acetonitrile (B) and eluted with 5% B for 5 min. The flow rate was 0.2 mL/min, the injection volume and detection wavelength were 2 μL and 210 nm, respectively, and the column oven was set at 40 ± 5 °C. The propionic acid and phosphoric acid were analytical-grade and purchased from Sigma-Aldrich (St. Louis, MO, USA), and HPLC (high performance liquid chromatography) grade water and acetonitrile were purchased from J.T. Baker (Center Valley, PA, USA).

2.3. Method validation

The quantitative analysis methodology was validated according to the ICH Guidelines for selectivity, linearity, precision, accuracy, limit of detection (LOD), and limit of quantification (LOQ). LOD and LOQ were determined from the standard deviation (σ) and the response of the slope (S) as follows: LOD = 3.3 × σ/S, LOQ = 10 × σ/S. The precision was evaluated for within-day (intraday) and between-day (interday) precisions. Intraday and interday variations were established by analyzing three different concentrations of the working solution (low, medium, and high) of 25 μg/mL, 100 μg/mL, and 200 μg/mL. The accuracy was determined by a recovery test that evaluated the sample concentrations adding three different levels of standard solution. The repeatability was determined using six replicates of the standard solution.

3. Results

3.1. Quantitative method development and validation

The analysis method of propionic acid from Scutellaria roots was assessed by UPLC using a reverse -phase C18 column as the stationary phase. The propionic acid from these instrument conditions showed fine chromatogram for quantification.

The specificity of the method was studied by comparing the chromatograms from the blank, standard, and sample solutions. The peak from the propionic acid standard appeared at 2.6 minutes, and the propionic acid in the samples was detected well in these conditions in which it was fully isolated from other compounds. The linearity was studied by regression analysis over six different concentrations. For the tested range (10–2000 μg/mL), the linearity was found to have an excellent fit with the regression curve correlation coefficient.
The quantification of propionic acid content. The developed method from this study using UPLC was successfully applied to the measurement of *S. baicalensis* roots, showing shorter analysis time, more convenient conditions, and lower LOD and LOQ values.

Propionic acid is widely used as a preservative in the food industry; consequently, the presence of propionic acid is viewed as a sign of the presence of artificial additives for antimicrobial effects. Propionic acid was reported to be present in fish and vegetables fermented by microorganisms, and organic acids including propionic acid were studied in a recent study, which included the quantification of propionic acid from 10 herbs for each five samples. Among the herbs included (Lycium chinensis, Astragalus radix, and Atractylodes rhizoma), propionic acid ranged from 2.8 μg/mL to 19.8 μg/mL. However, the study of the quantification of propionic acid and its distribution obtained from *Scutellaria* roots has not been reported yet.

This study is the first to detect propionic acid in *S. baicalensis* root. The content variation of 50 samples was then analyzed. Propionic acid was detected in 47 out of 50 samples. The samples collected from China showed a detection rate of 91.7%, and the samples collected from Korea showed a detection rate of 100%. This means that almost all commercial *S. baicalensis* contain propionic acid.

The Korean Food and Drug Administration and similar agencies from other countries, which strictly regulate propionic acid levels in *S. baicalensis* raw materials, have not considered the fact that propionic acid originally exists in the plant.

This study suggests that the quality specification currently used to identify the artificially added propionic acid content in *Scutellaria* roots should be corrected in consideration of the data obtained in this study, the natural occurrence of propionic acid.

### Conflicts of interest

The authors declare no conflict of interests.

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