

Development of an analytical method for simultaneous detection of greater celandine and *Coleus forskohlii* in dietary supplements

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Abstract

Greater celandine and *Coleus forskohlii* are designated as ingredients that require special attention (designated ingredients) under the Food Hygiene Act. In this study, we developed a method for the simultaneous quantitative analysis of coptisine and forskolin in dietary supplements containing the designated ingredients using ultra-high performance liquid chromatography (UHPLC). We also developed a simultaneous qualitative analysis method for coptisine, sanguinarine, forskolin, and isoforskolin in dietary supplements containing the designated ingredients using a UHPLC-quadrupole-Kingdon trap mass spectrometry with photodiode array detector. Extraction of samples was carried out by ultrasonication using methanol as the extraction solvent. An ODS column was used for quantitative and qualitative analyses. Quantitative analysis was performed on a gradient of 10 mmol/L phosphate buffer (pH 3.0)-acetonitrile, and qualitative analysis was performed on a gradient of 10 mmol/L ammonium formate buffer (pH 3.0)-acetonitrile. The recovery test results showed recoveries of between 97.23 and 102.14%; the standard deviation was within 2%, indicating good precision. In the application of our quantitative analysis method to commercial dietary supplements, maximums of 10.71 mg/bottle of coptisine and 27.73 mg/tablet of forskolin were detected. When a product containing 27.73 mg/tablet of forskolin was consumed as indicated on the label, the daily intake of forskolin was 110.92 mg. In the qualitative analysis, we confirmed the presence of coptisine, sanguinarine and forskolin in the dietary supplements. The isoforskolin content was confirmed except for one product that contained the minimum amount of forskolin.

Keywords : ultra-high performance liquid chromatography, quadrupole-Kingdon trap mass spectrometry, greater celandine, *Coleus forskohlii*, simultaneous analysis

I Introduction

Greater celandine is a perennial plant of the poppy family and is native to Europe, West Asia and North Africa. It contains alkaloids such as coptisine and sanguinarine, and has been reported to exhibit antimicrobial, antiviral, analgesic, anti-inflammatory, anticancer, and hepatoprotective effects^{1, 2)}. Dietary supplements containing greater celandine have been distributed in European countries with claims of liver cleansing and anti-tumor effects; however, there have been reports of liver dysfunction³⁻⁵⁾ following intake. Meanwhile, *Coleus*

forskohlii is a tropical plant of the Perillaceae family that is native to India, and contains forskolin as its main active constituent. Forskolin has been reported to activate adenylate cyclase, increase the concentration of intracellular cAMP^{6, 7)}, and activate lipolytic enzymes⁸⁾. Therefore, the dietary intake of *Coleus forskohlii* may be expected to have a weight loss effect, and dietary supplements containing *Coleus forskohlii* are widely distributed in Japan with the implicit claim of having a slimming effect. However, forskolin is thought to induce cholera-like diarrhea⁹⁾ due to fluid retention in the gastrointestinal tract, and diarrhea has been reported as a health