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A UPLC-ESI-Q-TOF method for rapid and reliable identification and quantification of major indole alkaloids in *Catharanthus roseus*

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ABSTRACT

We developed a novel ultra performance liquid chromatography-quadrupole time-of-flight (UPLC-Q-TOF) mass spectrometry method that allows sensitive, rapid, and reliable detection and identification of six representative indole alkaloids (vincristine, vinblastine, ajmalicine, catharanthine, serpentine, and vindoline) that exhibit physiological activity in *Catharanthus roseus* (*C. roseus*). The alkaloids were eluted on a C18 column with acetonitrile and water containing 0.1% formic acid and 10 mM ammonium acetate, and separated with good resolution within 13 min. Electrospray ionization-Q-TOF (ESI-Q-TOF) analysis was performed to characterize the molecules and their fragment ions, and the characteristic ions and fragmentation patterns were used as to identify the alkaloids. The proposed analytical method was verified in reference to the ICH guidelines and the results showed excellent linearity ($R^2 > 0.9988$), limit of detection (1 ng/mL to 10 ng/mL), limit of quantification (3 ng/mL to 30 ng/mL), intra-day and inter-day precisions, and extraction recovery rates (92.8% to 104.1%) for all components. The validated UPLC-Q-TOF method was applied to the analysis of extracts from the root, stem, and leaves of *C. roseus*, allowing the identification of six alkaloids by comparison of retention times, molecular ions, and fragmentation patterns with those of reference compounds. Sixteen additional indole alkaloids were tentatively identified by comparison of chromatograms to chemical databases and literature reports. The contents of bis-indole alkaloids (vincristine and vinblastine) were high in the aerial parts, while the contents of mono-indole alkaloids (ajmalicine, catharanthine, serpentine, and vindoline) were high in the roots. The present results demonstrate that the proposed UPLC-Q-TOF method can be useful for the investigation of phytochemical constituents of medicinal plants.

1. Introduction

Alkaloids are basic organic compounds containing nitrogen, and mainly occur as secondary metabolites in plants [1]. To date, structures of approximately 1200 alkaloids have been identified, and some of these alkaloids are characterized by their clear physiological activity in the human body, ranging from toxic to pharmacological effects, including arousal [2]. Indole alkaloids are derived from tryptophan and are commonly found in the *Apocynaceae* and *Rubiaceae* families of plants [2]. In particular, *Catharanthus roseus* (*C. roseus*), which belongs to the *Apocynaceae* family, produces approximately 130 indole alkaloids, known to contain pharmacologically active components [3]. The representative pharmacologically active compounds are vincristine, vinblastine, ajmalicine, serpentine, vindoline, and catharanthine, which have been shown to have anticancerous, antihypertensive, antihemophilic, hemostatic, and analgesic properties [4–7]. However, the yield of alkaloids obtained from *C. roseus* is very low, and significant efforts have been therefore devoted to increasing the yields including chemical synthesis and biosynthesis approaches. Nevertheless, most alkaloids used continue to be extracted from plants because synthetic efforts have not been practically viable due to their cost [8]. Therefore, determination of individual components present in target plants is critical for utilization and quality control of the active ingredients, and instrument-based analytical approaches for component analysis are expected to play an essential role in this objective.

Indole alkaloids are typically analyzed using HPLC (high performance liquid chromatography) or UPLC (ultra performance liquid chromatography)-based analytical methods involving coupling to a UVD (ultra violet detector), FLD (fluorescence detector), or MS detector [9]. HPLC-based methods are often preferred because the repeatability and reproducibility of other analytical strategies such as gas chromatography (GC) are significantly lower due to the relatively high melting

Abbreviations: *C. roseus*, *Catharanthus roseus*; HPLC, high performance liquid chromatography; UPLC, ultra performance liquid chromatography; UVD, ultra violet detector; FLD, fluorescence detector; Q-TOF, quadrupole-time-of-flight; CID, collision-induced dissociation; MRM, multiple reaction monitoring

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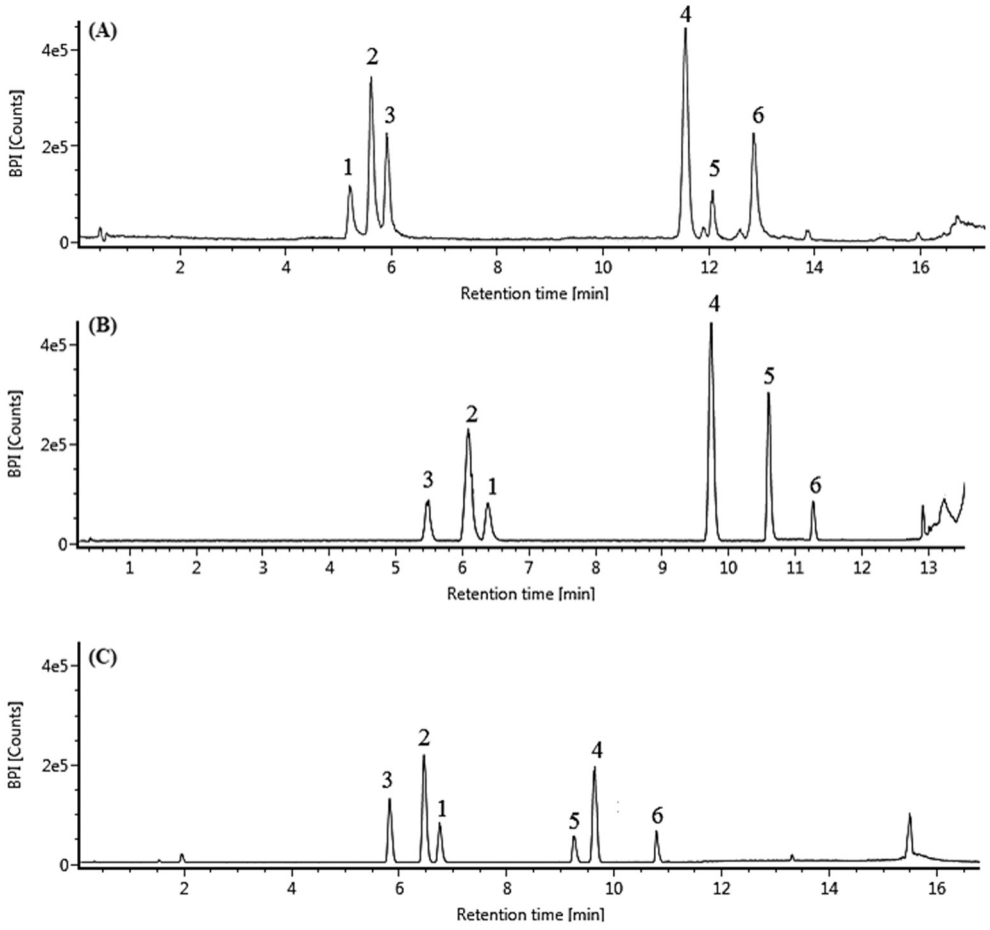

Fig. 1. Representative chromatograms of standard alkaloid mixture as a function of pH: (A) pH 4.5 (10 mM ammonium acetate with 0.1% acetic acid in water), (B) pH 3.3 (10 mM ammonium formate with 0.1% formic acid in water), and (C) pH 2.7 (0.1% formic acid in water); The peaks were identified as follows: (1) serpentine, (2) catharanthine, (3) ajmalicine, (4) vindoline, (5) vincristine, and (6) vinblastine.

points of certain bis-indole alkaloids (e.g., vincristine and vinblastine). In addition, FLD is not suitable for simultaneous analysis because of large differences in compound fluorescence; e.g., fluorescence of catharantine is nine times lower than that of ajmalicine and 360 times lower than that of serpentine [10]. Although UVD has been used as a universal detector for alkaloids, its sensitivity to bis-indole alkaloids is very low compared with its sensitivity for other alkaloids. Thus, it is typically very challenging and time-consuming to distinguish and quantify trace amounts of components present in plants. Significant research efforts have been devoted to the development of simple sample processing and fast and efficient analytical methods for the detection of medicinally relevant substances.

Recent advances in LC technology have led to the introduction of UPLC, as well as new analytical methods for the detection of alkaloids based on devices that incorporate mass spectrometers such as tandem MS (MS/MS) or Q-TOF (quadrupole-time-of-flight) MS. In comparison to HPLC, UPLC offers faster analysis, better resolution, and improved sensitivity [11]. The MRM (multiple reaction monitoring) mode used in MS/MS is a method that allows quantitative analysis based on fragment ions generated by collision-induced dissociation (CID), and provides better sensitivity and shorter analysis time than UVD or FLD. In particular, Q-TOF is a detector that can measure mass accurately to the level of 1 ppm (to four decimal places), and calculate the molecular formula of the expected compound based on measured data [12]. Furthermore, because the full-scan MS and the MS/MS spectra generated by CID are measured simultaneously, it is possible to identify unknown components by profiling their fragmentation patterns, which makes the MRM mode an optimal quantitative method [13]. Although UPLC-Q-TOF is more expensive than other methods, it has been receiving increasing attention as an optimal instrument for investigating constituents of medicinal plants containing alkaloids [14]. Chemical structure identification of active indole alkaloids using UPLC-Q-TOF has been reported previously [15,16]. However, while numerous studies have been conducted to quantitatively analyze indole alkaloids in *C. roseus* using HPLC, UPLC-DAD, or LC/MSMS, few studies have focused on qualitative and quantitative analyses using UPLC-Q-TOF. Moreover, few studies have reported analytical methods with the capacity to simultaneously measure multiple components. In this study, we developed and verified a highly sensitive, rapid, and reliable analytical UPLC-Q-TOF method for identification and detection of representative active indole alkaloids. Furthermore, the established UPLC-Q-TOF method was applied to *C. roseus* to identify and quantify indole alkaloids.

2. Materials and method

2.1. Chemicals and materials

Methanol, acetonitrile, ethyl acetate, and water (all solvents were of HPLC grade) were purchased from Merck (Darmstadt, Germany). Formic acid and ammonium formate (both of analytical grade) were purchased from Sigma-Aldrich. Vincristine (≥95%), catharanthine (> 98%), and ajmalicine (> 98%) were purchased from Sigma-Aldrich. Vindoline (≥98%), vinblastine, serpentine, and ajmalicine were purchased from ChemFaces (Wuhan, Hubei, China). Each of the standard materials was diluted with methanol to a concentration of 1000 ng/mL. Subsequently, a standard material mixture was prepared at a concentration of 1 μg/mL and was used in the construction of the calibration curve.

Catharanthus roseus was sourced from a herb farm in Cheongju-si (Korea) in September 2017. The sample was thoroughly dried at 100 °C in the dryer, and was divided into the root, stem, and leave sections and stored at −20 °C prior to analysis.