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An automatic versatile system integrating solid-phase extraction with ultra-high performance liquid chromatography–tandem mass spectrometry using a dual-dilution strategy for direct analysis of auxins in plant extracts

Qisheng Zhong[b], Xiongxiong Qiu[b], Caiyong Lin[b], Lingling Shen[b], Yin Huo[b], Song Zhan[b], Jinting Yao[b], Taohong Huang[b], Shin-ichi Kawano[b], Yuki Hashi[b], Langtao Xiao[c], Ting Zhou[a,*]

[a] School of Bioscience and Bioengineering, South China University of Technology, Guangzhou, 510006, China
[b] Shimadzu Global COE for Application & Technical Development, Guangzhou, 510010, China
[c] Hunan Provincial Key Laboratory of Phytohormones and Growth Development, Hunan Agricultural University, Changsha, 410128, China

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ABSTRACT

An automatic versatile system which integrated solid phase extraction (SPE) with ultra-high performance liquid chromatography–tandem mass spectrometry (UHPLC–MS/MS) was developed. Diverse commercial SPE columns can be used under an ambient pressure in this online system realized by a dual-dilution strategy. The first dilution enabled the direct injection of complex samples with minimal pretreatment, and the second dilution realized direct introduction of large volume of strong eluent into the UHPLC column without causing peak broadening or distortion. In addition, a post-column compensation mode was also designed for the matrix-effects evaluation. The features of the online system were systematically investigated, including the dilution effect, the capture of desorption solution, the column-head stacking effect and the system recovery. Compared with the offline UHPLC system, this online system showed significant advantages such as larger injection volume, higher sensitivity, shorter analysis time and better repeatability. The feasibility of the system was demonstrated by the direct analysis of three auxins from different plant tissues, including leaves of *Dracaena sanderiana*, buds and petals of *Bauhinia*. Under the optimized conditions, the whole analysis procedure took only 7 min. All the correlation coefficients were greater than 0.9987, the limits of detection and the limits of quantitation were in the range of 0.560–0.800 ng/g and 1.80–2.60 ng/g, respectively. The recoveries of the real samples ranged from 61.0 to 117%. Finally, the post-column compensation mode was applied and no matrix-effects were observed under the analysis conditions. The automatic versatile system was rapid, sensitive and reliable. We expect this system could be extended to other target analytes in complex samples utilizing diverse SPE columns.

1. Introduction

The development of hyphenated analysis systems is one of the predominant trends in modern analytical chemistry [1,2]. High-performance liquid chromatography (HPLC) is a mature technique commonly used in routine analysis [3]. However, despite the outstanding performance of modern chromatographic systems, sample preparation is still the most tedious and time-consuming step, which is recognized as the main bottleneck of the analytical process [4,5]. Thus, special coupling of sample pretreatment methods to HPLC such as solid-phase extraction (SPE)-HPLC [6,7], solid phase microextraction (SPME)-HPLC [8–14] and so on have been developed, which provide a number of advantages including reduced analysis time, high sample throughput and great reproducibility.

SPE is an attractive approach to the preparation of environmental and biological samples. Compared with liquid–liquid extraction (LLE) technique and SPME, SPE provides higher enrichment factor and better reproducibility [15]. Most importantly, SPE offers countless diverse choices of sorbents, ranging from the traditional

* Corresponding author. Tel.: +86 20 39380670; fax: +86 20 39380670.
E-mail address: tingzhou@scut.edu.cn (T. Zhou).

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reversed-phase sorbents to some new materials such as carbon nanotubes [16], electrospun nanofibers [17], ionic liquid-modified materials [18,19], restricted access media [20,21] and so on. In addition, SPE could be performed on-line by directly connecting to the chromatographic system. Hyphenated SPE-HPLC on-line systems are designed to improve not only the sensitivity and selectivity but also the precision due to minimal human participation [22]. Up to now, many different types of SPE-HPLC online systems have been developed. Kantiani et al. [23] used a fully automated online SPE-LC/ESI-MS–MS system to detect β-lactam antibiotics in bovine milk samples, and the limits of quantification (LOQs) were between 0.09 ng/mL and 1.44 ng/mL. Liu et al. [24] established a polymer microchips integrating SPE with HPLC system using reversed-phase polymethacrylate monoliths for the separation and enrichment of peptides, free fluorescein and labeled proteins, where the sensitivity was significantly improved.

After the domination of separation science by HPLC for about half a century, ultra-high performance liquid chromatography (UHPLC) has recently emerged as a more powerful separation approach [25]. The main differences between UHPLC and HPLC are the column and the pressure of the system. UHPLC uses the columns packed with sub-2 μm particles in ultra-high pressure conditions, while the columns used in HPLC are commonly packed with 5 μm particles under a much lower pressure [26]. In addition, the reduction of particle size down to sub-2 μm allows either speeding up of the analytical process while maintaining similar efficiency or a theoretical increase in efficiency for a shorter run time [27]. Furthermore, the combination of UHPLC with mass spectrometry (MS) detector appears to be a suitable approach that fulfills key requirements in terms of sensitivity, selectivity, and peak-assignment certainty for the rapid determination of analytes at low concentrations in complex matrices [26]. Thus, UHPLC has been well accepted as a powerful analytical tool for its fast separation, high sensitivity and improved throughput [28–30].

The on-line combination of SPE and UHPLC is expected to be an efficient method for the rapid and ultra-trace analysis of complex samples [31,32]. However, there are two bottlenecks during the achievement of SPE-UHPLC online system. One is the peak broadening and distortion caused by excessive volume of solvents with high elution strength. During SPE procedure, large volume of strong solvent is usually necessary for efficient SPE elution, which results in broad, flat-top shaped peaks with low plate counts in the following chromatographic analysis [30]. In fact, this volume overload problem is the main obstacle to online hyphenation of sample preparation techniques to HPLC, and the problem is much more serious for UHPLC due to its lower sample capacity which is limited by the narrower and shorter columns. Recently, an online analytical system integrating solid-phase-based extraction technique with UHPLC based on a fractionized sampling and stacking (FSS) strategy was established, which would address the volume overload problem of the online SPE-UHPLC system [33]. However, during FSS procedure, the valve needed to be manually switched 50 cycles. The other bottleneck is the high pressure of UHPLC system which normal SPE column could not stand. The pressure of UHPLC system which is much higher than that of HPLC system would cause serious damages to the SPE materials.

Auxin is one of the most crucial plant hormones, which regulates the growth and development of the plants [34]. The 3-indole acetic acid (IAA) is the first plant hormone used to stimulate rooting of cuttings. There are also several synthetic compounds with structure similar to that of IAA that elicits auxin-like physiological responses, such as 3-indole-propionic acid (IPA), 3-indole-butyric acid (IBA). Analysis of auxins has a great value for the study of biological process and its indepth application, such as modern agriculture and biotechnology [35]. However, plant hormones are difficult to analyze due to their lower concentrations in plant tissues which are very rich in interfering substances [36].

In this study, an automatic analysis online system coupling SPE with UHPLC–MS/MS using a dual-dilution strategy was developed. The dual-dilution strategy allowed the direct injection of complex samples and enabled direct introduction of large volume of strong eluent into the UHPLC column without causing peak broadening, which was the key ingredient for realizing this online system. Besides, a post-column compensation mode for the matrix-effects evaluation was designed with minor modification. In this dual-dilution SPE-UHPLC–MS/MS online system, common commercial SPE columns could be coupled with the UHPLC–MS/MS equipment, achieving automatic online analysis. To demonstrate the feasibility of the approach, this online system was applied to the direct analysis of three auxins in different plant tissues. And the features of the online system were investigated systematically.

2. Experimental

2.1. Reagents and samples

Standards of IAA, IPA and IBA were purchased from Dr. Ehrenstorfer GmbH (Augsburg .Germany). All standard solutions were prepared in methanol and were stored at −20 °C. Methanol, acetonitrile and formic acid of HPLC grade were purchased from CNW technologies (Shanghai, China). The other solvents of analytical grade were purchased from Guangzhou Chemical Reagents Factory (Guangzhou, China). Water used throughout the study was prepared with a Milli-Q Gradient A-10 water system (Millipore, Bedford, MA, USA).

Leaves of *Dracaena sanderiana*, buds and petals of *Bauhinia* were collected from Yuexiu Park in Guangzhou. After being cut into pieces, 1.00 g of each sample was added into 20 mL 80% (v/v) methanol aqueous solution. Then the mixture was homogenized by a homogenizer and extracted by ultrasonic wave, each for 10 min. The plant extracts were filtered with 0.45 μm membrane before analysis.

2.2. Apparatus

The online system was converted from the Shimadzu LC–MS/MS system (Shimadzu, Kyoto, Japan). A LCMS-8040 tandem mass spectrometer (Shimadzu, Shimadzu, Kyoto, Japan) was equipped with an electrospray ionization (ESI) interface. The LC system consisted of a CBM-20A controller, two LC-30AD pumps (Pump A&B, which could stand ultra-high pressure up to 130 MPa), two LC-20AD pumps (Pump C&D, which could only stand normal pressure up to 40 MPa), two vacuum degassers, an autosampler, a column oven, and a SPD-M20A photodiode array (PDA) detector. Pump D was equipped with a solvent switching valve for the alternation between the loading solvent and the conditioning solvent. Two six-port valves, V1 and V2, which could respectively resist the maximum pressure of 34 MPa and 130 MPa, were mounted inside the column oven. Two mixers, Mixer 1 and Mixer 2 were used for the dilution of the injected solution and the desorption solvent, respectively. An in-line filter was installed between V1 and V2. The SPE column was mounted between two ports (NO. 1 and NO. 4) of V1 for extraction. A stainless steel capture loop of 500 μL (Loop 1) was mounted between two ports (NO. 1 and NO. 4) of V2 for capturing of the target analytes. An in-line filter with 0.5 μm mesh size was installed between V1 and V2 to filter the desorption solution. Another valve (V3) was installed after the UHPLC column for the post-column compensation mode and a capture loop of 1 mL (Loop 2) was mounted between two ports (NO. 1 and NO. 4) of V3 for loading of the standard solvent for matrix-effects evaluation.