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Pre-column dilution large volume injection ultra-high performance liquid chromatography-tandem mass spectrometry for the analysis of multi-class pesticides in cabbages

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ABSTRACT

Pre-column dilution large volume injection (PD-LVI), a novel sample injection technique for reverse phase ultra-high performance liquid chromatography-tandem mass spectrometry (UHPLC–MS/MS), was developed in this study. The PD-LVI UHPLC–MS/MS system was designed by slightly modifying the commercial UHPLC–MS/MS equipment with a mixer chamber. During the procedure of PD-LVI, sample solution of 200 μL was directly carried by the organic mobile phase to the mixer and diluted with the aqueous mobile phase. After the mixture was introduced to the UHPLC column in a mobile phase of acetonitrile-water (15/85, v/v), the target analytes were stacked on the head of the column until following separation. Using QuEChERS extraction, no additional steps such as solvent evaporation or residue redissolution were needed before injection. The features of PD-LVI UHPLC–MS/MS system were systematically investigated, including the injection volume, the mixer volume, the precondition time and the gradient elution. The efficiency of this approach was demonstrated by direct analysis of 24 pesticides in cabbages. Under the optimized conditions, low limits of detection (0.00074–0.8 ng/kg) were obtained. The recoveries were in the range of 63.3–109% with relative standard deviations less than 8.1%. Compared with common UHPLC–MS/MS technique, PD-LVI UHPLC–MS/MS showed significant advantages such as excellent sensitivity and reliability. The mechanism of PD-LVI was demonstrated to be based on the column-head stacking effect with pre-column dilution. Based on the results, PD-LVI as a simple and effective sample injection technique of reverse phase UHPLC–MS/MS for the analysis of trace analytes in complex samples showed a great promising prospect.

1. Introduction

The sensitivity of liquid chromatography (LC), which is always presented by the quantitation of limit, is directly proportional to the sample volume loaded into chromatographic column [1]. However, large injection volume especially with strong elution solvent may influence the retention process and chromatographic parameters, such as efficiency, retention factor, peak shape among others [2]. Furthermore, sample preparation procedures based on solid phase extraction (SPE) or liquid phase extraction (LPE) with additional steps of solvent evaporation and residue redissolution in mobile phase makes the analysis process tedious and time-consuming and may cause significant errors [3]. It is expected that large volume injection (LVI) of strong elution solvent will not represent a critical parameter for the separation process. Therefore, a breakthrough in the injection volume of LC system would significantly improve the sensitivity and speed up the analysis.

At present, LVI for LC could be realized in several ways. The simplest situation is that the sample is dissolved in a weak elution solvent which could be injected directly without causing serious peak broadening or distortion [4,5]. Since water is a weak elution

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solvent under reverse phase-LC (RP-LC) condition, the large injection volume of water samples ranges from 250 μL to 1800 μL [2,6]. In a report about the determination of acrylamide in environmental and drinking waters, water samples were solvent exchanged into dichloromethane and 750 μL were injected directly onto a hydrophilic-interaction liquid chromatography column [7]. The second way is that a preconcentration step such as solid-phase extraction (SPE) [8,9] or a pre-column [10] is added after LVI, which is also called column-switching liquid chromatography [11]. Guo et al. [8] developed a method for determining eight chlorophenols by LVI online turbulent flow SPE-HPLC in urine samples, in which an aliquot of 1.0 mL urine sample could be analyzed directly after centrifugation and LODs were between 0.5 and 2 ng/mL. Malerod et al. [12] used a column switching system with a zwitter ionic-HILIC silica pre-column for LVI of aqueous peptide samples for characterization of post translational modifications. Galera et al. [13] investigated the potential of coupled-column liquid chromatography combined with LVI (5 mL of sample) and fluorescence detection for determination of beta-blockers in groundwater with LOQ of 1–7 ng/L. The third way for LVI is that using of a large volume injection of hydrophobic solvents as diluents for less hydrophobic solutes in reversed-phase liquid chromatography, which has been proven recently [14,15]. However, these solvents with high hydrophobicity were strongly adsorbed in the stationary phase and a part of the stationary phase adsorption centres were blocked by the hydrophobic solvent, which would influence the retention factor and peak shape of the dissolved solutes [16]. Another way for LVI is that using one line of a common commercial HPLC quaternary low pressure pump for direct on-column injection [17,18]. However, the obvious disadvantage of this approach is that the sample volume should be larger than 20 mL due to the large dead volume and the sample replacement was manual manipulation which was very inconvenient and laborious. In addition, all the LVI techniques mentioned above were operated under HPLC system rather than Ultra-high performance liquid chromatography (UHPLC) system.

UHPLC has recently emerged as a more powerful separation approach for its fast separation, high sensitivity and improved throughput [19–21]. Moreover, 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 [22,23]. And UHPLC combined with MS has been widely used to determine the contaminants and other analytes which are at trace levels in many kinds of samples [24,25]. Compared with HPLC, UHPLC uses the columns packed with much smaller particles (sub–2 μm) under a much higher pressure, allowing speeding up of the analytical process with a theoretical increase in efficiency [26]. However, the volume overload problem, which is the main obstacle to LVI technique for HPLC, is much more serious for UHPLC due to its lower sample capacity which is limited by the narrower and shorter columns [27–29]. Large volume of strong solvent with high elution strength which is usually necessary for efficient residue redissolution results in peak broadening and distortion with low plate counts in the following chromatographic analysis [30]. Therefore, there is a high need to develop a simple and robust LVI technique of strong sample solution for UHPLC analysis.

In this study, a novel sample injection technique for reverse phase (RP) UHPLC–MS/MS called pre-column dilution large volume injection (PD-LVI) was developed. During the procedure of PD-LVI, the sample solution with strong elution strength carried by organic mobile phase was diluted with aqueous mobile phase in the mixer. After being introduced to the UHPLC column, the target analytes were stacked on the head of the column until following separation. No solvent evaporation or residue redissolution were needed before injection. To demonstrate the feasibility of this approach, PD-LVI UHPLC–MS/MS system was applied to analysis of 24 pesticides in cabbages. The features PD-LVI UHPLC–MS/MS system and the mechanism of PD-LVI were systematically investigated.

2. Experiment

2.1. Reagents and samples

Standards of all pesticides were purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany). All standard solutions were prepared in methanol and were stored at −20 °C. Acetonitrile, methanol 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).

Chinese cabbages (*Brassica rapa pekinensis*) were bought from organic products district of Guangzhou Lotus Supermarket Chain Store and tested fresh.

2.2. Apparatus

The PD-LVI UHPLC–MS/MS system was converted from a Shimadzu UHPLC–MS/MS system, which was shown in Fig. 1A. The Shimadzu Nexera X2 UHPLC system (Shimadzu, Kyoto, Japan), which consisted of a CBM-20A controller, two LC-30AD pumps (Pump A&B, which could stand ultra-high pressure up to 130 MPa), a solvent mixer which was used for the dilution of the injected sample solution, a vacuum degassers (DGU-20A5), an autosampler (SIL-30AC) with 200 μL sample loop, a column oven (CTO-30A). An in-line filter was installed between autosampler and mixer. A LCMS-8050 tandem mass spectrometer (Shimadzu, Kyoto, Japan) was equipped with an electrospray ionization (ESI) interface. A Shimadzu workstation LabSolutions (Version 5.60) was used to control the LCMS system. The details of the analysis procedure were fully discussed in Section 3.

2.3. Sample preparation

The sample preparation used the Wondapak QuEChERS set (Shimadzu-GL), which was according to a related reference [31]. After being cut into pieces, an approximately 200 g portion of Chinese cabbage sample was weighed and homogenized (IKA T18, Germany) at 10,000 rpm for 1 min. A 15 g portion of homogenized sample was weighed into a 50 mL Teflon centrifuge tube, followed by the addition of 10 mL of acetonitrile/formic acid (99/1, v/v). After being vortexed for 1 min, the tube was added with 6 g $MgSO_4$ and 1.5 g NaOAc and was shaken again. Afterwards, the tube was centrifuged at 3500 rpm for 5 min and 1 mL of the acetonitrile layer was transferred to a microcentrifuge tube containing 150 mg $MgSO_4$, 50 mg primary secondary amine, 50 mg C_{18} powder. The mixture was shaken vigorously for 2 min and centrifuged at 13000 rpm for 2 min. Then, the supernatant was filtered through a 0.22 μm polytetrafluoroethylene filter and transferred to an autosampler vial.

2.4. PD-LVI UHPLC–MS/MS conditions

The separations were carried out on a Shim-pack XR-ODS III column (2.0 mm i.d. × 50 mm, 1.6 μm particle size) from Shimadzu. The mobile phase consisted of 0.02% formic acid in water with 2 mM ammonium acetate (A) and acetonitrile (B) under gradient elution. After the injection, the sample solution of 200 μL was driven to the mixer by mobile phase B and then diluted into 85% mobile phase A in the mixer. The gradient was started with this initial ratio (mobile phase B: mobile phase A = 15: 85, v/v). After 2.7 min (the precondition time), mobile phase A was decreased to 0% within 7.3 min