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Highly sensitive strategy for identification of trace chemicals in complex matrix: Application to analysis of monacolin analogues in *monascus*-fermented rice product

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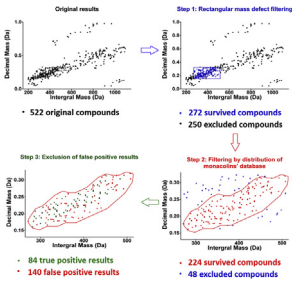
HIGHLIGHTS

- An HPLC-Chip-MS method was established to identify trace chemicals in complex matrix.
- A database/multi-step filtering method was developed to characterize monacolin analogues.
- A total of 84 monacolins including 16 new ones were identified in *monascus*-fermented rice product.

GRAPHICAL ABSTRACT



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ABSTRACT

Trace analysis of chemical analogues was always a hot topic and attracted the researchers' attentions. In this study, a database/multi-step filtering strategy was developed by using HPLC-Chip-MS system for comprehensive characterization of monacolin analogues in *monascus*-fermented rice product (MFRP). This strategy mainly included three steps, including chemical profile of MFRP by HPLC-Chip-MS, establishment of monacolin analogue database, multi-step filtering of monacolins based on modified mass defect filtering. All target compounds showed the symmetrical peak shapes and high MS response by using nanoflow HPLC-Chip-MS. According to the previous literature and experimental MS data, a database including 720 monacolin analogues was established. The original 522 ions in MFRP were automatically extracted by molecular feature extraction function. And then, through rectangular mass defect filtering and analogues distribution area filtering, 298 ions were further excluded. Finally, a total of 84 monacolins including 16 new compounds were unambiguously identified or tentatively characterized based on MS/MS information. In comparison with conventional mass defect filtering method, the proposed method was accurately able to exclude false positive results. The 438 false positive results were excluded in our study, while only 250 ones would be filtered out by using conventional method. This study provided a sensitive and powerful method for rapidly characterization of trace chemicals in complex matrix.

1. Introduction

Chemical analogues widely appeared in the complex matrix, such as natural products, metabolites, and biological samples [1,2].

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The same family of chemical compounds often shared the similar skeletons, substituent groups and substituent sites. It's even reported that hundreds of analogues were found in one herb. Qiu et al. reported that a total of 646 ginsenosides were identified from the stems and leaves of *Panax ginseng* [3]. Most of these analogues were trace and might possess potent activities or toxicities [4]. Therefore, characterizing the trace-level constituents has always fascinated researchers.

Liquid chromatography coupled with high resolution mass spectrometry (LC-HRMS) has been widespread applied to chemical analysis of complex samples [5,6]. To enhance the detection efficiency, many efforts for updating instrument were made [7,8]. HPLC-Chip-MS is a microfluidic chip-based technology for nanospray LC-MS, which was proposed in 2005 [9]. This allowed the advantages of high-sensitivity and low-sample consumption for trace analysis of complex samples [10–14].

Advances in LC-MS techniques enhanced the analytical performance [15,16]. However, manual inspection of chromatographic peaks one by one was low-effective, labor-intensive and error-prone for identification of chemicals in complex sample. Simple and rapid characterization methods have always been required. Mass defect filtering (MDF) is an efficient data processing technique based on HRMS. It could rapidly obtain the MS profile of targeted analytes of interest, by removing a large number of background interrupt signals with the narrow and well-defined mass defect window [17,18]. Tobias et al. used MDF for simplification and classification of lignin degradation products [19]. Xie et al. applied a MDF technique for rapid identification of ophiopogonins and ophiopogonones [20]. However, during the analysis of complex samples, conventional MDF method was also limited in resulting in some false positive results, like mistaking some high intensity fragment ions as molecular ions. Therefore, corresponding skeleton, substituents, and their filtering window should be careful optimization.

In this work, a database/multi-step filtering strategy based on the similarities of basic structure of analogues was proposed by using HPLC-Chip-QTOF MS. Trace analysis of monacolins in *monascus*-fermented rice product (MFRP) was taken as a case. Monacolin was a class of main active ingredients in MFRP, and monacolin K, also known as lovastatin, was the most representative inhibitor of hydroxymethylglutaryl coenzyme A (HMG-CoA) reductase for inhibiting cholesterin biosynthesis [21,22]. Other monacolins in MFRP may have the similar activity, but were often ignored. By using the proposed method, 84 potential trace monacolins in MRFP including 16 new ones were discovered.

2. Experimental

2.1. Drug preparations and chemical reagents

Reference substance of monacolin K was purchased from Must Biological Technology Co. Ltd. (Chengdu, China). Compactin was purchased from Meilune Biological Technology Co. Ltd. (Dalian, China). Dihydromonacolin K and dehydromonacolin K were imported from Toronto Research Chemicals (Toronto, Ontario, Canada). The purity of each compound was determined to be higher than 95% by HPLC. Their chemical structures were identified by comparison of their ^{1}H NMR, ^{13}C NMR and high-resolution MS data with references.

The LC/MS-grade acetonitrile was purchased from Merck (Darmstadt, Germany). Deionized water (18 MΩ cm) was purified using a Milli-Q water purification system from Millipore (Bedford, MA, USA). The HPLC-grade formic acid was purchased from ROE Scientific Inc. (USA). The AR-grade methanol was obtained from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China).

Nine batches of MFRPs (Xuezhikang capsules) were obtained from Peking University WBL Biotech Co., Ltd., China (Beijing, China). The batch numbers were listed in Table S1.

2.2. Sample preparation and standard solutions

The contents of 6 capsules from the same batch of MFRP were dumped, blended, ground, and screened through mesh size of 80. A total of 0.3 g MFRP powder was accurately weighed, and then extracted by ultrasonicating for 30 min with 10 mL 70% methanol for each sample. The 70% methanol extraction was filtered, and the filtrate was diluted 100 times by 70% methanol. Then the diluent was centrifuged (13000 rpm, 10 min). Finally, the supernatant was saved as the resultant sample in −80 °C for further analysis.

Standard solution of compactin, monacolin K, dihydromonacolin K, and dehydromonacolin K was prepared in the concentration 2.5, 10, and 20 μg mL^{-1} in 70% methanol and stored at 4 °C until use. A standard working solution of the mixtures of 4 standards at low, middle, high concentration levels was obtained by diluting stock solutions to desired concentrations.

2.3. HPLC-chip-MS conditions

An Agilent 6520 QTOF tandem mass spectrometer (Agilent Corp., Santa Clara, CA, USA) was applied to MS and MS/MS detection. The nanospray source on Nano LC-Chip was operated under the positive ion mode with the capillary voltage set at 1900 V. Other MS conditions were as follows: drying gas (N_2) temperature, 350 °C; drying gas flow rate, 10.0 L/min; fragmentor, 120 V; skimmer, 65 V; OCT RF V, 750 V. The mass range recorded *m/z* 100–1100. For MS/MS detection, the collision energy was set at 25 eV. The TOF mass spectrometer was calibrated using a Calib-Chip II (Agilent Corp., Santa Clara, CA, USA) and an Agilent TOF tuning-mix infused by a syringe pump at a flow rate 0.3 μL min^{-1} every day before analysis. Agilent MassHunter Workstation Acquisition Software Version B.05.01 and Qualitative Analysis Software Version B.06.00 were utilized for system control, data acquisition, and data processing.

For the sample enrichment and separation, an Agilent 1260 capillary LC system (Agilent Corp., Santa Clara, CA, USA) was employed for sample loading onto the enrichment column (Fig. S1), while an Agilent 1260 nano LC system (Agilent Corp., Santa Clara, CA, USA) was utilized to provide nano-flow mobile phase into analytical column (Fig. S1). In addition, a micro well-plate autosampler with a temperature control module was utilized for injection and to keep sample solutions at 4 °C during the analysis. UHC Chip II (Agilent Corp., Santa Clara, CA, USA), consisting of Zorbax 80SB-C18 bonded stationary phase (5 μm), including an enrichment column (500 nL) and an analytical column (150 mm × 75 μm) for HPLC-Chip-QTOF MS analysis.

The mobile phase of the capillary pump and nanofluidic pump was 0.1% (v/v) formic acid (A) and acetonitrile containing 0.1% (v/v) formic acid (B). The capillary pump was performed as isocratic elution with 5% B. A gradient program was established on the nanofluidic pump as follows: 0–2 min, 20% B; 2–10 min, 20%–25% B; 10–22 min, 25%–30% B; 22–30 min, 30%–35% B; 30–36 min, 35%–45% B; 36–53 min, 45%–60% B; 53–60 min, 60%–70% B; 60–85 min, 70%–90% B; 85–90 min, 90% B. The capillary pump and nanofluidic pump were set at microflow mode with the flow rates 4 μL min^{-1} and 0.6 μL min^{-1}. The operating mode of Agilent 1260 Chip Cube was forward flush. The connection of capillary tube, chip and 6-way μ-switch valve was shown in Fig. S1. Sample enrichment was operated at 0–2 min, whereas sample analysis was performed during 2–90 min. Data acquisition and data analysis was performed with MassHunter Acquisition Software version B.05.01