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Generic DART-MS platform for monitoring the on-demand continuous-flow production of pharmaceuticals: Advancing the quantitative protocol for caffeates in microfluidic biocatalysis

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ABSTRACT

Today, continuous processing is regarded as an effective on-demand production technique of pharmaceuticals. Homemade microreactors packed with immobilized lipase under continuous-flow conditions were first applied to tailor the production of high-value caffeic acid phenethyl ester (CAPE) from methyl caffeate (MC) and 2-phenylethanol (PE) in cyclohexane *via* transesterification; however, this method is challenging due to the lack of a rapid platform for monitoring caffeates in microfluidic biocatalysis. The reactants were directly analyzed using Direct Analysis in Real Time Mass Spectrometry (DART-MS), and the corresponding ionization parameters were investigated. Special ions produced from MC (parent ion *m/z* 192.87 and product ion *m/z* 133.44) and CAPE (parent ion *m/z* 282.93 and product ion *m/z* 178.87) were determined using DART-MS^2 in the negative ion mode. The peak areas of the select reaction monitoring (SRM) signals were calculated to develop the standard curves for quantitative analyses of the concentration. Reasonable linear regression equations of MC and CAPE were obtained in the range of 3.125–50.000 mg/L, with linear coefficients (R^2) of 0.9515 and 0.9973, limits of detection (LOD) of 0.005 and 0.003 mg/L, limits of quantification (LOQ) of 0.02 and 0.01 mg/L, and recovery ranges of 92.50–97.11% and 90.11–97.60%, respectively. The results using DART–MS^2 were in good agreement with those using conventional High-Performance Liquid Chromatography with a UV detector (HPLC-UV) and were successfully applied to monitor the kinetics constants and mass transfer coefficients in a continuous-flow packed bed microreactor. Thus, the DART-MS^2 method is an efficient tool for analyzing caffeates in microfluidic biocatalysis with limited sample preparation and short operating time.

1. Introduction

The pharmaceutical industry is constantly evolving, with impacts of new reactions and applications of established reactions on human health [1]. Continuous manufacturing of the Active Pharmaceutical Ingredients (APIs) and derived drug products is one of emerging lab techniques to support these efforts. It can improve synthetic process by employing integrated processing and central control, while it avoid the disadvantages of batch processing, including long production times and the potential for supply chain disruptions [2]. In addition, continuous manufacturing systems benefit from integrated processing and control, which translates to increased safety (no manual handling) and shorter processing times [3]. As a preliminary demonstration of an alternative approach of batch processing, we reported a continuous-flow synthesis of caffeates (a type of APIs usually found in fruits, vegetables, and herbs, have beneficial effects on a number of disease states [4,5]), including methyl caffeate (MC) [6] and caffeic acid phenethyl ester (CAPE) [7], in a microreactor using lipase-catalyzed esterification and transesterification, which decreased the time from 2–3 days to 2–3 h, while the temperature decreased by approximately 30 °C, and lipase could reused without a loss of activity for 20 cycles or 9 days. This method can be regarded as an attractive method to produce caffeates compared with extraction from natural sources and chemical synthesis [7]. However, CAPE is unstable and readily degraded into other by-products, particularly at elevated temper-

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atures [8]. Thus, a rapid and stable platform is urgently needed for on-demand monitoring of the continuous-flow production of APIs, such as caffeates, in microfluidic biocatalysis using a simple procedure.

Numerous studies have been published on the analysis of CA and its ester derivatives from foods and medicines using Liquid Chromatography with Photodiode Array and Mass Spectrometry (LC-PDA-MS), capillary gas chromatography, Fourier Transform Mid-Infrared Spectroscopy (FT-MIR), Nuclear Magnetic Resonance Spectrometry (NMR), Thin Layer Chromatography (TLC) and TLC–MS [4]. Due to its powerful separation efficiency and sensitive detection, LC–MS and High-Performance Liquid Chromatography (HPLC) have become popular and useful analytical tools in the field of caffeate research [4,9]. However, the separation, identification and quantitation of caffeates from lipase-catalyzed transesterification systems pose great technical challenges due to the presence of the caffeic group in their structures and coexistence with unwanted byproducts with similar structure [8]. In addition, the chromatographic method suffers from its inherent time constraint drawback [10]. The tedious HPLC protocol cannot be used to measure the enzymatic kinetics in a continuous-flow biocatalysis process. Thus, there is a need to explore more rapid and efficient methods.

Direct Analysis in Real Time Mass Spectrometry (DART-MS) is an ambient ionization technique in mass spectrometry, capable to screen for a number of compounds ranging from feedstocks, foods, cosmetics and medicines [11,12]. It can eliminate extensive sample preparation or chromatographic separation steps [12], allowing direct sampling for rapid screening [11]. So, DART-MS provides a promising alternative to traditional methods. To date, DART-MS has not been used for the monitoring of APIs, in a continuous-flow production using microfluidic biocatalysis. To the best of our knowledge, the monitoring of caffeates in an enzymatic reaction using DART-MS is investigated for the first time in this study.

The objective of this work was to develop a rapid and green approach to monitor caffeates from a continuous-flow biocatalysis process using DART-MS technology. The developed DART-MS was applied to identify caffeates including MC and CAPE, in the enzymatic transesterification from MC and PE, and then used to measure and compare kinetic models for lipase-catalyzed synthesis using a continuous-flow microreactor and a batch reactor.

2. Materials and methods

2.1. Materials and reagents

CA and CAPE standards were purchased from the National Institute for the Control of Pharmaceutical and Biological Products (NICPBP, Beijing, China) and Sigma-Aldrich Chemical Co. (St. Louis, MO, USA), respectively. The synthesis materials for CA and 2-phenylethanol (PE) were purchased from Nanjing Zelang Pharmaceutical Sci. & Tech. Co. Ltd. and Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China), respectively. Immobilized lipase Novozym 435 was purchased from Novo Nordisk A/S (Bagsvaerd, Denmark). HPLC grade methanol and acetonitrile were purchased from TEDIA Co. Ltd. (Fairfield, OH, USA). All other solvents and reagents used were analytical grade. Water was purified using an Elga Purelab Option-Q purification system (Elga Labwater, High Wycombe, Bucks, UK) and had a resistivity of greater than 18.0 MΩ cm.

2.2. Preparation of standard solutions

The stock standard solutions of MC and CAPE of 1.0 mg/mL were prepared by dissolving solid powders in 70% aqueous methanol (v/v). Working standard solutions ranging from 3.125 μg/mL to 50.000 μg/mL were prepared by diluting the stock standard solution with 70% aqueous methanol (v/v). All of the standard solutions were stored at 4 °C and equilibrated to room temperature before use.

2.3. On-demand biosynthesis of CAPE in a continuous-flow packed bed microreactor

An on-demand continuous-flow packed bed reactor was built in a stainless steel plate with the following dimensions: width (W) of 8 mm, height (H) of 500 μm, and length (L) of 75 mm. Novozym 435 was used as a biocatalyst, and cyclohexane was employed as the medium. One layer of Novozym 435 beads (90 mg) was incorporated into the channels and then sealed with a glass pane [7]. Cyclohexane containing a specific volume of MC and PE was driven through the packed bed microreactor by a syringe pump (LSP02-1B, Longer Pump Co. Ltd., Beijing, China) at a predefined flow rate. At regular time intervals, 10 μL aliquots were collected from the reaction mixture and were diluted using 990 μL of methanol for DART-MS and HPLC-UV analysis. All of the experiments were performed in triplicate.

2.4. DART-Mass analysis

The DART-MS analyses were performed on a TSQ Quantum Access MAX triple quadrupole mass spectrometer (Thermo Scientific, San Jose, CA, USA). The instrument operating parameters in SRM mode were set as follows: capillary temperature, 300 °C; tube lens offset, 60 V; skimmer offset, off; collision pressure, 0 mTorr (under SRM mode); and collision energy, −10 eV. The mass spectra were recorded across the range m/z 50–350. The instrument was calibrated according to the manufacturer's manual using polytyrosine dissolved in methanol. Fragmentation data for the selected precursor ions were obtained in the Q2 (collision cell) after adjusting the Collision-Induced Dissociation (CID) energy by colliding the precursor ion with argon atoms. The collision cell was supplied with argon gas of 99.999% purity, and the collision pressure was set at 1.5 mTorr. All data analyses and peak integrations were performed with the Thermo Xcalibur™ software suite [13].

A DART SVP ionization source (IonSense, Saugus, MA, USA) connected to the mass spectrometer through a VAPUR interface was used in this study. A small membrane pump (Vacuubrand, Wertheim, Germany) was used to create a vacuum in the VAPUR interface. The orientation of the DART source was aligned with the ceramic tube leading into the VAPUR interface before the inlet of the mass spectrometer. The orifice of the DART ion source and the ceramic ion transfer tube were separated by 1.0 cm. Working solutions (1.0 μL) were drawn in the full range of a 2.5 μL pipette (Eppendorf, Hamburg, Germany) and deposited on the tips of glass sample sticks. The glass sticks were allowed to dry until no liquid was observed. After drying, the sample sticks were secured on an engineered block, holding them on a 12 Dip-It glass tip linear rail that then passed through the gap between the DART ion source and the ceramic ion sampling tube to introduce the samples into the mass spectrometer. The optimized DART settings were as follows: negative ion mode; gas pressure, 0.3 MPa; gas temperature, 300 °C; and grid electrode (Mo 50 mesh) voltage, 100 V. A constant speed of 0.2 mm/s was used for the Dip-It tip rail system. The pressure of the membrane pump was set at 12.0 kPa, as recommended by the manufacturer. High-purity nitrogen (99.999%) was used as both the standby and running gas, which is conducive to the method and is economically advantageous [13].