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# Development of an analytical method coupling cell membrane chromatography with gas chromatography–mass spectrometry via microextraction by packed sorbent and its application in the screening of volatile active compounds in natural products

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ABSTRACT

Natural products (NPs) are important sources of lead compounds in modern drug discovery. To facilitate the screening of volatile active compounds in NPs, we have developed a new biochromatography method that uses rat vascular smooth muscle cells (VSMC), which are rich in L-type calcium channels (LCC), to prepare the stationary phase. This integrated method, which couples cell membrane chromatography (CMC) with gas chromatography–mass spectrometry (GC–MS) via microextraction by packed sorbent (MEPS) technology, has been termed VSMC/CMC-MEPS-GC–MS. Methodological validation confirmed its specificity, reliability and convenience. Screening results for *Radix Angelicae Dahuricae* and *Fructus Cnidii* obtained using VSMC/CMC-MEPS-GC–MS were consistent with those obtained using VSMC/CMC-offline-GC–MS. MEPS connection plays as simplified solid-phase extraction and replaces the uncontrollable evaporation operation in reported offline connections, so our new method is supposed to be more efficient and reliable than the offline ones, especially for compounds that are volatile, thermally unstable or difficult to purify. In application, senkyunolide A and ligustilide were preliminary identified as the volatile active components in *Rhizoma Chuanxiong*. We have thus confirmed the suitability of VSMC/CMC-MEPS-GC–MS for volatile active compounds screening in NP.

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## 1. Introduction

In modern drug development, new chemical entities are selected from hundreds of thousands of candidates [1]. The introduction of natural products (NPs) into screening sets has increased the diversity of chemical scaffolds compared with synthetic libraries and provided an indispensable source of novel lead compounds [2]. The inclusion of NP in libraries not only expands the scope of screening, but also enormously increases the workload. Classical strategies to identify potentially active components in NP include purification/identification, pharmacological assays in cells/tissues/animals, virtual docking and metabolic analysis. These techniques are supported by artificial derivatization and verification based on bioactivity and are costly and inefficient [3–7]. An understanding of the chemistry and pharmacology of the components of NPs is essential in choosing appropriate isolation and screening objects [8] (as in the discovery of artemisinin, from *Artemisia annua* L. [9]). Improvement of NP screening methods,

*Abbreviations:* BMC, bio-membrane chromatography; CMAC, cellular membrane affinity chromatography; CMC, cell membrane chromatography; FC, *Fructus Cnidii*; HCS, high content screening; HTS, high-throughput screening; IAMC, immobilized artificial membrane chromatography; IP, imperatorin; LC, liquid chromatography; LCC, L-type calcium channels; MEPS, microextraction by packed sorbent; NC, nicotine; NM, nimodipine; NP, natural products; OS, osthole; RAD, *Radix Angelicae Dahuricae*; RBA, radio-ligand binding assay; RC, *Rhizoma Chuanxiong*; RPLC, reverse-phase liquid chromatography; SCDE, supercritical carbon dioxide extraction; SD, Sprague-Dawley (rat); SPE, solid-phase extraction; SR, sorafenib tosylate; TIC, total ion current; Tris, Tris–hydroxymethyl aminomethane; VSMC, vascular smooth muscle cells.

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in terms of both specificity and efficiency, is still an important issue. Some modern strategies, such as high-throughput screening (HTS) and high-content screening (HCS) [10,11], are useful for accelerating screening but need pure compound samples, which presents a challenge with current NP purification techniques.

The introduction of biomembrane chromatography (BMC) has facilitated screening of NPs. BMC uses immobilized biomacromolecules as the stationary phase and combines features of high-performance liquid chromatography (HPLC) and affinity chromatography [12]. It can simulate interactions in a physiological environment and shows advantages in terms of dynamic characterization, efficiency and convenience compared with classical radio-ligand binding assays (RBA). Immobilized artificial membrane chromatography (IAMC) and cellular membrane affinity chromatography (CMAC) developed by Wainer *et al.* are two important examples of BMC. Both have good specificity and reproducibility [13,14]. Another important type of BMC is cell membrane chromatography (CMC) [15]. CMC maintains the biological characteristics of the cell membrane and has proved to be a reliable bioanalytical method. Biomacromolecules, including receptors, ion channels and enzymes, which are embedded in cell membranes, participate in cellular activities such as intercellular communication, transmembrane signal transduction and substance transport. Some of these biomacromolecules are important drug targets and the drug must act at the target site within the cell membrane. The chromatographic stationary phase that contains live cell membranes can mimic the physiological process and selectively bind active compounds in sample, making CMC a suitable technique for the identification of active components in complex sample matrices, such as NPs [16–19].

In this study, we focused on finding simpler purification procedures for potentially active volatile components of NP using CMC screening. Gas chromatography–mass spectrometry (GC–MS), the preferred method for analysis of complex volatile samples, allows rapid preliminary separation and qualitative identification [20]. Some methods that combine CMC with GC–MS in an offline mode have previously been reported [21,22]. Although such methods are feasible, they are time-consuming and labor-intensive. Thermal decomposition and volatilizing loss of volatile analytes during the concentration of CMC-retained fraction are also problems.

To overcome these shortcomings, we used microextraction by packed sorbent (MEPS) technology. MEPS is a promising method of sample preparation that has been introduced in recent years. MEPS is effectively a miniaturized version of solid-phase extraction (SPE) that uses a modified injection syringe and enrichment cartridge. The latter is a detachable syringe needle incorporating a metal cell filled with in-tube sorbent particles. Analytes can be applied to the cartridge in a large sample volume and eluted in a much smaller volume that can be injected directly into an LC or GC instrument. Analytes in low concentrations, such as organics in environmental water sample, can be quickly and efficiently concentrated using MEPS [23,24].

L-type calcium channels (LCCs) are voltage-dependent transmembrane $Ca^{2+}$ channels that are involved in vasoconstriction and vasodilation, making them an interesting therapeutic target for the treatment of cardiovascular diseases [25]. LCC are abundant on vascular smooth muscle cells (VSMCs), the main structural component of the blood vessel wall controlling vasomotion. The first report of an analytical method to detect volatile compounds in NPs by VSMC/CMC and GC–MS was published by Hou *et al.* [26]. This method adopted an inconvenient, offline process. In the present study, VSMC membranes were used to produce the stationary phase of the VSMC/CMC module. VSMC/CMC, MEPS and GC–MS were integrated to provide a new qualitative method for detecting active volatile compounds in NP that act on LCC. We aimed to improve on the existing CMC-offline-GC–MS method by simplifying the procedure and increasing efficiency.

## 2. Material and methods

### 2.1. Chemicals and materials

Sprague-Dawley (SD) rats were from the Laboratory Animal Center, College of Medicine, Xi'an Jiaotong University (Xi'an, China). Silica gel (ZEX-II, 5 μm diameter, 200 Å pore size) was from Meigao Co., Ltd. (Qingdao, China). Analytical-grade NaCl, KCl and $MgSO_4$ were from Hongyan Chemical Factory (Tianjin, China). Analytical-grade hydrochloric acid was from Beijing Chemical Works Co., Ltd. (Beijing, China). Tris–hydroxymethyl aminomethane (Tris) was from Sino-American Co. (Zhengzhou, China). HPLC-grade methanol was from Spectrum Chemical MFG Co. (Gardena, New Brunswick, USA). The reference substances of nimodipine (NM), nicotine (NC), imperatorin (IP) and osthole (OS) were from the National Institute for Food and Drug Control (Beijing, China). The reference substance of sorafenib tosylate (SR) was from An'ge Co., Ltd. (Nanjing, China). Samples of *Radix Angelicae Dahuricae* (RAD), *Fructus Cnidii* (FC) and *Rhizoma Chuanxiong* (RC) were from the Xiaozhai Branch of Wanbaiquan Pharmacy in Xi'an and were authenticated by the Department of Pharmacognosy, Xi'an Jiaotong University (Xi'an, China). Reference samples have been deposited in the specimen room at the Institute of Materia Medica, School of Pharmacy, Health Science Center, Xi'an Jiaotong University (Xi'an, China).

### 2.2. Instruments

The CMC column (10.0 mm length × 2.0 mm inside diameter) was from Biocomma Co., Ltd (Shenzhen, China). The RPL-10ZD column loading machine was from Replete Co., Ltd. (Dalian, China). The VSMC/CMC instrument, constructed on the basis of a conventional HPLC system, consisted of an LC-10AT VP pump from Shimadzu Co. (Kyoto, Japan), a Shimadzu SPD-M10A VP diode array detector (DAD), an HT-230A column oven from Heng'ao Co., Ltd (Tianjin, China) and a Rheodyne 7725i injection valve from IDEX Health & Science Co. (WA, USA). An MEPS C18 cartridge (8 μL volume, octadecyl silane sorbent, 45 μm diameter, 60 Å pore size), loaded onto a 100 μL MEPS syringe made by SGE Analytical Science Pty. Ltd. (Ringwood, Australia), was used for target component enrichment prior to GC–MS injection. The GC–MS unit was a Shimadzu GCMS QP-2010 Plus gas chromatograph–mass spectrometer with GCMS solution workstation software (Version 2.50 SU1) and a NIST 08 mass spectrogram library.

### 2.3. Preparation of samples

Stock solutions ($1\,mg\,mL^{-1}$) of NM, NC, SR, IP and OS were prepared in methanol, respectively. A standard solution of each compound ($0.2\,mg\,mL^{-1}$) was prepared by dilution of the stock solution with methanol. Aqueous solutions of NM, IP and OS were prepared by dissolving the compound (1 mg) in methanol (100 μL), and then slowly diluting with deionized water (250 mL) with rapid stirring to reach a final concentration of $4\,\mu g\,mL^{-1}$. Each solution was serially diluted with deionized water to give concentrations of 2, 1, 0.5 and $0.25\,\mu g\,mL^{-1}$. The supernatants from these aqueous solutions were collected after centrifugation (12,000 × *g*, 5 min) to prepare analog samples corresponding to the retained fractions eluted from the VSMC/CMC column. Supercritical carbon dioxide extraction (SCDE) of RAD, FC and RC was carried out in our own laboratory. All the extracts were respectively diluted to 0.1% (v/v) with methanol, and the supernatants from each extract solution

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