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On-line coupling of macroporous resin column chromatography with direct analysis in real time mass spectrometry utilizing a surface flowing mode sample holder

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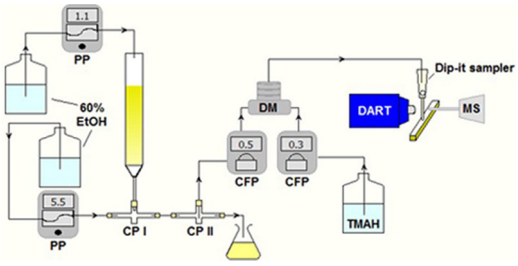
GRAPHICAL ABSTRACT



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ABSTRACT

A surface flowing mode sample holder was designed as an alternative sampling strategy for direct analysis in real time mass spectrometry (DART-MS). With the sample holder, the on-line coupling of macroporous resin column chromatography with DART-MS was explored and the new system was employed to monitor the column chromatography elution process of *Panax notoginseng*. The effluent from macroporous resin column was first diluted and mixed with a derivatization reagent on-line, and the mixture was then directly transferred into the ionization region of DART-MS by the sample holder. Notoginsenosides were methylated and ionized in a metastable helium gas stream, and was introduced into MS for detection. The on-line system showed reasonable repeatability with a relative standard deviation of 12.3% for the peak area. Three notoginsenosides, i.e. notoginsenoside R_1, ginsenoside Rb_1 and ginsenoside Rg_1, were simultaneously determined during the eluting process. The alteration of the chemical composition in the effluent was accurately identified in 9 min, agreeing well with the off-line analysis. The presented technique is more convenient compared to the traditional UPLC method. These results suggest that the surface flowing mode DART-MS has a good potential for the on-line process monitoring in the pharmaceutical industry.

1. Introduction

Macroporous resin column chromatography (MRCC) is widely used for the isolation and enrichment of bioactive components from complex sample matrices in the pharmaceutical industry [1]. Compared to other purification techniques, MRCC features the advantages of low cost, high adsorption capacity, high desorption ratio and easy regeneration. In most cases, the quality control of MRCC relies on the off-line HPLC or TLC analysis at regular time intervals [2,3]. However, due to the delay in such off-line analysis, it is hardly practical to achieve real-time monitoring and thus provide feedback control to the MRCC process.

Direct analysis in real time mass spectrometry (DART-MS) has evolved as a powerful analytical technique since its introduction in

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early 2005[4], and its applications to the quality control of natural products have been extensively explored [5–10]. DART-MS requires no or little sample pretreatment, and the direct and rapid detection makes it an ideal tool for process monitoring [11]. While DART-MS has been applied to off-line detection [12,13], on-line quality monitoring has seen little success due to the low-level automation in the original DART sampling strategy. The whole process was manually operated in a typical DART sampling method as follows. A glass rod was usually first dipped into the sample before it was placed in the gas stream for ionization [14]. In recent years, several commercial and home-made auto-samplers have been employed to deliver sample automatically into the ionization region of DART [15,16]. However, these approaches still lack the capability to perform in a continuous fashion, since every sample has to be individually deposited onto a different glass rod. An experienced analyst can, up till now, run a maximum of 96 samples completely automated per run. Consequently, for the practical use of DART-MS in the MRCC process, it is in great need to develop a new DART sampling method that allows for convenient continuous analysis.

A number of groups have studied the development of alternative sampling strategy for DART-MS. Eberherr et al. [17] developed a simple interface that enabled the on-line coupling of HPLC with DART-MS. A stainless steel or fused silica capillary was used to transfer an HPLC effluent directly into the DART ionization region. A stable liquid jet was obtained with a flow rate of 0.3–1.6 mL min^{-1} with an inner diameter of the capillary at 50–150 μm. Chang et al. [18] applied similar methodology to the HPLC analysis of jasmonic acid enantiomers, but only found peak tailing at a flow rate of 1.0 mL min^{-1}. The peak broadening was presumably caused by the residual liquid in the capillary tip. In order to reduce the broadening, a TEE junction was introduced between the HPLC column and fused silica capillary to split the effluent. The experiments showed that the effluent from the capillary was ionized efficiently without liquid residual at a splitting ratio of 12:1. In these systems, high pressure offered by HPLC facilitated the formation of a constant liquid stream in the capillary. In contrast to HPLC, MRCC is often performed under atmospheric pressure, where a stable liquid flow is hard to form without pressure assistance if a capillary is used to transfer the MRCC effluent. The MS inlet will thus risk of contamination by liquid drops formed in the end of the capillary. Thus far, no effort has yet been reported in combining atmospheric MRCC with DART-MS.

In the present work, we design a surface flowing mode sample holder to achieve the on-line coupling of MRCC with DART-MS, and the approach is evaluated on the column chromatography of *Panax notoginseng* (*P. notoginseng*). *P. notoginseng* is widely used for the prevention and treatment of cardiovascular diseases in traditional Chinese medicine. The main bioactive substances of *P. notoginseng* are notoginsenosides, including notoginsenoside R_1, ginsenoside Rb_1 and ginsenoside Rg_1 among others, as shown in many pharmacological studies [19]. Based on the chemical properties, such as polarity and molecular weight, of these notoginsenosides, macroporous resin D101 has frequently been selected to separate and purify them from *P. notoginseng* extracts [20,21].

2. Material and methods

2.1. Chemicals, samples and sorbent

Acetonitrile and acetic acid of HPLC grade were purchased from Merck (Darmstadt, Germany) and ROWE Scientific (Wilmington, DE, USA), respectively. Tetramethylammonium hydroxide (TMAH, 25% in methanol) and ethanol of analytical grade were obtained from Aladdin (Shanghai, China) and Lingfeng Chemical Reagent Co., Ltd. (Shanghai, China), respectively. Standards of notoginsenoside R_1, ginsenoside Rb_1 and ginsenoside Rg_1 (Fig. 1) were purchased from Ronghe Pharmaceutical Technology Development Co., Ltd. (Shanghai, China). Deionized water was prepared by a Milli-Q water purification system (Milford, MA, USA). *P. notoginseng* was obtained from Yunnan Medicinal Materials Co., Ltd (Yunnan, China), and macroporous resin D101 was supplied by the chemical plant of Nankai University (Tianjin, China).

2.2. Preparation of P. notoginseng extracts

P. notoginseng was pulverized and sieved through a 20-mesh size screen to give a powder. *P. notoginseng* powder (11.0 kg) was extracted three times with ethanol (60%, 144 L) at 50 °C. The extracts were combined, purified by filtration, and concentrated by drying down the ethanol solvent. The obtained extracts were diluted with deionized water (1:7, v/v). After standing still for 24 h, the mixture was filtered by vacuum filtration to give *P. notoginseng* extract for subsequent experiments.

2.3. Resin column chromatography

The column chromatography of *P. notoginseng* was performed on a glass column (25 cm × 14 mm i.d.) wet-packed with 13.5 g macroporous adsorption resin. The chromatographic column was pretreated as follows: the column was first activated with 95% ethanol and equilibrated with deionized water. Subsequently, *P. notoginseng* extract (5.0 mL) was loaded onto the pretreated column and washed with deionized water at 1.1 mL min^{-1} for 2 h. The notoginsenoside-rich extract was further eluted with 60% ethanol at the same flow rate for 2 h. Finally, the column was regenerated by 95% ethanol at a flow rate of 0.36 mL min^{-1} overnight.

2.4. On-line derivatization and detection system

Fig. 2 shows the on-line derivatization and the DART-MS detection device for the *P. notoginseng* chromatographic process. A peristaltic pump was used to deliver eluting solvent into the resin column (1.1 mL min^{-1}), and a second pump was employed to add the dilution solvent (60% ethanol) into the cross pipe I at a flow rate of 5.5 mL min^{-1}. After mixing, the diluted effluent flowed into the cross pipe II where the solution was delivered by a constant flow pump into the dynamic mixer at 0.5 mL min^{-1}. Simultaneously, a derivatization reagent (25% TMAH in methanol) was pumped into the dynamic mixer by another constant flow pump at 0.3 mL min^{-1}. Through a PEEK tube (100 cm × 0.5 mm i.d.), the mixture was then transferred onto the surface flowing-mode sample holder of DART. The sample holder was constructed on the basis of an Ionsense DIP-it sampler (Saugus, MA, USA). The DIP-it sampler consists of a hollow plastic handle and a glass tube. The PEEK tube carrying the sample solution was fixed into the inner side of the plastic handle, and two symmetrical holes (2 mm i.d.) were drilled in the lower end of the handle. Liquid samples from PEEK tube first flowed into the plastic handle. When the fluid level reached the drilled holes, the sample solution automatically effused through the holes and distributed over the glass tube. Continuous sampling was therefore achieved. A track support was utilized to carry the modified DIP-it sampler. For each 1.5 min, the track support structure was programmed to execute the predetermined movements as follows: (1) sweep of the glass tube through the ionization region at 0.2 mm s^{-1} for MS detection, (2) return to the starting point at a rate of 4 mm s^{-1}. Meanwhile, the fraction from the cross pipe II was carefully collected every 10 min, and was subjected to off-line UPLC analysis after filtration with a 0.22 μm filter membrane. Before and after each experiment, the flowing-mode sample holder was washed with 60% ethanol for at least 15 min.