Journal of Chromatography A, 1192 (2008) 17–24

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Separation of sanguinarine and chelerythrine in *Macleaya cordata* (*Willd*) *R. Br.* based on methyl acrylate-co-divinylbenzene macroporous adsorbents

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ARTICLE INFO

Article history:
Received 15 December 2007
Received in revised form 10 March 2008
Accepted 13 March 2008
Available online 19 March 2008

Keywords:
Sanguinarine
Chelerythrine
Macroporous adsorbent
Gradient eluent
Adsorption affinity

ABSTRACT

Sanguinarine (SAN) and chelerythrine (CHE) are known as major effective components in the quaternary benzo[c]phenanthridine isoquinoline alkaloids (QBA) fraction of *Macleaya cordata* (*Willd*) *R. Br.* but possess different biological activities. In this study, a method for the separation of SAN and CHE based on methyl acrylate-co-divinylbenzene (MA-co-DVB) macroporous adsorbents was established. The relationship between the polarities of the adsorbents and their adsorption–desorption behaviors towards SAN and CHE was investigated. The results showed that, among three selected commercial adsorbents and seven synthesized macroporous polymeric adsorbents with different MA content, the adsorbent No. 5 with 50% MA content provided the best separation power, and the two alkaloids were separated successfully in a gradient eluent process with 60% (v/v) ethanol aqueous and 80% ethanol aqueous contained 8% acetic acid. Dynamic adsorption and desorption tests had been performed in the column packed with the adsorbent No. 5 for optimizing the process parameters. Under the optimized conditions, the ratio of SAN and CHE transformed from 2:1 in the QBA fraction of *M. cordata* to 1:13 and 25:1 in the products obtained from the two-step gradient elution, and the recoveries of both SAN and CHE were nearly 90%.

1. Introduction

Macleaya cordata (*Willd*) *R. Br.*, one of the Macleaya genus plants in Papaveraceous family, has attracted much attention because of its bioactive alkaloid contents. The most important bioactive components of *M. cordata* are sanguinarine (SAN) and chelerythrine (CHE), associated with a small quantity of other alkaloids such as protopine and allocryptopine [1,2]. Both of SAN and CHE are members of the quaternary benzo[c]phenanthridine isoquinoline alkaloids (QBA). *M. cordata* has been used in traditional medicine (North America, Europe, China) owing to its biological activities [3], such as antimicrobial, antifungal, anti-inflammatory, adrenolytic, sympatholytic, local anesthetic effects and so on [4–6]. It is reported that the QBA fraction of *M. cordata* is able to treat the cervical carcinogenesis and thyroid cancer [7] according to the clinical research. The potential use of the alkaloids in *M. cordata* as anticancer drugs has attracted much interest.

In recent years, it has been realized that although SAN and CHE are structurally homologous (as illustrated in Fig. 1) [8,9], they do not have identical pharmacological activities [10]. SAN is an antiplatelet agent [11], and also is a potent inhibitor for Na-K-dependent ATPase, cholinesterase [12], NF-κB and mitogen-activated protein kinase phosphatase-1 [13,14]. Its chloride is capable of lowering the levels of both ICAM-1 and VCA-1 [15]. On the other hand, CHE is identified as an inhibitor of BclXL function [16], and a specific inhibitor of protein kinase C [17,18]. Furthermore, it is reported that the cytotoxicity of SAN is not the same as that of CHE [19]. All of the above makes it extraordinarily indispensable to do pharmacological and toxicological experiments on SAN and CHE, respectively, in order to investigate their definite bioactivities. Undoubtedly, it is important that a cost-effective method for the separation of SAN and CHE must be developed to supply sufficient samples to the in-depth research, and further applications in anticancer drugs.

Up to now, although the production of SAN from elicited plant cell culture has been reported [20], the most important resource of SAN and CHE is the extract from natural plants. A number of methods for the extraction of the QBA fraction of *M. cordata* have been available in the large-scale industrial production [21]. But the reports of the separation of SAN and CHE are few. The most effective process for the separation of analogue compounds like SAN and CHE is the chromatography method, such as preparative column chromatography (PCC), high-speed counter-current chromatography (HSCCC) [22–24] and so on. But when an amount of samples was prepared, the chromatography methods would consume a lot of time, manpower and material resources due to its relatively low handling capacity in one cycle, and are not suitable for the large-scale industrial production. Recently, separation and purification

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doi:10.1016/j.chroma.2008.03.039

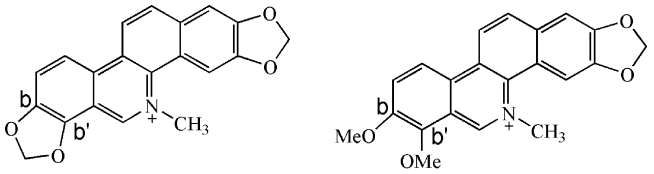

Fig. 1. Structures of SAN and CHE.

methods based on the macroporous polymeric adsorbents have been widely applied in pharmaceutical applications [25–30] for its simple procedure, high efficiency, low-cost and little pollution.

The separation of the macroporous adsorbents is based on differences in the affinity for the adsorbent towards the adsorbates, which depends on the molecular weight, polarity and so on [31]. The hydrophobic structures of SAN and CHE molecular are extremely similar, so the hydrophobic interactions between them and adsorbents are almost the same. On the other hand, the two alkaloids are both quaternary amine, thus adsorbents with special function groups, of which specific affinities are hydrogen-bonding interactions, electrostatic interactions, cannot achieve the separation of the two alkaloids. As shown in Fig. 1, the difference in the molecular structure between SAN and CHE is in the positions b and b′, where SAN has a cyclic ether group, and CHE has two methoxyl groups. As a result, the two alkaloids have different polarities. Adsorbates with different polarities can be adsorbed onto the adsorbents due to the synergistic effect of the hydrophobic and dipole interactions, and the adsorbate with a higher polarity in the system has a greater tendency to be desorbed by a polar desorption solution, owing to the easier breakage of the dipole interactions which depends on the magnitude of polarity. But when the hydrophibic interaction is too strong, the differences on the polarity cannot fully express.

In this study, three conventional adsorbents were selected and a series of methyl acrylate-co-divinylbenzene (MA-co-DVB) macroporous polymeric adsorbents were synthesized in order to separate SAN and CHE based on their different polarities. The hydrophobic interaction was weakened by the introduction of ester groups to the hydrophobic matrix of the synthesized adsorbents, and dipole interactions were lead inside. The total affinity contained hydrophobisc and dipole interactions were adjusted via adjusting the proportion between MA and DVB in synthesis course, and the resolution (R) was heightened as a result. Hereby, the difference on the polarity between SAN and CHE expressed in a gradient elution, and the two alkaloids were separated under the atmospheric pressure. The separation process was simply in manipulation, low-cost, pollution-free, and provided a method for the large-scale production of the individual alkaloids.

2. Experimental

2.1. Materials

Methyl acrylate (MA, analytical-reagent grade) and divinylbenzene (DVB, purity: 80%) were obtained from the Chemical Plant of Nankai University (Tianjin, China). 2,2′-Azobisisobutyronitrile (AIBN), toluene, liquid paraffin, ethanol, acetic acid, triethylamine and phosphoric acid were purchased from Tianjin Chemical Co. and were all in analytical-reagent grade.

The standards of SAN and CHE were purchased from J&K Chemical (Beijing, China). Acetonitrile of HPLC grade was purchased from Concord Technology (Tianjin, China). All solutions prepared for HPLC were filtered through 0.45 μm nylon membranes before use.

The QBA fraction of *M. cordata* (*Willd*) *R. Br.* was purchased from CAMAS Technologies (Broomfield, CO, USA), in which SAN and CHE were 37.6% and 18.2% in weight, respectively, analyzed by HPLC.

2.2. Pretreatment of commercial adsorbents

Macroporous adsorbents Amberlite XAD-4 and XAD-7 were purchased from Rohm and Haas (Philadelphia, PA, USA). Macroporous adsorbent ADS-17 was purchased from Nankai Hecheng S&T (Tianjin, China). The physical properties of the adsorbents were summarized in Table 1. The adsorbents were eluted with light petroleum (b.p. 60–90 °C) to remove the monomers and porogenic agents trapped inside the pore, and then dried at 60 °C under vacuum [32]. Prior to the adsorption experiments, weighed amounts of adsorbents were soaked in ethanol and subsequently washed by deionized water thoroughly [33].

2.3. Synthesis of the adsorbents with different MA content

The adsorbents were prepared using a suspension polymerization method. An organic solution composed of MA, DVB, porogenic agent (toluene, 16.7%, w/w, and liquid paraffin, 33.3%, w/w) and initiator AIBN (0.5%, w/w) was mixed with the aqueous solution composed of polyvinyl alcohol (1%, w/w) and NaCl (5%, w/w) in a 1000 mL three-necked flask equipped with a mechanical stirrer, a reflux condenser and a thermometer. The flask was heated by a programmed heater. The mixture was stirred to give a suspension of oil beads with a suitable size on the aqueous solution (100–120 rpm), then heated at 65 °C for 5 h and kept at 85 °C for 5 h. The copolymer beads were filtered out and washed with a large amount of hot water and ethanol in sequence, and finally packed in a Soxhlet extractor eluted with light petroleum (b.p. 60–90 °C) for 6–8 h. The beads were then taken out and air-dried.

The proportion between MA and DVB was changed in the synthesis process to obtain polymeric adsorbents with different hydrophobic affinities. The adsorbents were named No. 1, No. 2, No. 3, No. 4, No. 5, No. 6, No. 7, corresponding to 70%, 65%, 60%, 55%, 50%, 45%, 20% MA content in weight, respectively.

2.4. Determination of the adsorbents' physical parameters

2.4.1. Determination of pore structure parameters

Pore structure parameters of the adsorbents were measured using an automatic surface area and pore size analyzer (Autosorb-1MP, Quantachrome Instruments, Boynton Beach, FL, USA) based on the BET nitrogen adsorption method.

2.4.2. Determination of moisture content imbedded in the adsorbents

The hydrated adsorbents disposed of deionized water were weighed accurately and then dried in an oven at 110 °C until constant weight. The following equation was used to calculate the moisture content imbedded in the adsorbent.

$$\alpha = \frac{W_{\text{wet}} - W_{\text{dry}}}{W_{\text{wet}}} \times 100\% \quad (1)$$

where α is the moisture content imbedded in the adsorbent (%), W_{wet} is the weight of the hydrated adsorbent (g), W_{dry} is the weight of the dry adsorbent (g).

2.5. HPLC analysis of SAN and CHE

The standards of SAN and CHE were dissolved in methanol. A Waters HPLC system (Waters, Milford, MA, USA) consisting of Waters 510 HPLC pump and Waters 484 UV detector was used for