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Synthesis and study of a molecularly imprinted polymer for the specific extraction of indole alkaloids from *Catharanthus roseus* extracts

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ABSTRACT

Two molecularly imprinted polymers (MIP) for catharanthine and vindoline have been synthesized in order to specifically extract these natural indole alkaloids from *Catharanthus roseus* by solid-phase extraction (SPE). Each MIP was prepared by thermal polymerisation using catharanthine (or vindoline) as template, methacrylic acid (or itaconic acid) as functional monomer, ethylene glycol dimethacrylate (EDMA) as cross-linking agent and acetonitrile (or acetone) as porogenic solvent.

For catharanthine-MIP, a SPE protocol (ACN–AcOH 99/1 washing and MeOH–AcOH 90/10 elution) allows a good MIP/NIP selectivity (imprinting factor 12.6). The specificity of catharanthine-MIP versus related bisindole alkaloids was assessed by cross-reactivity study. The catharanthine-MIP specifically retained catharanthine and its N-oxide analogue but displayed a weak cross-reactivity for other Vinca alkaloids (vinorelbine, vincristine, vinblastine, vindoline, vinflunine). It appears that the catharanthine-like unit of these molecules are hardly trapped in catharanthine cavities located in the MIP, probably due to the sterical hindrance of the vindoline moiety. Finally, the MIP-SPE applied to *C. roseus* extract enabled quantitative recovery of catharanthine (101%) and the total removal of vindoline. Its capacity was determined and was equal to 2.43 μmol g^{-1}.

Vindoline is a weaker base than catharanthine, so the vindoline-MIP was achieved with a strong acidic monomer (itaconic acid) to increase vindoline–monomer interactions and a modified washing solvent (ACN–HCOOH 99/1) to reduce non-specific interactions. The influence of the amount of HCOOH (protic modifier) percolated during the washing step upon the elution yield and the imprinting factor for vindoline was investigated. This preliminary optimisation of the washing step, and in particular the number of moles of acid percolated, seems useful to emphasize the use of MIP in conditions of high selectivity or high yield. A compromise was obtained with an imprinting factor equal to 7.6 and an elution recovery of 33%. However MIP-vindoline failed to achieve a specific extraction of vindoline since catharanthine was also extracted probably because of strong non-specific interactions occurring between catharanthine and the sorbent.

1. Introduction

Several terpenoid indole alkaloids contained in the *Catharanthus roseus* leaves have shown therapeutic properties of great interest (Fig. 1). Thus, catharanthine and vindoline are the most abundant constituents in this plant extract (3.8 and 7.7% w/w, respectively). These two monomeric indole alkaloids are included in the structures of two natural bisindole alkaloids, vinblastine and vincristine, which are used in cancer chemotherapy. Furthermore, these two monomeric indole alkaloids are used for the semi-synthesis of two anti-cancer drugs (vinorelbine and vinflunine) [1–6]. Vinorelbine is recommended in the treatment of advanced human non-small-cell lung cancer and breast cancer [7]. Vinflunine, a fluorinated analogue of vinorelbine, is now in phase III trials, for the treatment of bladder cancer [8–10].

As catharanthine and vindoline are the direct precursors of vinblastine and vincristine antitumor molecules, their extraction from *C. roseus* leaves has widely been investigated by different methods developed during the last decade. Thus, centrifugal partition chromatography was applied successfully to a crude mixture of *C. roseus* alkaloids [11]. This separation technique is based on the difference in pK_a values between analytes. As catharanthine has a pK_a significantly higher than vindoline, it is possible to extract separately these two alkaloids by varying amounts of hydrochloric acid and triethylamine in the mobile or station-

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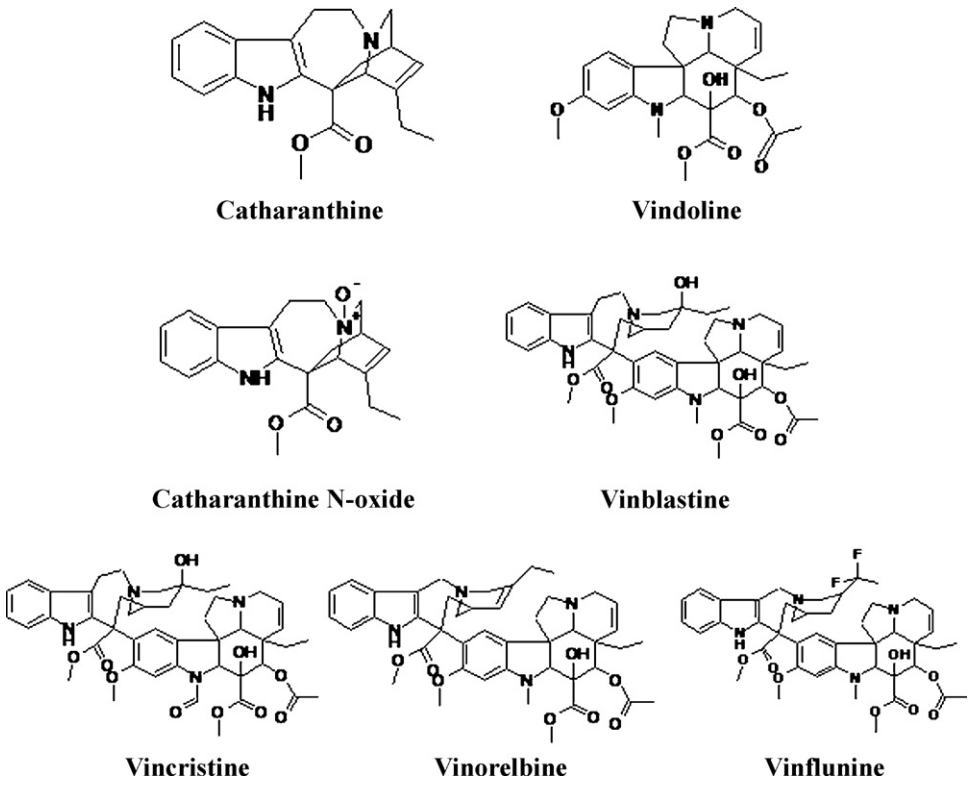

Fig. 1. Formulae of investigated Vinca alkaloids.

ary phases. The basic character of these two monomeric indole alkaloids has also been utilized during the solid–liquid extraction of dried leaves of *C. roseus* under acidic conditions [12,13]. Moreover, Verma et al. [14] have compared various quantitative extraction techniques such as supercritical fluid extraction, Soxhlet extraction, ultrasound-assisted solid–liquid extraction and hot water extraction. Catharanthine and vindoline were completely extracted by SFE with CO_2–methanol or by Soxhlet extraction with dichloromethane. The low recoveries obtained with other extraction techniques are due to the low solubility of the target molecules in the extraction solvents (water) or thermal degradation of molecules in the extraction conditions. Whatever the extraction technique employed, the selectivity of indole alkaloids extraction from the leaves of *C. roseus* is an important criterion.

In this study, we assessed the efficiency and specificity of new adsorbents, based on molecularly imprinted polymers (MIP) during solid–liquid extraction of catharanthine and vindoline from leaves of *C. roseus*. These highly cross-linked polymers display binding sites in cavities, able to recognize the template molecule which was introduced in the reactive mixture before polymerization. These cavities are complementary, both sterically and chemically, to the template molecule as well as to structural analogues, similarly to antibodies and enzymes having sites complementary of their antigens and substrates, respectively [15]. These tailor-made materials possess a high selectivity for a target molecule. SPE based on molecularly imprinted polymers have been already employed for the sample pretreatment of natural products prior to HPLC analysis, in particular triterpene acids in plant extracts [16,17].

To our knowledge, only Zhu et al. [18] developed a MIP dedicated to the specific extraction of a bis-indole alkaloid (vinblastine) from *C. roseus* extracts. The extraction was effective (recovery yield of 89% for vinblastine) and selective, as evidenced by the non-identification of matrix components into the MIP-SPE elution fractions.

In light of this first successful MIP-SPE of a bisindole alkaloid, we investigated the MIP solid-phase extraction of catharanthine and vindoline from a chloroform extract of *C. roseus* leaves.

An imprinted polymer of catharanthine was first synthesized by following a non-covalent approach. The methacrylic acid (MAA) has been selected as functional monomer to exchange hydrogen bonding/electrostatic interactions with basic molecules of catharanthine. After removal of the template, the imprinted polymer was used as SPE sorbent. The extraction protocol was developed on standard solutions of catharanthine and then on samples of *C. roseus* plant extracts. The main characteristic parameters of this polymer (selectivity, specificity, capacity, imprinting factor) were determined. Several Vinca alkaloids (catharanthine, catharanthine N-oxide, vindoline, vinorelbine, vinflunine, vinblastine and vincristine) were loaded on a synthesized catharanthine imprinted polymer to assess their selectivity and cross-reactivity.

By following a similar approach, two imprinted polymers of vindoline were synthesized by using two different monomers (methacrylic acid and itaconic acid) which differ by their acidic strength.

2. Experimental

2.1. Reagents

2.1.1. Reagents for the synthesis of MIPs

Methacrylic acid (MAA), itaconic acid (IA), ethylene glycol dimethacrylate (EDMA), 2,2′-azobisisobutyronitrile (AIBN), acetonitrile (>99.9% HPLC grade, ACN), acetone (HPLC grade) and chloroform (>99.9% HPLC grade) were provided by Aldrich (St.-Quentin-Fallavier, France). Before use, MAA was distilled under vacuum and AIBN was recrystallised from methanol.

The alkaloids (catharanthine sulfate, catharanthine N-oxide sulfate, vindoline, vinorelbine ditartrate, vinflunine ditartrate, vinblastine sulfate and vincristine sulfate) and a concentrated