Journal of Chromatography A, 1474 (2016) 59–70

Contents lists available at ScienceDirect

Journal of Chromatography A

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

Industrial case study on alkaloids purification by pH-zone refining centrifugal partition chromatography

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ARTICLE INFO

Article history:
Received 19 July 2016
Received in revised form 12 October 2016
Accepted 14 October 2016
Available online 22 October 2016

Keywords:
Centrifugal partition chromatography
Scale-up
pH-zone refining
Modelling
Alkaloids
Hydrodynamics

ABSTRACT

The industrial potential of pH-zone refining centrifugal partition chromatography has been evaluated by studying the purification of pharmaceutical ingredients at the pilot scale. For the first time, a scale up methodology based on both column capacity and mass transfer efficiency as invariants was developed. The purification of catharanthine and vindoline from an industrial crude extract of aerial parts of *Catharanthus roseus*, was used as a case of study. Toluene/CH_3CN/water (4/1/5, v/v/v) was selected as biphasic solvent system, triethylamine as retainer in the organic stationary phase and sulphuric acid as displacer in the aqueous mobile phase. The separation intensification was performed on a 36 mL CPC column equipped with 832 partition twin-cells. The combined effects of four parameters (displacer and retainer concentrations for intensive parameters, flow rate and rotational speed for extensive parameters) were studied by design of experiment in order to maximize both recoveries and productivities. Then, scale change was done on two larger columns (305 mL and 1950 mL of capacity) equipped with only 231 and 238 partition cells. For this step, it has been shown that the global mass transfer coefficient k_0a (the efficiency of a column design) and the stationary phase retention (the capacity of the column) were relevant and useful scale up invariants.

A CPC model based on acid-base equilibriums and interfacial mass transfer in continuously stirred tank reactors in series was used to predict fully separations on larger CPC column at the optimized operating conditions and to guide the CPC user in its scale-up strategy. The experimental validation on pilot CPC column, by injecting up to 150 g of *Catharanthus roseus* crude extract on the 1950 mL column highlighted the preservation of the separation quality, the non-linear character of the scale up in centrifugal partition chromatography and that a productivity of about 4 kg of processed crude extract per day can be reached by implementing developed methodology.

1. Introduction

Catharanthus roseus (L.) G. Don (Apocynaceae), the Madagascar periwinkle, contains a wide diversity of bioactive bis-indolomonoterpenic alkaloids, which have demonstrated highly interesting therapeutic properties [1–3]. Catharanthine and vindoline (Fig. 1) are the most abundant alkaloids in the aerial parts of this plant [4,5].

These two monomers are components of two natural dimers, vinblastine and vincristine, and two hemisynthetic ones, navelbin and vinflunin, which are used in anticancer chemotherapy [6,7]. The concentration of the natural bis-indole alkaloids being very low in the plant (0.001–0.003%) [8], intense research efforts have been made to find alternatives for the production of these drugs. For instance, the industrial production of vinblastine and vincristine, as well as the one of the semisynthetic dimers navelbin and vinflunin, is today generally achieved by hemisynthesis by using catharanthine and vindoline as precursors [9,10]. This method is efficient but requires selective and productive separation techniques allowing the purification of the precursor monomers from complex *Catharanthus roseus* crude extracts.

Centrifugal Partition Chromatography (CPC) is a separation technique, developed by Murayama et al. [11] and based on the par-

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http://dx.doi.org/10.1016/j.chroma.2016.10.039
0021-9673/

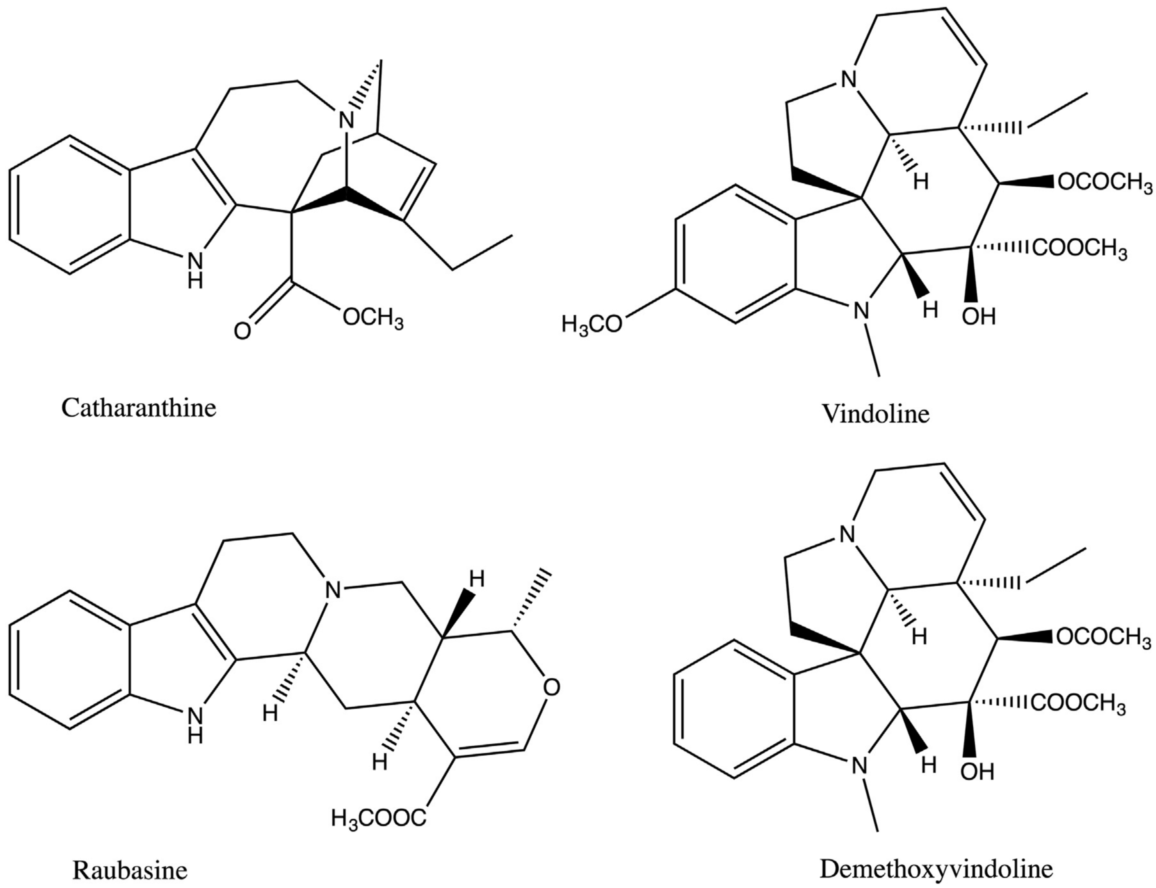

Fig. 1. Chemical structures of the target compounds.

titioning of solutes between at least two immiscible liquid phases [12]. A CPC column consists of a series of partition cells linked by ducts in cascade and arranged in a centrifuge (one axis, two rotary seals). One liquid phase, the stationary one, is maintained inside the column by the centrifugal acceleration field generated by the column rotation (50 to 800 times the gravity), while the other liquid phase, the mobile one, is pumped through it. There is thus no solid chromatographic support. As a consequence, CPC can generally support larger mass overload than silica-based chromatography, which normally should make it especially suitable for preparative or production applications. During the last decade, it was highlighted that high productivities can be reached with CPC, especially when used in the displacement mode [13–16]. The purification of indole alkaloids from *Catharanthus roseus* has already been studied at the preparative scale by using centrifugal partition chromatography in the pH-zone refining mode [17].

The "pH-zone refining" mode refers to a displacement mode dedicated to the separation of ionizable compounds [18] showing a dramatic polarity and solubility difference between their neutral and salt forms. It has been mainly applied to the purification of natural products, particularly alkaloids [19,20]. The principle of this technique is based on the use of a retainer introduced in the stationary phase and a displacer solubilized in the mobile phase in order to carry out the displacement chromatographic process. In the case of the purification of alkaloids in the descending mode (*i.e.* the lower aqueous phase with acidic displacer is the mobile phase and the upper organic phase containing a base – the retainer – is the stationary phase), the retainer must have a smaller apparent acidity constant K_a^{app} (defined as $K_a \times K_D$) than those of the alkaloids to deprotonate and force them to partition in the organic stationary phase [21]. The displacement process requires also a strong acid (sulphuric acid) in the mobile phase in order to displace the alkaloids as an isotachic train in the decreasing order of pK_a^{app}. Since the analytes are organized inside the column according to their respective acidity constants and hydrophobicity, the pH-zone refining mode can be a selective process. As a preliminary model of the process, the theoretical plates model (or Craig's model) was used and reactions were considered as instantaneous at the equilibrium [17,18,22]. In this model only thermodynamics had been taken into account when just a chemistry part (like displacer and retainer concentrations) was studied [23] with no evaluation of impact of operating conditions such as rotational speed or flow rate. Another model including mass transfer kinetics and interfacial reactions has been recently developed by Kotland, Chollet et al. ([24]) and it allowed the prediction of the concentration profile of the different species involved in the chromatographic process and the operating condition effects on the chromatogram for a binary separation (*i.e.* involving two alkaloids and an acidic displacer).

In the present study, the use of both mass transfer efficiency and column capacity as change scale invariants was studied to perform the scale up of an optimized separation by pH-zone refining in CPC. Scale-up of separations in CPC was historically considered to be simply proportional [14,16]. In recent works, scale change study integrating hydrodynamics aspects in CPC, when the latter is used in the displacement mode, showed that the scale change is not a linear phenomenon [25]. Another recent publication shows that a mastered scale-up can be performed by using mass transfer coefficient as similarity invariant (k_0a) [26]. This methodology will be implemented here and extended to chemical reactions (involving two alkaloid analytes, a retainer – considered as a third analyte – and a displacer), which occur during the pH-zone refining chromatographic process.