Separation Science and Technology
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713708471

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Online publication date: 08 July 2010

To cite this Article Boyadzhiev, L. and Yordanov, B.(2005) 'Pertraction of Indole Alkaloids from *Vinca minor* L', Separation Science and Technology, 39: 6, 1321 — 1329
To link to this Article: DOI: 10.1081/SS-120030485
URL: http://dx.doi.org/10.1081/SS-120030485

SEPARATION SCIENCE AND TECHNOLOGY
Vol. 39, No. 6, pp. 1321–1329, 2004

Pertraction of Indole Alkaloids from *Vinca minor* L.

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ABSTRACT

The liquid-membrane (pertraction) separation technique was applied for the recovery and preconcentration of the indole alkaloid vincamine from native acidic extracts of *Vinca minor* L. On the basis of the distribution coefficients of vincamine determined for eight couples of feed solution/membrane and membrane/strip solution, the most suitable pertraction three-phase system was found to be: native extract (acetate buffer)–trichloroethylene–hydrochloric acid solution. This system, applied in a glass laboratory pertractor, proved the possibility of the direct recovery and preconcentration of the alkaloid from the plant extract

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DOI: 10.1081/SS-120030485 0149-6395 (Print); 1520-5754 (Online)

without its alkalization. This permitted to integrate the processes of plant extraction and solute separation and preconcentration.

Key Words: Pertraction; Vincamine; Liquid membrane; Indole alkaloids.

INTRODUCTION

More than 40 alkaloids are present in the aerial parts of *Vinca minor*, the main of which is vincamine—an indole alkaloid of eburnamine type. Owing to its vasodilatating effect which improves the cerebral blood circulation and the assimilation of oxygen by the brain tissue, vincamine promotes the memory and the ability for mental concentration. It is therefore included in numerous medical preparations[1] and serves as a basis for several semisynthetic medicines with enhanced or modified physiological effect.

The recovery of vincamine from the dried plant leaves and its purification involve a sequence of separations, mainly extractive and chromatographic processes. Upon increasing the number of stages, the purity of the product increases, accompanied, however, by an increase in the product and solvent losses, the solvent regeneration expenses and the harmful ecological effects.

The pertraction or liquid membrane separation is a relatively novel and prospectful method for the selective separation and preconcentration of solutes from various liquid media.[2,3] The solute, usually present at a low concentration in the donor (feed) solution (F), is transferred to the acceptor (stripping) solution (R) by means of a liquid mediator called membrane (S). The latter is usually an organic solvent selectively dissolving the solute, while the donor and acceptor phases are aqueous solutions immiscible with the membrane liquid. The equilibrium conditions in the donor solution/membrane and membrane/acceptor solution couples promote the complete transfer of the solute from the donor to the acceptor solution. In this way some operations may be avoided and all processes may be performed in one apparatus.

Irrespective of the large number of publications in the field of liquid membrane processes there are only two studies on the use of liquid membranes for the recovery and purification of alkaloids. These studies deal with the recovery of nicotine from tobacco extracts using the emulsion membrane technique.[4,5] The membrane phase is a solution of *N*,*N*-di-2-octyl acetamide in sulfonated kerosene and the acceptor phase is 0.2 M sulfuric acid. The aim of the present study is to assess the applicability of pertraction for the recovery and preconcentration of the indolic alkaloid vincamine.

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MECHANISM OF VINCAMINE TRANSFER

Depending on the extraction process applied, vincamine and the accompanying alkaloids are present in the natural extract of *V. minor* either in the basic (B) or in the protonated form (BH^+). The former type is obtained when an organic solvent is used as an extractant; the latter, which is more frequent and more efficient—when the extraction process is carried out with an aqueous or an aqueous-alcoholic solution. For the further recovery and enrichment of the alkaloids from the native extracts of the second type, the acidic aqueous solution is alkalized to convert the protonated form to the basic one which is subsequently extracted with an organic solvent.

The use of an acetate buffer of pH about 4 as an extractant of the plant material[6] offers a possibility of shortening the above procedure. It this way vincamine is almost completely transferred to the organic phase from the acidic aqueous medium. Acetic acid is soluble not only in the vincamine aqueous donor solution, but also in the organic phase, where it is present exclusively in the non-dissociated form (H^+A^-). The latter partially exists in the aqueous solution as well. The protonated molecules of the alkaloid form with the acetic acid the ion-associated complex BH^+A^- according to the equation:

$$H^+A^-_{org} + BH^+_w \leftrightarrow BH^+A^-_{org} + H^+_w \quad (1)$$

This complex is also well soluble in the organic phase.

As stated by the author,[6] the process equilibrium depends on the concentration of acetic acid in the aqueous phase, on its solubility in the organic solvent used, and on the type and steric bonds of the alkaloid.

This scheme should be highly appropriate for the liquid-membrane recovery of the alkaloid. As the donor solution is not alkalized, it can be used after the removal of the alkaloid and some insignificant corrections, for the treatment of new raw material, i.e., the whole procedure can be performed as an integrated process.

In the present work an one-component membrane—trichloroethylene is used as a solvent of the complex (BH^+A^-). The acetic acid dissolved in the trichloroethylene binds vincamine according to the expression (1). Owing to its concentration gradient, the complex BH^+A^- is transferred across the membrane. At the second (membrane/acceptor) interface the complex is stripped into the acceptor phase R—an aqueous solution of a strong mineral acid. In this way a concentration gradient between the F- and R-phases is created which is the driving force of the whole process. In the strongly acidic R-phase most of the vincamine molecules are present as the neutral complex

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$B \cdot HCl$. Only a small part of them are in the protonated acidic form BH^+. A possible stripping mechanism is:

$$BH^+A^-_{org} + H^+_w + Cl^-_w \longleftrightarrow B \cdot HCl_w + H^+A^-_w \quad (2)$$

Acetic acid is practically not dissociated at this pH value and passes back into the membrane. The strong shift to the left of equilibrium (3) also contributes to the completeness of vincamine recovery:

$$B \cdot HCl_w \longleftrightarrow BH^+_w + Cl^-_w \quad (3)$$

After the appropriate membrane liquid was selected, the above described pertraction mechanism was experimentally proven.

EXPERIMENTAL

Materials

As a membrane phase the following organic solvents were used: chloroform (PPH POE, Gliwice, Poland), butylacetate, purum (Boron, Sofia, Bulgaria), 1-decanol, p.a. (Merck, Darmstadt, Germany), trichloroethylene, 99% (Valerus, Sofia, Bulgaria), carbon tetrachloride, p.a. (Gabex, Sofia, Bulgaria), 1,2-dichloroethane, purum (Valerus, Sofia, Bulgaria), hexane, p.a. (Valerus, Sofia, Bulgaria) and diisopropyl ether, p.a. (Merck, Darmstadt, Germany).

The acetate buffer was prepared from NaOH, p.a. (Khimsnab, Dimitrovgrad, Bulgaria) and acetic acid, p.a. (IChV-SU, Sofia, Bulgaria).

The acceptor solutions were prepared from hydrochloric acid, purum (Marvel, Plovdiv, Bulgaria).

Ammonium carbonate, p.a., (Boron, Sofia, Bulgaria) and methanol, super gradient (Labscan, Dublin, Ireland) were used for vincamine analysis.

The donor solution (F) was prepared by dissolving dry natural extract of *V. minor*—a yellowish-green powder with vincamine content of 0.042% (Elektra Extraction, Sofia, Bulgaria). The HPLC analysis was performed with an external standard of pure vincamine (>99%), (Covex, Madrid, Spain).

Method of Analysis

The concentrations of vincamine in the acetic acid-containing aqueous phase and in the organic phase were determined by HPLC using a 30 cm C18 Nucleosil 100-5 column, a UV-detector "Knauer" at a wavelength of 220 nm and an integrator "Shimadzu." The eluent was an 1 : 3 mixture

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of 0.01 N $(NH_4)_2CO_3$ and methanol at a flow rate of 0.30 mL min^{-1}. The hydrochloride complex of vincamine in the acceptor solution was destructed by solution alkalization. Ten milliliters of the corresponding phase were evaporated to dryness and after determining the dry weight of the residue it was dissolved in 10 mL of methanol. The samples were centrifugated for approx. 5 min at 3500–4000 min^{-1} and 20 μL portions were injected for analysis.

The equilibrium studies were performed by mixing 100 mL of the above-mentioned extract with 100 mL of the organic solvent for 30 min in a separatory funnel. After phase separation, 60 mL of the clear organic phase were taken—10 mL for analysis and 50 mL for back extraction with 50 mL of 0.7 N HCl in a separatory funnel under the same conditions. The pertraction of vincamine was performed in a glass laboratory pertractor of the bulk type "vessel in vessel," shown in Fig. 1. The organic phase S, which is heavier than the aqueous phases, fills the bottom part of the apparatus up to a level above the lower edge of the inner cylinder 2 whose bottom is open. The acceptor phase (R) fills the inner cylinder 2 on top of the organic phase while the donor phase (F) fills the space between the inner cylinder 2 and the outer cylinder 1. During the pertraction, the inner cylinder is set in rotation by

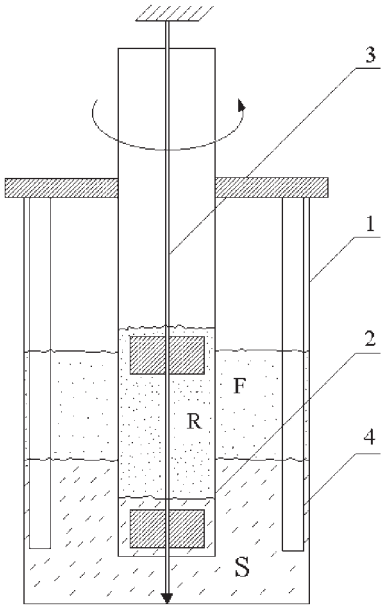

Figure 1. Laboratory glass pertractor: 1—Outer vessel. 2—Inner vessel. 3—Immobile axis. 4—Baffles.

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means of an electromotor. An immobile stirrer 3 is fixed along the vertical axis of the apparatus. The baffles 4 mounted close to the wall of the outer vessel favor the mixing of the F and S phases. In this way all phases both inside and outside cylinder 2 are simultaneously stirred by its rotation. The volume of the membrane phase was 230 mL, that of the donor phase—200 mL, and of the acceptor phase—50 mL. The F/S interface area was 49.73 cm^2 and that of the S/R interface—10.81 cm^2. The rotation velocity of the cylinder was 2 sec^{-1}.

RESULTS AND DISCUSSION

Equilibrium Studies

The equilibrium studies revealed the distribution coefficients of vincamine in the two-phase systems organic solvent/acetate buffer (Table 1). Diisopropyl ether, butylacetate, decanol, and hexane yielded distribution coefficients below 0.1 and were not suitable as membrane phases for the vincamine pertraction. Higher distribution coefficients were obtained with the chlorinated hydrocarbons (chloroform, dichloroethane, trichloroethylene, and carbon tetrachloride) which permitted their use as membrane liquids. The distribution coefficient of chloroform exceeds those of the other chlorinated hydrocarbons by at least two orders of magnitude.

The choice of the membrane liquid also depends on the completeness of solute stripping into the mineral acid used as an acceptor phase. The distribution coefficients of vincamine between the selected organic liquids and the acceptor phase are shown in Table 2.

Obviously, high values of the distribution coefficient are undesirable in this case, as they would lead to insignificant back extraction of the solute and lower efficiency of the overall process. From this point of view, trichloroethylene would be the most appropriate membrane phase, irrespective of the relatively low distribution coefficient of vincamine in the first pertraction stage.

Table 1. Distribution coefficient K of vincamine in the systems organic membrane/donor phase.

System	$K = C_S/C_F$
Chloroform/acetate buffer	>100
Dichloroethane/acetate buffer	0.96
Trichloroethylene/acetate buffer	0.78
Carbon tetrachloride/acetate buffer	0.75

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Table 2. Distribution coefficient K of vincamine in the systems organic membrane/acceptor phase.

System	$K = C_S/C_R$
Chloroform/0.7 N HCl	24
Dichloroethane/0.7 N HCl	0.23
Trichloroethylene/0.7 N HCl	0.02
Carbon tetrachloride/0.7 N HCl	0.33

Pertraction Studies

The donor solution (F) used in the above experiments was 0.5 M acetate buffer of pH 4 ($CH_3COOH + CH_3COONa$) containing 63 g L^{-1} of dry extract of *V. minor*. The initial concentration of vincamine in this solution was 0.0268 g L^{-1}.

As mentioned above, the acceptor phase was 0.7 N HCl and the membrane liquid was trichloroethylene. The experiments were performed under continuous stirring and samples were periodically taken for analysis. The solute concentrations were calculated taking into account the changes in the phase volumes after sampling.

Figure 2 presents the vincamine concentrations in all three phases as a function of time. At the end of the experiment, the concentration of vincamine in the acceptor phase (R) is higher by a factor of 90 towards the other two phases and by a factor of about 3.5 towards its initial concentration in the donor phase. Finally, 92.6% of the total vincamine content are transferred to the acceptor phase.

It should be noted that the prolonged duration of the experiment is due to the use of the bulk liquid membrane technique which is characterized by small interface areas and non-intense hydrodynamics resulting in low mass transfer coefficients.

CONCLUSION

The distribution coefficients of vincamine in the two-phase systems membrane–donor solution and membrane–acceptor solution were determined. Eight organic solvents were tested as membrane liquids: 1-decanol, butylacetate, diisopropyl ether, hexane, chloroform, 1,2-dichloroethane, trichloroethylene, and carbon tetrachloride. Chloroform was the best extractant of the alkaloid. From the point of view of the whole separation process, however, trichloroethylene was chosen as the membrane liquid.

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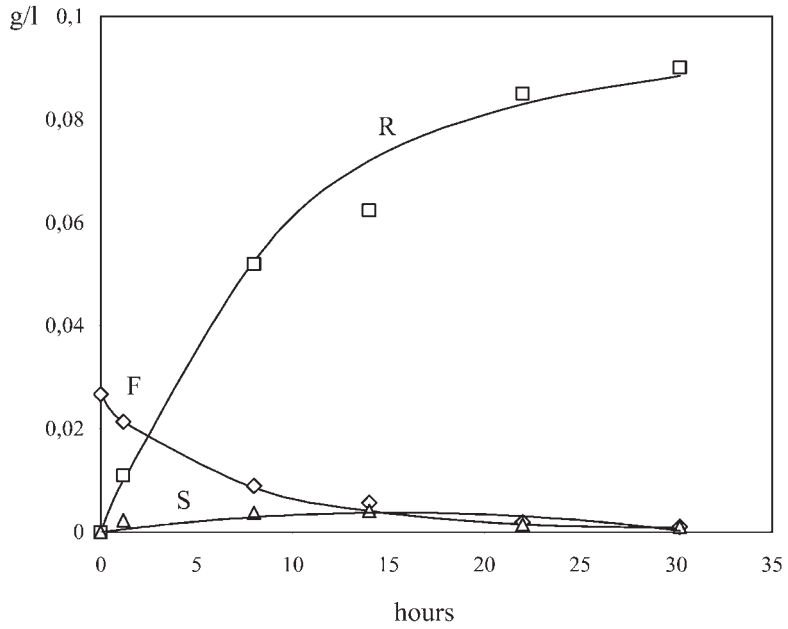

Figure 2. Change of the concentration of vincamine in the donor (F), membrane (S), and acceptor (R) phases depending on time. Initial phase ratios, F : S : R = 4 : 4.6 : 1. Rotation speed, 2 sec^{-1}.

The experiments performed in a glass laboratory pertractor of the bulk type "vessel in vessel" yielded a high recovery extent (over 92%) with simultaneous preconcentration of the alkaloid. The combination of liquid phases used permits the integration of the pertraction process with an efficient alkaloid extraction from the raw material using an acidic aqueous solution.

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Received April 2003
Revised October 2003