Claire Lopez[1]
Bérengère Claude[1]
Philippe Morin[1]
Martine Pelissou[2]
Richard Pena[2]
Jean-Paul Max[2]
Jean-Paul Ribet[2]

[1]Institut de Chimie Organique et Analytique, Université d'Orléans -CNRS FR 2708- UMR 6005, Orléans, France
[2]Département de Chimie Analytique, Institut de Recherche Pierre Fabre, Castres, France

Received January 6, 2011
Revised May 13, 2011
Accepted May 15, 2011

Research Article

Synthesis and study of a molecularly imprinted polymer for specific solid-phase extraction of vinflunine and its metabolite from biological fluids

A molecularly imprinted polymer (MIP) was synthesized in order to specifically extract vinflunine, an anticancer agent, and its metabolite (4-*O*-deacetylvinflunine) from bovine plasma and artificial urine by solid-phase extraction (SPE). Vinorelbine, a non-fluorinated analogue of vinflunine, was selected as a template for MIP synthesis. The selectivity of MIP versus the template (vinorelbine) and other alkaloids (catharanthine, vinblastine, vincristine, vinflunine and 4-*O*-deacetylvinflunine) was shown by a SPE protocol carried out with non-aqueous samples. A second protocol was developed for aqueous samples with two consecutive washing steps (AcOH–NH_2OH buffer (pH 7, $I = 10$ mM)–MeOH mixture 95:5 v/v and ACN–AcOH mixture 99:1 v/v) and an elution step (MeOH–AcOH mixture 90:10 v/v). Thus, MIP-SPE of bovine plasma brought high recoveries, 81 and 89% for vinflunine and its metabolite, respectively. This protocol was slightly modified for artificial urine samples in order to obtain a good MIP/NIP selectivity; furthermore, elution recoveries were 73 and 81% for vinflunine and its metabolite, respectively. Repeatability was assessed in both biological matrices and RSD (%) were inferior to 4%. The MIP also showed a suitable linearity (r^2 superior to 0.99), between 0.25 and 10 μg/mL for plasma, and between 1 and 5 μg/mL for artificial urine.

Keywords: Biological fluids / Metabolite / Molecularly imprinted polymer / SPE / Vinflunine
DOI 10.1002/jssc.201100015

1 Introduction

Vinca alkaloids, such as vinblastine and vincristine, are naturally occurring anticancer agents [1–5]. These molecules are dimers resulting from the coupling of two monomeric indole alkaloids, catharanthine and vindoline, present in *Catharanthus roseus* leaves (Fig. 1). These bisindole alkaloids were the first agents used for the treatment of diabetes [6]. Vinorelbine and vindesine are more recent semi-synthetic analogues. These agents are primarily used in combination with other cancer chemotherapeutic drugs for the treatment of a variety of cancers [7–9], including leukemias, lymphomas, advanced testicular cancer, breast and lung cancers, and Kaposi's sarcoma [10]. Thus, vinorelbine is recommended in the treatment of malignancies, advanced human non-small-cell lung cancer and advanced breast cancer [11, 12]. Otherwise, vinflunine is a new fluorinated Vinca alkaloid obtained by the use of superacid chemistry [13, 14]. Vinflunine was identified in preclinical studies as having *in vivo* antitumour activity against a large panel of experimental tumour models, with tumour regressions being recorded in human renal and small cell lung cancer tumour xenografts [15]. Its overall level of activity was superior to that of vinorelbine in many of the experimental models used [16, 18]. Therefore, these data provided a favourable preclinical profile for vinflunine, supporting its promising candidacy for clinical development, such as therapies in the case of bladder cancers [19, 20].

Various analytical methods such as high-performance liquid chromatography (HPLC) were set up to quantify vinorelbine and vinflunine in biofluids. Thus, HPLC with UV [21, 22], fluorescence [23–25] or electrochemical [26] detections were used to analyze vinorelbine and its main metabolite, 4-*O*-deacetylvinorelbine, in plasma and tumour cells. The very sensitive and specific liquid chromatography-tandem mass spectrometry (LC-MS/MS) was also successfully used to analyze vinorelbine, vinflunine and their metabolites in human blood, plasma, urine and faeces [27–29].

However, the analysis of compounds at low concentrations in complex matrices often requires sample pretreatments

Correspondence: Dr. Bérengère Claude, Institut de Chimie Organique et Analytique, Université d'Orléans, -CNRS FR 2708- UMR 6005, BP 6759 Rue de Chartres 45067 Orléans, France
E-mail: berengere.claude@univ-orleans.fr
Fax: +33-2-38417281

Abbreviations: AIBN, 2,2′-azobisisobutyronitrile; **EDMA**, ethylene glycol dimethacrylate; **IF**, imprinting factor; **MAA**, methacrylic acid; **MIP**, molecularly imprinted polymer

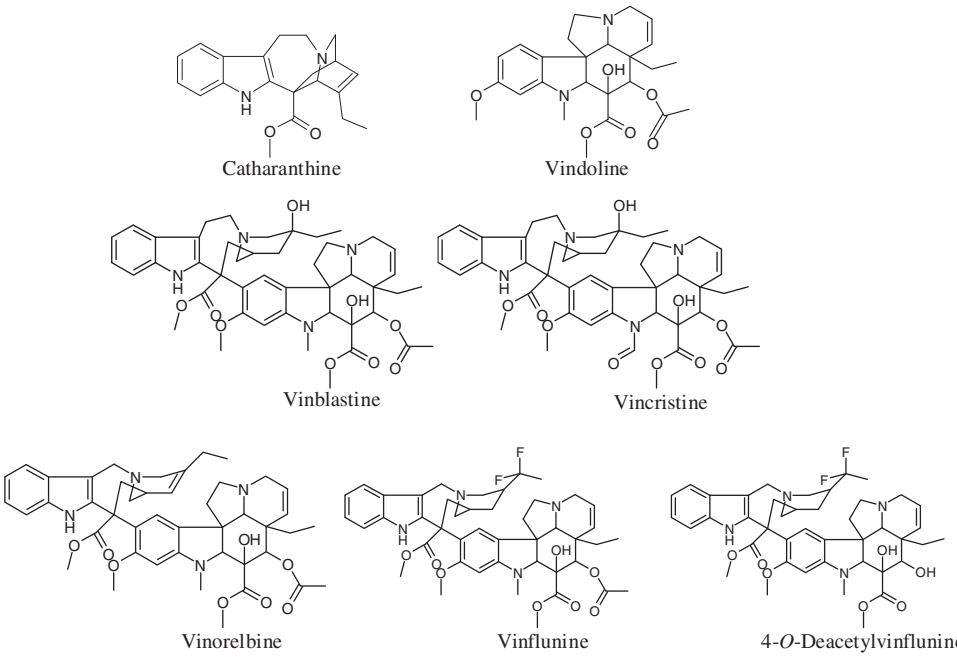

Figure 1. Chemical structures of investigated Vinca alkaloids.

in order to extract and preconcentrate the analytes. Liquid–liquid (LLE) or solid-phase extractions (SPE) are often used for this purpose.

Thus, Ragot et al. employed LLE with diethyl ether and dichloromethane to extract vinorelbine and vinorelbine *N*-oxide from human serum [28]. Zhao et al. extracted vinflunine from rat plasma by ethyl acetate [30]. Vendrig et al. achieved a selective SPE of vinblastine and vindesine from plasma or urine using cyanopropyl silica phase as sorbent [31]. Moreover, cation exchange benzene sulfonyl phases have been used to achieve the quantitative SPE of Vinca alkaloids from human and animal plasma [32].

Commercial supports proved their ability to quantitatively extract target analytes by SPE despite a lack of specificity. For a better specificity of sample preparation, new synthetic supports such as molecularly imprinted polymers (MIP) have been recently developed. A MIP is obtained by copolymerization of a functional monomer in the presence of a template molecule. Functional monomer is pre-arranged around a template molecule in porogenic solvent. Generally, the template interacts with the functional monomer by non-covalent bonds such as hydrogen bonds, ionic interactions or Van der Waals bonds. After removal of the template, this highly cross-linked polymer presents binding site cavities, which are complementary, both sterically and chemically, to the template molecule as well as to the structural analogues. Thus, MIP allows the rebinding of the template molecule as well as structurally similar compounds with good specificity.

Nowadays, a lot of MIP applications have dealt with biological samples [33–38]. In 2006, Chapuis et al. synthesized and evaluated a MIP to selectively extract an alpha-blocker, alfuzosin, from human plasma [39]. The SPE procedure was developed in dichloromethane (porogenic solvent) for the selective extraction of alfuzosin from human plasma with an extraction recovery of 60%.

Since these promising results, numerous applications of MIP-SPE pretreatment of biological samples have been proposed. Extensive efforts have focused on improving the selectivity and the capacity of MIPs in order to specifically extract target analytes from complex aqueous samples [40]. Thus, new monomers and polymerization methods were introduced to increase the aqueous compatibility of these imprinted polymers as well as the analyte accessibility to the specific MIP sites. However, the total removal of the template from the MIP sorbents remains a critical step usually carried out by Soxhlet extraction. However, the leakage of remaining template molecules in the SPE elution fraction still occurs and is a hindrance to quantification. Among the means suggested to get round this difficulty, the use of a dummy molecule as template is widespread [41].

Considering the therapeutic value of Vinca alkaloids, the performance of MIP supports for the specific extraction of vinblastine has been shown by Zhu et al. [42]. More recently, two MIPs of catharanthine and vindoline have also been synthesized in our laboratory in order to specifically extract these natural indole alkaloids from a plant extract [43]. In these previous studies, alkaloids were always extracted from organic samples whereas the present work aims to use MIP with aqueous samples.

In light of the successful MIP-SPEs in biological fluids, we investigated the MIP-SPE of vinflunine and its metabolite (4-*O*-deacetylvinflunine) from bovine plasma and artificial urine. An imprinted polymer of vinorelbine (template), a dummy molecule, was synthesized by following a non-covalent approach. A SPE protocol was developed on

standard solutions of alkaloids and afterwards adapted for real and complex aqueous samples. The main characteristic parameters of this polymer such as selectivity, specificity and linearity were investigated.

2 Materials and methods

2.1 Reagents

2.1.1 Reagents for the synthesis of MIPs

Methacrylic acid (MAA), ethylene glycol dimethacrylate (EDMA), 2,2′-azobisisobutyronitrile (AIBN), magnesium sulphate, acetonitrile (ACN) and chloroform ($CHCl_3$) (> 99.9% HPLC grade, ACN) were purchased from Aldrich (St.-Quentin-Fallavier, France) and ammonium hydroxide 28% from Fluka (St.-Quentin-Fallavier). Before use, MAA was distilled under vacuum and AIBN was re-crystallized from methanol.

Vinorelbine ditartrate was provided by the Institut de Recherche Pierre Fabre (Castres, France).

2.1.2 Reagents for sample preparation

Methanol (MeOH), ACN, ethyl acetate (AcOEt) were of HPLC grade and obtained from SDS (Carlo Erba, Val de Reuil, France). Glacial acetic acid (AcOH) 99.8% was purchased from Fluka (St.-Quentin-Fallavier). Hydroxylamine (50 wt%) was purchased from Aldrich. Deionized water (18 MΩ cm) was prepared with an Elgastat UHQ II system (Elga, Antony, France).

The acetic acid–hydroxylamine (pH 7) buffer solutions (10 and 20 mM ionic strength) were prepared with the help of Phoebus software (Analis, Namur, Belgium).

The analytes (vinflunine ditartrate, vinblastine sulphate, vincristine sulphate, 4-*O*-deacetylvinflunine, vindoline and catharanthine sulphate) and bovine plasma aliquots were provided by the Institut de Recherche Pierre Fabre.

2.2 Preparation of the MIP

Vinorelbine ditartrate was first neutralized in order to use its base form as template resulting in an increase of analyte–polymer interactions. So, a 193-mg amount of vinorelbine ditartrate was first dissolved in 10 mL of ammonium hydroxide solution (10 mM). Then, an 8-mL volume of chloroform was added and the organic phase became cloudy. A small quantity of ammonium hydroxide solution (1 M) was added drop by drop until pH of the upper aqueous phase was equal to 10. The solution was vortexed during 30 s and the aqueous phase was discarded. The organic phase was washed with water, dried with magnesium sulphate and reduced to dryness at room temperature under a stream of nitrogen. The basic form of vinorelbine was recovered as light yellow oil and stored at −18°C in darkness.

The imprinted polymer (MIP) was prepared in ACN (porogenic solvent) via bulk polymerization. Amounts of 0.4 mmol vinorelbine (template) and 1.6 mmol MAA (functional monomer) were dissolved in 2250 μL of ACN (porogenic solvent). Then, 8.0 mmol EDMA (cross-linker) and 30 mg AIBN (initiator) were added to the solution. The pre-polymerization solution was shaken and sonicated during 5 min. Then, the glass tube was cooled in an ice bath and deoxygenated with a stream of nitrogen during 5 min. The tube was sealed and the polymerization was carried out at 60°C for 16 h in a thermostat-controlled water bath, until the polymerization was completed. Then, the rigid bulk polymer was ground into powder with a pestle in a mortar. The particles were wet-sieved through a 45-μm metal sieve and washed in a Soxhlet apparatus with methanol–glacial acetic acid mixture 60:40 v/v to remove the template molecules trapped in the polymer matrix, and with pure methanol to eliminate residual acetic acid. The extraction was carried out until vinorelbine was not detected at 262 nm by HPLC-UV. Then, fine particles were eliminated by repeated sedimentation in acetone. Finally, the 25–45 μm range size particles were dried under vacuum and stored at ambient temperature.

The non-imprinted polymer was prepared under the same conditions but in the absence of the template.

2.3 SPE procedures using MIP

The extraction was achieved by using a 12-Port Visiprep vacuum manifold (Supelco, Sigma-Aldrich, St.-Quentin-Fallavier). One hundred and twenty milligram of polymer was packed into a 3-mL-volume SPE cartridge (Isolute IST, Hengoed, UK).

2.3.1 Sample preparation

For each alkaloid (catharanthine, vindoline, vinblastine, vincristine, vinflunine and 4-*O*-deacetylvinflunine), a stock solution (1000 μg/mL) was prepared by dissolving 3 mg of compound in 3 mL methanol and stored at −18°C in darkness. Working solutions (1 or 2 μg/mL) were prepared fresh daily by diluting the stock solution with a suitable solvent.

2.3.2 Treatment of plasma samples and preparation of artificial urine

A 2-mL aliquot of bovine plasma was spiked by vinflunine and its metabolite (concentration 1 μg/mL) and then diluted 1:1 with ACN in order to precipitate proteins. After shaking (3 min) and centrifugation (4000 rpm, 5 min at 5°C), 1 mL supernatant was collected and directly percolated through the MIP or the NIP.

The artificial urine compounds (sodium chloride, potassium chloride, calcium chloride, urea and creatinine) were purchased from Aldrich. Thus, a synthetic mixture

containing NaCl (8.39 g/L), KCl (5.73 g/L), $CaCl_2 \cdot 2H_2O$ (0.632 g/L) salts, urea (15 g/L) and creatinine (1 g/L) dissolved in AcOH–NH_2OH buffer (pH 7, 10 mM ionic strength) was prepared to mimic urine.

2.3.3 SPE protocols

The MIP and NIP sorbents were conditioned with methanol (6 mL) and ACN (6 mL). A third specific conditioning step was performed only for plasma and artificial urine samples.

2.3.3.1 SPE protocol for organic samples (protocol A)

A small volume (1 mL) of Vinca alkaloid standard solution (1 μg/mL dissolved in ACN) was loaded on the MIP and NIP cartridges. Then, 2 mL of ACN–AcOH mixture 99:1 v/v was percolated through the cartridge during the washing step. The elution was carried out with 4 mL MeOH–AcOH mixture 90:10 v/v. Each SPE fraction was evaporated at ambient temperature under a gentle stream of nitrogen until dryness. The residues were further dissolved in 1 mL of mobile phase and analyzed by HPLC.

This SPE protocol was thereafter applied to several alkaloids (vinflunine, 4-*O*-deacetylvinflunine, vinblastine, vincristine, catharanthine and vindoline).

2.3.3.2 SPE protocol for plasma and artificial urine samples (protocol B)

Protocol B1 (dedicated to plasma samples): This SPE protocol was applied to bovine plasma spiked with vinflunine and its metabolite, diluted in ACN 50:50 v/v and centrifuged in order to discard proteins by precipitation.

After percolation of 6 mL ACN–water 50:50 v/v mixture, the sample supernatant (1 mL) was loaded and two consecutive washing steps (2 mL AcOH–NH_2OH buffer (pH 7, 10 mM ionic strength)–MeOH mixture 95:5 v/v and 2 mL ACN–AcOH mixture 99:1 v/v) were carried out. The elution was performed with 4 mL MeOH–AcOH mixture 90:10 v/v.

Protocol B2 (dedicated to artificial urine samples): After percolation of 6 mL AcOH–NH_2OH (pH 7, 10 mM ionic strength) buffer, the SPE protocol involved the loading of 1 mL artificial urine spiked with vinflunine and 4-*O*-deacetylvinflunine (2 μg/mL) on both MIP and NIP cartridges. Then, two successive washing steps (6 mL AcOH–NH_2OH buffer (pH 7, 20 mM ionic strength)–MeOH 95:5 v/v and 4 mL ACN–AcOH 99:1 v/v) were carried out. Between the two washing steps, the cartridge was dried (2 min) in order to eliminate any residual water from the cartridge. Finally, alkaloids were eluted with 4 mL of MeOH–AcOH mixture 99:1 v/v.

Elution fractions were evaporated at ambient temperature under a gentle stream of nitrogen until dryness. The residues were further dissolved in 1 mL of mobile phase and then analyzed by HPLC.

2.4 Linearity study

Several aliquots of a diluted spiked plasma (vinflunine concentration range: 0.25–10 μg/mL) were loaded on MIP and NIP cartridges in order to determine SPE recoveries and to assess the MIP linearity. The same approach was used with the artificial urine samples (vinflunine concentration range: 1–5 μg/mL).

2.5 Apparatus and analytical conditions

The separation was performed on a (250 × 4.6 mm id, 5 μm) reversed-phase HPLC column XTerra MS C18 (Waters, Milford, MA, USA). All experiments were carried out on an Agilent 1100 Series system (Waldbronn, Germany). The mobile phase was a mixture of ACN and acetic acid–ammonia buffer (pH 10, 10 mM ionic strength prepared with the help of Phoebus) 75:25 v/v. The flow rate was 1 mL/min and the column was thermostated at 25°C. The volume injection was 20 μL. UV signal was recorded at 214 and 262 nm from an Agilent photodiode-array detector (1100 Series). Data were collected and analyzed using Agilent software (Chemstation, rev A 08.03).

3 Results and discussion

3.1 Synthesis of vinorelbine-MIP

The imprinted polymer of vinorelbine was obtained by copolymerization of MAA (functional monomer) and EDMA (cross-linker) in the presence of vinorelbine.

MAA ($pK_a = 4.66$) is frequently used as functional monomer for basic template imprinting. Indeed, its carboxylic acid function is assumed to interact with the tertiary amine function ($pK_a = 7.6$ and 5.4) of vinorelbine molecule. According to the nature of the solvents, different kinds of template–MAA interactions may occur simultaneously, such as hydrogen bonds in aprotic solvents and ionic interactions in polar organic solvents. To favour both hydrogen bonds and ionic interactions, ACN was chosen as pre-polymerization solvent.

Vinorelbine was selected as template because of its vinflunine-like structure. As vinorelbine is a UV-sensitive molecule, a thermal polymerization was achieved at 60°C. The reaction time was limited to 16 h as a trade-off between the yield of polymerization and the risk of thermal degradation of template molecules.

The molar ratio of template/monomer/cross-linker was set at 1:4:20 (mole/mole/mole). The volume of the solvent was a compromise between the solubility of the template–-monomer pre-polymerization mixture and the final porosity of the polymer. So, the monomer to ACN volume ratio was approximately 3:5 v/v.

Successive extractions with methanol–acetic acid 60:40 v/v mixture were carried out to eliminate vinorelbine from

the imprinted polymer. Nevertheless, a large amount of vinorelbine was still present. Thus, the residual template molecules were removed from the polymer matrix by washing the polymer in Soxhlet apparatus [42]. The extraction process was repeated until vinorelbine was no longer detected by HPLC. Then, particles were washed using methanol during 8 h in order to eliminate the residual acetic acid.

The MIP was characterized by Brunauer-Emmet-Teller (BET) surface area with a Micromeritics ASAP 2020 apparatus (Norcross, GA, USA). The MIP average surface area was 315 m^2/g which is not significantly different from other MIPs (270–295 m^2/g) prepared by Shi et al. [44] in similar conditions (MAA as monomer, EDMA as cross-linker and ACN as porogenic solvent). According to this report, accessibility of analyte molecules to imprints was easier and their retention better with a high MIP surface area. In comparison with MIP surface areas reported in the literature, it seems that the surface area of vinorelbine-MIP was high enough to favour analyte mass transfer and retention.

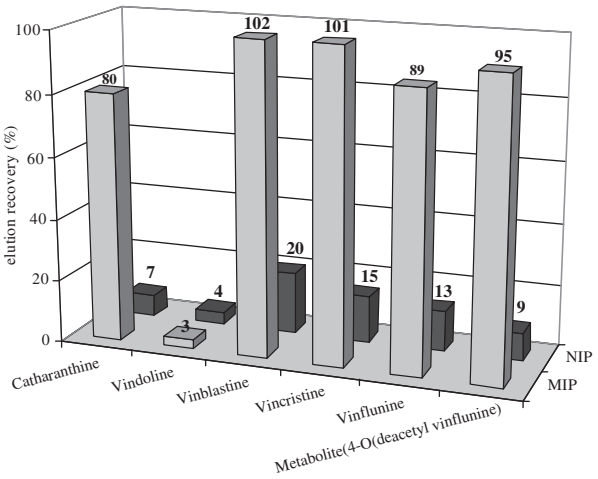

Figure 2. Elution recoveries of catharanthine, vindoline, vinblastine, vincristine, vinflunine and vinflunine metabolite on vinorelbine-MIP and NIP.

3.2 Evaluation of vinorelbine-MIP by SPE (MIP-SPE)

3.2.1 Set-up of a MIP-SPE protocol–cross-reactivity

The SPE protocol (protocol A) was firstly developed for the specific extraction of vinflunine from a standard solution. The MIP and NIP cartridges were first conditioned with methanol to activate the polymer surface and then with ACN. A 1-mL-volume aliquot of alkaloid standard solution (1 μg/mL in ACN) was loaded on both MIP and NIP cartridges. Then, a 2-mL volume of ACN–AcOH mixture 99:1 v/v was percolated through the cartridge in order to decrease non-specific interactions, mainly ionic interactions and hydrogen bonds. Therefore, the vinflunine retention greatly decreased on NIP only (Fig. 2), demonstrating that vinflunine was mainly retained by specific interactions in MIP cavities. In the last SPE step, the analytes were eluted by 4 mL MeOH–AcOH 90:10 v/v mixture. Thus, the recovery of vinflunine was higher (89%) on MIP rather than on NIP (13%), which proved the good affinity and selectivity of MIP for vinflunine.

The MIP ability for the extraction of other Vinca alkaloids (catharanthine, vindoline, vincristine, vinblastine and 4-*O*-deacetylvinflunine) was also investigated through a cross-reactivity study. The chemical structures of indole and bisindole alkaloids are given in Fig. 1. The previous SPE protocol was applied to related Vinca alkaloids on MIP and NIP (Fig. 2). The protic and acidic nature of the elution solvent mixture allowed to get high-elution MIP recoveries: 80, 102, 101 and 95% for catharanthine, vinblastine, vincristine and 4-*O*-deacetylvinflunine, respectively. In contrast, elution NIP recoveries were still low (20%) for these alkaloids. However, vindoline was only slightly retained by MIP (3%) due to its weak basic strength which induces little electrostatic interactions with the polymer matrix. These results confirmed the retention and the selectivity of vinorelbine-MIP only for molecules containing the catharanthine-like unit.

The imprinting factor (IF) was calculated as $IF = n_{MIP}/n_{NIP}$, where n_{MIP} and n_{NIP} were the recovered amounts of analytes after the elution step on the MIP and NIP polymers, respectively. The synthesized non-covalent vinorelbine-MAA polymer showed a good MIP/NIP selectivity with IF values of 11, 5, 7, 7 and 11 for catharanthine, vinblastine, vincristine, vinflunine and 4-*O*-deacetylvinflunine, respectively.

3.2.2 MIP-SPE applied to aqueous samples

Although a high MIP selectivity has been obtained for organic samples of vinflunine, the main objective consisted of the selective extraction of vinflunine from plasma and artificial urine. These complex samples contain many ionic compounds such as sodium and calcium. These monovalent and divalent cations were supposed to be competitors for ionic interactions and hydrogen bonds between target analyte molecules and MIP [41]. In order to assess the extraction performance of vinorelbine-MIP with respect to biofluids, a NH_2OH–AcOH (pH 7, 75 mM ionic strength) buffer solution was spiked with calcium (1.25 mM) or sodium (140 mM) at average plasma concentrations. Both solutions constituted solvents for vinflunine (1 μg/mL) samples. After MIP-SPE conditioning, 1 mL of sample was percolated according to the SPE protocol previously described as protocol B1.

Whatever was the cation nature, more than 90% of vinflunine were recovered from the MIP during the elution step, as reported in Fig. 3. Moreover, the MIP/NIP selectivity was very satisfactory since the IF was equal to 23 and 29 for samples spiked with calcium and sodium,

respectively. Thus, these experiments proved the ability of the MIP-SPE protocol to extract vinflunine from biological fluids such as bovine plasma.

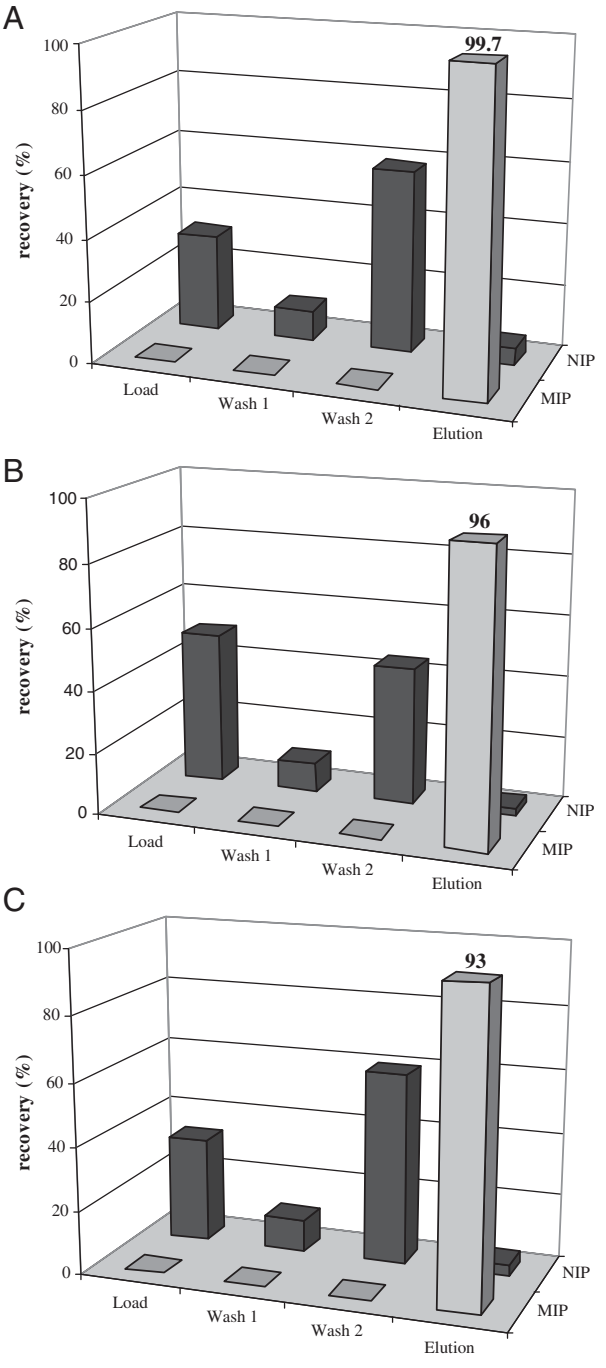

Figure 3. Study of the impact of human plasma salts versus vinflunine recovery during MIP-SPE. Sample solvent: (A) NH_2OH–AcOH buffer (pH 7, 75 mM ionic strength). (B) NH_2OH–AcOH buffer (pH 7, 75 mM ionic strength) with Ca^{2+} (1.25 mM). (C) NH_2OH–AcOH buffer (pH 7, 75 mM ionic strength) with Na^+ (140 mM). Load: 1 mL of buffer containing vinflunine (1 μg/mL). Washing steps: wash no. 1: 2 mL AcOH–NH_2OH buffer (pH 7, 10 mM ionic strength)/MeOH 95:5 v/v. Wash no. 2: 2 mL ACN/AcOH 99:1 v/v. Elution step: 4 mL MeOH/AcOH 90:10 v/v.

3.2.3 Extraction of vinflunine from bovine plasma

To test the selectivity of MIP-SPE on biological samples, a bovine plasma sample was spiked with vinflunine and its metabolite (2 μg/mL). Since plasma could not be directly percolated through the MIP, due to protein that could prevent vinflunine retention, the sample was diluted twice in ACN in order to disrupt vinflunine-protein interactions and to remove proteins by precipitation. After centrifugation, one milliliter of supernatant was loaded on MIP and NIP cartridges and then the SPE protocol B1 was applied. Thus, 81% ± 3% ($n = 3$) of vinflunine was recovered in the elution MIP fraction versus 40% ± 6% ($n = 3$) for NIP. The MIP/NIP selectivity was also significant for vinflunine metabolite, for which MIP and NIP recoveries were equal to 89% ± 3% ($n = 3$) and 15% ± 8% ($n = 3$), respectively. The chromatogram of the MIP elution fraction displayed two main peaks corresponding to the target analytes and a small sample matrix peak (Fig. 4), which confirms the MIP high selectivity. As expected, the ethyl acetate LLE from plasma was less efficient than MIP-SPE with a lower recovery (70%) of vinflunine and a larger sample matrix peak.

3.2.4 Extraction of vinflunine and its metabolite from artificial urine

The SPE protocol B1 has been slightly modified for application to artificial urine samples because washing steps were not efficient enough to eliminate urinary salts providing a low MIP/NIP selectivity. Several SPE parameters (volume and ionic strength of the first washing solution; volume of the second washing solution) were changed, as reported in Section 2.3.3. The new protocol B2 was applied to an artificial urine sample spiked with vinflunine and its metabolite (2 μg/mL). Satisfactory recoveries for vinflunine (73% ± 4% ($n = 3$)) and 4-*O*-deacetylvinflunine (81% ± 2% ($n = 3$)) were obtained on MIP, whereas the recoveries measured on NIP were rather low (null for vinflunine and 21% for 4-*O*-deacetylvinflunine). These results highlighted the efficiency of MIP for

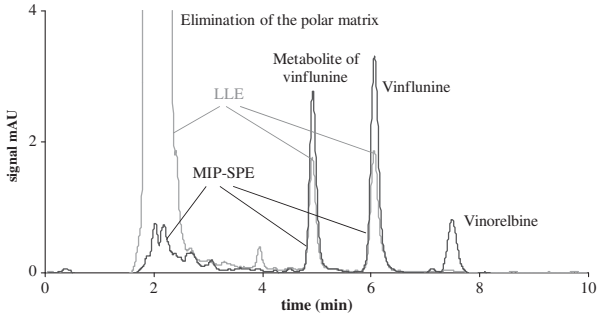

Figure 4. Superimposed chromatograms provided by HPLC-UV analyses of bovine plasma spiked with vinflunine and its metabolite (2 μg/mL) after MIP-SPE (in black) and after LLE with AcOEt (in grey). Column: X-Terra MS C18, 250 × 4.6 mm, 5 μm; mobile phase: ACN/AcOH–NH_3 buffer (pH 10, 10 mM ionic strength) 75:25 v/v; flow: 1 mL/min; detection: 262 nm. Injection volume: 20 μL.

Table 1. Linearity of HPLC-UV signal plotted versus concentration of alkaloids in bovine plasma ($n = 3$) and artificial urine ($n = 1$)

Fluid	Concentration range (μg/mL)	Alkaloid	Slope	Intercept	r^2
Bovine plasma	0.25–10	Vinflunine	13.45	0.078	0.995
		4-*O*-Deacetylvinflunine	15.35	0.759	0.999
Artificial urine	1–5	Vinflunine	14.37	0.016	0.993
		4-*O*-Deacetylvinflunine	14.28	−0.482	0.993

the selective extraction of vinflunine and its metabolite from artificial urine. The repeatability was similar to that previously reported for carbamazepine extracted by MIP-SPE in urine (RSD = 3.1% ($n = 3$)) [45].

3.2.5 Linearity study

3.2.5.1 Plasma

Six solutions of vinflunine and 4-*O*-deacetylvinflunine (0.25–10 μg/mL concentration range) were prepared fresh daily in bovine plasma. The selected concentration range surrounded the highest vinflunine concentration (1.8 μg/mL) in human plasma, which is usually reached during the first hour following drug administration [32].

The MIP-SPE protocol B1 was applied to each solution and also to a blank plasma sample. Each extraction was repeated during three consecutive days. The HPLC-UV signal was plotted versus the analyte concentration in plasma. The curves were linear with determination coefficients higher than 0.99 (Table 1).

For each SPE, the recoveries of vinflunine and 4-*O*-deacetylvinflunine were calculated. The results obtained were satisfactory for 4-*O*-deacetylvinflunine (mean recovery equal to 86.4% ± 11.8% ($n = 18$)) and for vinflunine (mean recovery equal to 88.7% ± 13.6% ($n = 15$)). SPE performed on blank samples did not show any peak on the chromatogram.

3.2.5.2 Urine

The linearity of the MIP-SPE/HPLC-UV was also assessed for artificial urine samples spiked with vinflunine and its metabolite at five levels of concentration (1, 2, 3, 4 and 5 μg/mL). This concentration range was selected according to the hypothesis that concentrations of excreted drugs in urine were equal or inferior to plasma concentrations. MIP-SPE protocol B2 was applied to each sample. The calibration curves were linear with determination coefficients higher than 0.99 (Table 1). Mean recoveries of vinflunine and its metabolite were equal to 64.8% ± 3.8% ($n = 5$) and 68.6% ± 3.1% ($n = 5$), respectively.

3.2.6 Influence of loaded sample volume upon recovery

The influence of loaded sample volume upon recovery was studied by loading different volumes (1–40 mL) of urine samples, spiked with vinflunine and its metabolite. The same amount of alkaloid (2 μg) was systematically percolated through 120-mg MIP cartridge. Whatever the loaded volume, elution recoveries were similar (77% ± 5.1% ($n = 7$) for vinflunine and 83% ± 4.9% ($n = 7$) for its metabolite). These results proved that the MIP was able to quantitatively extract low concentrations of analytes, i.e. 0.05 μg/mL, dissolved in a high volume of artificial urine, i.e. 40 mL.

The high-volume sample capacity of MIPs has already been observed by several authors [46, 47] with environmental aqueous samples. It was demonstrated that sample volumes as high as 50, 100 or 400 mL of river or drinking water could be loaded on MIP cartridges without any recovery decrease of the herbicide target analytes [48–50].

4 Concluding remarks

An imprinted polymer of vinorelbine has been synthesized by a non-covalent approach in ACN solvent. This polymer showed good MIP/NIP selectivity and high-elution recoveries in organic solutions towards alkaloids whose chemical structures contained a catharanthine-like unit, such as vinflunine, vinblastine and vincristine.

The analysis of aqueous samples led to high extraction recovery and satisfactory MIP/NIP selectivity for vinflunine and its metabolite. Besides, the addition of calcium or sodium ions to aqueous samples did not modify the performance of the MIP. Specific and quantitative MIP-SPEs of complex samples (bovine plasma, artificial urine) were successfully achieved.

The MIP-SPE of vinflunine and its metabolite from plasma and artificial urine followed by HPLC-UV provided a good linearity of the chromatographic signal versus the analyte concentration in samples.

Moreover, the capacity of MIP remained constant regardless of the loaded sample volume.

The authors have declared no conflict of interest.

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