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Optimization of the separation of *Vinca* alkaloids by nonaqueous capillary electrophoresis

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Abstract

A rapid method for the determination of *Vinca* alkaloids by nonaqueous capillary electrophoresis with diode array detection has been developed. A group of 11 alkaloids (catharanthine, vinorelbine, anhydrovinblastine, vinflunine, vindoline, 4-*O*-deacetylvinorelbine, 4-*O*-deacetylvinflunine, vindesine, vinblastine, 4′-deoxy-20′,20′-difluorovinblastine, vincristine) could be readily separated within 10 min. The compounds were separated using a capillary of 38 cm effective length, a running buffer composed of 50 m*M* ammonium acetate and 0.6 *M* acetic acid in a methanol–acetonitrile (75:25, v/v) mixture. A constant voltage of 25 kV with a ramp time of 1 min and a 344.7×10^3 Pa pressure, applied simultaneously to inlet and outlet buffer vials, were used during sample analysis. Five of these alkaloids were selected for optimization of the separation and for validation studies with respect to specificity, linearity, range, limits of quantification and detection and then accuracy. The feasibility of the assay was demonstrated by analyzing a commercial sample of vinorelbine (Navelbine®, ampoule at 10 mg/ml of vinorelbine base). The results were compared with a high-performance liquid chromatography method.

Keywords: Nonaqueous capillary electrophoresis; Pharmaceutical analysis; Alkaloids

1. Introduction

The indole alkaloids vinblastine and vincristine, initially extracted from the common Madagascar periwinkle (*Catharanthus roseus* G.Don, an Apocynaceae) have been in use for 30 years as anticancer agents [1]. These are binary alkaloids composed of one indole sub-unit (cleavamine, resulting from the rearrangement of catharanthine) and one indoline sub-unit (vindoline). The first product of this type was vinblastine, marketed in 1963, followed by vincristine in 1964. These two alkaloids were extracted from the *Catharanthus roseus* leaves, but only very small quantities could be obtained, since this plant has an alkaloid content of around only 0.005%. In 1983, a new synthetic antitumoral binary alkaloid appeared on the market: vindesine, which was obtained by hemisynthesis using vinblastine. Vinorelbine, which appeared on the French market in 1989, was the second non-natural medicinal product

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in this group. At that time vinorelbine was the only medicinal product obtained by coupling the two monomeric alkaloids, catharanthine and vindoline. Since 1974, an original chemical reaction, the Polonovski–Potier (see Ref. [2]) reaction, has been used to couple catharanthine *N*-oxide with vindoline, thus producing anhydrovinblastine. Vinorelbine could then be obtained, directly from anhydrovinblastine using *N*-bromosuccinimide and then silver tetrafluoroborate [3–5]. More recently, Fahy et al. [6] explored an original chemical approach based on the special reactivity provided by superacidic chemistry. Under these unusual conditions, starting from vinorelbine, they obtain vinflunine. This compound, like the other *Vinca* alkaloids inhibited microtubule assembly at micromolar concentrations [7,8], but exerted markedly superior in vivo antitumour activities against a panel of 13 murine and human tumour models compared to the parent compound, vinorelbine [9–11].

High-performance thin-layer chromatography [12–14], gas chromatography [15], supercritical fluid chromatography [16], radioimmunoassay [17–19] and high-performance liquid chromatography [20–31] were widely used in the analysis and the quantification of *Vinca* alkaloids. Recently, capillary electrophoresis (CE) was used as an interesting alternative with regard to its high efficiency, specificity and very high resolution.

Several papers have reported on the analysis of *Vinca* alkaloids by CE. In 1996 Chu et al. [32] employed CE for the first time in the separation and quantification of vincristine and vinblastine in *Catharanthus roseus* leaves. They used capillary zone electrophoresis (CZE) with a running buffer composed of 0.2 *M* ammonium acetate, pH 6.2, and an applied voltage of 10 kV. The addition of methanol as an organic modifier was found to increase the column efficiency, but to reduce the resolution for the vinblastine/vincristine pair. However these authors claimed that for the separation of other alkaloids, that co-exist in the plant samples, resolution was improved dramatically by the addition of organic modifiers such as methanol. In 1997, Unger et al. [33] described the analysis of various alkaloids such as vinblastine and vincristine by employing capillary electrophoresis and on-line combined CE–mass spectrometry (CE–MS). An excellent separation of four different groups of alkaloids was obtained using a 1:1 (v/v) mixture of 100 m*M* ammonium acetate in water, adjusted with acetic acid to pH 3.1, and acetonitrile. Later, Unger [34] investigated the influence of structure on the electrophoretic mobility of indole alkaloids. He demonstrated that the electrophoretic mobility was mostly dependent on the size and shape of the solvated analyte ions. More recently Zongyu and Jue [35] reported the quantitative determination of vindesine sulfate by CE using micellar electrokinetic chromatography (MEKC). The running buffer solution was 50 m*M* borax buffer (pH 7.7) added with 25 m*M* of sodium dodecyl sulfate. The internal standard was nicotinamide and the method showed good linearity between 58 and 580 μg/ml.

An aqueous electrolyte solution was initially used to separate the indole alkaloids mentioned in the Experimental. The electrolyte tested was potassium hydrogenphosphate at different pH where poor resolution was observed. Efforts to improve the separation by adding different organic solvents such as acetonitrile and methanol were found to be invalid.

However, there have been no reports on the application of nonaqueous capillary electrophoresis (NACE) method for the analysis of *Vinca* alkaloids. The use of nonaqueous solvents as electrophoretic media offers many advantages, in comparison with aqueous media, such as low joule heating and provides different selectivity which can be used to improve separation. This paper describes a newly developed NACE method which makes possible the separation of a complex mixture of *Vinca* alkaloids.

2. Experimental

2.1. Chemicals and reagents

Catharanthine sulfate (batch OP373A), vinorelbine ditartrate (batch 527), anhydrovinblastine dihydrochloride (batch FRE02227AO), vinflunine ditartrate (batch BHU0615900), vindoline (batch OP444), 4-*O*-deacetylvinorelbine sulfate (batch BHU0805400), 4-*O*-deacetylvinflunine ditartrate (batch LBR0202700), vinblastine sulfate (batch R12182), 4′-deoxy-20′,20′-difluorovinblastine ditartrate (batch BHU07143000), vincristine sulfate (batch 513) were

supplied by Plantes et Industrie Pierre Fabre Santé (Gaillac, France). The chemical purity of these compounds, evaluated by high-performance liquid chromatography, was >98% (w/w). Vindesine sulfate (Eldisine®) was obtained from Lilly. The developed formulas of all the above-mentioned compounds are shown in Fig. 1. Methanol (spectroscopy grade), acetonitrile (HPLC grade), acetic acid (>99.5%, w/w), analytical grade potassium hydroxide and sodium hydroxide were purchased from SDS (Peypin, France). The internal standard 4-(2-keto-1-benzimidazolinyl) piperidine and boric acid were obtained from Acros (NJ, USA). Ammonium acetate was obtained from Aldrich (Milwaukee, WI, USA) and potassium dihydrogen phosphate was analytical grade and was purchased from Merck (Darmstadt, Germany). Orthophosphoric acid was obtained from Prolabo (Manchester, UK). High-purity water was prepared by the Purelab1 water purification system (USF ionpure Ransbach-Baumbach). The capillary electrophoresis solvents were filtered through 0.45 μm GHP Acrodisc 13 mm Syringe filters (Gelman Laboratory, Ann Arbor, MI, USA).

The pK_a values of the indole alkaloids vinorelbine (7.60 and 5.40), vinflunine (7.55 and 5.37) and vindoline (5.5) were determined by an automatic potentiometric titrator (Mettler DL25, Viroflay, France) at ambient temperature using an internal procedure.

2.2. Capillary electrophoresis analysis

All capillary electrophoresis experiments were carried out with a Beckman P/ACE system MDQ equipped with an on-column photodiode array detector (Beckman Instruments, Fullerton, CA, USA). Instrument control, data collection and analysis were performed with the P/ACE system MDQ software version 2.2 running on an IBM 300 GL computer. Uncoated fused-silica capillaries, obtained from Beckman, of 75 μm I.D.×375 μm O.D. with a total length of 48 cm and an effective length of 38 cm were used. The capillary was thermostated at 20 °C and the autosampler temperature was maintained at 10 °C. Each day, the capillary was conditioned by flushing with 0.1 *M* sodium hydroxide, followed by deionized water and acetonitrile for 5 min each and finally with the running buffer, 10 min at 1.4×10^5 Pa. Prior to each analysis, the capillary was rinsed with the buffer solution for 3 min at 1.4×10^5 Pa. For storage, the capillary was washed with acetonitrile and water and then stored dry. Samples were injected hydrodynamically at 2.8×10^3 Pa for 2.5 s at the capillary inlet, unless otherwise indicated, and detected at 214 nm.

Optimal separations were obtained using a 75:25 (v/v) methanol–acetonitrile mixture containing 50 m*M* ammonium acetate and 0.6 *M* acetic acid. A constant voltage of 25 kV (current ~70 μA) with a ramp time of 1 min and a 3.5×10^5 Pa pressure, applied simultaneously to inlet and outlet buffer vials to avoid electric break-down, were used during sample analysis. The electrolyte solutions were filtered and then sonicated before use. The electroosmotic flow (EOF) was tested with methanol.

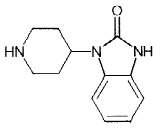
Separate stock standard solutions of *Vinca* alkaloids were prepared by dissolving ~10 mg of each compound in 10-ml volumetric flasks with 50 μl of acetic acid and methanol. The analyzed solutions were prepared by diluting the respective stock solutions with methanol so as to obtain a final concentration of 100 μg/ml.

2.3. High-performance liquid chromatography analysis

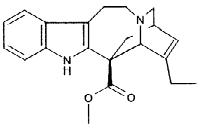
Chromatographic analyses were performed on a Merck–Hitachi Lachrom HPLC apparatus consisting of an L-7100 pump, L-7450 diode array detector, L-7200 autosampler and L-7360 oven. The integration of chromatograms were realized with a Merck HSM D-7000 integrator. The column employed was a 250×4.6 mm, 5 μm particle size, XTerra RP-18 (Waters).

Two mobile phases were used at a flow-rate of 1 ml/min: (I) acetonitrile–water–potassium dihydrogen phosphate (300:700:6.8, v/v/w) adjusted at pH 5 with a 10% (w/v) aqueous solution of orthophosphoric acid, (II) acetonitrile–water–boric acid (550:450:3.1, v/v/w) adjusted at pH 10 with an aqueous solution of potassium hydroxide. Chromatography was performed at 35 °C and the eluate was monitored at 214 nm.

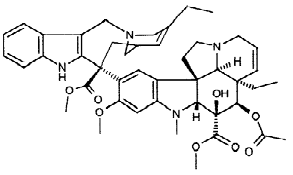
Separate stock standard solutions of vindoline, catharanthine, vinorelbine, anhydrovinblastine and vinflunine were prepared at a concentration of



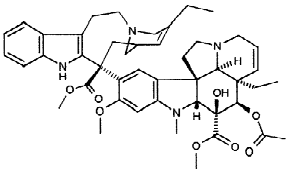
4-(2-keto-1-benzimidazolinyl) piperidine , I.S (1)



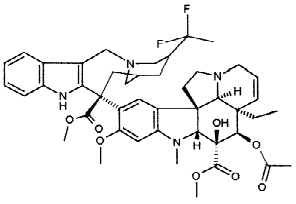
Catharanthine (2)



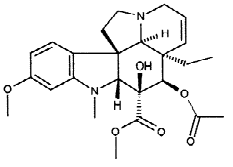
Vinorelbine (3)



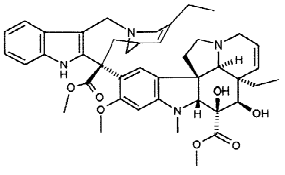
Anhydrovinblastine (4)



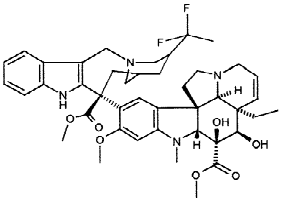
Vinflunine (5)



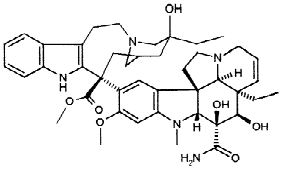
Vindoline (6)



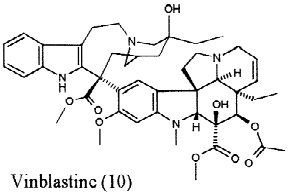
4-O-deacetylvinorelbine (7)



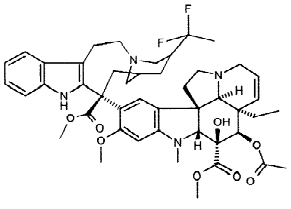
4-O-deacetylvinflunine (8)



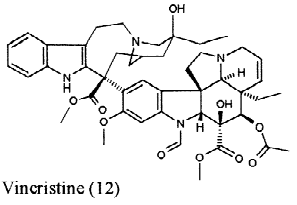
Vindesine (9)



Vinblastine (10)



4'-Deoxy-20',20'-difluorovinblastine (11)



Vincristine (12)

Fig. 1. Formulas of the investigated compounds.

0.2 mg/ml in a mixture of acetonitrile–water (50:50, v/v). The injection volume was 5 μl. Twenty μl of a solution including all compounds above-mentioned at a concentration of 0.04 mg/ml was also analyzed. Injectable ampoules of vinorelbine ditartrate equivalent to 10 mg/ml of vinorelbine base were assayed. The limits of detection (LOD) and quantification (LOQ) were evaluated with mobile phase II.

3. Results and discussion

3.1. Capillary electrophoresis analysis

The ammonium acetate–acetic acid pair in a methanol–acetonitrile mixture is a widely used nonaqueous buffer [36–40]. Acetonitrile and methanol exhibit relatively low UV absorbance and low viscosity which result in short analysis time and high efficiency. Moreover, selectivity manipulation can be achieved simply by varying the proportions of these two solvents.

Five alkaloids (catharanthine, vinorelbine, anhydrovinblastine, vinflunine, vindoline) were selected in order to predict the optimum conditions for the separation. The influence of methanol–acetonitrile ratio, concentration of ammonium acetate and acetic acid, applied voltage and capillary temperature on the electrophoretic separation was studied.

3.1.1. Composition of acetonitrile–methanol mixture

Different acetonitrile–methanol mixtures containing 50 m*M* ammonium acetate and 0.5 *M* acetic acid were prepared. The acetonitrile percentage in methanol was varied from 0 to 90%. Pure acetonitrile was not selected as nonaqueous solvent due to the very low solubility of ammonium acetate in this solvent.

As illustrated in Fig. 2A, the composition of the acetonitrile–methanol mixture induced different migration behaviors for the investigated alkaloids. The variation of methanol–acetonitrile ratio affected electrophoretic mobilities and selectivity. Between 10 and 40% acetonitrile the difference of mobility was the most marked. Nevertheless, anhydrovinblastine and vinflunine were not well separated. The best separation of these two compounds was obtained with 25% acetonitrile in methanol (Fig. 2B).

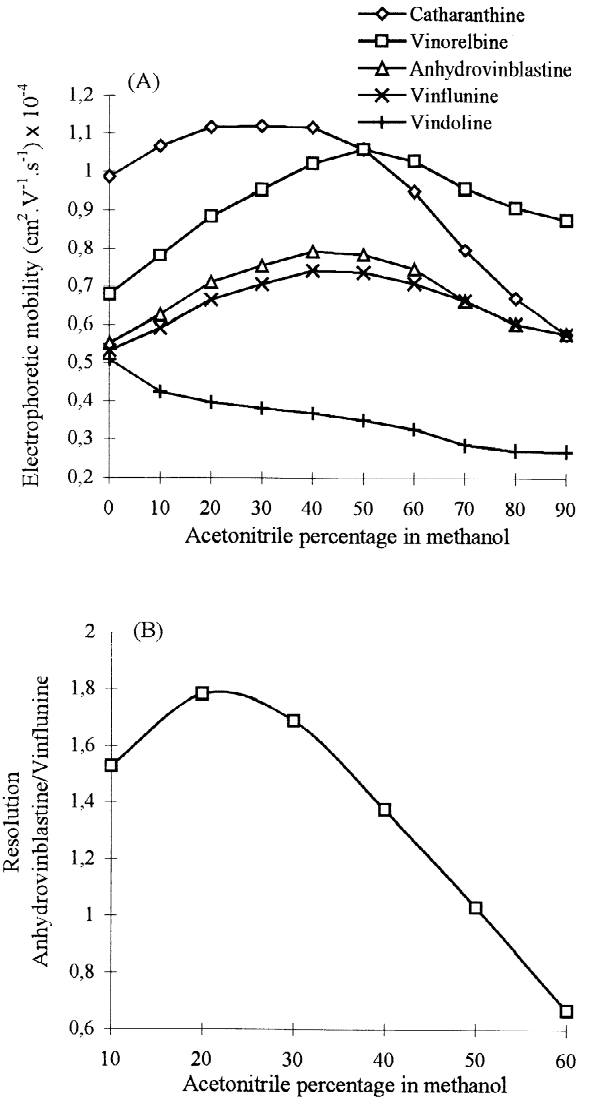


Fig. 2. Effect of acetonitrile percentage in methanol (A) on the electrophoretic mobility of the five studied *Vinca* alkaloids and (B) on the resolution between anhydrovinblastine and vinflunine. Experimental conditions: electrolyte 50 m*M* ammonium acetate and 0.5 *M* acetic acid in different acetonitrile–methanol mixtures, uncoated fused-silica capillary 48 cm (38 cm injector to detector)×75 μm I.D., UV detection at 214 nm, applied voltage 25 kV, capillary temperature 25 °C.

3.1.2. Concentration of acetic acid

Effects of varying the concentration of acetic acid between 0.2 and 1.8 *M* was also investigated, while keeping a constant concentration of ammonium acetate (50 m*M*) in a methanol–acetonitrile (75:25,

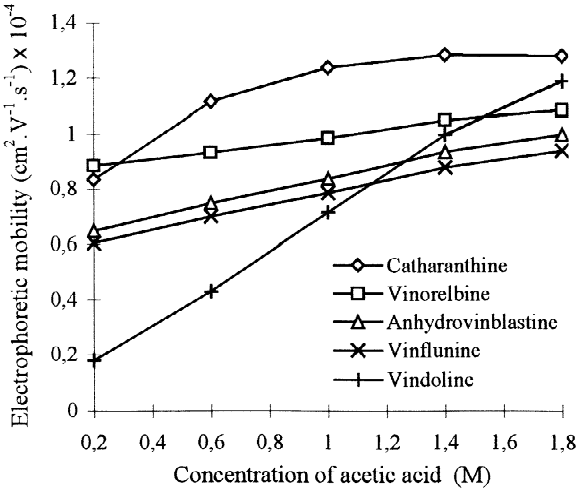


Fig. 3. Effect of the concentration of acetic acid on the electrophoretic mobility of the compounds. Electrophoretic medium: 50 m*M* ammonium acetate and various amounts of acetic acid in acetonitrile–methanol (25:75, v/v) mixtures. Other CE conditions as in Fig. 2.

v/v) mixture. This parameter was found to have a crucial effect on selectivity based on charge-to-size ratios. Fig. 3 shows the electrophoretic mobility modification by a small variation of the acetic acid concentration, mainly for the two monomer alkaloids, vindoline and catharanthine. Thus an acetic acid concentration of 0.6 *M* allowed complete separation of the five alkaloids and their impurities.

3.1.3. Electrolyte ionic strength

Electrophoretic media with a methanol–acetonitrile (75:25, v/v) mixture containing 0.6 *M* acetic acid and various concentrations of ammonium acetate, ranging from 10 to 70 m*M*, were prepared so as to evaluate the influence on the separation of the electrolyte ionic strength. We found that an increase in the buffer concentration improved resolution. Moreover, the electric current increased from 20 to 90 μA producing Joule heating and evaporation problems. Consequently electrophoretic media with a concentration of ammonium acetate greater than 70 m*M* were not studied.

3.1.4. Applied voltage and capillary temperature

High voltage was required in CE to reduce the analysis time. Therefore, a voltage between 10 and 30 kV was applied successively. It was observed that an increase in the applied voltage increased the separation efficiency up to a certain limit. A voltage higher than 20 kV affected resolution, particularly for the anhydrovinblastine/vinflunine pair, with peak broadening occurring as a consequence of the diffusion phenomenon with Joule effect. However, a constant voltage of 25 kV was selected for a complete separation of all compounds in a short time with an acceptable electric current (~70 μA).

The influence of the capillary temperature was also studied with optimized electrophoretic medium between 15 and 30 °C. As temperature increased, migration time decreased due to a reduction of the electrolyte viscosity. Separation was found to be affected slightly by temperature variations. A temperature of 20 °C was selected so as to obtain a resolution of 2 for the anhydrovinblastine/vinflunine pair.

3.2. High-performance liquid chromatography

Mobile phases in the pH range of 3 to 10 (pH 3, 4, 5, 7, 9, 10) were tested on the XTerra RP-18 column. Among these different eluents, three achieved separation of the five studied *Vinca* alkaloids (pH 5, 9 and 10). The most suitable separations were obtained with mobile phases buffered at pH 5 (system I) and pH 10 (system II). In order to compare UV traces obtained by HPLC and CE, the detection wavelength was set to 214 nm, that used for CE measurements. The maximum absorption wavelength was 216 nm for all compounds, except for catharanthine whose value was 227 nm.

Samples analyzed by HPLC were dissolved in a mixture of acetonitrile–water (1:1, v/v) since an alkaline buffer would damage analytes in solution.

The column was thermostated at 35 °C to decrease the retention of compounds, and so, the time of analysis.

Elution order was reversed between pH 5 and pH 10 mobile phases for these two pairs of derivatives: catharanthine/vindoline and vinorelbine/anhydrovinblastine (Fig. 4A and B).

Values of asymmetry (Table 1) were included in the range (1.1/1.4) and were similar for the two HPLC systems. Good resolution, greater than 2, was observed for all compounds, except for anhydrovin-

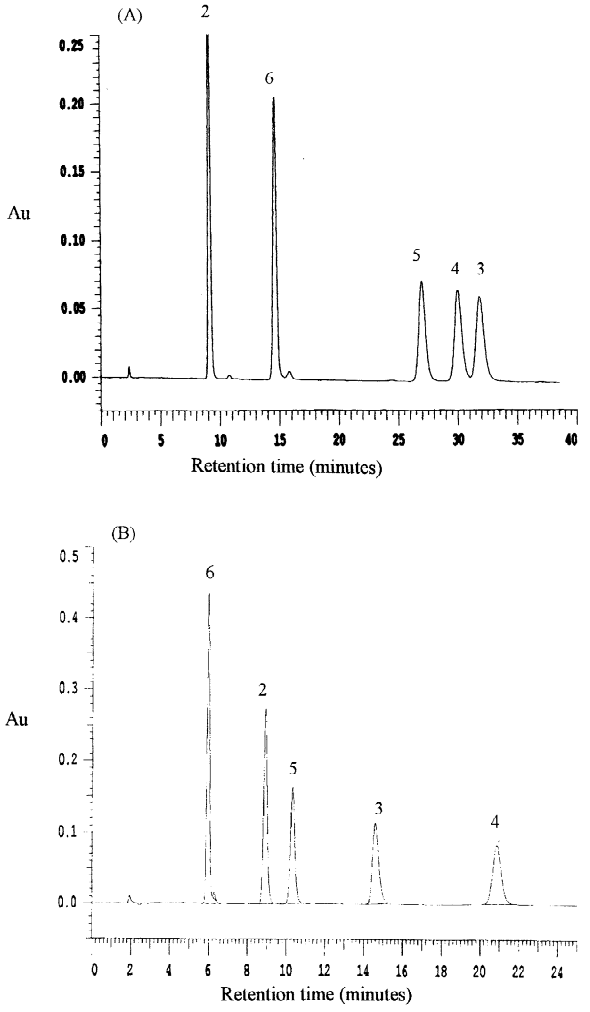


Fig. 4. HPLC chromatograms of *Vinca* alkaloids. Conditions: XTerra RP-18, 5 μm, 250×4.6 mm I.D., column maintained at 35 °C; mobile phase acetonitrile–water–potassium dihydrogen phosphate (300:700:6.8, v/v/w) adjusted at pH 5 (A) and acetonitrile–water–boric acid (550:450:3.1, v/v/w) adjusted at pH 10 (B) at a flow-rate of 1.0 ml/min; UV detection at 214 nm. Peak identification: 2, catharanthine; 3, vinorelbine; 4, anhydrovinblastine; 5, vinflunine; 6, vindoline.

blastine and vinorelbine peaks which had a value of 1.58 (system I–pH 5) which did not permit baseline separation.

Irrespective of the pH of the mobile phase, the number of theoretical plates per metre was around 40,000–50,000 for all compounds.

According to these results, the most suitable eluent system to separate the five studied *Vinca* alkaloids in a time less than 25 min was system II with the mobile phase buffered at pH 10. This HPLC system was retained for further investigations (LOD, LOQ, accuracy).

3.3. Performances comparison of CE and HPLC

In order to evaluate the performances of CE and HPLC methods developed, parameters such as specificity, linearity, accuracy, limits of detection and quantification were studied.

3.3.1. Specificity

To demonstrate specificity, a series of structurally similar compounds were added to the five studied *Vinca* alkaloids. Three pharmaceuticals, vindesine, vincristine and vinblastine; two degradation products, 4-*O*-deacetylvinflunine and 4-*O*-deacetylvinorelbine; one reaction intermediary, 4′-deoxy-20′,20′-difluorovinblastine.

Only two components of the *Vinca* alkaloid complex mixture were not separated by CE. Vinblastine co-migrated with vinflunine. The developed method allowed baseline separation of 10 alkaloids in less than 10 min (Fig. 5). To achieve this result and particularly to improve resolution of the last three analytes (vincristine, vindoline and 4′-deoxy-20′,20′-difluorovinblastine) the concentration of acetic acid was increased from 0.6 to 0.8 *M*.

Chromatographic resolution of vincristine and 4′-deoxy-20′,20′-difluorovinblastine was obtained by HPLC. On the other hand, vinblastine, vindesine, deacetylvinorelbine and deacetylvinflunine were not separated. A poor efficiency was obtained with the latter three molecules due to an unsuitable chromatographic system. The aim of the HPLC study was to obtain a fast isocratic separation. Under these conditions, HPLC did not allow complete separation of the complex mixture. The chromatogram of different compounds eluted in less than 25 min is shown in Fig. 4B.

3.3.2. Linearity

The linearity of the CE method was evaluated by analyzing standard solutions containing the five studied *Vinca* alkaloids. Nine solutions diluted to various concentrations ranging from ~5 to 125 μg/ml were prepared in order to determine the relation-

Table 1
HPLC chromatographic parameters

Analytes	Eluent system I–pH5				Eluent system II–pH 10			
	t_r (min)	A_s	R_s	N	t_r (min)	A_s	R_s	N
Catharanthine	9.11	1.29	–	40,500	9.01	1.12	10.36	46,000
Vindoline	14.63	1.08	12.49	50,300	6.00	1.19	–	38,500
Anhydrovinblastine	29.97	1.27	2.83	46,000	21.21	1.11	9.46	44,200
Vinorelbine	31.81	1.20	1.58	44,300	14.62	1.37	8.25	39,900
Vinflunine	26.96	1.21	16.15	46,000	10.47	1.13	3.86	39,500

t_r, Retention time; N, number of theoretical plates (European Pharmacopoeia); R_s, resolution (European Pharmacopoeia); A_s, peak asymmetry.

ship between the peak area and the concentration of the analyte. A basic compound, 4-(2-keto-1-benzimidazolinyl) piperidine was used as internal standard to provide results free from injection system variations. Each solution was spiked with a constant concentration of 132 μg/ml. A nine-point calibration curve for each alkaloid was established by plotting analyte/internal standard peak area ratio against the analyte concentration. The results, given in Table 2, show that linear responses were obtained over the investigated concentration range for all compounds with correlation coefficients (r^2) greater than 0.9990.

3.3.3. Limits of detection (LOD) and quantification (LOQ)

For all alkaloids, the LOD and the LOQ were estimated by considering the amount of analyte

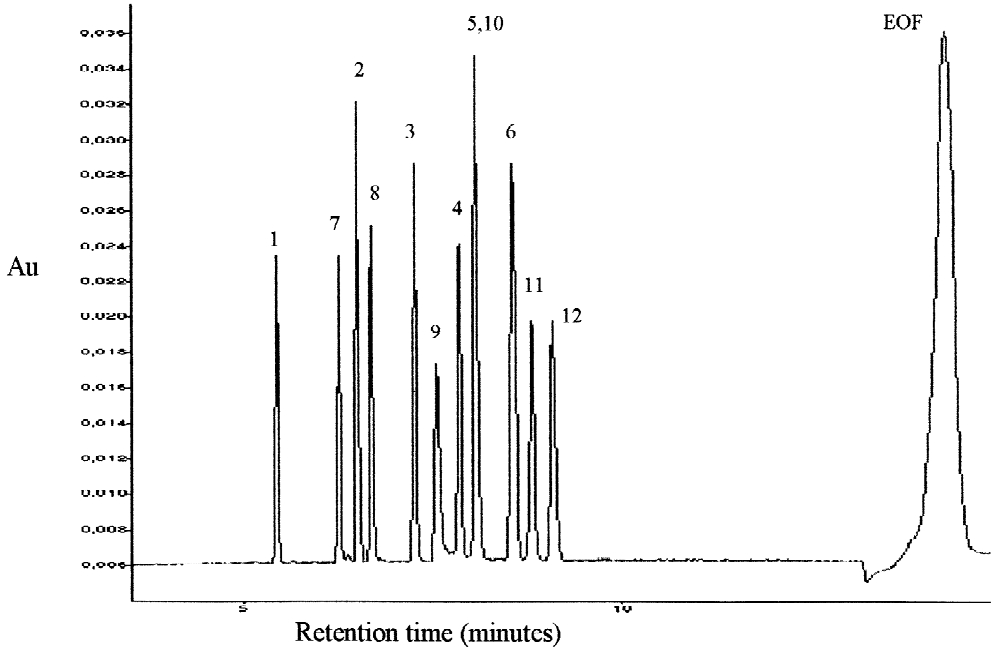


Fig. 5. Electropherogram of a complex mixture of *Vinca* alkaloids. Electrophoretic medium: 25:75 (v/v) acetonitrile–methanol mixture containing 50 m*M* ammonium acetate and 0.8 *M* acetic acid. Applied voltage 25 kV, capillary temperature 20 °C. Peak identification: 1, internal standard; 2, catharanthine; 3, vinorelbine; 4, anhydrovinblastine; 5, vinflunine; 6, vindoline; 7, 4-*O*-deacetylvinorelbine; 8, 4-*O*-deacetylvinflunine; 9, vindesine; 10, vinblastine; 11, 4′-deoxy-20′,20′-difluorovinblastine; 12, vincristine.

Table 2
Linearity data

Analytes	Concentration range (μg/ml)	Slope (SD)	Intercept (SD)	Correlation coefficient (r^2)
Catharanthine	6.29–125.80	0.0817 (0.0001)	0.085 (0.065)	0.9990
Vindoline	5.46–109.20	0.0846 (0.0004)	0.020 (0.024)	0.9998
Anhydrovinblastine	5.25–105.00	0.0712 (0.0006)	0.006 (0.034)	0.9995
Vinorelbine	5.33–160.60	0.0565 (0.0006)	0.040 (0.033)	0.9993
Vinflunine	5.43–108.60	0.0593 (0.0005)	0.027 (0.030)	0.9995

which gave a signal-to-noise ratio of 3 and 10, respectively.

In CE, samples were injected hydrodynamically 10 s at 2.8×10^3 Pa so as to introduce a larger volume inside the capillary. Ten seconds was the longest injection time that it was possible to use since, after that, vinflunine and anhydrovinblastine were not totally resolved.

HPLC conditions for this study were identical to those described above, except for the injection volume which had a value set to 100 μl.

The values of LOD and LOQ are summarized in Table 3 for the two methods. The worst LOD and LOQ were observed for vinorelbine and vinflunine, respectively, in HPLC and CE. The best limits were obtained in HPLC, with factors ranging from 2.5 to 14.

3.3.4. Accuracy

Accuracy was evaluated for only one compound, vinorelbine, by analyzing a 10 mg/ml commercial vial of Navelbine® according to developed methods. The control solution was prepared by diluting the aqueous solution of Navelbine 100 times with a methanol–acetic acid (99.5:0.5, v/v) mixture for CE and with an acetonitrile–water (50:50, v/v) mixture for HPLC (Table 3).

4. Conclusion

A nonaqueous capillary electrophoresis method was optimized and evaluated in comparison with a HPLC method for the determination of *Vinca* alkaloids. The results demonstrate that the CE technique was a good alternative for assaying these compounds in pharmaceutical formulations and appears to offer some advantages over HPLC in terms of analysis time and resolution. In addition, the nonaqueous solvent developed appears appropriate for the coupling of CE to electrospray mass spectrometry. This is a promising feature in terms of the identification and analysis of *Vinca* alkaloids in biological fluids.

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Table 3
Performances comparison of capillary electrophoresis and high-performance liquid chromatography methods

Analytes	LOD (μg/ml)		LOQ (μg/ml)		Accuracy (%±RSD, $n=3$)	
Catharanthine	***0.04***	0.01	***0.15***	0.05	–	–
Vindoline	***0.14***	0.01	***0.46***	0.03	–	–
Anhydrovinblastine	***0.15***	0.04	***0.50***	0.14	–	–
Vinorelbine	***0.13***	0.05	***0.43***	0.17	***100.7±0.5***	101.5±0.3
Vinflunine	***0.17***	0.02	***0.55***	0.06	–	–

CE, in bold.

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