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Sensitive determination of vinorelbine and its metabolites in human serum using liquid chromatography-electrospray mass spectrometry

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Abstract

A liquid chromatography-electrospray mass spectrometry method was developed for the quantitation of vinorelbine (VNB) and two metabolites, vinorelbine *N*-oxide (VNO) and deacetyl vinorelbine (DAV) in human serum. The limits of quantitation (LOQ) reached 0.5 ng/ml for both VNB and VNO and 1 ng/ml for DAV. The method was proved linear in the range of LOQs up to 1000 ng/ml, and extraction recovery was 80% on average for the three compounds. It was applied to the pharmacokinetic monitoring of vinorelbine and, for the first time, to the detection of VNO in the serum of patients suffering from non-small-cell lung cancer. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Vinorelbine; Vinorelbine *N*-oxide; Deacetylvinorelbine; LC-MS

1. Introduction

Vinorelbine (5′-noranhydrovinblastine, VNB) is a semi-synthetic vinca alkaloid, tested in the treatment of a variety of malignancies and currently indicated for advanced human non-small-cell lung cancer (NSCLC) and advanced breast cancer [1–3]. Chemically (Fig. 1), this drug differs from its analogues by alterations on the catharanthine moiety [4,5]. Its mechanism of action is only partially known but it is assumed that it acts, like vinblastine and vincristine, as an antimicrotubule agent arresting all divisions in mitosis [6,7]. In comparison with other vinca alkaloids, it was estimated that VNB showed reduced neurotoxicity. Its dose-limiting toxicity is neutropenia [8]. After i.v. administration, the drug is rapidly distributed into the peripheral tissues reflecting its intense tissue uptake, due to the concentration of alkaloids in cells in general and the lipophilicity of VNB in particular [9,10]. The extensive distribution of VNB into lung tissues may explain its particularly high activity in NSCLC, as a single agent therapy or in combination with other antitumour drugs. Indeed, 3 h after administration, VNB levels were much higher (up to 300-fold) in lung tissue than in serum [11]. During blood transport, VNB mainly binds to α1-acid glycoprotein and low density lipoproteins. When determined in sera col-

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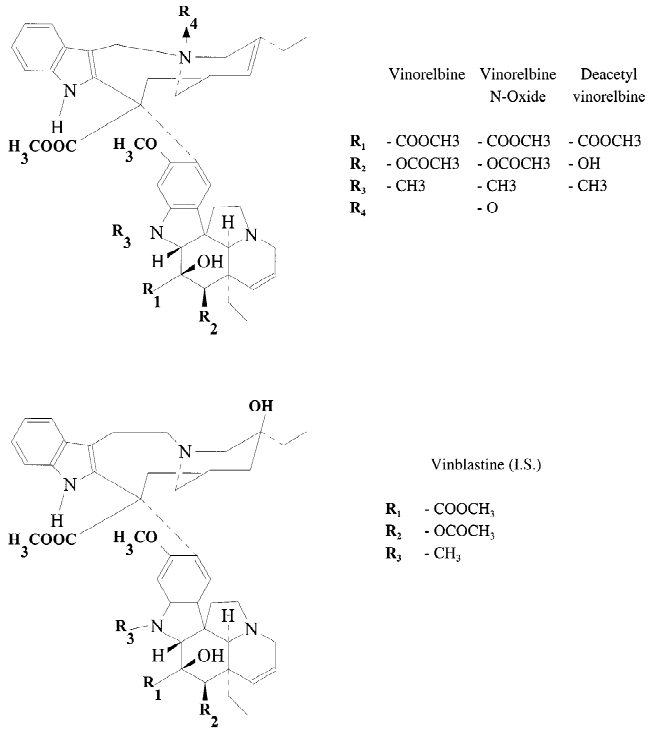

Fig. 1. Chemical structure of vinorelbine, vinorelbine *N*-oxide, deacetyl vinorelbine and vinblastine (I.S.).

lected from cancer patients, the unbound fraction of VNB was at least 13.5% [12]. Little is known about the biotransformation of VNB. However, there is some evidence for the existence of a biotransformation in plasma, which might have pharmacological and toxicological implications in clinical practice [13]. Some preclinical data obtained in vitro and in vivo suggested the existence of two metabolites [14,15]: deacetyl vinorelbine (DAV) and vinorelbine *N*-oxide (VNO). The former was found in urine fractions, representing 0.25% of the injected dose [16], but to the best of our knowledge, has not been detected in human serum up to now. Although DAV showed the same activity and toxicity as the parent drug [14], it cannot account for a large part of the overall activity of VNB. VNO does not exert any antiproliferative activity in vitro and, so far, had never been detected in any biological fluid (serum or urine) of treated patients [17]. Several clinical pharmacokinetic studies of VNB were performed [15,16,18,19], using different analytical techniques, but the investigation of the pharmacokinetics of its metabolites has been limited by the lack of a suitable, specific analytical method. Initially, radioimmunoassays [20,21] have been reported, the major drawback of which was the lack of specificity between the parent drug and the eventual metabolites. Owing to the low serum concentrations of VNB (below 5 ng/ml, 24 h after a usual dose of 30 mg/m^2 i.v. weekly), a specific and very sensitive detection method was thus required for clinical pharmacokinetics. The separative techniques published so far for VNB, and sometimes its metabolites, mainly involved HPLC with UV [17,22], fluorescence [23–26] or electrochemical [27,28] detection. Some of them concerned the qualitative identification of metabolites [24], or the quantitation of the sole VNB in serum or in plasma [25,26,28], or in serum and urine [27], or the determination of VNB and DAV in serum and urine [22]. The best LOQs reached were 1 ng/ml for VNB in plasma [27] and 2.5 ng/ml for DAV in serum [22], the latter being

still insufficient to detect DAV in any clinical samples. Moreover, neither quantitative nor sensitive enough methods have been described for VNO, allowing its detection in any biological matrices.

The aim of the present study was to design a sensitive, specific and fully validated method using liquid chromatography-electrospray mass spectrometry (LC-ES-MS) for the simultaneous determination of VNB, DAV and VNO in human serum. This method was intended to be applied to routine therapeutic drug monitoring (TDM), as well as to the study of VNB metabolism in human, particularly the detection of its putative *N*-oxide metabolite in serum.

2. Experimental

2.1. Chemicals, reagents and solutions

VNB was supplied by Pierre Fabre Laboratories (Castres, France) as a pure commercial form, Navelbine®, whereas deacetyl VNB and VNB *N*-oxide were kindly provided as pure standards by the same laboratory. Vinblastine, used as the internal standard (I.S.), was obtained from Lilly-France Laboratories (Saint Cloud, France). Acetonitrile, methanol, diethyl ether and dichloromethane of analytical grade, as well as sodium carbonate and sodium hydrogencarbonate, were purchased from Prolabo (Fontenay-sous-bois, France). Formic acid and ammonium formate (99% pure for both) were from Sigma (St. Louis, MO, USA). Deionised water was prepared on a Milli-Q laboratory plant (Millipore, Bedford, MA, USA). For sample extraction, Extrelut-3 extraction cartridges (Merck, Darmstadt, Germany) were used.

A 0.5 *M*, pH 9.5 carbonate–hydrogencarbonate buffer was prepared by mixing a solution of 0.5 *M*, pH 9.5 sodium carbonate, with an equal amount of a solution of 0.5 *M*, pH 9.5 sodium hydrogencarbonate.

Methanolic solutions (1 g/l) of VNB, VNO, DAV and the I.S. were prepared and kept at −20°C in the dark. The drugs were stable for at least 3 months in these conditions. Working solutions of the analytes at concentrations of 0.01, 0.1, 1 and 5 mg/l, and of the I.S at 2.5 mg/l, were freshly prepared each day by appropriate dilution in methanol.

2.2. Sample preparation

Fresh blood samples were centrifuged for 15 min at 2500 *g* and serum was frozen at −20°C until analysis. To 500 μl of serum in a 15-ml glass tube were added 50 μl of I.S. (2.5 mg/l) and 500 μl of 0.5 *M*, pH 9.5 carbonate–hydrogencarbonate buffer. The tube was briefly vortex-mixed (10 s) and the mixture was deposited on an Extrelut-3 extraction cartridge. After 10 min impregnation, elution of the analytes was performed using 6 ml of diethyl ether into a clean 10-ml glass tube. The solvent was evaporated at 30°C under a gentle stream of nitrogen, then a complementary elution of the analytes was obtained with 6 ml of diethyl ether–dichloromethane (50:50, v/v), collected in the same tube. After evaporation, the definitive extract was redissolved in 25 μl of a mixture of acetonitrile and 2 m*M*, pH 3.0 ammonium formate (70:30, v/v), of which 2 μl were injected into the chromatographic system.

2.3. HPLC Conditions

The HPLC system included a Series 200 micro-LC, high pressure gradient chromatographic pump and a Series 200 autosampler (Perkin-Elmer, Foster City, CA, USA). The reversed-phase chromatographic column was a Nucleosil C_{18}, 5 μm (150×1 mm I.D.) (LC-Packings, Polymer Laboratories, Marseille, France); its temperature was maintained at 35°C using a HPLC-column oven (Waters corporation, Milford, MA, USA). The mobile phase was a gradient of acetonitrile (37% for 2 min, increased to 60% in 6 min, maintained for 4 min and finally decreased to 37% in 0.5 min) in 2 m*M* ammonium formate (pH 3.0), delivered at a flow-rate of 50 μL/min.

2.4. Mass spectrometry

A Sciex (Toronto, ON, Canada) API-100 mass spectrometer was used, equipped with an electrospray-type ionisation device. High-purity nitrogen was used as nebulization and curtain gas. Mass spectrometric detection was performed in the positive ionization mode. For optimising ionization and ion transmission conditions of VNB, its metabolites and the I.S., pure solutions of each compound in the

mobile phase were infused separately, at 5 μl/min, into the ion source. During this experiment, a mass range from m/z 50 to 1000 was monitored with a resolution of 0.1 a.m.u. Then, for the quantitation of each analyte, particular ions were selected: the protonated molecule $[M+H]^+$ as the quantification ion and one or two main fragments as confirmation ions (Table 1). In order to obtain the highest possible intensity for quantitation and confirmation ions, fragmentation energy (''orifice voltage'') was optimised for each of them, as reported in Table 1. Acquisition was made in the selected ion monitoring mode, with a dwell time varying between 150 and 300 ms depending on the considered ion. The main other parameter settings of the ionspray source were as follows: nebulization gas-flow at 1.55 l/min; curtain gas-flow at 1.36 l/min; ionspray voltage at 5500 V.

2.5. Validation procedures

Extraction recoveries were determined in triplicates at concentrations of 5, 50 and 500 ng/ml for VNB, VNO and DAV. For a given concentration of drugs, three serum samples were spiked with the I.S. and the appropriate amount of drugs, while three others only had the I.S. added. After extraction and evaporation, the first three samples were reconstituted with 25 μl of mobile phase and the last three with 25 μl of mobile phase containing the nominal amount of VNB, VNO and DAV. Recoveries were calculated by comparison of the analyte/I.S. peak area ratios of the extracted samples with those of the corresponding unextracted standard solutions, representing a 100% recovery.

For the rest of the analytical validation, the guiding principles of the American Association of Pharmaceutical Scientists [29] were followed. These recommendations can be briefly summarised as follows: the within-day precision should be studied at least at three concentrations; the intermediate (day-to-day) precision should be assessed using at least five determinations of 5–8 concentrations (excluding blank values); within-day and day-to-day experiments should yield a precision relative standard deviation (RSD) of less than 15% and a deviation (mean relative error) of less than 15% from the nominal value at every concentration studied, except for the limit of quantitation (LOQ) where 20% is acceptable for both parameters.

Accordingly, within-day precision was assessed at concentration levels of 0.5, 5 and 50 ng/ml for both VNB and VNO and of 1, 5 and 50 ng/ml for DAV, by extraction and analysis on the same day of six

Table 1
Retention times, selected ions and related orifice voltages and limits of detection of vinorelbine, vinorelbine *N*-oxide, deacetyl vinorelbine and vinblastine (I.S.)

Compound	Retention time (min)	Selected ions[a] (a.m.u)	Orifice voltage (V)	Relative intensity (%)	LOD (ng/ml)
Vinorelbine	9.2	**779.4**	45	100	0.25
		658.4	40	25	
		390.4	40	15	
Vinorelbine *N*-oxide	9.1	**795.4**	50	100	0.50
		272.0	60	5	
Deacetyl-vinorelbine	7.4	**737.4**	30	100	0.75
		616.4	40	60	
		357.4	70	25	
Vinblastine (I.S.)	7.1	**811.6**	20	100	N.D.[b]
		272.0	60	80	

[a] Quantifying ions are in bold characters.
[b] N.D.=Not determined.

drug-free serum samples fortified with the three compounds for each level. Intermediate precision was estimated by analysing each day for 6 days a set of calibrating samples spiked at 0, 0.5, 1, 2, 5, 10, 20, 50, 200, 500 and 1000 ng/ml of VNB, VNO and DAV. The calibration graph of the peak area ratio (y) of the drug-to-I.S. versus concentrations (x) of the calibration standards were fitted by linear least squares regression, using different weights ($1/x$ and $1/x^2$) in order to optimize the correlation coefficient and to minimize the relative errors of the back-calculated concentrations. The limit of detection, defined as the lowest concentration yielding a signal-to-noise ratio higher than three, was determined by analysing samples spiked at 0.25, 0.5, 0.75 and 1 ng/ml of each analyte.

3. Results

The chromatographic retention times for VNB and its metabolites are reported in Table 1. DAV is chromatographically well resolved, eluting earlier than the other two analytes. Though the retention times of VNB and VNO were very similar and the chromatographic peaks not totally resolved on the total ion chromatogram, their specific identification and quantitation were possible using chromatograms reconstructed from their characteristic ions (Fig. 2). For each compound, the ratio between confirmation and quantitation ions was automatically compared to its theoretical value by the quantitation software used (TurboQuan, Sciex, Toronto, ON, Canada). The selectivity of MS detection is very valuable for the

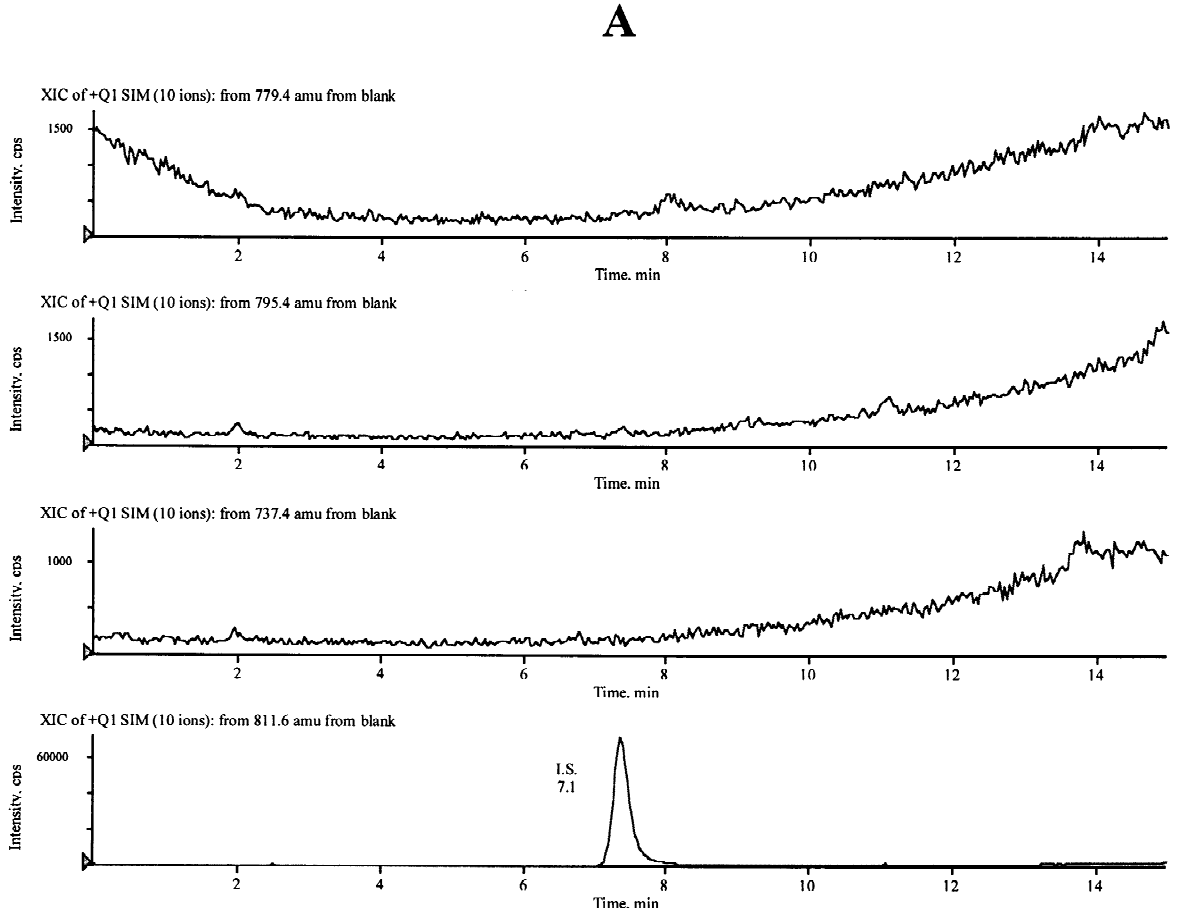

Fig. 2. Extracted ion chromatograms of extracts from (A) a blank serum and (B) a serum spiked at 1 ng/ml of VNB, VNO and DAV.

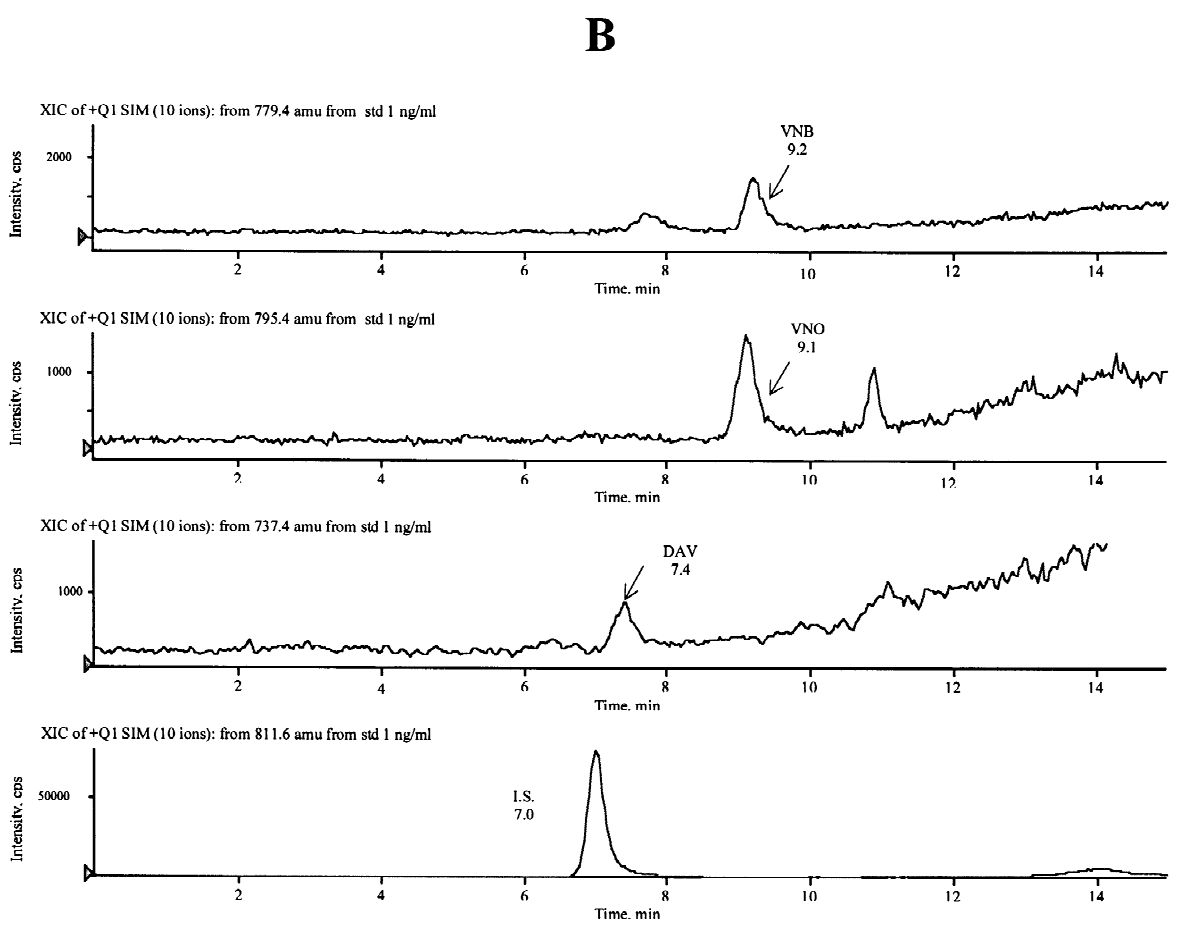

Fig. 2. (*continued*)

monitoring of the often polymedicated cancer patients. Moreover, no interference was noted on the chromatograms of blank serum samples from healthy volunteers or patients. The full-scan mass spectra of each analyte are presented in Fig. 3. Extraction recoveries at 5, 50 and 500 ng/ml were, respectively: 80.5±2.9%, 79.6±7.0% and 78.5±1.6% for VNB; 76.7±7.1%, 77.9±1.5% and 77.1±4.8% for DAV; 78.7±6.3%, 78.0±8.4% and 77.9±5.3% for VNO. The extraction recovery of the I.S. was 79.6±8.6%. The detection limits were 0.25 ng/ml for VNB, 0.5 ng/ml for VNO and 0.75 ng/ml for DAV, using a 0.5-ml sample volume.

Intra-assay precision and accuracy were very satisfactory for all the concentrations tested, as shown in Table 2. C.V. values were between 4.2 and 9.7% for all three drugs and the mean relative error was less than 11% at all concentrations except for VNO, for which it reached 13.1% at 500 ng/ml. The between-day precision C.V. values (Table 3), ranged between 3.2 and 9.8%, except for the LOQs where 14.8 and 19.6% were found for VNB and VNO, respectively. The between-day mean relative error was less than 10% at all concentrations. The limits of quantitation could then be assessed as 0.5 ng/ml for both VNB and VNO and 1 ng/ml for DAV. Linearity was verified from their respective LOQ up to 1000 ng/ml using $1/x$ weighting, which gave the best accuracy at the lower end of the curves. The correlation coefficients (r) were greater than 0.998 for all the curves and the three compounds.

This method was applied to the determination of

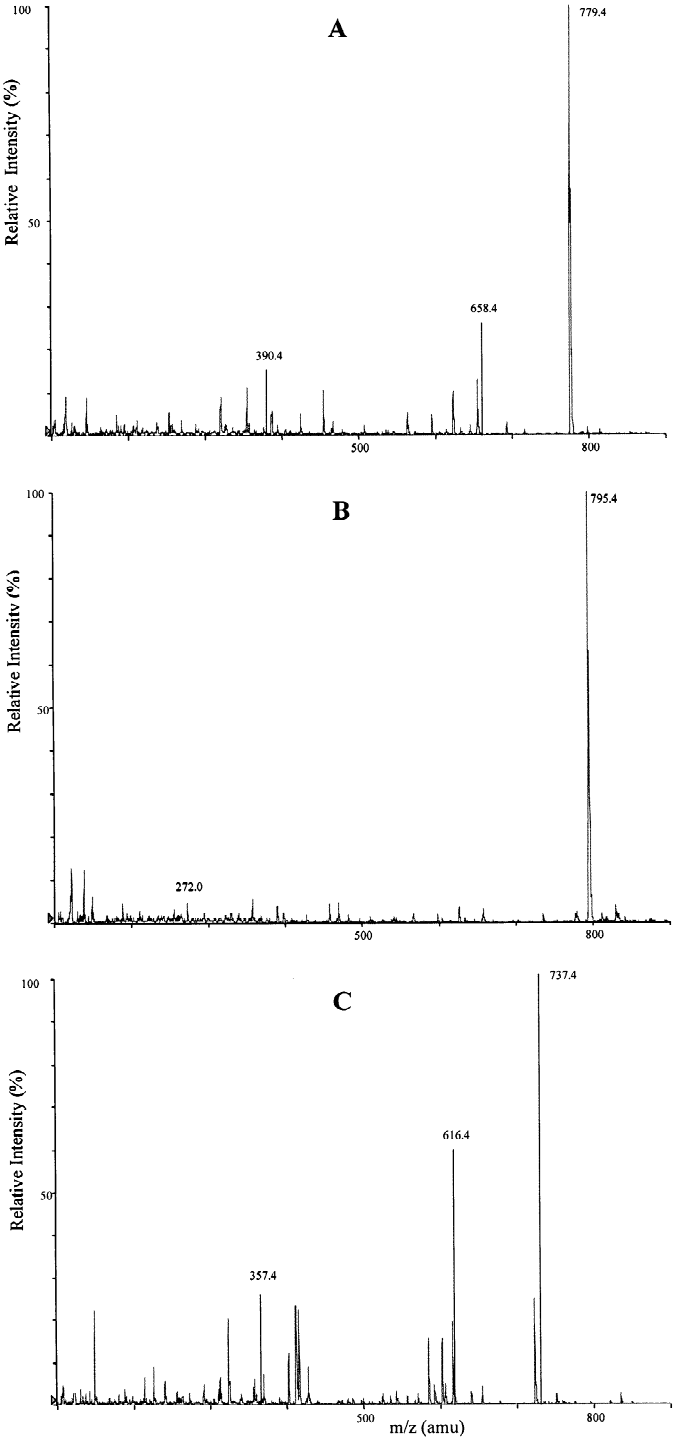

Fig. 3. Mass spectra of (A) vinorelbine, (B) vinorelbine *N*-oxide and (C) deacetyl vinorelbine.

Table 2
Intra-assay precision and accuracy for the determination of vinorelbine, vinorelbine *N*-oxide and deacetyl vinorelbine in human serum

Added concentration (ng/ml)	Mean measured concentration ($N=6$) (ng/ml)	Precision (C.V.%)	Mean relative error[a] (%)
Vinorelbine			
0.5	0.5	9.2	3.5
50.0	49.4	7.8	1.2
500.0	444.2	4.2	11.2
Vinorelbine N-oxide			
0.5	0.5	9.7	5.2
50.0	49.8	6.4	0.5
500.0	434.5	6.0	13.1
Deacetyl vinorelbine			
1.0	1.1	8.6	10.2
50.0	49.2	9.1	1.6
500.0	449.1	4.6	10.2

[a] Mean relative error=|mean measured value−theoretical value|×100/theoretical value.

VNB and its metabolites in blood samples of patients with NSCLC. Fig. 4 shows the selected ion chromatograms of a blood sample obtained from a 67-year-old male patient, who received a 47-mg dose of Navelbine® as a 20-min intravenous infusion. The concentrations found in this sample, collected 1 h after the end of infusion, were 39 and 3.6 ng/ml for VNB and VNO, respectively. In a preliminary series of three patients in whom blood samples were collected at 1, 6 and 24 h after infusion of Navelbine® according to a limited sampling strategy with Bayesian estimation [30], we were able to detect *N*-oxide in all the samples collected at 1 h and in two of those collected at 6 h, but not in samples collected at 24 h. DAV could not be detected in any of these samples (Table 4).

4. Discussion

Many methods have been proposed for the determination of vinca-alkaloids in biological fluids, but to our knowledge, no technique has been published for the determination of VNB and its two putative metabolites simultaneously by HPLC coupled to MS. VNB was generally determined by HPLC, with different detection techniques. The first method published was based on UV detection [22] and claimed to be able to determine VNB and DAV in serum and urine; the LOQs were 2.5 ng/ml in serum and 5 ng/ml in urine, for both compounds. However, UV detection is not very selective, nor very sensitive and in this case failed to detect DAV in serum of patients treated with vinorelbine and included in a pharmacokinetic study. Nevertheless, this technique was adapted by Levêque et al. [17] to screen for VNB, DAV and VNO in several biological fluids; though an LOD of 1 ng/ml was reached for VNB, linearity ranges were reported as 25–1000 ng/ml in plasma (which suggests an LOQ of 25 ng/ml) and 5–1000 ng/ml in urine. No quantitative validation data were given for VNO by the authors. Other systems of detection have been employed, such as fluorescence and electrochemical detectors, which have offered potential advantages in terms of selectivity or sensitivity. Several years ago, our team [27] investigated the use of an electrochemical, amperometric detector. VNB was the sole analyte quantitated, with an LOQ of 1 ng/ml. The coulometric mode has been evaluated by Mouchard et al. [28] for the determination of VNB in rabbit plasma; it yielded the same LOQ value. Fluorescence detection has been used by several teams [23–26]. This mode of detection is at least as sensitive as UV detection and more robust. The method developed by Debal et al. [23] for cell culture medium has been adapted by Robieux et al. [26] to improve sensitivity. The latter authors obtained an LOD of 1 ng/ml and an LOQ of

Table 3
Intermediate precision, accuracy and linear regression parameters of vinorelbine, vinorelbine *N*-oxide and deacetyl vinorelbine determination in human serum

Added concentration (ng/ml)	Mean measured concentration ($N=6$) (ng/ml)	Precision (C.V%)	Mean relative error[a] (%)
Vinorelbine			
0.5	0.50	14.8	0.5
1.0	1.05	9.0	4.7
2.0	1.91	8.0	4.7
5.0	4.84	9.3	3.2
10.0	10.06	5.2	0.6
20.0	21.55	8.3	7.8
50.0	45.99	7.0	8.0
200.0	198.36	3.2	0.8
500.0	516.66	6.2	3.3
1000.0	993.04	4.7	0.7
Calibration curve:			
Slope	0.009±0.001		
Intercept	0.001±0.003		
Correlation coefficient	0.998±0.002		
Vinorelbine N-oxide			
0.5	0.56	19.6	12.8
1.0	1.00	6.3	0.03
2.0	1.90	6.2	5.1
5.0	4.98	7.9	0.5
10.0	10.21	4.9	2.1
20.0	20.08	9.7	0.4
50.0	46.50	8.0	7.0
200.0	192.48	7.6	3.8
500.0	528.56	4.5	5.7
1000.0	985.41	4.3	1.5
Calibration curve:			
Slope	0.008±0.002		
Intercept	−0.003±0.003		
Correlation coefficient	0.998±0.001		
Deacetyl vinorelbine			
1.0	1.16	8.9	15.6
2.0	2.14	8.4	6.9
5.0	5.02	9.5	0.3
10.0	9.60	8.6	4.0
20.0	20.11	9.8	0.6
50.0	45.19	6.8	9.6
200.0	183.70	7.8	8.2
500.0	531.80	8.1	6.4
1000.0	1010.70	3.2	1.1
Calibration curve:			
Slope	0.003±0.001		
Intercept	−0.002±0.001		
Correlation coefficient	0.998±0.003		

[a] Mean relative error=|mean measured value−theoretical value|×100/theoretical value.

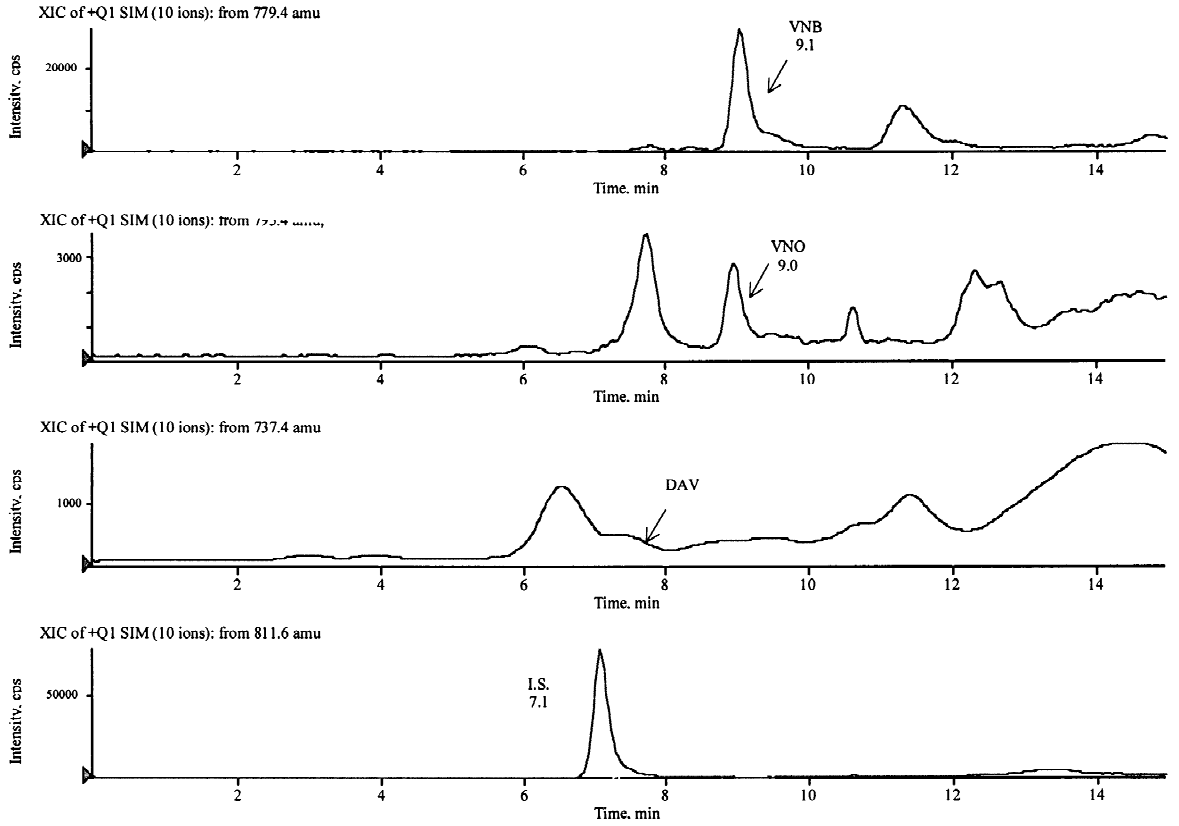

Fig. 4. Extracted ion chromatograms of a serum sample collected 1 h after i.v. infusion of 47 mg of Navelbine® in a NSCLC patient.

2 ng/ml for VNB, i.e., slightly worse than the previous methods. Moreover, as found by Van Tellingen et al. [27] and Mouchard et al. [28], to improve the quantitation accuracy for low VNB concentrations, two calibration graphs were necessary for the determination of high and low levels.

Table 4
Serum concentrations of vinorelbine, vinorelbine *N*-oxide and deacetyl vinorelbine at 1, 6 and 24 h after 20-min i.v. infusions of Navelbine® in three patients

Patient	Dose (mg)	Time post-dose (h)	VNB (ng/ml)	VNO (ng/ml)	DAV (ng/ml)
Patient 1	53.7	1	17.4	4.6	<LOQ
		6	4.1	1.2	<LOQ
		24	0.9	<LOQ	<LOQ
Patient 2	47.0	1	39.0	3.6	<LOQ
		6	9.3	0.5	<LOQ
		24	1.1	<LOQ	<LOQ
Patient 3	44.0	1	23.1	0.6	<LOQ
		6	4.0	<LOQ	<LOQ
		24	1.1	<LOQ	<LOQ

The suitability of our method for clinical samples was demonstrated by the determination of VNB and its metabolites in blood samples of several patients. The presence of VNO in serum is an interesting finding elicited by this technique. We were able to detect VNO in all the serum samples collected at 1 h after the end of infusion. Compared with VNB, its concentration was always much lower (up to ten times less). The concentrations in the samples collected later were certainly too low to be detectable, despite an LOD of 0.5 ng/ml. The consequences of the presence of VNO in blood are unknown, but *N*-oxides are often reactive, i.e., toxic molecules. VNO might therefore occasionally be responsible for some toxic effects in some patients, inasmuch as its concentration seems to vary greatly between individuals. The specificity of MS detection and the detection of VNO in different patients at concentrations compatible with the pharmacokinetics of a metabolite, dismiss the probability of an artefact. No quantitative method for VNO had been previously described in the literature. It has been screened in biological fluids only by Levêque et al. [17], who could not find it. Anyway, given the low serum concentrations found in some samples herein, it is possible that the detection of VNO has been limited by the lack of sensitivity of the method used. Our extraction procedure also favoured the detection of *N*-oxide. Most of the liquid–liquid extraction procedures for VNB in biological fluids involved diethyl ether, except one paper that reported an ion-pair extraction [24] for the determination of VNB in tumour cells. In our experiments, although diethyl ether was able to extract VNB and DAV, it was not satisfactory for the extraction of VNO. Several organic solvents were evaluated to improve the recovery of this oxided metabolite. The use of dichloromethane in a second elution was essential to obtain a good extraction recovery of VNO. Finally, we could not detect DAV in any of these serum samples, despite an LOD of 0.75 ng/ml. This is in accordance with previous studies [16] though these did not achieve such LODs. This implies either that this metabolite does not exist in humans or that its circulating concentrations are extremely low.

The present procedure is the first one using liquid chromatography coupled to mass spectrometry for the simultaneous determination of VNB and VNO in human serum. Though it is the most sensitive method published to date and doubtlessly one of the most specific owing to MS, it failed to detect any DAV in serum samples of cancer patients treated with vinorelbine. We currently use it, together with a Bayesian pharmacokinetic estimation procedure previously described [30], for TDM and dose adaptation of Navelbine® using only three blood samples.

References

[1] X.J. Zhou, R. Rahmani, Drugs 44 (Suppl. 4) (1992) 1.
[2] C. Toso, C. Lindley, Am. J. Health-Syst. Pharm. 52 (1995) 1287.
[3] A. Depierre, E. Lemarie, G. Dabouis, G. Garnier, P. Jacoulet, J.C. Dalphin, Am. J. Clin. Oncol. 14 (1991) 115.
[4] F. Guéritte, A. Pouilhès, P. Mangeney, R.Z. Andriamialisoa, N. Langlois, Y. Langlois, P. Potier, Eur. J. Med. Chem. 18 (1983) 419.
[5] P. Mangeney, R.Z. Andriamialisoa, J.Y. Lallemand, N. Langlois, Y. Langlois, P. Potier, Tetrahedron 35 (1979) 2175.
[6] R.A. Bender, E. Hamel, K.R. Hande, in: B.A. Chabner, J.M. Collins (Eds.), Cancer Chemotherapy, Principles and Practice, JB Lippincott, Philadelphia, 1990, p. 253.
[7] S. Binet, A. Fellous, V. Meininger, Semin. Oncol. 16 (Suppl. 4) (1989) 5.
[8] H.A. Burris III, S. Fields, Semin. Oncol. 21 (Suppl. 10) (1994) 14.
[9] A.M. Lengsfeld, J. Dietrich, B. Schultze-Maurer, Cancer Res. 42 (1982) 3798.
[10] W.D. Singer, R.H. Himes, Biochem. Pharmacol. 43 (1992) 545.
[11] D. Levêque, E. Quoix, P. Dumont, G. Massard, J.G. Hentz, A. Charloux, F. Jehl, Cancer Chemother. Pharmacol. 33 (1993) 176.
[12] S. Urien, F. Brée, F. Breillout, G. Bastian, A. Krikorian, J.P. Tillement, Cancer Chemother. Pharmacol. 32 (1993) 231.
[13] P. Bore, R. Rahmani, J. van Cantfort, C. Focan, J.P. Cano, Cancer Chemother. Pharmacol. 23 (4) (1989) 247.
[14] A. Krikorian, R. Rahmani, M. Bromet et al., Semin. Oncol. 16 (Suppl. 4) (1989) 21.
[15] W.A. Wargin, V.S. Lucas, Semin. Oncol. 21 (Suppl. 10) (1994) 21.
[16] D. Levêque, F. Jehl, Clin. Pharmacokinet. 31 (3) (1996) 184.
[17] D. Levêque, F. Jehl, E. Quoix, H. Monteil, Xenobiotica 23 (11) (1993) 1325.
[18] P. Marquet, G. Lachâtre, J. Debord, B. Eichler, F. Bonnaud, G. Nicot, Eur. J. Clin. Pharmacol. 42 (1992) 545.
[19] I. Robieux, R. Sorio, E. Borsatti, R. Cannizzaro, V. Vitali, P. Aita, A. Freschi, E. Galligioni, S. Monfardini, Clinical Pharmacology and Therapeutics 59 (1996) 32.
[20] R. Rahmani, M. Martin, J. Barbet, J.P. Cano, Cancer Res. 44 (1984) 5609.

[21] P. Bore, R. Rahmani, J. van Cantfort, C. Focan, J.P. Cano, Cancer Chemother. Pharmacol. 23 (4) (1989) 247.
[22] F. Jehl, J. Debs, C. Herlin, E. Quoix, C. Gallion, H. Monteil, J. Chromatogr. B 525 (1990) 225.
[23] V. Debal, H. Morjani, J.M. Millot, J.F. Angiboust, B. Gourdier, M. Manfait, J. Chromatogr. B 581 (1) (1992) 93.
[24] S.J. Van Belle, M. De Smet, C. Monsaert, F. Geerts, G.A. Storme, D.L. Massart, J. Chromatogr. B 576 (2) (1992) 351.
[25] O. van Tellingen, A. Kuijpers, J.H. Beijnen, M.R. Baselier, J.T. Burghouts, W.J. Nooyen, J. Chromatogr. B 573 (2) (1992) 328.
[26] I. Robieux, V. Vitali, P. Aita, A. Freschi, R. Lazzarini, R. Sorio, J. Chromatogr. B 675 (1996) 183.
[27] G. Nicot, G. Lachâtre, P. Marquet, F. Bonnaud, J.P. Vallette, J.L. Rocca, J. Chromatogr. B 528 (1990) 258.
[28] C. Mouchard-Delmas, B. Gourdier, R. Vistelle, J. Chromatogr. B 663 (1995) 390.
[29] V.P. Shah, K.K. Midha, S. Dighe, I.J. Mc Gilveray, J.P. Skelly, A. Yacobi, T. Layloff, C.T. Viswanathan, C.E. Cook, R.D. McDowall, K.A. Pittman, S. Spector, J. Pharm. Sci. 81 (1992) 309.
[30] C. Sabot, P. Marquet, J. Debord, N. Carpentier, L. Merle, G. Lachâtre, Eur. J. Pharmacol. 54 (1998) 171.