Available online at www.sciencedirect.com
ScienceDirect
Journal of Pharmaceutical and Biomedical Analysis 44 (2007) 144–149
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
www.elsevier.com/locate/jpba

A high performance liquid chromatography method for vinorelbine and 4-*O*-deacetyl vinorelbine: A decade of routine analysis in human blood

C. Puozzo [a,*], H.L. Ung [b], G. Zorza [a]

[a] *Institut de Recherche Pierre Fabre, Oncology Pharmacokinetic Department, 2 rue Christian d'Espic, 81106 Castres, France*
[b] *Institut de Recherche Pierre Fabre, Centre Expérimental de Pharmacocinétique de Campans, Bel Air de Campans, 81106 Castres, France*

Received 30 August 2006; received in revised form 5 January 2007; accepted 9 January 2007
Available online 16 January 2007

Abstract

A sensitive high performance liquid chromatographic method was developed and validated for the simultaneous quantification of vinorelbine and its active metabolite, 4-*O*-deacetyl vinorelbine, in human biological fluids. These two compounds together with vinblastine, used as internal standard, were extracted from blood and urine by a liquid–liquid process using diethyl ether, and followed by a back-extraction in acidic conditions. Then, they were analysed through a cyano column and detected in ultraviolet at 268 nm. The assay linearity was validated up to 2000 ng/ml. The lower limit of quantification was set at 2.5 ng/ml. The between-run precision and accuracy were always higher than 94%. Biological samples were stable when stored at −80 °C over 2 years. The long-term reproducibility and the suitability of this analytical method were demonstrated within the last decade through the analysis of about 7000 samples during the clinical development of i.v. and oral formulations of vinorelbine.

Because vinorelbine binds mainly to platelets and blood cells and because this binding is rapidly reversible and highly influenced by environmental conditions, drug concentration in plasma may be highly influenced by the sampling conditions and the centrifugation process used to separate blood cells from plasma. Therefore, this method was developed in blood and then used for sample analyses in routine. The major benefit was that it was easy for nurses to directly collect blood instead of plasma and that reduced volume of sampling could be withdrawn from frail patients. Furthermore, the analysis in blood enabled to quantify vinorelbine and 4-*O*-deacetyl vinorelbine concentrations for a longer period of time, which resulted in a more accurate evaluation of pharmacokinetic parameters.
© 2007 Elsevier B.V. All rights reserved.

Keywords: Vinorelbine; 4-*O*-Deacetyl vinorelbine; HPLC; Human blood

1. Introduction

Vinorelbine (VRL), nor-5′-anhydrovinblastine bitartrate, is a vinca-alkaloid obtained by chemical semi synthesis. The intravenous formulation (Navelbine® i.v.) was marketed for non-small cell lung cancer and advanced breast cancer indications. Then, an oral formulation (Navelbine® oral) was launched in several European countries. Although vinorelbine is not an anti-neoplastic drug that requires therapeutic drug monitoring, an easy to use, reliable and sensitive bioanalytical method is a major issue to characterize its pharmacokinetics in patients.

Several methodologies based on different approaches have been published. The very early method involved a radioimmunoassay technique [1] and was used in initial clinical trials [2–4]. Then, more specific techniques based on high performance liquid chromatography were successively developed.

Separation was generally carried out using reversed phase columns followed by UV [5,6], fluorescence [7–9] or electrochemical detection [5,10]. Liquid–liquid extraction with a one- or two-step procedure [11,5,6,9], solid phase extraction [12,13], or ion-pair extraction [14] enabled to separate vinorelbine from biological fluids.

All these different methods were developed in plasma, urine and scarcely in bile. They were mainly used in phase I clinical trials that included pharmacokinetics on a limited number of patients [15], hence variable vinorelbine pharmacokinetic parameters [4,16], and inconsistencies between studies. This resulted in unreliable comparison between groups of patients when pooling data. The absence of cross-validation between methods did not allow to appraise the respective contribution of analytical bias *versus* patient variability [17,18].

* Corresponding author at: Institut de Recherche Pierre Fabre, Oncology Pharmacokinetic Department, 2 rue Christian d'Espic, 81106 Castres Cedex, France. Tel.: +33 5 63 71 46 37; fax: +33 5 63 71 46 40.
E-mail address: christian.puozzo@pierre-fabre.com (C. Puozzo).

0731-7085/$ – see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.jpba.2007.01.011

It was therefore necessary to set up and validate one method only that was going to improve the knowledge of the i.v. vinorelbine pharmacokinetics, and that will enable to secure a development of the oral formulation.

The interest focused rapidly on the development of a method in whole blood instead of plasma. The *in vitro* distribution of VRL demonstrated that this drug was mainly bound to blood cells and more particularly to platelets (78%) [19]. Because this binding was rapidly reversible, it was evidenced that blood to plasma distribution was highly influenced by the processing of samples. Therefore, while vinorelbine concentration in blood will remain constant, that in plasma will vary depending on differences in the centrifuge conditions (internal data).

Little, although controversial information was available on the VRL metabolism [5,17,20]. Since a different metabolism profile might have been observed between i.v. and oral administration, this method was developed in order to quantify both the parent compound and its active metabolite, 4-*O*-deacetylvinorelbine (DVRL) that was previously detected in urine [17], scarcely in plasma but never in blood [21].

The challenge was to have an easy, highly reproducible and cost effective method that could be used reliably in large numbers of biological samples. This paper presents the characteristics of this LC method.

2. Experimental

2.1. Chemical and reagents

Ammonium acetate and sodium hydroxide were supplied by Merck (Nogent sur Marne, France). Acetonitrile and diethyl ether from SDS (Peypin, France) were respectively LC and analytical grade. Sodium bicarbonate and phosphoric acid 85% were provided by Prolabo (Fontenay sous Bois, France). Deionised water was obtained from Milli-Q system, Millipore (Saint Quentin en Yvelines, France). Vinorelbine bitartrate, 4-*O*-deacetyl vinorelbine sulfate and vinblastine sulfate were obtained from Pierre Fabre Medicament (Castres, France).

2.2. Instruments

Chromatographic analysis was performed using a high-pressure pump model 305 Gilson (Villiers le Bel, France) and an automatic thermostated injector model 717 Waters (Saint Quentin en Yvelines, France).

The detection was done using a UV detector SM4000 Finnigan (Orsay, France) equipped with the acquisition data integrator Millenium 2.1® software from Waters (Saint Quentin en Yvelines, France).

2.3. Analytical conditions

HPLC separations were carried out on a Spherisorb CN column (100 mm × 4.6 mm I.D., dp = 3 μm) from Varian Chrompack (Les Ulis, France) set at 25 °C protected with a Spherisorb CN precolumn (10 mm × 2 mm I.D., dp = 5 μm). The mobile phase was a mixture of acetate buffer, 40 mM, obtained with 3.1 g of ammonium acetate in 1000 ml of water and adjusted with phosphoric acid 85% to pH 3.0, and acetonitrile (55/45, v/v). The mobile phase was filtered through a 0.45 μm membrane filter before the run. The column was initially equilibrated for at least 3 h with the mobile phase at a flow rate of 0.5 ml/min.

Separation was achieved by isocratic solvent elution at a flow rate of 1 ml/min. The detection wavelength was set at 268 nm. Backpressure of the system was about 2000 p.s.i. (1 psi = 6894.76 Pa).

2.4. Preparation of the calibration curves and quality control (QC) samples

In order to prevent any adsorption of vinca-alkaloids on glass materials, only silicone-coated materials were used. Furthermore, when split samples had to be prepared, the biological sample aliquot was first introduced in the tube, followed by the addition of the chemical reference.

Stock aqueous solutions (1 mg/ml) of VRL and DVRL were prepared separately by weighing the appropriate amount of compounds and by dissolving them in 40 mM acetate buffer, pH 3.0. These stock solutions were then successively diluted and finally pooled in order to obtain working solutions in the concentration range of 0.05–4 μg/ml for each analysis.

A stock solution of vinblastine (VBL, internal standard) was similarly prepared and a reference solution of 1 μg/ml was obtained by subsequent dilutions. All the solutions were stored at +4 °C, and were brought to room temperature before use. Stock solutions were prepared every 3 weeks while working solutions were prepared every week.

Reference solutions were prepared separately for standard calibration and QC samples.

For preparation of blood and urine, calibration curve samples, 50 μl mixture of VRL and DVRL working solutions were added to 1 ml of human control blank sample in order to obtain final concentrations of 2.5, 10, 50 and 200 ng/ml. All the calibration samples were freshly prepared on the day of analysis.

For QC preparation, appropriate amount of working solution mixtures were added to 1 ml aliquots of human control blood or urine in order to achieve final concentrations of 10, 50 and 150 ng/ml. Series of QC were regularly prepared in one batch, aliquoted and then stored at −80 °C until daily use.

2.5. Blood sample preparation

In a glass tube, to 1 ml blood sample aliquots thawed at room temperature were added 50 μl of IS, 50 μl of 40 mM acetate buffer, pH 3.0, 1 ml of saturated $NaHCO_3$ aqueous solution and 200 μl of NaOH 2N. After a brief vortex mixing, 3 ml of diethyl ether were added. The tubes were shaken for 15 min on a back and forth shaker and then centrifuged for 10 min at 2400 × *g*. From the supernatant, 2.5 ml of the organic layer were transferred into conical bottom screw capped tubes then the extraction process was repeated with another 3 ml of diethyl ether. The two organic layers were combined and 200 μl of 40 mM ammonium acetate buffer, pH 3.0 were added. The tubes were gently shaken for 10 min on a back and forth shaker and then cen-

trifuged for 10 min at 2400 × *g*. The organic layer was discarded and the aqueous layer transferred into conical bottom vials. A 80 μl aliquot of the solution was injected on LC system.

2.6. Urine sample preparation

In order to prevent adsorption of the compounds on the wall, the glass tubes were first wetted with 1% albumin aqueous solution, i.e. 500 μl of albumin solution were added into the tubes and walls were wetted by rolling the tubes. Then, 50 μl of VBL solution was added to 1 ml urine sample as well as 1 ml of saturated $NaHCO_3$, and 200 μl of NaOH 4N. The content of the tubes was briefly mixed and the next steps of the extraction and back-extraction process were similar to those previously described for blood.

When VRL or DVRL concentrations were out of range of the calibration curve, appropriate dilution of samples with control urine was carried out before reprocessing the samples.

2.7. Method validation

Blood or urine samples were quantified using the ratio of peak area of VRL or DVRL to that of the IS. Peak area ratios were plotted against concentrations. The calibration curves were calculated through a linear least squared regression model with a weighting factor of 1/C.

Accuracy, precision and linearity of the method were demonstrated through within- and between-run validations. Ten replicates at each calibration curve value were processed for the within-run validation. The between-run validation was carried out over 3 days. Mean errors and S.D. values were calculated from the theoretical and experimental concentrations in order to assess the precision and the accuracy of the method.

Recoveries of VRL, DVRL and VBL were calculated by comparing the HPLC signals obtained from spiked biological samples with those from directly injected similar concentrations in aqueous solutions.

The lower limit of quantification of the method was defined as being the concentration level which presented an error ≤ 20%, between theoretical and calculated values during the between-run analysis.

2.8. Stability of biological samples

The short-term stability was assessed over 24 h in whole blood using QC samples (two levels in duplicate) stored at room temperature and at 4 °C. The long-term stability was explored with QC samples, prepared as batch aliquots and stored for 18 months either at −80 °C or −20 °C. Aliquots were regularly thawed and processed in order to compare observed experimental values with those initially determined just after QC preparation.

2.9. Daily data analysis and long-term reproducibility

QC and test biological samples were calculated from the equation of the regression line of the calibration curves.

The daily run was accepted if no more than two QC sample values were out of range (six QC randomly located into the run protocol), and if they were not at the same QC level. QC samples values were accepted if they were ± 15% for the high and middle level and ±20% for the low QC level [22].

The long-term reproducibility of the method was assessed over a 3-year period of clinical pharmacokinetic investigations. R.S.D. and bias were calculated from observed and theoretical concentrations for VRL and DVRL. The data of daily QC runs were plotted in order to illustrate any bias tendency over a period of use.

3. Results and discussion

3.1. Analytical issues

During the set-up and the validation of the present method, derived from that of Jehl et al. [11], the attention initially focused on the extraction step observed as being highly variable and not enough specific, particularly for clinical samples. This was a critical issue for VRL measurement, as described by Tellingen et al. [23]. Extraction recoveries were highly influenced by small variations of the pH in the matrix whereas suitable results were obtained when buffering blood or urine through the addition of an equal volume of saturated $NaHCO_3$ solution in combination with NaOH solution. Better control of the pH resulted in improved recoveries, which were more particularly documented in clinical samples. The liquid–liquid extraction with diethyl ether enabled to quantify both VRL and DVRL at low concentrations. Both compounds were predominantly non-ionised and were therefore soluble in diethyl ether, as reported for plasma by Robieux et al. [8]. Other solvents, such as chloroform or methylene chloride, were previously rejected because they either resulted in a poor recovery of both VRL and DVRL when used alone or required a preliminary deproteinisation step [23]. Initially, when using once 6 ml of diethyl ether the recovery in blood was poor (30–40%) whereas two subsequent extraction processes (i.e. 2 × 3 ml of solvent) resulted in better recoveries.

The extraction from matrices required also a strong clean up of the samples. Kobayashi et al. [6], and Robieux et al. [8] have described a disturbed signal baseline and poor stability of the extracted compounds when the solvent diethyl ether was evaporated to dryness in order to concentrate the extract before injection. By using an acidic back-extraction with ammonium acetate buffer, the compounds became protonated and concentrated in the aqueous layer that will be injected in the chromatographic system.

After having controlled the extraction process, some unexpected variability was discovered during pharmacokinetic data modelling, and more particularly at low concentration values.

Tellingen et al. [23] reported that drug adsorption to the sampling material was a general feature of vinca-alkaloids and that the extent of adsorption was higher at low concentrations. We carried out an internal experiment using tritiated radiolabelled VRL to evaluate the potential of VRL to be adsorbed onto sil-

icone coated glass tube. Highly variable adsorption of VRL on ordinary glass tubes was confirmed, resulting in a mean loss of drug ≤ 50%, whereas there was no adsorption on silicone coated tubes. Consequently, as a preventive measure all the reference solutions as well as the QC samples and the clinical samples were prepared, collected and stored in silicone coated glass tubes. Nevertheless, VRL adsorption on ordinary glass tubes is reversible. They could be used in routine for sample process provided that a sufficient amount of organic solvents was added [18]. In urine samples, the addition of 0.5 ml of 1% serum albumin was likely to prevent the VRL adsorption on the glass material. Because VRL concentrations in urine are much higher (100 to 1000-fold) than in blood, the addition of albumin might probably be sufficient.

3.2. Performances of the method

No endogenous interfering peaks were observed in extracted control blood and urine samples at the respective retention times of VRL, DVRL and VBL, illustrating the specificity against endogenous compounds. All compounds were rapidly eluted (less than 10 min). Relative retention times of the three compounds were as follows: $RRT_{VBL} = 0.68$; $RRT_{DVRL} = 0.77$; $RRT_{VRL} = 1.0$. (see Fig. 1)

The best fit of the calculated concentrations towards the theoretical values was observed with the weighted l/C least squared regression analysis. The response was linear within the range 2.5–2000 ng/ml and 2.5–1000 ng/ml for VRL and DVRL, respectively. For routine analysis VRL and DVRL calibrations were plotted in blood and urine up to 200 ng/ml. The mean extraction recoveries of both compounds were higher than 75% and the extraction recovery of VBL was higher than 86%. The within-run R.S.D. and the between-run R.S.D. were below 5.6% for both VRL and DVRL (Table 1). Mean bias illustrating method's accuracy was below 5.9% for VRL and below 6.3% for DVRL. The signal-to-noise ratio observed at 2.5 ng/ml was at least 20:1 for both VRL and DVRL (see Fig. 1). Furthermore at this LLOQ, the between-run R.S.D. were lower than 4.8% for VRL and than 6.6% for DVRL.

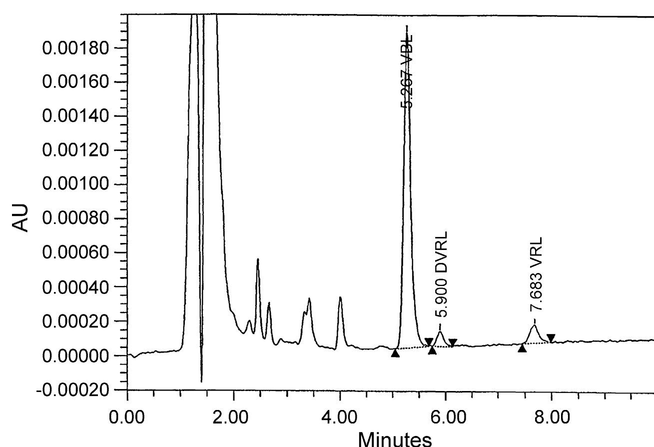


Fig. 1. Typical chromatogram from a blood control sample spiked with 2.5 ng/ml of VRL and DVRL.

Table 1
Intra-assay and inter-assay precision and accuracy of VRL and DVRL

	C_{theor} (ng/ml)	Blood		Urine	
		R.S.D. (%)	Bias (%)	R.S.D. (%)	Bias (%)
VRL					
Within run	2.5	5.4	3.2	5.0	3.4
	10	4.2	−2.0	2.9	−3.4
	50	2.5	−1.2	1.9	−0.3
	200	2.2	0.4	1.3	0.2
Between-run	2.5	3.6	5.8	4.7	5.3
	10	5.4	−2.2	3.2	−3.2
	50	5.5	−4.8	2.2	−2.9
	200	5.4	1.2	1.2	0.8
DVRL					
Within run	2.5	3.6	5.8	5.4	2.3
	10	5.4	−2.2	4.9	−3.1
	50	5.5	−4.8	2.8	0.8
	200	5.4	1.2	1.1	−0.1
Between-run	2.5	4.0	4.1	6.5	6.2
	10	4.4	−1.8	4.9	−3.9
	50	3.7	−3.1	3.3	−3.2
	200	4.1	0.8	1.7	0.9

3.3. Clinical experience

The use of blood [20] is an obvious benefit to nurses and patients: twice less volume of sampling and no sample processing to separate plasma. Furthermore, VRL concentrations being higher in blood, they reached later the analytical lower limit of quantification (LLOQ). This resulted in an improved accuracy on exposure calculation (see Fig. 5) [24]. In plasma, 39% of patients reached the LLOQ at 24 h, 33% at 48 h and 22% at 72 h compared to 17%, 45% and 36% in blood, respectively. This demonstrated that VRL concentrations were measurable for a longer period of time on blood than in plasma.

The present method in blood enabled to quantify with accuracy VRL and DVRL.

A typical blood pharmacokinetic profile in patient obtained through this method is presented on Fig. 6. Gauvin et al. [25] detailed a method close to ours, but only VRL analysis was described and no metabolites were observed. In our studies, two additional peaks to those of VRL and DVRL were also detected in blood and were attributed to new metabolites (see Fig. 2). According to their respective relative retention time (RRT) they were labelled P6 and P11 eluted at RRT = 0.65 and 0.91, respectively. These substances were not initially available as reference chemicals, but rodents were observed to extensively metabolise VRL. Aliquots of a large pool of blood collected from mouse treated with VRL were used as a reference to control elution settings. Then, a LC–MS/MS method was developed to identify all the chemical structures of VRL metabolites [26]. The two peaks, P6 and P11, were attributed to more than two metabolites whereas DVRL was confirmed to be the peak previously labelled P9 [20,27,28].

The present HPLC method has never detected VRL 6′-oxide (NO-VRL), once described as a putative VRL metabolite [15].

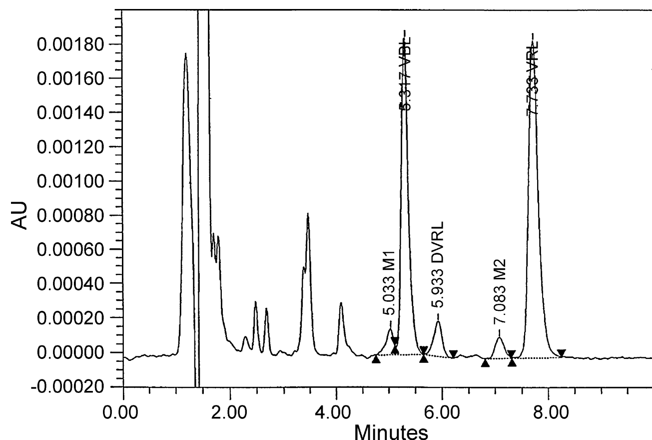


Fig. 2. Typical chromatogram from a blood patient sample collected after oral administration of 60 mg/m^2 vinorelbine displaying VRL, DVRL and other metabolic peaks.

As previously reported, diethyl ether is not a performant solvent to extract NO-VRL [21]. We demonstrated that NO-VRL could be newly formed during the extraction process with old diethyl ether through peroxide residues contained in the organic solvent. Nevertheless, NO-VRL structure was confirmed as a VRL metabolite [26] although it was very scarcely detected in patients at trace levels in bile or faeces, and never in blood whatever the dose level [27]. Furthermore, this metabolite was demonstrated to be inactive [29].

3.4. Long-term reproducibility

In order to assess the long-term reproducibility of this HPLC method, the stability behaviour was thoroughly investigated under several stress conditions. These included short experiments to validate the analytical process from biological sample collection up to sample analysis as well as long-term stability assessment of frozen samples. Results proved that biological samples remained stable when stored for more than 4 h at room temperature (about 20 °C) and for more than 24 h at 4 °C (maximal deviation up to 7.4% for VRL and DVRL). Moreover, no degradation was observed when blood samples were stored over 18 months at −20 °C and −80 °C (Fig. 3) and −80 °C for urine samples. The long-term reproducibility was also checked through QC day-to-day analysis during a 3-year study period.

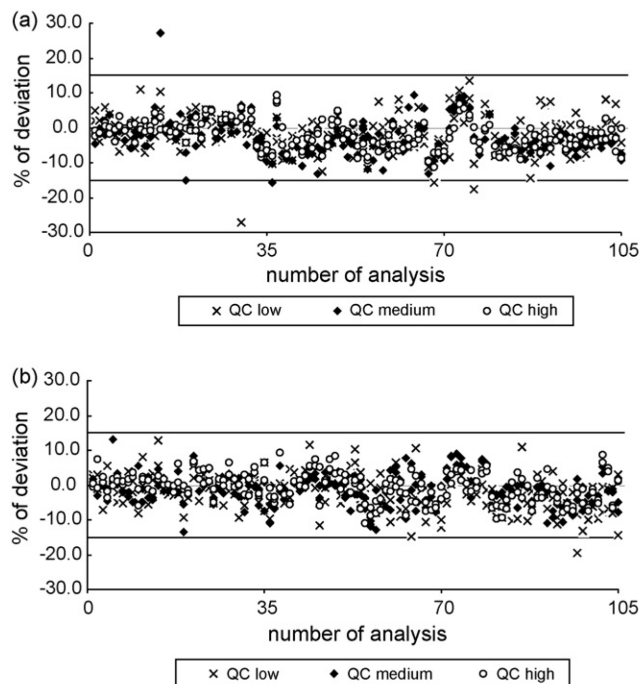


Fig. 4. Long-term reproducibility of the HPLC method in whole blood: day to day QC analysis ($n \geq 400$).

An illustration of this long-term reproducibility is presented in Fig. 4 for both VRL and DVRL in human blood. The R.S.D. and the mean bias were lower than 5.5 and 7.2%, respectively. More than 98% of the QC samples (416 out of 420) assayed daily with the clinical samples were within the acceptance limits fixed previously for the validation of the analytical series. Under these conditions, reanalysis of human blood samples reached comparable concentration values to those previously collected.

Approximately 600 patients or courses representing about 7000 samples of blood were assayed during the development period of i.v. and oral formulation of VRL. In both media, this method enabled to accurately quantify VRL and DVRL, then providing suitable data for the development of a

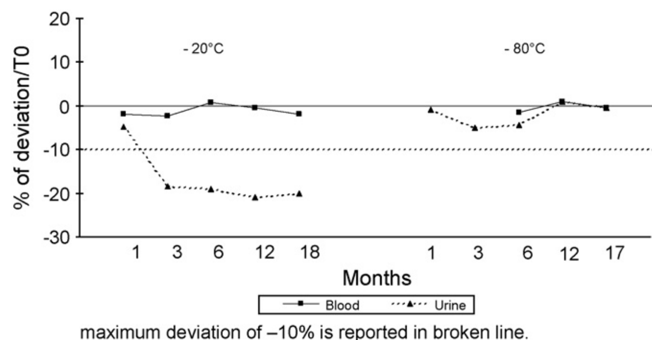


Fig. 3. Stability assessment of vinorelbine in spiked control samples.

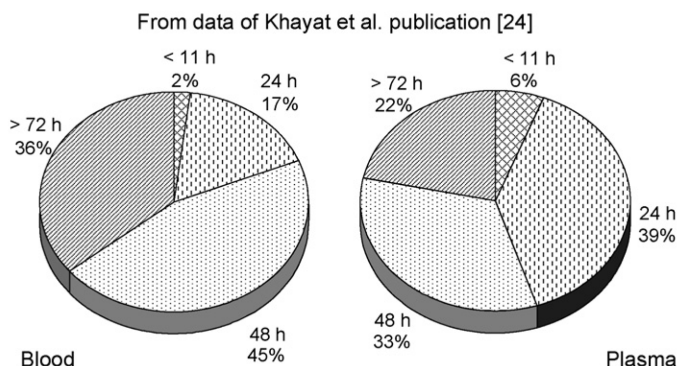
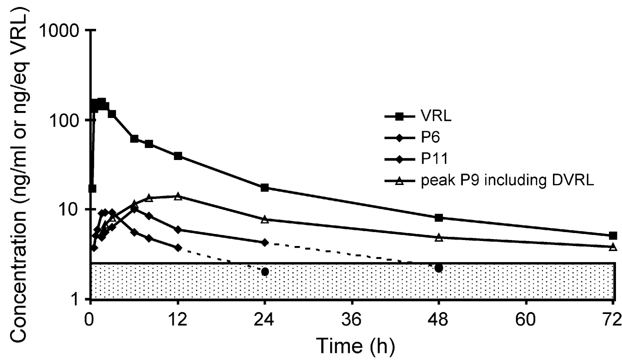


Fig. 5. Suitability of pharmacokinetic profiles: last sampling time (T_{last}) at which vinorelbine was quantified.



Concentrations are expressed on log scale.
The grey area represents the limit of quantification (below 2.5 ng/ml)
Dotted lines fitted the extrapolation of P6 and P11 PK profile to the next point detected (not quantifiable).

Fig. 6. Example of a blood profile for vinorelbine and its metabolites.

robust database for population pharmacokinetic and pharmacokinetic/pharmacodynamic analyses [30,31].

The main advantages from the development of such a method in blood are ethical (lower volume withdrawn from patients), practical (less handling by nurses) and scientific (longer detection and higher accuracy of concentration measurements). After 10 years of using this method, we are confident of its long-term reproducibility and its suitability for patient monitoring. Furthermore, it is easy and cost effective to set up.

References

[1] R. Rahmani, M. Martin, J. Bardet, J.P. Cano, Cancer Res. 44 (1984) 5609–5613.
[2] R. Rahmani, R. Bruno, A. Iliadis, Cancer Res. 47 (1987) 5796–5799.
[3] R. Rahmani, F. Guéritte, M. Martin, Cancer Chemother. Pharmacol. 16 (1986) 223–228.
[4] P. Boré, R. Rahmani, J. Van Canfort, Cancer Chemother. Pharmacol. 23 (1989) 247–251.
[5] G. Nicot, G. Lachatre, P. Marquet, F. Bonnaud, J.P. Valette, J.L. Rocca, J. Chromatogr. 528 (1990) 258–266.
[6] S. Kobayashi, T. Sakai, P.D. Dalymple, S.G. Wood, L.F. Chasseaud, Arzneim. Forsch. /Drug Res. 43 (1993) 1367–1377.
[7] O.V. Tellingen, A. Kuijpers, J.H. Beijnen, M.R.P. Baselier, J.J.M. Burghouts, W.J. Nooyen, J. Chromatogr. 573 (1992) 328–332.
[8] I. Robieux, V. Vitali, P. Aita, A. Freshci, R. Lazzarini, R. Sono, J. Chromatogr. B. 675 (1996) 183–187.
[9] V. Debal, H. Morjani, J.M. Millot, J.F. Angiboust, B. Gourdier, M. Manfait, J. Chromatogr. 581 (1992) 93–99.
[10] C. Mouchard-Delmas, B. Gourdier, R. Vistelle, J. Chromatogr. B. 663 (1995) 390–394.
[11] F. Jehl, J. Debbs, C. Herlin, E. Quoix, C. Gallion, H.S. Monteil, J. Chromatogr. 525 (1990) 225–233.
[12] D.E.M.M. Vendrig, J. Teeuwsen, J.J.M. Holthuis, J. Chromatogr. 424 (1988) 83–94.
[13] D.E.M.M. Vendrig, J. Teeuwsen, J.J.M. Holthuis, J. Chromatogr. 434 (1988) 145–155.
[14] S.P.J. VanBelle, M. DeSmet, C. Monsaert, F. Geerts, G.A. Storme, D.L. Massart, J. Chromatogr. 576 (1992) 351–357.
[15] D. Leveque, F. Jehl, Clin. Pharmacokinet. 31 (1996) 184–197.
[16] E.K. Rowinsky, D.A. Noé, D.L. Trump, J. Clin. Oncol. 12 (1994) 1754–1763.
[17] F. Jehl, E. Quoix, D. Lévêque, G. Pauli, F. Breillout, A. Krikorian, H. Monteil, Cancer Res. 51 (1991) 2073–2076.
[18] O. Van Tellingen, J.H.M. Sips, J.H. Beijnen, A. Bult, W.J. Nooijen, Anticancer Res. 12 (1992) 1699–1716.
[19] S. Urien, F. Bree, F. Breillout, G. Bastian, A. Krikorian, J.P. Tillement, Cancer Chemother. Pharmacol. 32 (1993) 231–234.
[20] T. Schilling, H.H. Fiebig, S. Kerpel-Fronius, B. Winter-Halter, P. Variol, P. Tresca, B. Heinrich, A.R. Hanauske, Investigational New Drugs 14 (1996) 371–378.
[21] S. Ragot, F.L. Sauvage, A. Rousseau, D. Genet, J.L. Dupuy, N. Tubiana-Mathieu, P. Marquet, J. Chromatogr. B. 753 (2001) 167–178.
[22] V.P. Shah, K.K. Midha, S. Dighe, I.J. McGilvray, J.P. Skelly, A. Jacobi, T. Layloff, C.T. Viswanathan, C.E. Cook, R.D. McDowall, S. Spector, J. Pharm. Sci. 81 (1992) 309–312.
[23] O.V. Tellingen, J.H. Beijnen, W.J. Nooyen, J. Pharm. Biomed. Anal. 9 (1991) 1077–1082.
[24] D. Kahayat, O. Rixe, R. Brunet, A. Goupil, R. Bugat, J.L. Harrousseau, N. Ifrah, C. Puozzo, Cancer Chemother. Pharmacol. 54 (2004) 193–205.
[25] A. Gauvin, F. Pinguet, S. Poujot, C. Astre, F. Bressolle, J. Chromatogr. B. 748 (2000) 389–399.
[26] J.C. van Heugen, J. De Graeve, G. Zorza, C. Puozzo, J. Chromatogr. A. 926 (2001) 11–20.
[27] C. Puozzo, G. Zorza, R. Guimbaud, J.C. Van Heugen, J. De Graeve, E. Chatelut, P. Variol, P. Canal, J. Fahy, R. Bugat, Proceedings of the 91st annual meeting of the American Association for Cancer Research, San Francisco, March 2000, 2000, p. 280.
[28] C. Focan, F. Kreutz, I. Leroy, G. Blanchot, J.C. Van Heugen, J. Aerts, C. Puozzo, Proceedings of the 92nd annual meeting of the American Association for Cancer Research, New Orleans, March 2001, 2001, p. 383.
[29] J. Soudon, G. Zorza, J.C. van Heugen, J. De Graeve, M. Vincenti, T. Imbert, C. Puozzo, Proceedings of the 92nd annual meeting of the American Association for Cancer Research, New Orleans, March 2001, 2001, p. 541.
[30] P. Variol, L. Nguyen, B. Tranchand, C. Puozzo, Eur. J. Clin. Pharmacol. 58 (2002) 467–476.
[31] L. Nguyen, B. Tranchand, C. Puozzo, P. Variol, Br. J. Clin. Pharmacol. 53 (2002) 459–468.