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Note

Determination of navelbine and desacetylnavelbine in biological fluids by high-performance liquid chromatography

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Navelbine (5′-noranhydrovinblastine) (Fig. 1) is a new antitumour drug of the vinca alkoloids group, closely related to vinblastine, and is currently under clinical investigation. Preclinical evaluation revealed a good antitumour activity against L 1210 and P 388 murine leukemias [1,2]. In patients, a weekly

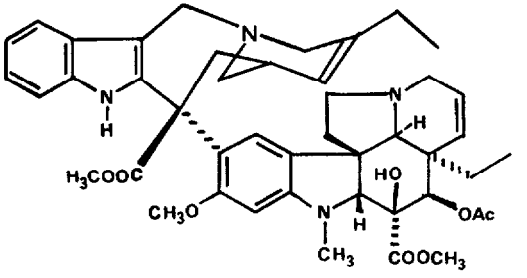

Fig. 1. Structure of navelbine.

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intravenous administration of navelbine at a dose of 30 mg/m^2 has shown a good activity in non-operable non-small cell lung cancer and in advanced breast cancer [3,4]. The pharmacokinetic properties of this new drug are currently under investigation [5–8], mostly by means of radioimmunoassay or radioactive determination following the administration of tritiated navelbine [8]. There is some evidence for the existence of an in plasma circulating biotransformed form, which may have pharmacological and toxicological implications in clinical practice [8].

As further pharmacokinetic investigations are needed, we developed a rapid, sensitive and accurate high-performance liquid chromatographic (HPLC) method for the measurement of both navelbine and desacetylnavelbine, a potential metabolite, in biological fluids, using UV detection.

EXPERIMENTAL

Reagents and chemicals

Navelbine and desacetylnavelbine were kindly provided by Pierre Fabre Labs. (Paris, France) as pure powders. Vinblastine, the internal standard, was obtained from Eli Lilly (Saint-Cloud, France).

Acetonitrile, methanol, diethyl ether, ammonium acetate, sodium dihydrogenphosphate and hydrochloric acid were all of analytical grade (E. Merck, Darmstadt, F.R.G.). Water was obtained daily from a Milli-Ro–Milli-Q system (Millipore, Molsheim, France).

Stock solutions of 100 μg/ml vinblastine, navelbine and desacetylnavelbine were prepared in water and stored at −20°C. Under these conditions, the solutions are stable for at least three months.

Extraction procedure

Serum. A 100-μl volume of the stock solution of internal standard (vinblastine at 1 μg/ml) was added to 1 ml of each serum sample to be analysed, in a screw-capped glass tube. After mixing on a vortex mixer, 1 ml of 66 m*M* phosphate buffer (pH 7) and 3 ml of diethyl ether were added. The tube was then gently shaken by rotation for 30 min (20 rpm). After 10 min centrifugation at 1000 *g*, the supernatant was transferred to another glass tube and evaporated to dryness under a stream of nitrogen at 37°C. The dry residue was then dissolved in 120 μl of methanol–hydrochloric acid (pH 2) (20:80, v/v). A 50-μl aliquot was injected into the chromatograph.

Urine. Urine samples were prepared exactly as described for serum samples, except that the volume of diethyl ether was 5 ml instead of 3 ml.

Calibration curve and linearity

The calibration curve was prepared by spiking normal sera or urine samples with increasing amounts of navelbine and desacetylnavelbine. The linearity in

serum was assessed with concentrations ranging from 2.5 to 1000 ng/ml (2.5, 5, 10, 25, 50, 100, 250, 500, 1000 ng/ml). In urine, it was assessed from 5 ng/ml to 10 μg/ml. In both cases, the ratio of the peaks areas for navelbine or desacetylnavelbine to vinblastine was plotted against navelbine or desacetylnavelbine concentration to check for linearity, and the correlation coefficient was calculated.

Chromatography

The isocratic liquid chromatograph consisted of a 126 programmable solvent-delivery module (Beckman, Fullerton, CA, U.S.A.), a Model 210 sample injection valve with a variable loop (Beckman) and a Model 166 programmable wavelength detector (Beckman) set at 268 nm. Chromatograms were processed by a Model 450 chromatographic data system (Beckman).

Chromatographic separations were performed on a 250 mm × 4 mm I.D. cyano analytical column, 5 μm particle size (SGE, Paris, France).

Navelbine and its desacetyl derivative were eluted using a mobile phase consisting of 55% acetonitrile in 40 m*M* ammonium acetate (final concentration). The pH was adjusted to 3 with hydrochloric acid. The mixture was delivered at 1 ml/min.

Detection limit

The limit of detection was defined as the lowest concentration of navelbine and desacetylnavelbine resulting in a signal-to-noise ratio of 4.

Reproducibilities

Serum. Both within- and between-day reproducibilities were tested for navelbine and desacetylnavelbine. Two concentrations of each drug were included in this study, the first high (1000 ng/ml) and the second low (25 ng/ml). Ten aliquots of each sample were tested on the same day and the resulting coefficients of variation (C.V.) indicated the within-day reproducibilities. Aliquots of the same samples were tested once a day for ten days and the resulting C.V. indicated the between-day reproducibilities.

Urine. Both within- and between-day reproducibilities in urine were tested at 50 and 1000 ng/ml.

Selectivity

Interference studies were carried out with many substances that could be coadministered with navelbine: analgesics, salicylate, carbamazepine, phenytoin, antibiotics (β-lactams, aminoglycosides, quinolones, glycopeptides), theophylline, digitoxin, furosemide and quinidine.

RESULTS

Chromatograms

Typical chromatograms obtained from samples of various body fluids are shown in Figs. 2–4. Fig. 2 shows chromatograms of an extracted blank serum and the same serum containing navelbine, desacetylnavelbine and vinblastine as the internal standard. As can be seen, the vinca alkaloids are completely resolved from endogenous peaks. Retention times are as follows: navelbine, 5.6 min; vinblastine, 4.4 min; desacetylnavelbine, 5.0 min. The three compounds are well resolved, with selectivity factors ranging from 1.18 to 1.43. Fig. 3 shows chromatograms obtained from a serum sample from a patient who received a 30 mg/m^2 body area dose of navelbine, and supplemented with the internal standard.

The same considerations hold for the chromatograms from urine samples (Fig. 4), which appear as 'clean' chromatograms showing well resolved alkaloid peaks. Many drugs were included in the interference study. All chromatograms were carefully checked for skewed peaks, shouldering peaks or tailing peaks. None of these chromatographed solutes resulted in interference with the vinca alkaloids.

Reproducibilities

Within- and between-day reproducibilities for serum and urine navelbine and desacetylnavelbine concentrations are shown in Table I.

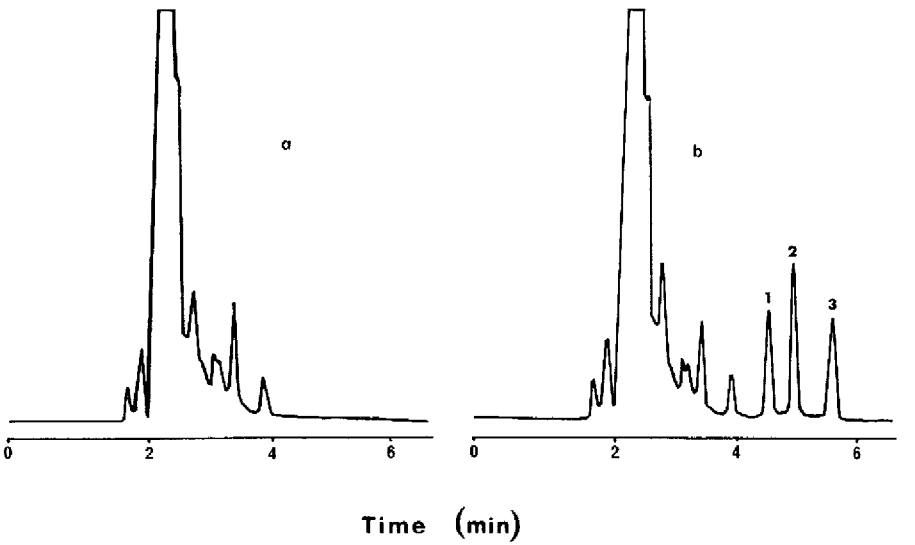

Fig. 2. HPLC of (a) human blank serum and (b) human serum spiked with the internal standard (1) at 100 ng/ml, desacetylnavelbine (2) at 100 ng/ml and navelbine (3) at 100 ng/ml. Detector sensitivity, 0.01 a.u f.s.

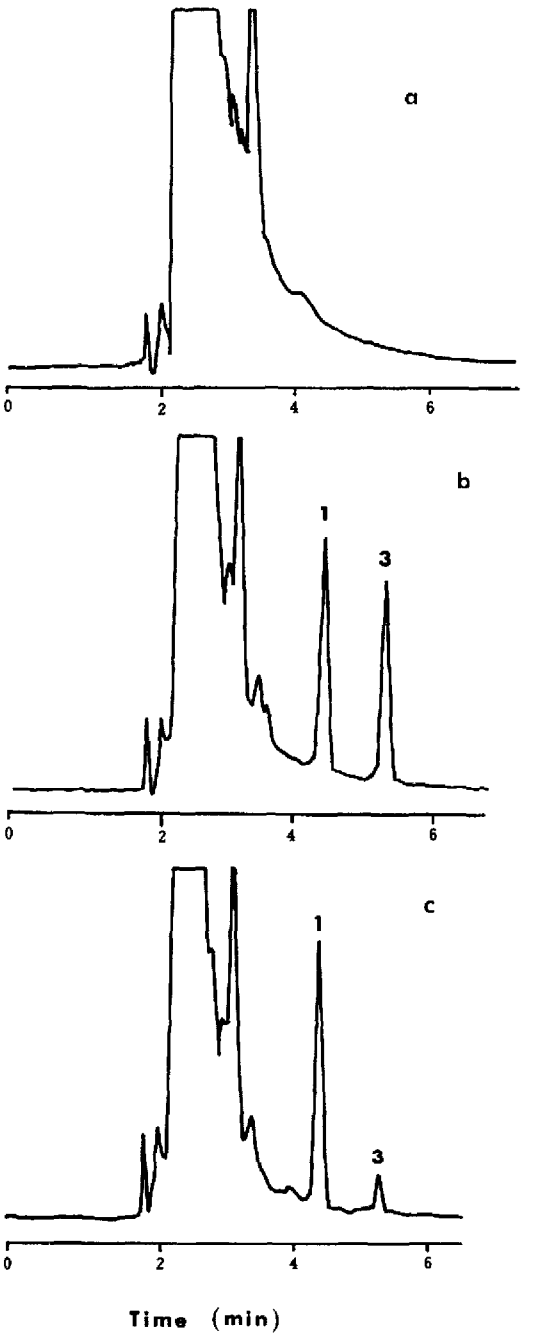

Fig. 3. HPLC of (a) a patient serum before treatment, (b) the same patient serum spiked with the internal standard and sampled 30 min after a single navelbine intravenous dose of 30 mg/m^2 body area and (c) the same patient serum sampled 4 h after navelbine administration. Peaks: 1 = internal standard; 3 = navelbine. Detector sensitivity, 0.01 a.u.f.s.

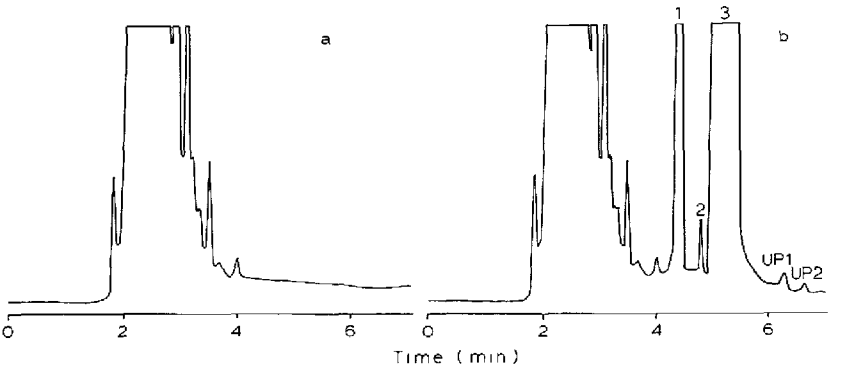

Fig. 4. HPLC of (a) a patient urine sampled before treatment and (b) the same patient urine spiked with the internal standard and sampled 1 h after a single navelbine intravenous dose of 30 mg/m^2 body area. Peaks: 1 = internal standard; 2 = desacetylnavelbine; 3 = navelbine (12.5 μg/ml); UP1 = unknown peak1; UP2 = unknown peak 2. Detector sensitivity, 0.01 a.u.f.s.

TABLE I

PRECISION OF THE CHROMATOGRAPHIC ASSAY

Sample	Concentration (μg/ml)	Coefficient of variation (%)	
		Navelbine	Desacetylnavelbine
Within-day			
Serum	1	1.9	1.7
	0.025	2.5	2.3
Urine	1	7.8	4.9
	0.050	3 5	3.5
Between-day			
Serum	1	3.5	2.9
	0.025	2.5	3.4
Urine	1	7.8	6.9
	0.050	8.1	7.7

The same C.V. were calculated without an internal standard. As expected, values were somewhat higher, indicating the necessity for an internal standard. For example, without vinblastine as internal standard, the within-day C.V. ranged from 7.3 to 21% and the between-day C.V. from 11 to 33%.

Linearity

The linearity study was carried out with concentrations ranging from 2.5 to 1000 ng/ml in serum and from 5 to 5000 ng/ml in urine. The coefficients of correlation between the navelbine/internal standard peak-area ratio and navelbine concentration were 0.9933 and 0.9958, respectively. The equations re-

sulting from the analysis of the regression plot were $y=0.0116x-0.2$ and $y=0.0132x-1.7$, respectively. Similar results were obtained with desacetylnavelbine ($r=0.9981$, $y=0.0121x-0.1$ for serum and $r=0.9971$, $y=0.0139x-1.5$ for urine).

Limit of detection

In serum and urine, the detection limit of navelbine was 2.5 and 5 ng/ml, respectively. The same values were obtained for the desacetyl metabolite.

Selectivity

Under the conditions developed for the analysis of navelbine and its desacetyl metabolite, none of the substances included in the interference studies resulted in any co-elution with navelbine, desacetylnavelbine or the internal standard. There are two reasons for this: either the substances tested for interferences were not extracted from serum by the sample preparation procedure (e.g. β-lactams) or, when co-extracted, the substances did not co-elute because they required different analytical conditions (stationary or mobile phase).

Extraction recoveries

The extraction recoveries ($n=10$) from plasma spiked with both navelbine (10 and 500 ng/ml) and desacetylnavelbine (10 and 500 ng/ml) were 67.3 ± 1.2 and $66.8 \pm 0.9\%$ and 68.1 ± 1.7 and $67.9 \pm 2.1\%$, respectively.

DISCUSSION

HPLC proved to be particularly suitable for the monitoring of vinca alkaloids. Many methods have been proposed for vinblastine [11,13–15], vincamine [9,12] and vincristine [10,11,14], but to our knowledge no technique has been published for the determination of navelbine by HPLC with UV detection, this drug generally being determined by other methods, such as radioimmunoassays. The applied HPLC method should be very selective for the monitoring of plasma and urine samples, because cancer patients who are treated with vinca alkaloids often receive extensive medication. We measured navelbine concentration during seven days following intravenous administration of 30 mg/m^2 body area in ca. fifteen patients included in a pharmacokinetic study. Most of these patients were polymedicated and no interference appeared in the chromatograms. The detection limit of 2.5 ng/ml appeared to be suitable for such studies.

Fig. 5 shows a typical serum concentration–time curve for these patients. Concentrations are measurable up to 96 h. The proposed method allows the determination of desacetylnavelbine, a potential metabolite of navelbine. The preliminary pharmacokinetic studies of humans [6,8] revealed that the uri-

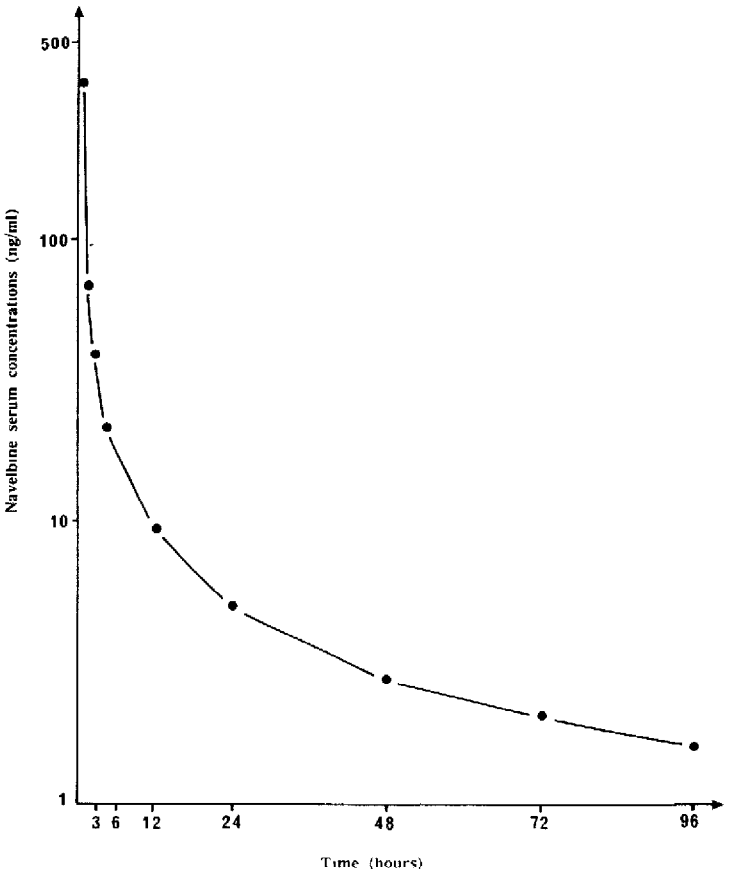

Fig. 5. Plot of navelbine serum concentration versus time during 96 h following a single navelbine intravenous administration of 30 mg/m^2 body area.

nary excretion of navelbine is very low (ca. 20% of administered dose); thus, an important hepatic and/or metabolic clearance may occur. Rahmani and coworkers [5–8] showed the existence of one or more unidentified metabolites in blood, the excretion of which could be by the biliary route. The use of HPLC should prove to be very effective in such a situation, as desacetylnavelbine could be one of these metabolites. Among all the patients that we studied, we detected desacetylnavelbine in almost all of their urines. Compared with navelbine, the concentration of desacetylnavelbine was always much lower (nanograms rather than micrograms). Nevertheless, no desacetylnavelbine could be detected in blood from the same patients, probably because of the very low circulating concentrations of this metabolite. Of great interest was the appearance of two additional chromatographically well resolved peaks, named UP1 and UP2 by us (for unknown peak), eluting later than navelbine in the

urine of two of our patients (Fig. 3). This indicates the presence of other potential metabolites, less polar than desacetylnavelbine, the structures of which remain to be elucidated.

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