Journal of Chromatography, 345 (1985) 309—321
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMBIO. 2811

HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC DETERMINATION OF VINCA-ALKALOIDS IN PLASMA AND URINE

M. DE SMET

Vrije Universiteit Brussel, Farmaceutisch Instituut, Laarbeeklaan 103, B-1090 Brussels (Belgium)

S.J.P. VAN BELLE and G.A. STORME

Oncology Centre VUB, Eenheid Kanker, Academisch Ziekenhuis Jette, Laarbeeklaan 101, B-1090 Brussels (Belgium)

and

D.L. MASSART*

Vrije Universiteit Brussel, Farmaceutisch Instituut, Laarbeeklaan 103, B-1090 Brussels (Belgium)

(First received May 20th, 1985; revised manuscript received July 23rd, 1985)

SUMMARY

A liquid chromatographic method is described for separating and determining vinblastine, vincristine and vindesine in plasma and urine. The drugs are extracted from the biological material using an ion-pair extraction, with sodium octylsulphate as counter-ion at pH 3. The extracts are injected on a reversed-phase system with a cyano column as stationary phase and a mobile phase composed of acetonitrile—phosphate buffer, pH 3 (65:35, vol. %). Stability studies are carried out for stock solutions of the drugs in water at different temperatures and pH values. The stability of these compounds in plasma is also investigated in the presence of an antioxidant. The method is applied to determine drug levels of vindesine and vinblastine in preliminary pharmacokinetic studies, using vincristine as the internal standard.

INTRODUCTION

Vincristine, vinblastine and its semi-synthetic derivative vindesine (desacetyl-

0378-4347/85/$03.30

vinblastine amide) are anti-neoplastic agents, originally obtained from extracts of the plant *Vinca rosea*. These drugs are currently used in several chemotherapeutic treatments but are still under investigation for their anti-invasive and anti-metastatic effects [1—3]. Since in vitro studies [2] have shown that the plasma levels needed to obtain an anti-invasive activity may be achieved with clinically employed doses, it would be of interest to develop a method for drug monitoring in biological material [4, 5].

Pharmacokinetic investigations of these agents have been limited by the lack of sensitive analytical techniques with suitable selectivity. Until now, these drugs have generally been determined by methods such as radioimmunoassay (RIA) [6—10] or by enzyme-linked immunosorbent assay [11]. Other techniques have also been applied, such as thin-layer chromatography [12] and high-performance liquid chromatography (HPLC) [12—17].

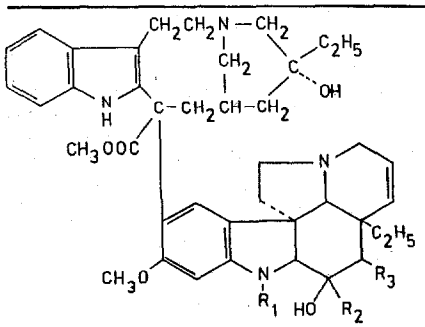
Some data [18] suggest that the metabolites have a longer residence in the body than the parent drug itself and that the kinetics of these metabolites could be significantly different from the parent drug. As the RIA techniques cannot discriminate between the parent drug and the metabolites, we developed an HPLC method to investigate the pharmacokinetic behaviour of vinblastine and vindesine, using vincristine as the internal standard.

High-performance liquid chromatography is a widely used method for the determination of drugs and their metabolites in biological materials. A few HPLC methods have been reported for the determination of the vinca-alkaloids in crude materials [13, 14], in preformulation studies [15] and in neoplastic tissues [16]. Other workers have used HPLC to separate vinca-alkaloids from by-products obtained during the semi-synthesis [12, 13]. Some HPLC procedures require gradient elution [14, 16], which should be avoided when the procedure is used in routine application.

This paper reports on an HPLC method for the quantitation of vinblastine, vincristine and vindesine in plasma and urine. A survey of the chemical

TABLE I

CHEMICAL STRUCTURE OF THE VINCA-ALKALOIDS



Compound	R_1	R_2	R_3
Vinblastine	$-CH_3$	$-COOCH_3$	$-COOCH_3$
Vindesine	$-CH_3$	$-CONH_2$	$-OH$
Vincristine	$-CHO$	$-COOCH_3$	$-COOCH_3$

structure of the vinca-alkaloids is given in Table I. The vinca-alkaloids are extracted using an ion-pair extraction procedure, which is generally applied in our laboratory for all determinations of basic drugs [19, 20]. A cyano column is used as the stationary phase for the chromatography since it is particularly suited for the chromatography of pharmaceutical compounds [21].

Attention has also been paid to the stability of vindesine and vinblastine in water and in biological fluids. In the first instance, we are interested in the pharmacokinetics of the latter two drugs, using vincristine as the internal standard.

EXPERIMENTAL

Apparatus

A Varian 5060 liquid chromatograph, equipped with a Rheodyne loop injector (100-μl sample loop) and a Varian UV-100 variable-wavelength detector, is used. The chromatograms are recorded and integrated with a Varian CDS 401 chromatographic data system. The column is a stainless-steel column (250 × 4 mm I.D.) packed with LiChrosorb CN with a particle size of 5 μm. A guard column (30 × 4 mm I.D.) packed with LiChrosorb CN (particle size 5 μm) is used. The guard column is replaced when ca. 200 plasma or urine samples have been injected. The mobile phase is composed of acetonitrile—phosphate buffer, pH 3 (65:35, vol. %). The absorbance of the eluent is determined at 220 nm. The flow-rate is set at 1.5 ml/min. All experiments are carried out at ambient temperature.

All laboratory glassware is treated with a silylating reagent to avoid adsorption of sample material. Surfasil (a dichlorooctamethyltetrasiloxane silylating agent, obtained from Pierce, Rockford, IL, U.S.A.) diluted with acetone, is used. Plasma and urine samples of patients are stored in polystyrene tubes.

Standards and reagents

Vinblastine sulphate (Velbe®), vindesine sulphate (Eldisine®), vincristine sulphate (Oncovin®) and desacetylvinblastine are of pharmaceutical purity and were obtained from Eli Lilly (Brussels, Belgium) or Eli Lilly (Indianapolis, IN, U.S.A.).

Acetonitrile and dichloromethane are of liquid chromatographic grade and chloroform is pro analysis quality (all from Merck, Darmstadt, F.R.G.). Double-distilled water, which is further purified with a Water-I system (Gelman Sciences, Ann Arbor, MI, U.S.A.), was used to prepare the phosphate buffer solutions for the extraction and for the mobile phase.

A phosphate buffer of pH 3 with an ionic strength (μ) of 0.4, containing $5 \cdot 10^{-2}$ *M* sodium octylsulphate, is used for the extraction.

For the mobile phase, a phosphate buffer of pH 3 with an ionic strength of 0.06 is prepared. Sodium octylsulphate, phosphoric acid and $NaH_2PO_4 \cdot H_2O$ are pro analysis quality and are obtained from Merck.

Extraction procedure

Plasma. A 4-ml volume of plasma is pipetted into a centrifuge tube equipped with a PTFE-covered screw cap. Deproteinization is carried out by dropwise addition of 8 ml of acetonitrile and by continual vortexing. After centrifuga-

tion for 30 min, the supernatant is pipetted into another centrifuge tube and the acetonitrile is evaporated in a water-bath at 60°C under a gentle stream of nitrogen. A 10-ml aliquot of phosphate buffer at pH 3 (μ = 0.4), containing $5 \cdot 10^{-2}$ *M* sodium octylsulphate and 5 ml of chloroform, is added and shaken for 30 min in a shaking bath. After centrifugation, 4 ml of the organic phase are put into a reacti vial and evaporated to dryness at 30°C under a gentle stream of nitrogen. The residue is dissolved in 200 μl of dichloromethane and 100 μl are injected into the HPLC system.

Urine. A 4-ml volume of urine is pipetted into a centrifuge tube equipped with a PTFE-covered screw cap. A 10-ml aliquot of phosphate buffer, containing $5 \cdot 10^{-2}$ *M* sodium octylsulphate and 5 ml of chloroform, is added and shaken in a shaking bath for 30 min. After centrifugation, 4 ml of the chloroform phase are put into a reacti vial and evaporated to dryness at 30°C under a gentle stream of nitrogen. The residue is dissolved in 200 μl of dichloromethane and 100 μl are injected into the chromatographic system.

Quantitation is carried out by intrapolation on an extracted standard curve in plasma or in urine (peak area of drug/peak area of internal standard versus concentration of drug).

For determination of the extraction recovery from plasma or from urine, quantitation is performed by comparison with an unextracted standard curve in water.

Preparation of standard curves in plasma and in urine

Stock solutions of the vinca-alkaloids are prepared in double-distilled water in the following concentrations: vinblastine, 200 μg/ml; vindesine, 100 μg/ml; and vincristine, 20 μg/ml. Standard solutions of vinblastine and vindesine in water are prepared from the stock solutions to spike drug-free plasma in the following concentrations: 1, 2, 4, and 8 μg/ml. Vincristine is used as internal standard and a standard solution containing 8 μg/ml is prepared in water. Standard curves are prepared in the following way: to 4 ml of drug-free plasma, 100 μl of a standard solution and 100 μl of internal standard are added by vortexing and further treated as described above in the extraction procedure. For each standard curve, a blank plasma is also prepared. Standard curves of vindesine and vinblastine for urine are prepared in the same way as for plasma. The stock solutions are stored at 4°C between use.

Stability of the vinca-alkaloids in water at different temperatures and pH values

Two series of stock solutions of vinblastine (100 μg/ml) and vindesine (100 μg/ml) were prepared in water and kept at different temperatures during a period of two months. Ascorbic acid (0.5 mg/ml) is added to one series, providing a pH of 3. The other series is kept without ascorbic acid at a neutral pH. The solutions are kept at 4, 20 and 55°C for two months. The different stock solutions are injected into the HPLC system at time t = 0 and after 24 h, one week, two weeks, one month and two months of storage. An ascorbic acid blank solution is also treated in the same way. All injections were done in duplicate.

Stability of the vinca-alkaloids in frozen plasma

The stability of vinblastine and vindesine in frozen plasma was investigated both in the presence of ascorbic acid and without ascorbic acid. Two series of drug-free plasma are treated in the following way: to one series, vinblastine or vindesine (100 ng/ml) is added and to the other series, vinblastine or vindesine (100 ng/ml) and ascorbic acid (0.5 mg/ml) are added. The plasma samples are then frozen, extracted as described above and analysed by HPLC at time t = 0 and after 24 h, 48 h, one week, two weeks and one months of storage. On the day of analysis, 100 μl of internal standard solution (8 μg/ml vincristine) are added before extraction. The amount of vinblastine or vindesine is determined by intrapolation on a freshly extracted standard curve in plasma.

Stability of the vinca-alkaloids following extraction

Experiments were carried out in which the stability of the vinca-alkaloids was assessed following three post-extraction storage treatments. The first treatment involves the preservation of the chloroform phase at 4°C for 24 and 48 h before injection into the HPLC system; the second storage treatment involves keeping the evaporated extract at 4°C for 24 and 48 h; the third treatment involves dissolving the evaporated chloroform extract in dichloromethane and storing it for 24 and 48 h at 4°C. These storage procedures are carried out with plasma samples spiked with 100 ng/ml vinblastine or vindesine and with 100 μl of internal standard solution (8 μg/ml). Quantitation is done by intrapolation on a freshly extracted standard curve in plasma.

Preliminary pharmacokinetic studies of vinblastine and vindesine

Blood and urine samples were taken from patients with a proven malignant disease, who were treated for the first time with vinblastine or with vindesine. The drug was administered by an intravenous bolus injection of 6 mg/m^2 vinblastine sulphate or 3 mg/m^2 vindesine sulphate.

Blood samples were obtained at regular time intervals: 0, 2, 4, 6, 10, 15, 20, 30, 45, 60 and 90 min, and 2, 4, 8 and 12 h. The blood samples were collected in heparinized tubes. After centrifugation, the plasma samples were stored at −20°C until analysis.

TABLE II

EXTRACTION RECOVERY OF THE VINCA-ALKALOIDS FROM PLASMA AND URINE

Drug	Matrix	Concentration (ng/ml)	Recovery (%) (n = 6)	Coefficient of variation (%)
Vinblastine	Plasma	25	99.2	7.6
		100	102.7	4.5
	Urine	25	100.0	4.0
		100	97.3	4.1
Vindesine	Plasma	25	100.4	5.3
		100	103.0	1.3
	Urine	25	98.5	9.5
		100	101.6	4.1
Vincristine	Plasma	200	102.3	5.3
	Urine	200	97.6	3.3

Urine was collected for 24 h after administration, to determine cumulative excretion. The urine samples were also stored at −20° C.

RESULTS AND DISCUSSION

Extraction procedure

The extraction recoveries of the vinca-alkaloids from plasma and urine are given in Table II. For vinblastine and vindesine, the recovery is determined at two concentration levels (25 and 100 ng/ml), while the extraction recovery for vincristine is carried out at only one concentration (200 ng/ml), which is the concentration used as internal standard. The extraction results in a complete recovery of the vinca-alkaloids from plasma or urine, with acceptable coefficients of variation (C.V.).

The extraction procedure for plasma is more time-consuming than for urine because a deproteinization step with acetonitrile is necessary before the addition of the ion-pairing reagent, in order to avoid interactions between the plasma proteins, the drugs and the counter-ion [20], which otherwise would result in poorer extraction recoveries. For the determination of the vinca-alkaloids in urine, sodium octylsulphate is immediately added to the matrix because the amount of protein in urine is negligible. The calibration curves (peak area of solute/peak area of internal standard versus concentration of solute) are linear in the studied concentration range.

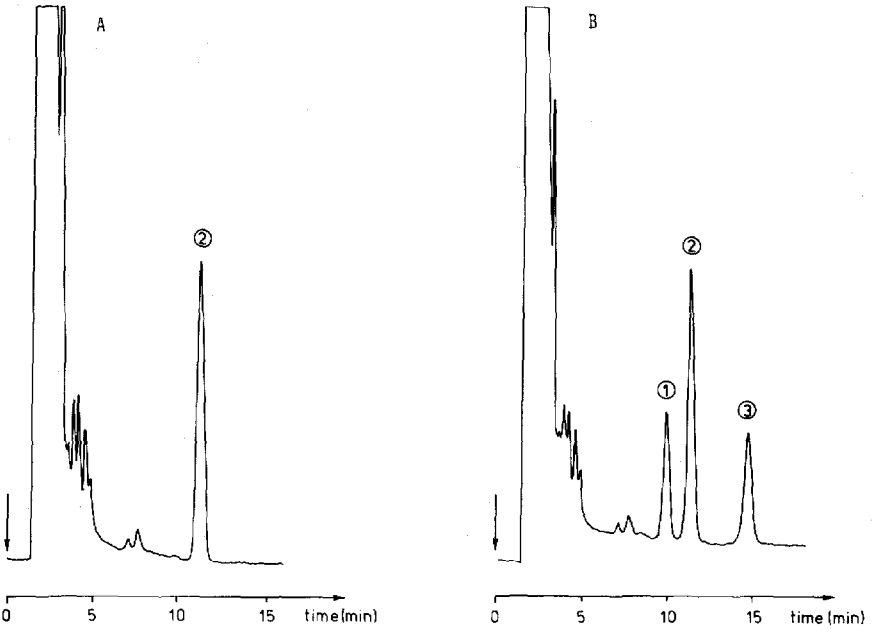


Fig. 1. Chromatogram of (A) a blank plasma extract spiked with the internal standard (200 ng/ml) and (B) a plasma extract spiked with vindesine (100 ng/ml), vinblastine (100 ng/ml) and the internal standard (200 ng/ml). Mobile phase: acetonitrile—phosphate buffer, pH 3 (65:35, vol. %). Flow-rate 1.5 ml/min; detection 220 nm, a.u.f.s. 0.01. Peaks: 1 = vindesine; 2 = vincristine; 3 = vinblastine.

TABLE III

RELATIVE RETENTION OF OTHER DRUGS

Drug	Vin-desine	Vin-cristine	Vin-blastine	Drug	Vin-desine	Vin-cristine	VIn-blastine
Vindesine	1.00	0.88	0.69	Paracetamol	0.20	0.17	0.13
Vincristine	1.13	1.00	0.78	Indomethacin	0.22	0.20	0.15
Vinblastine	1.44	1.29	1.00	Diclofenac	0.26	0.23	0.17
Alizapride	0.36	0.32	0.25	Naloxone	0.35	0.31	0.23
Metoclopramide	0.44	0.39	0.30	Tilidine	0.44	0.38	0.29
Domperidone	0.39	0.34	0.27	Pentazocine	0.46	0.40	0.30
Amitriptyline	0.49	0.44	0.34	Morphine	0.30	0.26	0.20
Nortriptyline	0.47	0.42	0.32	Dexamethasone	0.21	0.19	0.14
Imipramine	0.49	0.44	0.34	Prednisone	0.21	0.19	0.14
Papaverine	0.44	0.39	0.29	Digitoxin	0.23	0.20	0.15
Doxepin	0.46	0.41	0.31	Sulphathiazole	0.22	0.19	0.14
Diazepam	0.23	0.20	0.16	Amoxycillin	0.23	0.20	0.15
Bromazepam	0.22	0.19	0.16	Doxycycline	0.31	0.27	0.24
Nitrazepam	0.23	0.20	0.16	Caffeine	0.25	0.21	0.19
Oxazepam	0.22	0.19	0.16	Ascorbic acid	0.20	0.18	0.14
Chlordiazepoxide	0.33	0.30	0.23	Acetylsalicylic acid	0.21	0.19	0.15
Melitraceen	0.50	0.44	0.35				
Flupentixol	0.51	0.45	0.35				

Chromatography

Fig. 1 gives a chromatogram of (A) an extracted blank plasma sample spiked with the internal standard (200 ng/ml vincristine) and (B) an extracted plasma sample spiked with 100 ng/ml vindesine and vinblastine and the internal standard (200 ng/ml vincristine). The vinca-alkaloids are completely resolved from the plasma components. Vindesine elutes first, followed by vincristine and vinblastine.

When the vinca-alkaloids are determined in urine, no interference is observed from urine compounds (see Fig. 3a and b).

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 an extensive medication (i.e. antibiotics, anti-emetics, benzodiazepines, antidepressives, etc.), which can interfere in the analysis. Therefore, many possibly interfering drugs were injected into the HPLC system and their relative retention with respect to the vinca-alkaloids is given in Table III. None of the chromatographed solutes interfere with the vinca-alkaloids and all of them elute with a shorter retention time. Therefore, the combination of the cyano column and a mobile phase composed of acetonitrile and phosphate buffer at pH 3 seems to be a suitable HPLC system for the determination of the vinca-alkaloids in routine application.

Precision and detection limit

Replicate spiked samples (n = 6) were analysed to evaluate the within-day precision, determined at two concentration levels (25 and 100 ng/ml) for plasma and urine. For vinblastine in plasma, coefficients of variation of 5.9 and 5.6%, respectively, were found, while the coefficients of variation for vindesine in plasma were 5.2 and 4.4%, respectively. The within-day precision for vinblastine in urine was 4.0 and 3.1%, respectively, and for vindesine in urine the coefficients of variation were 5.8 and 4.0%, respectively. The detection limit was estimated to be 6 ng/ml at a signal-to-noise ratio of 3.

TABLE IV

STABILITY OF STOCK SOLUTIONS OF VINBLASTINE AT DIFFERENT pH AND TEMPERATURES

Time of storage	Remaining vinblastine (%)					
	Without ascorbic acid (neutral pH)			With ascorbic acid (pH 3)		
	4°C	20°C	55°C	4°C	20°C	55°C
0	100	100	100	100	100	100
24 h	100	95	99	100	96	96
1 week	100	90	96	97	90	87
2 weeks	99	87	90	93	85	58
1 month	98	85	85	92	81	44
2 months	96	76	75	90	71	38

TABLE V

STABILITY OF STOCK SOLUTIONS OF VINDESINE AT DIFFERENT pH AND TEMPERATURES

Time of storage	Remaining vindesine (%)					
	Without ascorbic acid (neutral pH)			With ascorbic acid (pH 3)		
	4°C	20°C	55°C	4°C	20°C	55°C
0	100	100	100	100	100	100
24 h	100	99	99	100	99	97
1 week	99	96	97	98	98	91
2 weeks	98	95	97	98	97	68
1 month	97	94	88	94	86	42
2 months	95	74	71	90	69	33

Stability studies

A first experiment was carried out in which stock solutions of vinblastine and vindesine in water, separately, were preserved with and without ascorbic acid. The results of these stability studies are given in Tables IV and V for vinblastine and vindesine, respectively. The percentages given in the tables are the remaining percentages of vinblastine and vindesine with regard to the freshly prepared solutions. These results are obtained by comparing the peak areas. A faster degradation was observed when the solutions were kept at lower pH and at higher temperatures. Therefore, stock solutions of the vinca-alkaloids for spiking plasma or urine samples were kept without ascorbic acid at 4°C between use and were freshly prepared every two weeks.

A second experiment was performed to investigate the stability of vinblastine and vindesine in frozen plasma samples and also to see if the addition of an antioxidant (ascorbic acid) to plasma is necessary. The addition of ascorbic acid to plasma did not influence the pH. For all plasma samples, recoveries not significantly different from 100% are found after one month of

storage. These percentages are obtained by comparing peak areas. We can therefore conclude that the addition of ascorbic acid to plasma samples before they are stored at −20°C is not necessary and that vinblastine and vindesine are stable in frozen plasma for at least one month, on the condition that they are kept in siliconized glassware or in polystyrene tubes.

A third experiment assesses the stability of vinblastine, vincristine and vindesine after extraction and being stored at 4°C in the organic phase (chloroform phase), after evaporation of that solution and after evaporation and reconstitution in dichloromethane. Keeping the plasma extracts at 4°C results in a loss of approximatively 10% every 24 h for the evaporated extracts and a loss of 15% when stored in chloroform or in dichloromethane. These results are obtained by comparing peak areas. However, this is compensated by the use of an internal standard, since an equal loss is also observed for these peak areas.

Preliminary pharmacokinetic studies

The usefulness of the proposed method was tested in human plasma and urine samples collected from patients, following the intravenous administration of vinblastine or vindesine.

Fig. 2 represents a plot of the plasma concentration of vinblastine versus time. The peak level is rapidly obtained due to the manner of administration and a fast decline is observed in the first hour. In the blood samples taken at 8 and 12 h after administration, the plasma concentration of the parent drug reaches a level below the detection limit.

The analysis of the corresponding urine samples results in the discovery of a metabolite of vinblastine. This metabolite has the same retention time as desacetylvinblastine and, therefore, we conclude that this substance is formed by metabolization of vinblastine. Fig. 3d illustrates a chromatogram of a

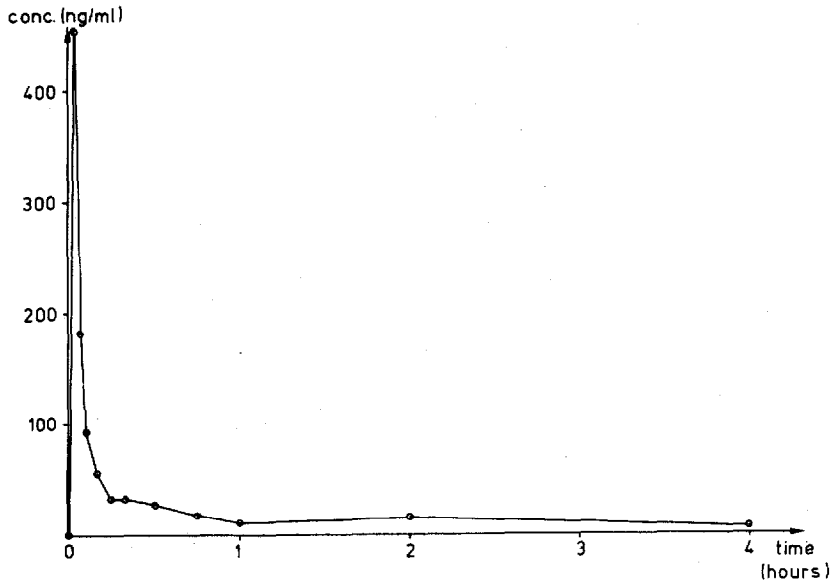


Fig. 2. Plasma concentration—time curve for a patient who received an intravenous injection of 6 mg/m² of vinblastine.

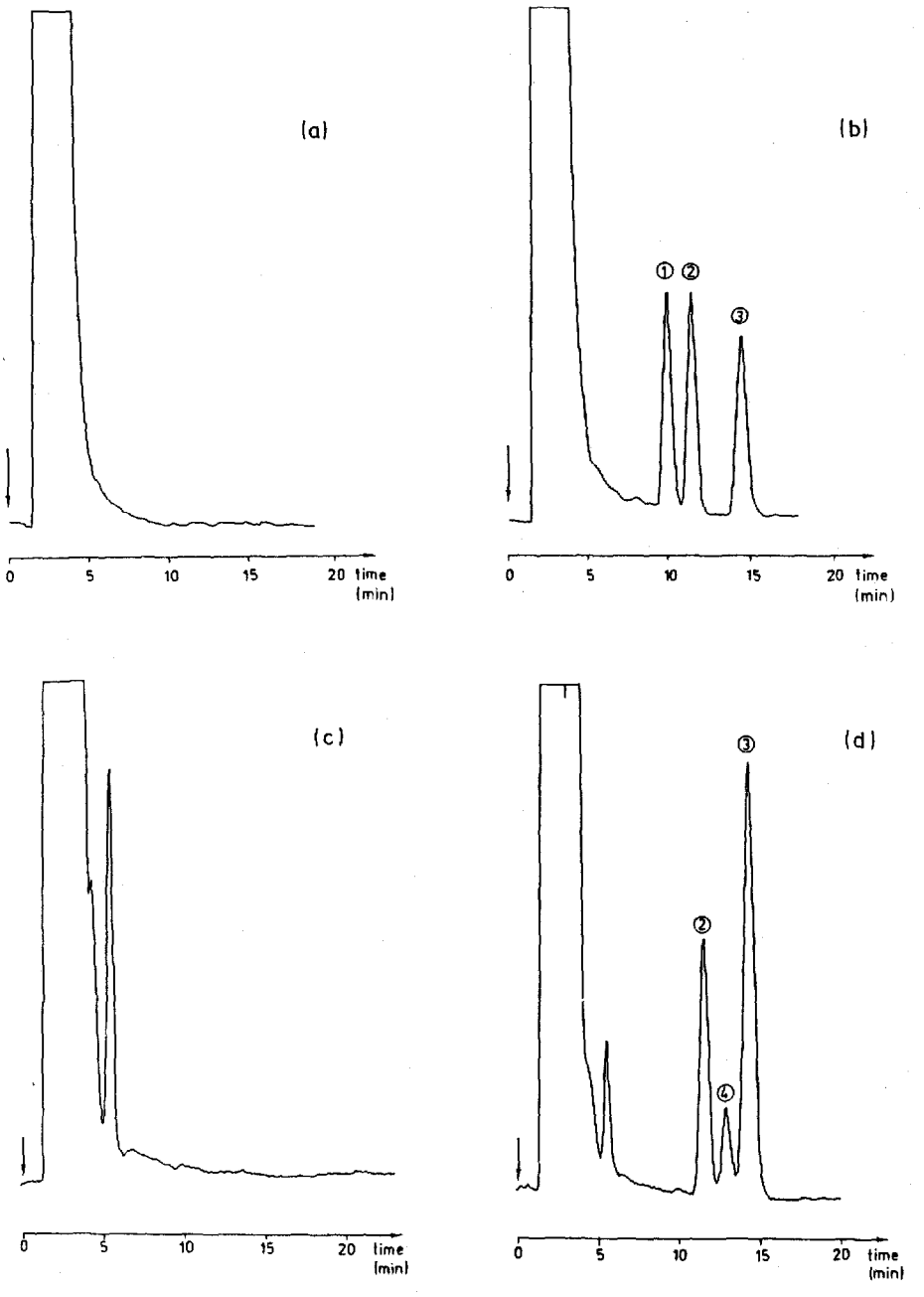


Fig. 3. Chromatogram of (a) a blank urine and (b) a urine sample spiked with vindesine (200 ng/ml), vincristine (200 ng/ml) and vinblastine (200 ng/ml). Chromatograms of urine extracts of the same patient as in Fig. 2: (c) a blank urine and (d) an extracted urine. Peaks: 1 = vindesine; 2 = vincristine (internal standard); 3 = vinblastine; 4 = desacetylvinblastine. Same chromatographic conditions as in Fig. 1.

urine sample, where desacetylvinblastine elutes between the internal standard (vincristine) and the parent drug. The metabolite is also extracted from urine when ion-pair extraction is used. The extraction recovery of desacetylvin-

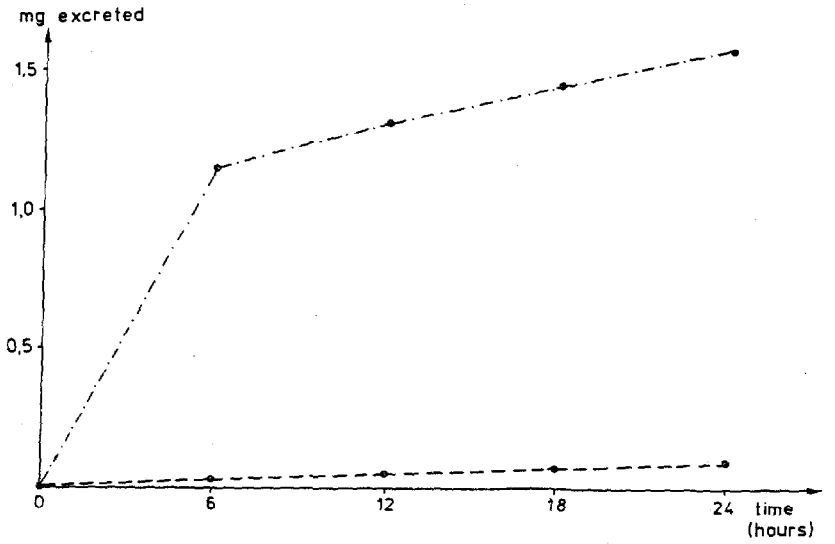


Fig. 4. Cumulative urinary excretion plot of vinblastine (- • - • -) and desacetylvinblastine (- - -).

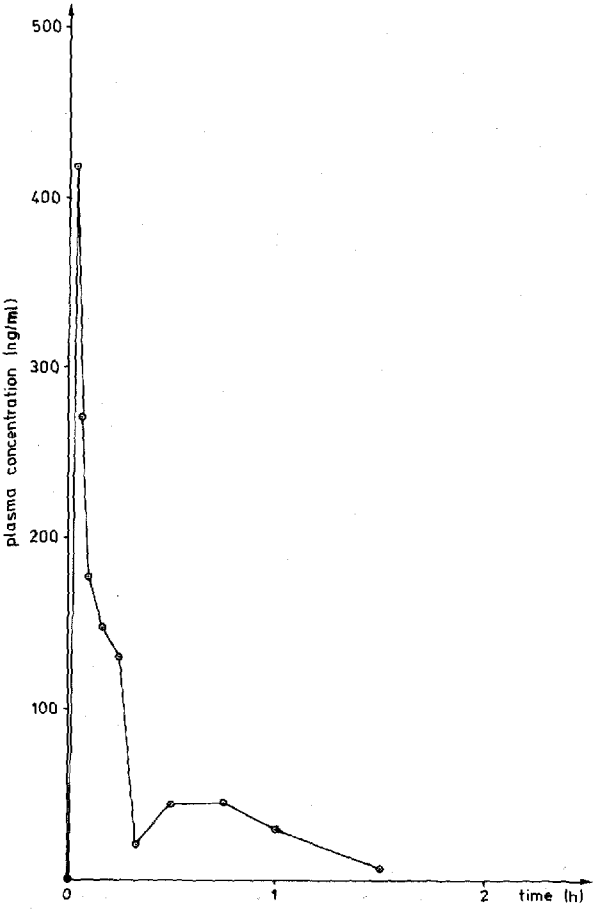


Fig. 5. Plasma concentration—time curve for a patient who received an intravenous injection of 3 mg/m² vindesine.

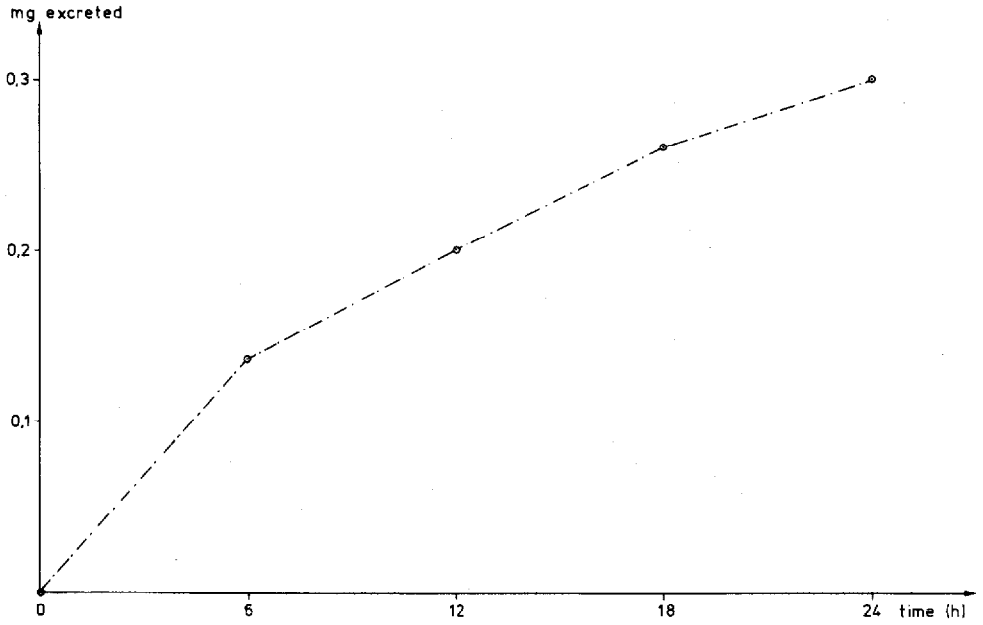


Fig. 6. Cumulative urinary excretion plot of vindesine.

blastine from urine is 99.2 ± 5.3% at 25 ng/ml level and 92.6 ± 2.6% at the 100 ng/ml level. The within-day precision for desacetylvinblastine in urine is determined at the 25 and 100 ng/ml level: the coefficients of variation were 5.2 and 4.3%, respectively.

The cumulative excretion of vinblastine and desacetylvinblastine is shown in Fig. 4. The urine samples have not been deconjugated, so the amount of vinblastine and desacetylvinblastine found represents only the fraction excreted as the unconjugated drug. The remaining amount can be excreted as glucuronide or sulphate conjugates. This will be further investigated.

The curves of vinblastine and desacetylvinblastine have the same profile of excretion, namely a faster rate in the first 6 h and becoming slower in the following time intervals. Approximately 16% of the total dose administered (10 mg of vinblastine) is excreted after 24 h, 15% as the parent drug and 1% as the metabolite, both as the free drug.

Figs. 5 and 6 represent the plasma concentration—time plot and the cumulative excretion in urine of a patient receiving a total dose of 5.4 mg of vindesine. The urine also has not been deconjugated. The plasma—concentration profile behaves in the same way as for vinblastine. However, the urinary excretion rate of vindesine seems to be slower compared to vinblastine (approximately 5% of the total dose administered is excreted after 24 h). However, these results are preliminary and a larger set of patient samples has to be investigated to confirm these preliminary conclusions.

CONCLUSION

A new HPLC method was developed for the determination of vinca-alkaloids (vinblastine, vincristine and vindesine) in plasma and urine. This method

involves ion-pair extraction, providing an almost complete recovery of the drugs from plasma or urine, and a selective reversed-phase HPLC system using a cyano column as the stationary phase. The whole procedure is suitable for routine application.

The method is expected to be a valuable tool for further elucidation of the human pharmacokinetics of vinca-alkaloids. A metabolite of vinblastine was detected in urine samples. In this paper, the method is applied to the preliminary pharmacokinetics of vinblastine and vindesine but it can also be used for the pharmacokinetic studies of vincristine. In this case, vinblastine or vindesine can be added as the internal standard.

ACKNOWLEDGEMENTS

The authors thank Mrs. K. Decq for skilful technical assistance, Eli Lilly (Belgium) for supplying the standards and F.G.W.O. for financial assistance.

REFERENCES

1 G. Atassi, P. Dumont and M. Vanderdris, Invasion Metastasis, 2 (1982) 217.
2 M.M. Mareel, G.A. Storme, G.K. De Bruyne and R.M. Van Cauwenberghe, Eur. J. Cancer Clin. Oncol., 19 (1982) 199.
3 G.A. Storme, D. Schallier, J. De Greve, S. Van Belle, G. De Wasch and G. Dotremont, in K. Helleman and S. Eccles (Editors), Treatment of Metastasis: Problems and Prospects, Taylor and Francis, London, 1985, p. 25.
4 R.L. Nelson, R.W. Dyke and M.A. Root, Cancer Treat. Rev., 7 (1980) 17.
5 J. Wells, R.J. Berry and A.H. Lain, Eur. J. Cancer, 12 (1976) 793.
6 R.L. Nelson, R.W. Dyke and M.A. Root, Med. Ped. Oncol., 10 (1982) 115.
7 R.J. Owellen, M.A. Root and F.O. Hains, Cancer Res., 37 (1977) 2603.
8 J.J. Langone, M.R. D'Onofrio and H. Vunakis, Anal. Biochem., 95 (1979) 214.
9 V.S. Sethi, S.S. Burton and D.V. Jackson, Cancer Chemother. Pharmacol., 4 (1980) 183.
10 R. Rahmani, J. Barbet and J.P. Cano, Clin. Chim. Acta, 129 (1983) 57.
11 M.P. Hacker, J.R. Dank and W.B. Ershler, Cancer Res., 44 (1984) 478.
12 R.L. Hussey and W.M. Newlon, J. Pharm. Sci., 67 (1978) 1319.
13 S. Görög, B. Herényi and K. Jovánovics, J. Chromatogr., 139 (1977) 203.
14 M. Verzele, L. De Taeye, J. Van Dyck, G. De Decker and C. De Pauw, J. Chromatogr., 244 (1981) 95.
15 J.E. Bodnar, J.R. Chen, W.H. Johns, E.P. Mariani and E.C. Shinal, J. Pharm. Sci., 72 (1983) 535.
16 J.A. Houghton, P.M. Torrance and P.J. Houghton, Anal. Biochem., 134 (1983) 450.
17 Atta-ur-Rahman, M. Bashir, M. Hafeez, N. Perveen, J. Fatima and N. Mistry, Med. Plant Res., 47 (1983) 216.
18 R.E. Crabteex and R.L. Nelson, unpublished results.
19 G. Hoogewijs and D.L. Massart, J. Pharm. Biomed. Appl., 2 (1984) 449.
20 G. Hoogewijs and D.L. Massart, J. Pharm. Biomed. Appl., 3 (1985) 165.
21 M. De Smet, G. Hoogewijs, M. Puttemans and D.L. Massart, Anal. Chem., 56 (1984) 2662.