Journal of Chromatography, 414 (1987) 91–100
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMBIO. 3415

SOLID-PHASE EXTRACTION OF VINBLASTINE AND VINCRISTINE FROM PLASMA AND URINE: VARIABLE DRUG RECOVERIES DUE TO NON-REPRODUCIBLE COLUMN PACKINGS

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(First received July 10th, 1986; revised manuscript received September 5th, 1986)

SUMMARY

A sensitive and selective high-performance liquid chromatographic (HPLC) method for the determination of vinblastine and vincristine in plasma and urine is described. The drugs are isolated from 1.0 ml of the biological fluid with a solid-phase extraction column (Bond-Elut Diol®). The HPLC method was combined with electrochemical detection at +850 mV versus an Ag/AgCl reference electrode. The detection limit is 100 pg for vinblastine and 250 pg for vincristine with a signal-to-noise ratio of 3, which permits the determination of these compounds in biological fluids at the nanogram level. Evaluation of the isolation method revealed that the drug recoveries and the reproducibility of the extraction procedure depend on the batch number of the solid-phase extraction column used.

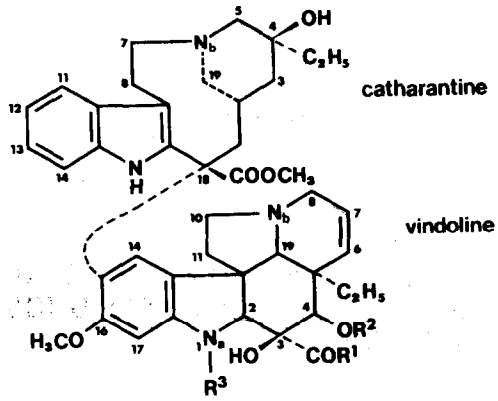
INTRODUCTION

Vinblastine and vincristine are dimeric catharanthus alkaloids used in the chemotherapy of various neoplastic diseases. Vinblastine and vincristine are very similar in their structure (Fig. 1) and in their biological action, but different in their clinical toxicity, activity and pharmacokinetic behaviour [1–3].

To study the clinical pharmacokinetics of these compounds, several high-per-

*Deceased May 31st, 1986.

0378-4347/87/$03.50



	$R_{1(3)}$	$R_{2(4)}$	R_3
VINCRISTINE	$COOCH_3$	$OCOCH_3$	CHO
VINBLASTINE	$COOCH_3$	$OCOCH_3$	CH_3

Fig. 1. Structures of vincristine and vinblastine.

formance liquid chromatographic (HPLC) methods have been developed for their determination in biological fluids using UV detection in combination with liquid scintillation counting [4–6] or using UV detection alone [7]. Radioactively labelled drugs in combination with thin-layer chromatography [8,9] and radioimmunoassay [10–12] have also been used for quantitation in biological matrices. Because of the laborious sample clean-up or poor selectivity of these methods, there is a need for a comparatively simple, fast, selective and sensitive method for the determination of these compounds in biological fluids.

This paper describes the development of an assay using liquid–solid extraction prior to HPCL in combination with electrochemical detection. Evaluation of the method revealed that the drug recovery and reproducibility are dependent on the batch number of the solid-phase column.

EXPERIMENTAL

Apparatus and chromatographic conditions

The chromatogrpahic system consisted of an M6000 A solvent delivery system equipped with a U6K septumless injector (loop volume 200 μl) or a Waters Intelligent Sample Processor (Model 710) automatic injector. A Hypersil ODS (150×3.9 mm I.D., particle size 5 μm) (Shandon, Runcorn, U.K.) column was used at ambient temperature.

The analytical column was protected by a laboratory-made guard column (20×3.9 mm I.D.), dry-packed with LiChrosorb RP-8 (5–20 μm) (Merck, Darmstadt, F.R.G.). The mobile phase was methanol–10 m*M* phosphate buffer (pH 7.0) (65:35, w/w). The solvents were filtered with a Millipore filter (0.2

μm) and after mixing were degassed by sonification for 5 min. The flow-rate was 1.0 ml/min for all experiments.

The electrochemical detector was of the wall-jet type and was developed in our laboratory [13]. The detector was equipped with a glassy carbon electrode (diameter 3 mm, Metrohm EA 286/1) and connected to a Metrohm 641 VA potentiostat. The chromatograms were recorded on a flat-bed recorder (Kipp & Zonen, Delft, The Netherlands). The glassy carbon electrode was cleaned daily by polishing with 0.3-μm aluminium oxide powder (Metrohm EA 1086). To shorten the stabilization period of the electrochemical detector the potential was adjusted to +990 mV for 15 min, after which the potential was decreased to the measuring potential of +850 mV versus an Ag/AgCl reference electrode.

Chemicals and solutions

Vinblastine sulphate (Velbe®) and vincristine sulphate (Oncovin®) were supplied by Eli Lilly Nederland.

Methanol (Merck, pro analysi) and water were distilled from glass before use. Other chemicals were of analytical-reagent grade and were used without further purification.

Standard solutions of vinblastine (1.00 mg of Velbe in 5.0 ml of methanol) and vincristine (10.00 mg of Oncovin in 5.0 ml of methanol) were prepared in polypropylene tubes and stored at −18°C. These solutions were diluted with methanol or the mobile phase as required.

Drug-free plasma and urine samples from volunteers were stored at −18°C.

Extraction procedure

Vincristine was used as the internal standard (I.S.) in the determination of vinblastine and vice versa. To 1.0 ml of plasma or urine 5–20 μl of the I.S. solution (1–200 ng/μl in methanol) and 10 μl of 1.8 *M* sulphuric acid were added (resulting pH=5–6). The plasma samples were mixed on a vortex mixer for 5 s and centrifuged for 10 min at 2500 *g*. The vinca alkaloids were isolated by means of liquid–solid extraction using a Bond-Elut Diol extraction column (1 ml capacity) (Analytichem International). Different batches of this type of column were used: Nos. 25061, 140257 and 141488. The Bond-Elut column was first washed with 5 ml of distilled methanol and 0.05 *M* tetramethylammonium bromide in 0.09 *M* sulphuric acid (pH 2). After transferring the biological fluid on to the column, the fluid was passed through the column using a Vac-Elut processing station (Analytichem International), applying a pressure of 2.5–50 cmHg. After the column had been washed consecutively with 2 ml of 0.025 *M* phosphate buffer (pH 8.5) and 5 ml of methanol–water (10:90, v/v) the column was dried by purging with air (63.5 cmHg pressure). The vinca alkaloids were eluted with 500 μl of methanol. The eluate was collected in a conical polypropylene tube (capacity 1.5 ml). After mixing on a vortex mixer for 5 s, the methanol was evaporated under nitrogen at ambient temperature. The residue was dissolved in 20–100 μl of the mobile phase by vortexing for 30 s. A volume of 10–50 μl was injected into the chromatographic system.

Because of the wide concentration range found for vinblastine and vincristine

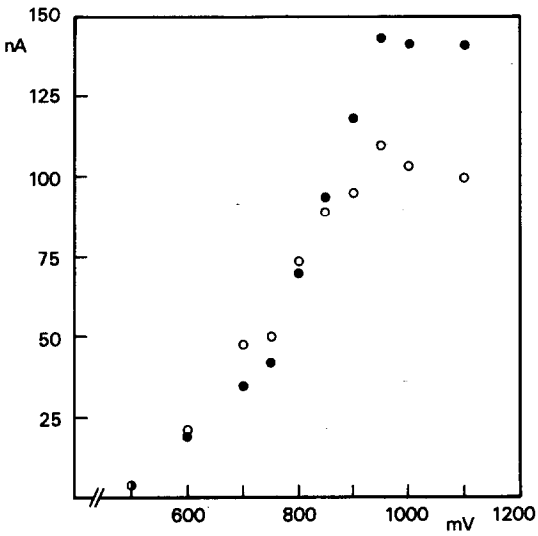


Fig. 2. Hydrodynamic voltammogram obtained after repetitive injection of 200 ng of vinblastine (●) and 200 ng of vincristine (○).

in the various biological matrices, different calibration graphs in the concentration range 1–1000 ng/ml in plasma and 2.5–1000 ng/ml in urine were used (Table II). The calibration graphs were obtained by spiking drug-free plasma and urine samples with vinblastine and vincristine dissolved in methanol (a maximum of 25 μl methanol was added).

Drug recovery and reproducibility

Intra-assay precision was determined at different concentrations (10 and 100 ng/ml in plasma and 50 ng/ml in urine) for both drugs and with columns from different batches.

The extraction recovery was calculated by comparing the peak-height ratios of the drug to be determined and the external standard (added to the eluate after the column extraction) with those of methanolic standards, dried under nitrogen and dissolved in the mobile phase.

RESULTS AND DISCUSSION

Electrochemical detection of the vinca alkaloids

Preliminary cyclic voltammetric experiments carried out at the same glassy carbon electrode as used for the electrochemical detection showed that the oxidation current of both compounds increases with increasing pH. The pH of the mobile phase was chosen to be as high as possible with regard to the stationary phase.

Fig. 2 shows the current–potential curves for vinblastine and vincristine obtained after repetitive injection of 200 ng of both compounds. It can be seen that a plateau is reached at +900 mV for both compounds. At potentials above

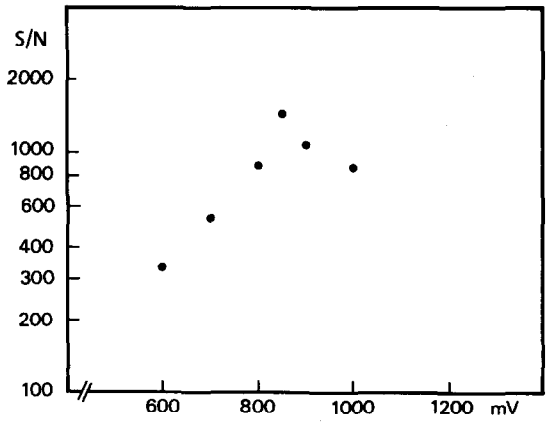


Fig. 3. Dependence of the signal-to-noise ratio (S/N) of vinblastine on detection potential.

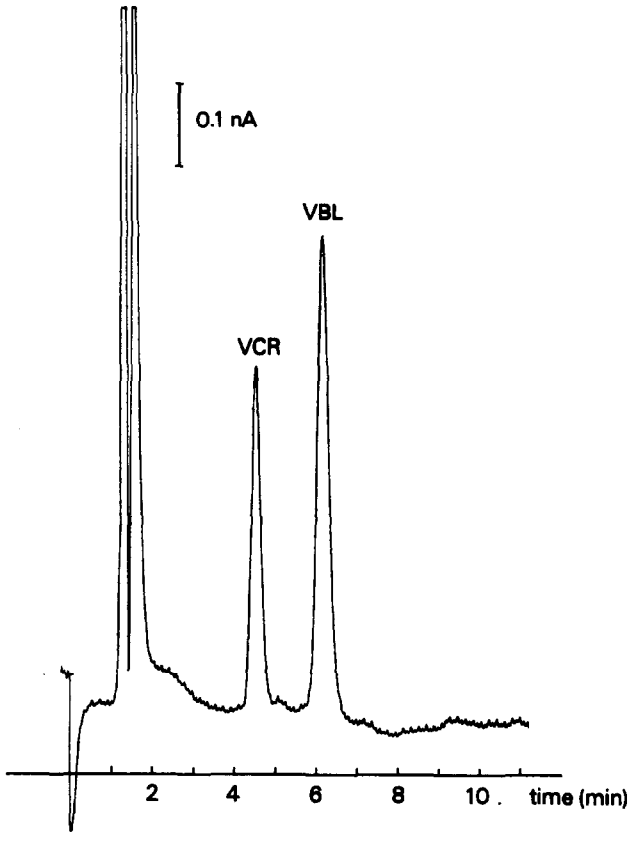


Fig. 4. Chromatogram of a mixture of vincristine (VCR, 1.5 ng) and vinblastine (VBL, 1.9 ng) in 10 μl of methanol.

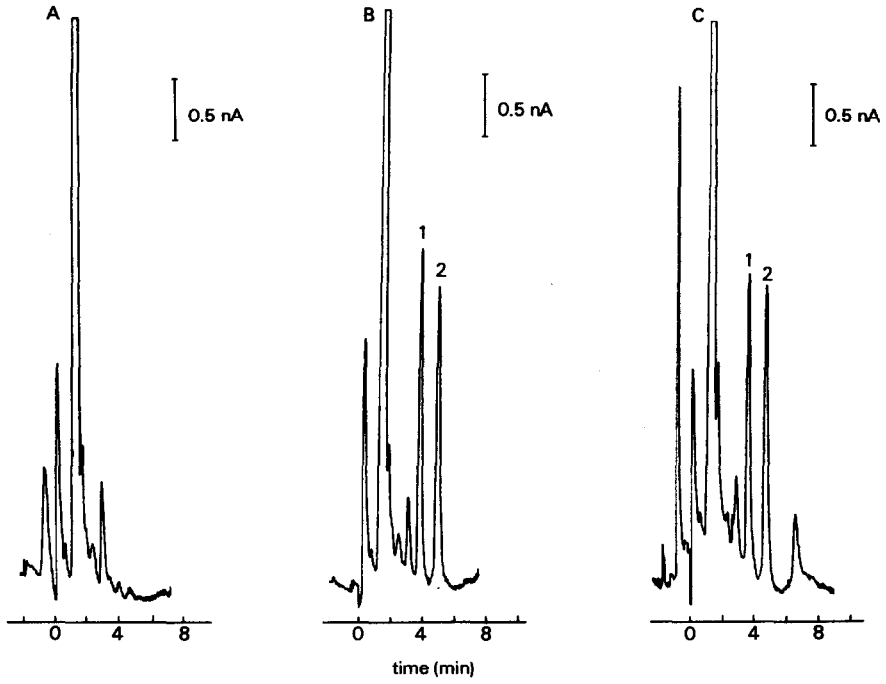


Fig. 5. Chromatograms of plasma extracts using vincristine as internal standard. (A) An extract of 1.0 ml of blank plasma. (B) An extract of 1.0 ml of plasma spiked with 48.4 ng/ml vinblastine. (C) An extract of 1.0 ml of patient's plasma containing 44.0 ng/ml vinblastine. Peaks: 1 = vincristine; 2 = vinblastine.

+900 mV a slight change in potential results in only a small change in current. Normally a detection potential is chosen that lies at the plateau in order to reduce the variation in current due to variation in the potential of the reference electrode. As the background current and noise increase exponentially above +750 mV [13], it is necessary to choose the detection potential carefully in order to achieve maximum sensitivity. Evaluation of the noise and signal current showed that a maximum signal-to-noise ratio is achieved at a detection potential of +850 mV (Fig. 3). This potential was chosen as the detection potential for all experiments. Despite the fact that +850 mV lies on the steep slope of the potential–current curve, a low relative standard deviation (R.S.D.) of the peak-height ratio of 1.3% was obtained after repetitive injection ($n=90$) of a mixture of 20 ng of vinblastine and 20 ng of vincristine dissolved in 10 μl of the mobile phase.

Detector response and calibration

The precision of the chromatographic system was determined by repetitive injection ($n=6$) of 5 and 50 ng of vinblastine dissolved in 10 μl of methanol. The R.S.D.s of the measured peak heights were 1.8 and 2.8%, respectively. The calibration graphs were linear over a concentration range of 1–2000 ng for vinblastine and vincristine with correlation coefficients of 0.9986 and 0.9994, respectively. The intercepts on the ordinates of both graphs were not significantly different

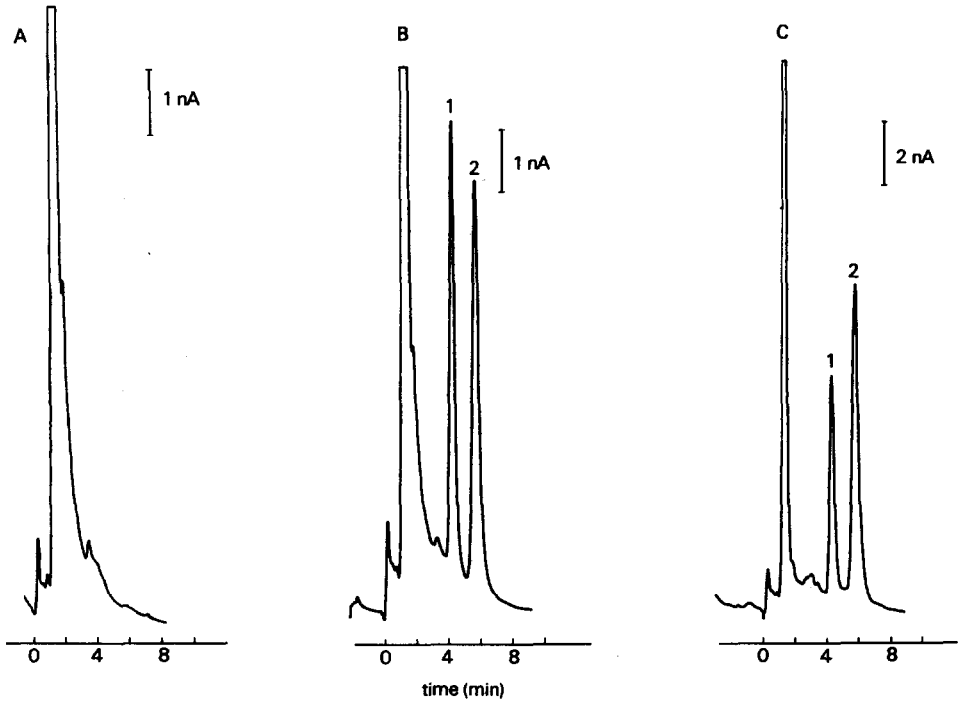


Fig. 6. Chromatograms of urine extracts using vincristine as internal standard. (A) An extract of 1.0 ml of blank urine. (B) An extract of 1.0 ml urine spiked with 242 ng/ml vinblastine. (C) An extract of 1.0 ml of patient's urine containing 393 ng/ml vinblastine. Peaks: 1 = vincristine; 2 = vinblastine.

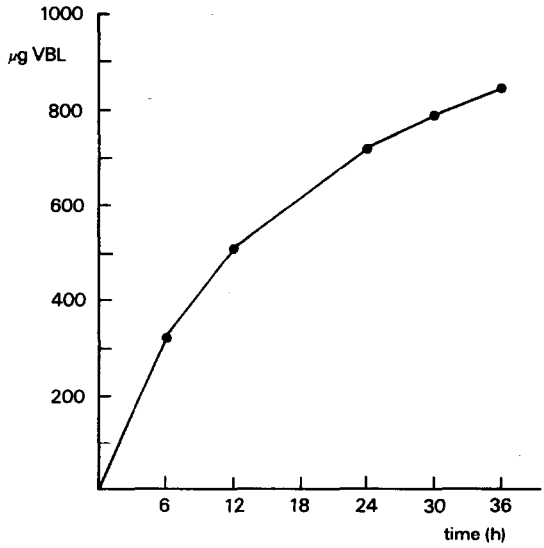


Fig. 7. Cumulative excretion curve of vinblastine (VBL) after intravenous administration of 16 mg of vinblastine to a patient.

from zero ($p<0.05$). The detection limit at a signal-to-noise ratio of 3 was 100 pg (110 fmol) for vinblastine and 250 pg (204 fmol) for vincristine. Fig. 4 presents a chromatogram obtained after injection of a mixture of vinblastine and vincristine.

Chromatography

Several methanol–buffer mixtures were investigated as possible mobile phases. The best results were obtained with the mixture described above. The aqueous part of the mobile phase was buffered not higher than pH 7 in order to obtain an optimum sensitivity and to prevent damage to the column.

The chromatographic system used permits the elution of both compounds at nanogram levels within 7 min (Fig. 4) and baseline resolution is achieved. Figs. 5 and 6 show chromatograms of extracts of plasma and urine samples containing vinblastine; vincristine was used as the internal standard. These chromatograms were obtained using the batch of solid-phase extraction columns with the highest drug recovery (No. 25061). The chromatographic system used gives a good separation between plasma components and drugs. The combination of the relatively polar bonded phase (diol column) used for the isolation of the compounds from biological fluids and the non-polar C_{18} bonded phase in the analytical column gives a high selectivity. The described method is also suitable for the determination of the vinca alkaloids in urine, but the determination limit of vinblastine and vincristine in urine is higher (2.5 and 10 ng/ml, respectively).

Drug recovery, linearity, intra-assay and inter-batch variability of the extraction procedure

Different Bond-Elut extraction columns, e.g. C_2, C_{18} and Diol, were tested for the isolation of vinca alkaloids from biological fluids. It was found that the diol column gave a high drug recovery and, in combination with an analytical C_{18} column, less interfering peaks in the chromatogram in comparison with other combinations. The retention of the vinca alkaloids on the diol column is probably based on the interactions of the protonated/deprotonated species with the free silanol groups and diol groups. On addition of sulphuric acid to the biological fluid and acidification of the column the vinca alkaloids are protonated and are probably retained on the column owing to interaction of the protonated nitrogen atoms with the free silanol groups (ion-exchange effect). During the subsequent washing procedure with the phosphate buffer of pH 8.5 the vinca alkaloids are deprotonated. The retention of the compounds is now assured by the lipophilic interactions of the bonded phase with the deprotonated alkaloids. This last interaction is strong, as is demonstrated by the fact that the column can be washed with 5 ml of 10% methanol. The pre-washing of the extraction column with tetramethylammonium bromide solution permits the elution of the vinca alkaloids with 500 μl of methanol. Without this pre-treatment of the extraction column, about 2 ml of methanol are necessary to elute the same amount of drug and in that event increased amounts of interfering compounds are eluted.

The strong retention of the vinca alkaloids on the diol column batch No. 25061 makes it possible to wash the column with several fluids of different pH and

TABLE I

RECOVERIES AND RELATIVE STANDARD DEVIATIONS OF ANALYSIS OF VINBLASTINE AND VINCRISTINE IN SPIKED SAMPLES USING BOND-ELUT DIOL COLUMNS OF DIFFERENT BATCHES

N.D. = not determined.

Column batch No.	Compound	Concentration (ng/ml)	Matrix	Recovery (%)	R.S.D.* (%)	R.S.D.** (%)	Amount of I.S. or E.S. (ng)	*n*
25061	Vinblastine	10	Plasma	74.4	7.9	3.8	11	6
		50	Urine	75.0	4.3	1.3	49	6
		100	Plasma	82.5	6.8	4.3	122	6
	Vincristine	10	Plasma	75.0	4.3	1.3	9	6
		50	Urine	83.2	3.0	1.3	48	6
141488	Vinblastine	100	Plasma	17.2	24.3	9.6	146	6
	Vincristine	100	Plasma	7.3	16.9	3.1	62	6
140257	Vinblastine	100	Plasma	83.6	N.D.	N.D.	146	1
	Vincristine	100	Plasma	53.1	N.D.	N.D.	62	1

*When an external standard is used.
**When an internal standard is used.

contents of methanol, leading again to fewer interfering peaks from plasma or urine. Table I (top) shows the extraction recovery of the vinca alkaloids in plasma and urine determined at several concentration levels using a diol column of batch No. 25061. The recovery was not quantitative but high enough and with acceptable R.S.D.s. The results in Table I also show that a better R.S.D. is obtained when an internal standard (added before the column extraction) is used, and that it is not necessary to use a deproteinization step in order to achieve a high recovery.

Table II presents data for the calibration graphs used for the determination of the vinca alkaloids in the range 1–1000 ng/ml. Because of the wide range of the clinically active concentrations it is necessary to use three calibration graphs. The calibration graphs were all linear, with acceptable correlation coefficients. The intercepts on the ordinate proved to be not significantly different from zero ($p < 0.05$) for all the graphs.

Table I also shows the extraction recoveries and R.S.D.s obtained with diol columns of batch Nos. 141488 and 140257. On comparing the results with those for the column of batch No. 25061 can be concluded that the recovery of vinblastine and vincristine decreases dramatically and the extraction becomes less reproducible when batches of columns other than No. 25061 are used. Recovery studies revealed that part (ca. 20%) of the protonated alkaloid is eluted with the biological fluid that is forced through the column. Another part (ca. 30%) is eluted with the phosphate buffer and approximately 20% is washed from the column by the 10% methanol–water mixture. This results in an low overall recovery of vinblastine and vincristine in the methanolic fractions.

The cause of the low recovery obtained with some batches is unknown. After

TABLE II

DATA FOR CALIBRATION GRAPHS OF VINBLASTINE AND VINCRISTINE IN DIFFERENT MATRICES USING A BOND-ELUT DIOL COLUMN OF BATCH No. 25061

Compound	Concentration range (ng/ml)	Matrix	I.S.	Amount of I.S. (ng)	Correlation coefficient (r^2)	n
Vinblastine	100 -1000	Plasma	Vincristine	541	0.9996	5
	10 - 100			49	0.9985	5
	1 - 10			5	0.9942	5
	100 -1000	Urine		541	0.9999	5
	10 - 100			49	0.9983	5
	2.5- 10			5	0.9890	4
Vincristine	100 -1000	Plasma	Vinblastine	484	0.9991	5
	10 - 100			48	0.9893	4
	1 - 10			5	0.9774	4
	100 -1000	Urine		484	0.9981	5
	10 - 100			48	0.9969	5

receiving the columns they were stored and activated under identical circumstances. It is therefore assumed that the chemical properties of the columns differ from batch to batch.

The method is sensitive enough to determine the vinblastine levels in plasma and urine from patients (Figs. 5C and 6C).

Fig. 7 shows the cumulative excretion curve of vinblastine after a short infusion of 16 mg of vinblastine into a patient. Within 36 h 5.3% of the adminsitered dose was excreted unchanged in the urine.

In conclusion, the determination of the vinca alkaloids at the nanogram level in biological fluids is possible when solid-phase diol columns are used. This study emphasizes again that although liquid–solid extractions are rapid and easy to perform, their applicability depends on a constant quality of the solid-phase extraction columns used.

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