Cancer Chemother Pharmacol (1982) 7:141–145

Cancer Chemotherapy and Pharmacology

Pharmacokinetics of VP16-213 Given by Different Administration Methods

M. D'Incalci[1], P. Farina[1], C. Sessa[2], C. Mangioni[2], V. Conter[3], G. Masera[3], M. Rocchetti[1], M. Brambilla Pisoni[4], E. Piazza[4], M. Beer[5], and F. Cavalli[5]

[1] Istituto di Ricerche Farmacologiche "Mario Negri", Via Eritrea 62, I-20100 Milano
[2] 1ª Clinica Ostetrica Ginecologica, Università, I-20100 Milano
[3] Clinica Pediatrica, Università, I-20100 Milano
[4] Clinica Medica V, Università, I-20100 Milano, Italy
[5] Ospedale S. Giovanni, CH-6500 Bellinzona, Switzerland

Summary. *Plasma pharmacokinetics of VP16-213 were investigated after a 30–60 min infusion in 14 adult patients and six children. In adults the elimination half-life ($T_{1/2}$ β), plasma clearance (Cl_p) and volume of distribution (Vd) were respectively 7.05 ± 0.67 h, 26.8 ± 2.4 ml/min/m², and 15.7 ± 1.8 l/m²; in children 3.37 ± 0.5 h, 39.34 ± 6.6 ml/min/m², and 9.97 ± 3.7 l/m². After repeated daily doses no accumulation of VP16-213 was found in plasma. The unchanged drug found in the 24 h urine after administration amounted to 20–30% of the dose.*

In eight choriocarcinoma patients plasma levels of VP16-213 were measured after oral capsules and drinkable ampoules. The bioavailability compared to the i.v. route was variable, mean values being 57% for capsules and 91% for ampoules. In one further patient, with abnormal d-Xylose absorption results, VP16-213 was not detectable in plasma after the oral ampoule dose.

Steady state levels investigated in three patients after 72 h continuous VP16-213 infusion (100 mg/m²/24 h) were around 2–5 μg/ml. Levels of VP16-213 were undetectable in CSF after i.v. or oral administration.

Introduction

VP16-213 (VP16) (NSC-141540) is a semisynthetic podophyllotoxin-derivative reported to be active in some experimental and human malignancies [3, 5, 11, 12, 14, 15, 17]. Pharmacokinetic studies could help rationalize and improve the clinical efficacy of this compound, whose antitumor effect is known to be dosage-schedule dependent in animal models and appears increasingly to be so in man too [5, 15]. Some clinical pharmacokinetic studies are available but apart from the questionable specificity of the methods used, they report data on only small numbers of patients after i.v. administration, whereas the drug can also be given orally. This need for better knowledge of the pharmacokinetics of VP16 prompted different groups to develop analytical assays with the required specificity, sensitivity, and reliability for clinical research studies [1, 9, 10, 18, 19]. Using an HPLC method developed in our laboratory [9], we have investigated VP16 kinetics after i.v. administration to a considerable number of adult patients and in children and compared the bioavailability of the drug given orally in capsules or drinkable ampoules. Some preliminary data on plasma levels of VP16 given in 72 h continuous infusion are also reported.

Send offprint requests to M. D'Incalci at the above address

Materials and Methods

Patients. Twelve adult patients were suffering from gestational choriocarcinoma relapsing from previous chemotherapy or with metastatic involvement of distant organs including abdominal cavity, lung or CNS. One of these patients (No. 13) and three other patients who received 2 h continuous infusion had lung cancer. Another patient (No. 10) had ovarian dysgerminoma. Six children were suffering from acute leukemia refractory to conventional chemotherapy. All patients had normal liver and renal function.

Drug Treatment. VP16 for clinical use was supplied by Bristol-Myers, New York, NY. The oral capsules or ampoules were given to patients fasted from 12 h before to 8 h after VP16 treatment. All patients were given VP16 for 5 consecutive days and the courses were repeated every 4 weeks. The pharmacokinetic studies were performed in the 24 h after the first daily dose of the cycle. Patients 4 and 6 were also investigated after the 5th daily i.v. dose of the first course. Patients 3 and 6 received first the 5-day i.v. course, then after 4 weeks a 5-day oral capsules course, then after another 4 weeks a 5-day oral ampoules course. Patients 1, 2, and 7 received first the 5-day i.v. course, after 4 weeks a 5-day oral ampoules course and after another 4 weeks a 5 day oral capsules course. Patients 4, 5, and 8 received first the 5-day oral capsules

0344-5704/82/0007/0141/$01.00

course, after 4 weeks a 5-day i.v. course and after another 4 weeks a 5-day oral ampoules course.

Sample Collection. Blood samples of 3 ml were collected from a forearm vein at intervals from 0–24 h after infusion or oral administration, immediately put into heparinized tubes and spun down at 2,000 rpm. From patients 1, 2, 3, and 8, 8 ml of CSF were taken by lumbar puncture between 4–17 h after the i.v. drug and in patients 1 and 3 again 3–4 h after oral capsule administration. The volume was replaced with saline containing 10 mg of methotrexate. Five ml of CSF were sent to the laboratory for β-HCG determination and the other 3 ml were stored like the plasma samples at −20° C for drug assay. A part of the 24 h urine was also stored at −20° C until analysed.

Drug Assay. The method, described in detail elsewhere [9], can be summarized as follows: after diisoporopyl ether washing of plasma (1 ml), urine (0.5 ml) or CSF (2.5 ml), extraction is carried out with chloroform using VM26 as internal standard. The organic phase is dried under vacuum and the residue is redissolved in 100 µl of mobile phase; 5–20 µl of this solution was injected into a Waters Model 440 HPLC equipped with a 254 nm absorbance detector. Separation was achieved with an isocratic solvent system of water (45%) and methanol (55%) at a flow rate of 1 ml/min using a 25 cm long Lichrosorb RP-8 (5 µm) column purchased from Merck, Darmstadt, FRG.

A calibration curve was plotted using VP16 concentrations of 0.5, 1, 5, and 20 µg/ml. Extraction recovery was 79 ± 3%. The lower limit of sensitivity was 0.5 µg/ml of plasma and the coefficient of variation of the method was less than 10%.

Pharmacokinetic Calculations. The following formulae were used for pharmacokinetic evaluation:

AUC = Area under the plasma concentration vs. time curve $0 \rightarrow \infty$ extrapolated to infinity (trapezoidal method)

F = Bioavailability, i.e., ratio of AUC $0 \rightarrow \infty$ after oral to that after intravenous administration in the same subject

VD = Apparent volume of distribution: $\frac{\text{Dose} \times F}{\text{AUC} \times \beta}$

F = 1 in the case of i.v. administration

β = Apparent first order rate constant for elimination of drug from the body

Cl_p = Plasma clearance: $\frac{\text{Dose} \times F}{\text{AUC } 0 \rightarrow \infty}$

F = 1 in the case of i.v. administration

A 3-compartment open model after oral administration was applied for data of patients 1, 2, 3, 4, and 6 and a 2-compartment model for patients 5, 7, and 8. The 2-compartment open model was applied after i.v. administration. All data were processed using a non linear fitting program.

Results

After 30–60 min infusion VP16 disappears from plasma following a biphasic pattern. Tables 1 and 2 show the pharmacokinetic parameters respectively in adults and children. As indicated by the Cl_p and $T_{1/2}\beta$ values the elimination of VP16 appears to be faster in children, particularly when after 100 mg/m^2, than in adults. Figure 1 shows the virtually identical VP16 plasma levels after the 1st and 5th daily i.v. doses of the 5-day course, indicating no drug accumulation in plasma. Urinary elimination of the unchanged VP16 in the 24 h after treatment accounted for 20–30% of the dose (data not shown).

Table 3 summarizes the pharmacokinetic and bioavailability data in the eight patients given capsules or drinkable ampoules. The peak plasma level was achieved in 0.5–4 h, then rapidly declined, corresponding to the distribution phase, followed by a slower elimination rate. The elimination half-life of VP16 was 4.7 ± 0.78 after capsules and 5.98 ± 1.0 after ampoules. The bioavailability of capsules and ampoules was respectively 57 (29-137)% and 91 (48–149)% of that found after i.v. administration.

In patient No. 9, who did not absorb d-Xylose normally, no VP16 was detectable in plasma after the oral ampoule dose and the lack of absorption of VP16 was confirmed when the investigation was repeated 1 month later. VP16 was not detectable in CSF sampled 3, 4, 6, and 17 h after 60 min infusion in four patients and 3 and 4 h after oral capsules.

As shown in Figure 2 after 72 h continuous infusion of 100 mg/m^2/24 h VP16, plasma levels of 2–5 µg/ml were reached approximately 2–3 h from the beginning and were maintained until the end of the infusion period, after which thus declined at the same elimination rate as after rapid infusion.

Discussion

As previously described by Creaven and Allen [2, 7] VP16 disappears biphasically from patients' plasma, but the elimination rate found in this study was faster than reported by these authors. The mean elimination half-life ($T_{1/2}\beta$) after i.v. administration was about 7 h, compared to 11.5 h described by Creaven and Allen [2, 7]. This discrepancy could be due to the different analytical method used. Creaven et al. determined ^{3}H-VP16 after labelled drug treatment and the radioisotopic method may be less specific, possibly not distinguishing some chloroform extractable metabolites from the parent drug. More recent data obtained measuring VP16 by HPLC are in fact closer to the present results [11, 14, 15]. As the $T_{1/2}\beta$ is much shorter than the interval between subsequent doses (24 h), it is not surprising that no drug accumulates in plasma after five consecutive daily doses.

The faster [9, 16] disappearance of VP16 in children is likely to be related to the faster drug biotransformation or elimination already described for other compounds. The kinetic differences could to some extent, explain why children appear to tolerate

Table 1. Pharmacokinetic parameters of VP16 after i.v. administration to adult patients

Patient	Age (years)	Dose (mg/m^2)	A (μg/ml)	α (min^{-1})	$T_{1/2}\alpha$ (min)	B (μg/ml)	β (min^{-1})	$T_{1/2}\beta$ (h)	AUC $0 \rightarrow \infty$ (μg/ml × min)	Cl_p ($ml/min/m^2$)	Vd (l/m^2)
1	38	100	10.21	0.0086	81	3.63	0.0019	6.0	3,049	32.8	17.3
2	29	100	12.37	0.0599	12	7.86	0.0018	6.4	4,641	21.5	12.0
3	30	100	11.27	0.0082	84	2.91	0.0019	6.0	3,072	32.5	17.1
4	28	100	10.64	0.0073	95	3.85	0.0015	7.7	4,079	24.5	16.3
5	33	100	8.99	0.0075	92	4.11	0.0012	9.6	4,723	21.2	17.6
6	31	100	12.38	0.0082	85	1.43	0.0017	6.8	2,391	41.8	24.9
7	26	100	4.93	0.0099	70	5.60	0.0020	5.8	3,351	29.8	14.9
8	40	100	4.37	0.0130	53	6.34	0.0022	5.2	3,270	30.6	13.9
9	29	100	13.64	0.0064	108	7.07	0.0016	7.2	6,832	14.6	5.6
10	12	100	17.93	0.0094	74	5.88	0.0023	5.0	4,607	21.7	9.4
11	40	100	6.92	0.0572	12	9.06	0.0026	4.4	3,616	27.6	10.6
12	32	100	11.40	0.0094	74	8.62	0.0019	6.0	5,960	16.8	8.8
13	55	100	6.63	0.0222	31	2.86	0.0014	8.2	2,291	43.6	31.2
14	45	200	26.07	0.0057	122	5.51	0.0008	14.4	12,306	16.2	20.3

A = Intercept of fastest disposition slope with ordinate
α = Fastest disposition rate constant
$T_{1/2}\alpha$ = Distribution half life
B = Intercept of slowest disposition slope with ordinate
β = Slowest disposition rate constant
$T_{1/2}\beta$ = Biological terminal half life

Table 2. Pharmacokinetic parameters of VP16 after i.v. administration to children

Patient	Age (years)	Dose (mg/m^2)	A (μg/ml)	α (min^{-1})	$T_{1/2}\alpha$ (min)	B (μg/ml)	β (min^{-1})	$T_{1/2}\beta$ (h)	AUC $0 \rightarrow \infty$ (μg/ml × min)	Cl_p ($ml/min/m^2$)	Vd (l/m^2)
15	4.4	95	6.77	0.0943	7	8.13	0.0069	1.7	1,324	71.7	10.4
16	3.7	100	12.55	0.0522	13	9.90	0.0048	2.4	2,503	39.9	8.3
17	4.8	108	14.33	0.0330	21	9.85	0.0048	2.4	2,631	41.0	8.5
17	4.8	216	21.27	0.0099	70	8.40	0.0023	5.0	6,055	35.7	15.5
18	9	200	41.32	0.0107	65	13.15	0.0022	5.2	10,080	19.8	9.0
19	7	200	67.67	0.0120	58	8.12	0.0026	4.4	9,471	21.1	8.1
20	9.5	200	16.35	0.0573	12	17.66	0.0046	2.5	4,332	46.2	10.0

Table 3. Pharmacokinetic parameters of VP16 after oral capsules (cp) or ampoules (amp)

Patient	Dose (mg/m^2)		Peak level (μg/ml)	Time (min)	A (μg/ml)	α (min^{-1})	$T_{1/2}\alpha$ (min)	B (μg/ml)	β (min^{-1})	$T_{1/2}\beta$ (h)	AUC $0 \rightarrow \infty$ (μg/ml × min)	F (%)	Vd (l/m^2)
1	cp	214	18.9	120	16.96	0.0072	96	11.20	0.0016	7.2	8,940	137	20.5
	amp	143	15.1	150	36.15	0.0083	83	5.72	0.0012	9.6	6,488	149	27.4
2	cp	200	21.6	35	14.68	0.0111	62	9.22	0.0018	6.4	5,939	64	12.0
	amp	133	9.1	60	–	–	–	9.21	0.0021	5.5	4,306	70	10.3
3	cp	200	6.9	150	6.79	0.0078	89	6.36	0.0022	5.2	3,028	49	14.7
	amp	133	10.6	160	11.70	0.0061	114	3.40	0.0015	7.7	3,705	91	21.7
4	cp	186	6.6	50	8.77	0.0262	26	4.29	0.0018	6.4	2,479	33	13.7
	amp	125	10.6	120	8.84	0.0065	107	7.94	0.0014	8.2	6,172	121	17.5
5	cp	193	12.32	45	–	–	–	9.63	0.0022	5.2	4,244	46	9.5
	amp	129	10.36	120	–	–	–	10.62	0.0018	6.4	5,554	91	11.7
6	cp	207	5.0	60	5.43	0.0099	70	2.47	0.0022	5.2	1,445	29	18.9
	amp	138	8.0	30	11.38	0.0100	69	1.11	0.0013	8.9	1,590	48	32.0
7	cp	182	9.85	45	–	–	–	9.74	0.0041	2.8	2,124	35	7.3
	amp	121	8.41	30	–	–	–	6.44	0.0025	4.6	2,640	65	11.9
8	cp	150	4.8	240	–	–	–	8.50	0.0021	5.5	3,042	62	14.6

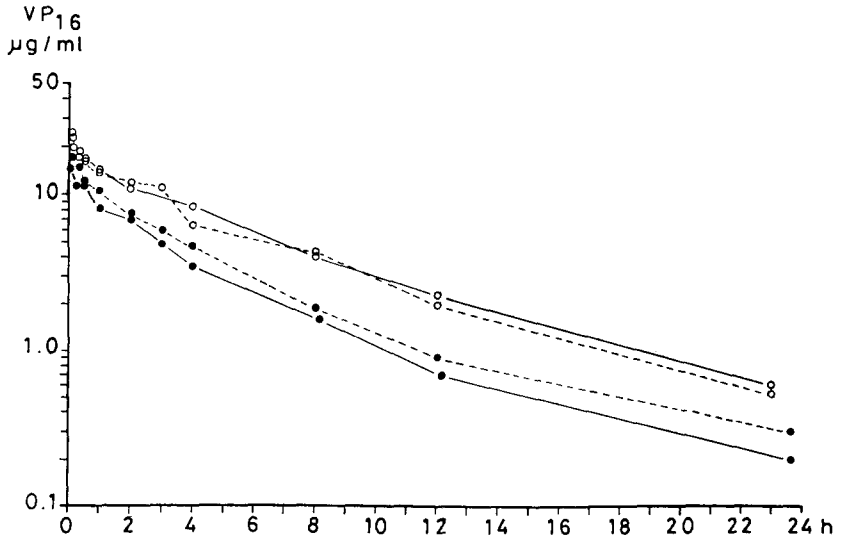

Fig. 1. VP16 plasma levels after 1st (———) and 5th (– – –) i.v. daily dose (100 mg/m²/day) in patient 3 (●) and patient 9 (○)

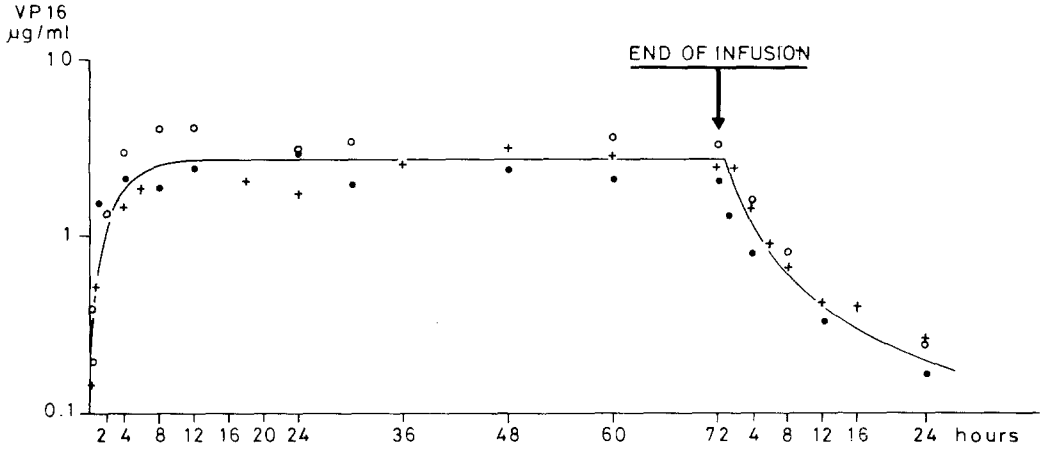

Fig 2. VP16 plasma levels in three patients receiving 72 h continuous infusion (100 mg/m²/24 h)

VP16 treatment better. Even though it cannot be statistically demonstrated, it is suspected that the elimination half-life of VP16 grows longer at larger doses. This tendency was observed in children, patient No. 17 showing VP16 half-lives of 2.4 and 5 h after 108 and 216 mg/m². In addition, only adults given 200 mg/m² had a $T_{1/2}\beta$ more than double the mean $T_{1/2}\beta$ of the other 13 subjects given 100 mg/m². The possibility of dose-dependent kinetics of VP16 cannot be excluded and requires further studies.

After oral administration the bioavailability of VP16 appeared to be variable. Previous studies indicate that a double oral dose is necessary to produce bone marrow toxicity equivalent to that arising after i.v. administration [4], suggesting that systemic bioavailability of the drug given p.o. is 50%. The present findings suggest that in some cases doubling the dose may not be sufficient to reach the same levels as after i.v. treatment, but in others much higher drug concentrations are reached, possibly resulting in severe toxicity. The observation of bioavailability higher than 100% in patient 1, after oral capsules and ampoules, further suggests that the elimination of VP16 is not proportional to the plasma concentration. When a higher oral than i.v. dose is given, if the absorption is efficient plasma levels should be higher than after the i.v. dose; this could result in a slower elimination rate. Patient 1 had in fact an elimination half-life of 6 h after the i.v. dose and 7.2 and 9.6 h after the oral capsules and ampoules. Therefore, when the i.v. route cannot be used, oral administration should be carefully followed by monitoring plasma drug levels to permit appropriate dose adjustment in each patient. It also appears advisable to test the absorption of d-Xylose before starting oral VP16 treatment, particularly in

patients who have previously received other chemotherapy known to cause gastrointestinal toxicity, altering absorption [13].

When tolerable doses of VP16 are given by continuous infusion, steady state levels reported to be highly cytotoxic on different cell lines [7a, 11] are achieved; this offers a rationale for investigating the clinical efficacy of this method of administration.

As previously described, there is poor CSF penetration by VP16, and this is in good agreement with distribution data in mouse indicating very low drug levels in the brain [6].

Acknowledgements. We would like to thank our patient Sandra Pacini for her enormous willingness to cooperate in this study, often giving up her precious time to help us. ML "Verga" Association for research and treatment of childhood leukemia is greatly acknowledged for the support given to the Dept. of Hematology of the Pediatric Clinic of University of Milan. We thank Ms. J. Baggott and Ms. Anna Mancini for preparation of the manuscript.

References

1. Allen LM (1980) Analysis of 4′-Demethylepipodophyllotoxin-9-(4,6-0-ethylidene-β-D-glucopyranoside) by high-pressure liquid chromatography. J Pharm Sci 69: 1440
2. Allen LM, Creaven PJ (1975) Comparison of the human pharmacokinetics of VM26 and VP16, two antineoplastic epipodophyllotoxin glucopyranoside derivatives. Eur J Cancer 11: 697
3. Arnold AM (1979) Podophyllotoxin derivative VP16-213. Cancer Chemother Pharmacol 3: 71
4. Brunner KW, Sonntag RW, Ryssel HJ, Cavalli F (1976) Comparison of the biologic activity of VP16-213 given iv and orally in capsules or drink ampules. Cancer Treat Rep 60: 1377
5. Cavalli F, Sonntag RW, Jungi F, Senn HJ, Brunner KW (1978) VP16-213 monotherapy for remission induction of small cell lung cancer: A randomized trial using three dosage schedules. Cancer Treat Rep 62: 473
6. Colombo T, Broggini M, Torti L, Erba E, D'Incalci M (1981) Pharmacokinetics of VP16 in Lewis lung carcinoma bearing mice. Cancer Chemother Pharmacol (in press)
7. Creaven PJ, Allen LM (1975) EPEG, a new antineoplastic epipodophyllotoxin. Clin Pharmacol Ther 18: 221

7a. D'Incalci M, Erba E, Vaghi M, Morasea L (1982) In vitro cytotoxicity of VP16-213 on primary tumor and metastasis of levis lung carcinoma. Eur J Cancer (in press)

8. Evans WE, Sinkule JA, Horvath A, Crom WR, Dow LW, Rivera G (1981) Clinical pharmacology of VM26 (NSC 122819) and VP16 (NSC 1415-0) in children with cancer. Proc Am Ass Cancer Res 22: 174
9. Farina P, Marzillo G, D'Incalci M (1981) High-performance liquid chromatography determination of 4′-demethyl-epipodophyllotoxin-9-(4,6-0-ehylidene-β-D-glucopyranoside (VP16-213) in human plasma. J Chromatogr 222: 141
10. Holthuis JJM, Pinedo HM, Van Oort WJ (1981) A sensitive high-performance liquid chromatographic method for determination of the antineoplastic agents VP16-213 and VM26 in biological fluids. Anal Chim Acta 130: 23
11. Issell BF, Crooke ST (1979) Etoposide (VP16-213). Cancer Treat Rev 6: 107
12. Newlands ES, Ragshawe KD (1980) Anti-tumor activity of the epipodophyllin derivative VP16-213 (etoposide: NSC 141540) in gestational choriocarcinoma. Eur J Cancer 16: 401
13. Pinkerton CR, Glasgow JFT, Bridges JM, Welshman SG (1981) Enterotoxic effect of methotrexate: Does it influence the drug's absorption in children with acute lymphoblastic leukaemia? Br Med J 282: 1276
14. Rivera G, Avery T, Pratt C (1975) 4′-Demethylepipodophyllotoxin 9-(4,6-0-2-thenylidene-β-D-glucopyranoside) (NSC-122819; VM26) and 4′-demethylepipodophyllotoxin 9-(4,6-0-ethylidene-β-D-glucopyranoside) (NSC-141540; VP16-213) in childhood cancer: Preliminary observations. Cancer Chemother Rep 59: 743
15. Rozencweig M, Von Hoff DD, Henney JE, Muggià FM (1977) VM26 and VP16-213: A comparative analysis. Cancer 40: 334
16. Snodgrass W, Walker L, Heideman R, Odom LF, Hays T, Tubergen DG (1980) Kinetics of VP16 epipodophyllotoxin in children with cancer. Proc Am Ass Cancer Res 21: 333
17. Stähelin H (1973) Activity of a new glycosidic lignan derivative (VP16-213) related to podophyllotoxin in experimental tumors. Eur J Cancer 9: 215
18. Strife RJ, Jardine I, Colvin M (1980) Analysis of the anticancer drugs VP16-213 and VM26 and their metabolites by high-performance liquid chromatography. J Chromatogr 182: 211
19. Strife RJ, Jardine I, Colvin M (1981) Analysis of the anticancer drugs etoposide (VP16-213) and teniposide (VM26) by highperformance liquid chromatography with fluorescence detection. J Chromatogr 224: 168

Accepted July, 1981