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Note

Quantification of teniposide in human serum by high-performance liquid chromatography with electrochemical detection

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Teniposide or 4'-demethylepipodophyllotoxin 9-(4,6-O-2-thenylidene-β-D-glucopyranoside) (VM 26, I) is a semi-synthetic derivative of podophyllotoxin and has major cytotoxic activity in mitosis [1] as well as the premitotic interval [2] of the cell cycle It has gained in importance because of its effectiveness against a variety of human tumours [3], especially ovarian carcinoma [4, 5] Its pharmacokinetics in ovarian carcinoma is well established [6] and it shows low concentrations in malignant ascites, requiring very sensible analytical methods Several high-performance liquid chromatographic (HPLC) assays [7—9] have been described, including UV absorption or spectrofluorometry, which provide detection limits of I from 0 05 to 0 5 μg/ml Lower quantification and detection limits can be achieved by using an HPLC method with electrochemical detection [10]

A simple, rapid and reproducible HPLC technique with electrochemical detection is described for the measurement of I in biological samples using etoposide or 4'-demethylepipodophyllotoxin-9-(4,6-O-ethylidene-β-D-glucopyranoside) (VP 16-213, II) as internal standard

EXPERIMENTAL

Chemicals and reagents

I and II were provided by Sandoz Labs (Basel, Switzerland) Methanolic solutions of these compounds were kept at 4°C for one month without any degradation

Methanol was chromatographic grade (Merck, Darmstadt, F R G),

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ammonium acetate, glacial acetic acid, chloroform and ethyl acetate were obtained from Merck.

All mobile phases were filtered through a 0 5-μm Millipore filter (type FH) immediately before use

Apparatus

The chromatographic system consisted of a high-pressure pump (solvent delivery system, Model 6000A, Waters Assoc , Milford, MA, U S A.) and an injection device (Model U6K, Waters Assoc). The electrochemical detector comprised a Faraday cage enclosing a TL5 glassy carbon electrode and an LC4 controller, both from Bioanalytical Systems (West Lafayette, IN, U S A) The oxidative potential was set at +900 mV against an Ag/AgCl reference electrode

A Waters μBondapak C_{18} reversed-phase column (30 × 3 9 mm I.D , particle size 10 μm) was eluted isocratically at a flow-rate of 1 5 ml/min at room temperature

Chromatograms were traced on a PE Servotrace recorder at a speed of 0 5 cm/min.

Mobile phase

The mobile phase comprised methanol—ammonium acetate buffer—acetic acid (54 45 1). The ammonium acetate concentration was varied from 0 to 0.3 *M*

Preparation of biological samples

Three extraction procedures were compared

(i and ii) Plasma samples (1 ml) were extracted with 2 × 3 ml of chloroform (i) or ethyl acetate (ii) and shaken for 10 min After centrifugation (1500 *g* for 10 min), 5-ml aliquots of the organic layer were removed and evaporated to dryness at 50°C under a stream of nitrogen, the residue was reconstituted with 200 μl of mobile phase and 20 μl were injected into the column

(iii) Plasma samples (1 ml) were passed through a reversed-phase C_{18} Sep-Pak cartridge (Waters Assoc), previously equilibrated with 3 ml of methanol and 10 ml of distilled water The cartridge was then washed with 10 ml of phosphate buffer (0.02 *M*, pH 7 3) and the epipodophyllotoxins were eluted with 3 ml of pure methanol After evaporation, the residue was reconstituted as above

Four sera, containing different amounts of I (1, 5, 7 and 10 μg/ml) and 5 μg/ml II as internal standard, were extracted in triplicate according to the three methods.

Calibration and reproducibility

Calibration curves were obtained by analysing spiked serum from healthy volunteers with various concentrations of I in methanolic solution.

To assess the reproducibility of the method, three 20-ml serum samples obtained from volunteers not receiving epipodophyllotoxins were spiked with 1, 5 and 10 μg/ml I and 5 μg/ml II. Each of these three samples was vortexed, separated into 1-ml aliquots and frozen at −20°C Over ten days, two samples of each serum were extracted, each day, and each extract was analysed in triplicate Mean values, standard deviations and coefficients of variation were calculated simply.

RESULTS AND DISCUSSION

Mobile phase composition

The peak heights of the two compounds (I and II) were measured as a function of ammonium acetate concentration in the mobile phase (Fig 1) Detection efficiencies for I and II were maximum at a concentration of 250 m*M* Using the mobile phase methanol—250 m*M* ammonium acetate buffer—acetic acid (54 45 1), the retention times of II and I were 4 1 ± 0 2 and 8 3 ± 0 3 min, respectively These retention times are significantly decreased in comparison with those obtained by Sinkule and Evans [10]

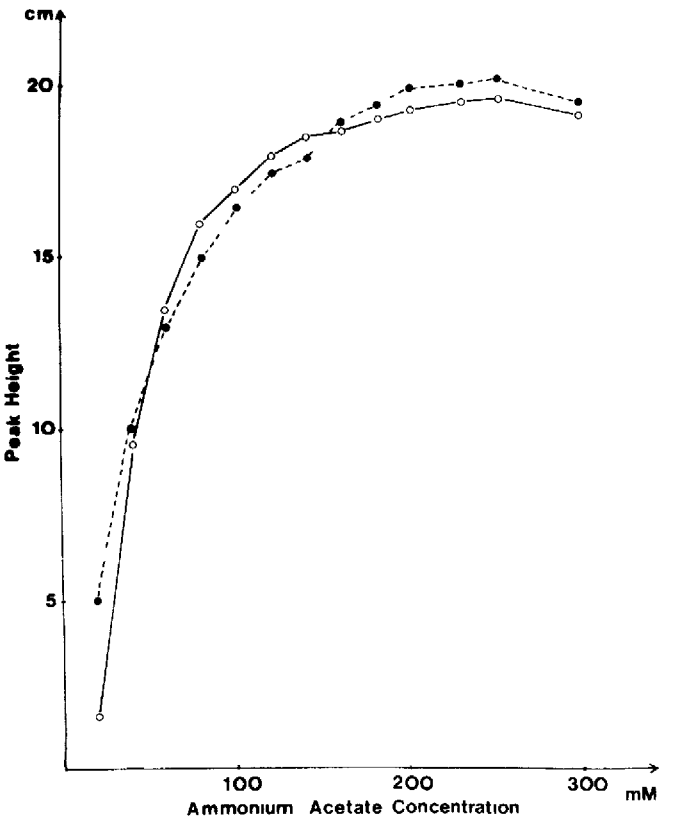

Fig 1 Influence of ammonium acetate concentration in the mobile phase on peak heights of II (VP 16-213) (○) and I (VM 26) (●)

Fig 2a represents a chromatogram of a control plasma sample, while Fig 2b shows a plasma sample containing 520 ng/ml I, analysed at a sensitivity of 10 nA There was an absence of interfering peaks in the control plasma for all the subjects measured

Extraction procedures

The recovery of I isolated from serum according to the procedures described above was calculated by comparing the peak heights obtained with those measured by direct injection of the same amounts dissolved in a mobile phase

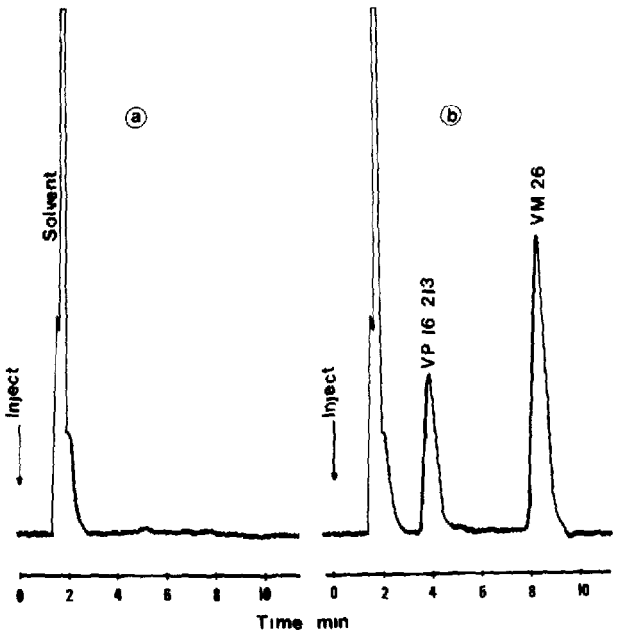

Fig 2 Representative chromatograms of (a) blank serum extract and (b) an extract of serum from a patient 24 h after intravenous administration of 45 mg of I (VM 26). HPLC conditions injection volume, 20 μl, mobile phase, methanol—250 m*M* ammonium acetate buffer—acetic acid (54 45 1), flow-rate, 1 5 ml/min, C_{18} μBondapak column (10 μm), oxidative potential, +900 mV, sensitivity, 10 nA, recorder at a speed of 0 5 cm/min

TABLE I

RECOVERY OF I ADDED TO SERUM AND EXTRACTED BY CHLOROFORM, ETHYL ACETATE OR THE SEP-PAK C_{18} PROCEDURE

Concentration of I added (μg/ml)	Recovery (mean ± S D , n = 3) (%)		
	Chloroform	Ethyl acetate	Sep-Pak C_{18}
1	95 4 ± 1 2	86 3 ± 0 9	68 2 ± 2 3
5	94 5 ± 0 9	89 1 ± 1 6	63 6 ± 2 1
7	94 8 ± 1 3	96 1 ± 2 3	66 6 ± 1 8
10	95 3 ± 1 5	92 6 ± 1 5	68 5 ± 2 3

From Table I, it can be seen that the extraction procedure with chloroform gives the greater extraction percentages (> 90% in all cases) These results agree with those of most other authors [7—9] However, ethyl acetate [10], diisopropyl ether [11] and 1,2-dichloroethane [12] have also been used.

Calibration and sensitivity

Calibration curves were linear over the range 20 ng/ml to 20 μg/ml These calibration curves were used for calculating the concentration of I in samples from patients by the internal standard method.

The lowest amount of I detectable, defined as five times the noise level, at a sensitivity of 0 1 nA, was 200 pg. This amount, for 20 μl injected into the

loop column, corresponds to a concentration of 10 ng/ml, which is comparable to that described by Sinkule and Evans [10] and by Littlewood et al. [12] for II.

TABLE II

REPRODUCIBILITY OF I ASSAY, WITHIN-DAY AND BETWEEN-DAY

Concentration of I added (μg/ml)	Coefficient of variation (%)	
	Within-day (n = 6)	Between-day (n = 10)
1	4 22	6 20
5	2 90	4 26
10	3 62	5 20

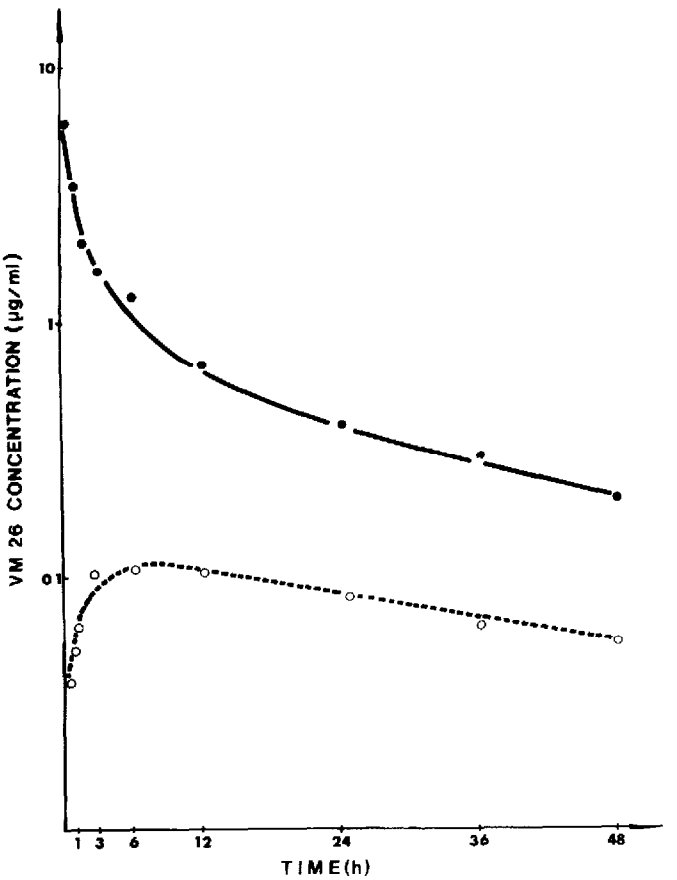

Fig 3 Concentration of I (VM 26) as a function of time in serum (—) and ascites (- - -) obtained from a patient receiving 45 mg of I intravenously

Reproducibility

A summary of the reproducibility studies is presented in Table II Using II as internal standard and the chloroform extraction procedure, the coefficients of variation were highest at the lowest concentrations and decreased with increasing concentration They ranged from 2.90 to 4 22% for within-day reproducibility and from 4.26 to 6 20% for between-day reproducibility

Preliminary clinical application of the method

The method described was tested on serum and ascites samples from a patient suffering from a serous ovarian epithelioma with microscopically proved malignant ascites, and receiving a 30-min infusion of 30 mg/m^2 I Fig 3 shows the plasma and ascites concentration—time courses

The decrease in blood level appears to be triphasic, as described by Creaven [13], and ascites concentrations are very low, as demonstrated by D'Incalci [6], but electrochemical detection allows their determination

The method described is as sensible as the method of Sinkule and Evans [10] Moreover, it appears to be more rapid and more highly reproducible The metabolite (hydroxy acid derivative) is insoluble in chloroform and therefore cannot interfere with I or II determination Thus, one can underline its suitability of further clinical or experimental investigations

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REFERENCES

1 H Stahelin and G Poschmann, Oncology, 35 (1978) 217
2 A Krishan, K Paika and E Frei, J. Cell Biol , 66 (1975) 521
3 B F Issel, Cancer Chemother Pharmacol , 7 (1982) 73
4 E Spremulli, J J Schulz, V J Speckhart and G L Wampler, Cancer Treat Rep , 64 (1980) 147
5 C Sessa, M D'Incalci, F Landoni, N Colombo and C Mangioni, Cancer Treat Rep , 68 (1984) 815
6 M D'Incalci, C Rossi, C Sessa, R Urso, M Zucchetti, P Farina and C Mangioni, Cancer Treat Rep , 69 (1985) 73
7 J J Holthuis and W J van Oort, Anal Chim Acta, 130 (1981) 23
8 R J Strife, I Jardine and M Colvin, J Chromatogr , 182 (1980) 211
9 I Jardine, R J Strife and M Colvin, Cancer Chemother Pharmacol , 7 (1982) 237
10 J A Sinkule and W E Evans, J Pharm Sci , 73 (1984) 164
11 P Farina, G Marzillo and M D'Incalci, J Chromatogr , 222 (1981) 141
12 T J Littlewood, A L Hutchings, D P Bentley and B P Spragg, J Chromatogr , 336 (1984) 434
13 P J Creaven, Cancer Chemother Pharmacol , 7 (1982) 151