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Note

High-performance liquid chromatographic determination of etoposide in plasma using electrochemical detection

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Etoposide [4′-demethylepipodophyllotoxin 9-(4,6-O-ethylidene-β-D-glucopyranoside), I] is a semisynthetic derivative of podophyllotoxin and is increasingly used in the treatment of a variety of malignant conditions [1]. Etoposide inhibits nucleoside transport within cells and thereby interferes with DNA and RNA synthesis. In addition breaks in DNA strands and inhibition of protein synthesis have been demonstrated [2].

The pharmacokinetics of I were demonstrated initially by Allen and Creaven [3] using the tritium-labelled drug, and subsequently by a number of investigators using high-performance liquid chromatography (HPLC) [4—6]. These chromatographic methods have included ultraviolet absorption and spectrofluorometry which provide detection limits from 0.03 to 0.5 μg/ml etoposide in plasma. Electrochemical detection, however, enables these limits to be decreased at least ten-fold [6]. A simple, rapid assay for I with electrochemical detection using teniposide [4′-demethylepipodophyllotoxin 9-(4,6-O-thenylidene-β-D-glucopyranoside), II] as internal standard in plasma is described which significantly decreases the retention times for I and II previously described using electrochemical detection [6, 7].

EXPERIMENTAL

The system comprised a Constametric Model III pump (Laboratory Data Control), an ESA Model 5100A electrochemical detector, a Model 5010 analytical cell and a Model 5020 guard cell. A Waters Assoc. 30 cm × 4.9 mm I.D. stainless-steel column containing 5-μm Bondapak phenyl was used.

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Reagents

Methanol was liquid chromatographic grade (Fisons) and 0.05 *M* phosphate buffer (pH 7.0) was prepared using glass-distilled water. The mobile phase was filtered through a 0.22-μm Millipore membrane immediately before use. 1,2-Dichloroethane (AnalaR) was supplied by BDH and pure I and II were kindly provided by Bristol Myers (U.K.).

High-performance liquid chromatography

The isocratic mobile phase was methanol—phosphate buffer (60:40) at a flow-rate of 1 ml/min. The potential difference across the analytical and guard cells was set at 800 mV.

Extraction procedure

Plasma (1 ml) containing 10 μg each of I and II was placed in a 100 mm × 18 mm "Quickfit" glass tube and 4 ml of 1,2-dichloroethane were added. The solution was mixed for 10 min using an Eschmann Rotamix and then centrifuged at 650 *g* for 5 min. The organic phase was removed and evaporated to dryness in a water bath at 90°C. The residue was reconstituted with 0.4 ml of the mobile phase and 20-μl aliquots were applied to the liquid chromatograph.

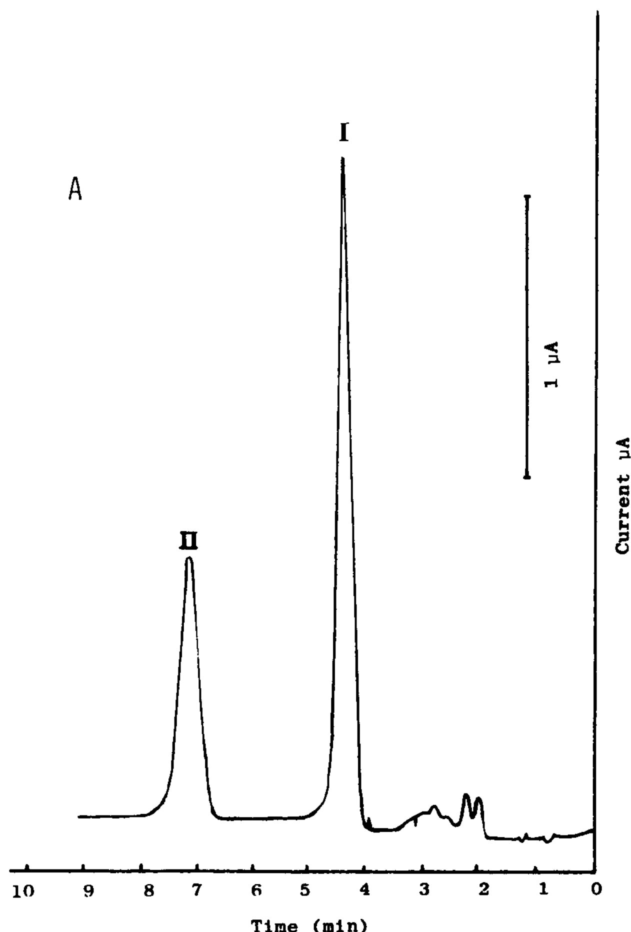

Fig. 1.

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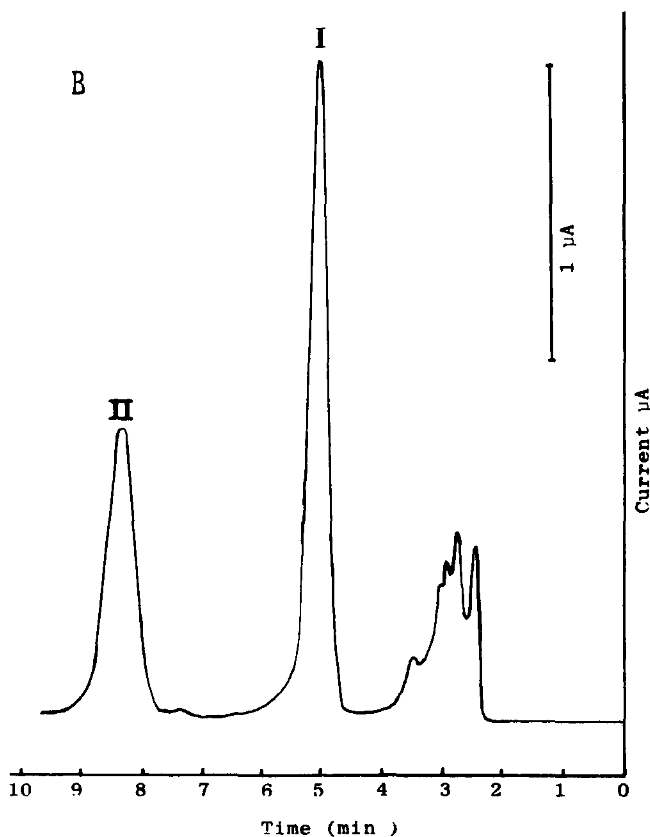

Fig. 1. (A) High-performance liquid chromatogram of human plasma spiked with 10 μg each of etoposide and teniposide. (B) High-performance liquid chromatogram of plasma after the administration of etoposide to the patient.

RESULTS AND DISCUSSION

Optimum conditions for chromatography were found to be different from those cited by Evans et al. [7]. A mobile phase of water—acetonitrile—acetic acid (74:25:1) at a flow-rate of 1 ml/min produced retention times for I and II of 13 and 20 min, respectively, whereas using a mobile phase of methanol—phosphate buffer (60:40) retention times of 5 and 9 min were obtained. Differing proportions of methanol relative to the phosphate buffer were investigated. A ratio of 60:40 produced optimum chromatography with short retention times and good resolution between I and II (Fig. 1).

A number of organic solvents including 1,2-dichloroethane, dichloromethane, chloroform and ethyl acetate were investigated for the extraction of I and II from plasma. The extraction percentage [8] was greater than 90% for each solvent (Table I) but 1,2-dichloroethane produced the fewest interfering peaks on the chromatogram. The oxidative potential of 800 mV was determined from analysis of the voltametric curves of I and II which demonstrate that oxidation for both these compounds is a two-electron process with the second electron released at 650 mV and a plateau reached at 800 mV.

TABLE I

EXTRACTION PERCENTAGES OF I AND II FOR DIFFERENT SOLVENTS

Organic solvent	Percentage extraction	
	I	II
1,2-Dichloroethane	93	94
Dichloromethane	92	93
Chloroform	93	92
Ethyl acetate	92	93

Pools of plasma containing 1, 5, 10, 20, 30 and 50 μg/ml of I were prepared and 1-ml aliquots of each pool were assayed as described above after addition of a similar concentration of II. Ten samples from each pool were assayed in duplicate and the results obtained from the peak height ratios of I and II produced a linear calibration curve (correlation coefficient $r = 0.9987$) for 2—500 ng of I applied to the column.

The lower limit of detection of I was 0.005 μg/ml. The coefficients of variation were found to be 6.93%, 4.37%, 1.84%, 4.68%, 1.60% and 1.49% for concentrations of I of 1, 5, 10, 20, 30 and 50 μg/ml, respectively.

In order to estimate the recovery the peak height ratio of I and II following extraction from plasma was compared to the ratio obtained following extraction from water. Recoveries were 110%, 106%, 104%, 102% and 101% for concentrations of 1, 10, 20, 30 and 50 μg/ml, respectively.

Using this simple, reproducible assay complete resolution of I and II was achieved in 9 min which offers an improvement over the retention times for I of 12 min previously described using electrochemical detection [7] and in addition detection limits were nearly ten-fold less then those described using ultraviolet absorption.

ACKNOWLEDGEMENT

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