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Note

High-performance liquid chromatography of etoposide in plasma and urine

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Etoposide (VP16-213) is a semi-synthetic podophyllotoxin with activity against a variety of solid tumours and haematological malignancies [1—3]. It is one of the most active agents against small-cell lung carcinoma [1, 2, 4] and germ cell tumours [5]. Over the past few years several high-performance liquid chromatographic (HPLC) assays have been developed for the measurement of etoposide in biological fluids [6—12] and most of these employ the closely related epipodophyllotoxin teniposide (VM26) as internal standard. However, the large capacity factor and poor efficiency of teniposide combine to give extended run times (Fig. 1). To shorten analysis time an assay using diphenylhydantoin (DPH) as internal standard has been developed. Methylphenytoin (MPPH) was used in patients receiving DPH as an anticonvulsant.

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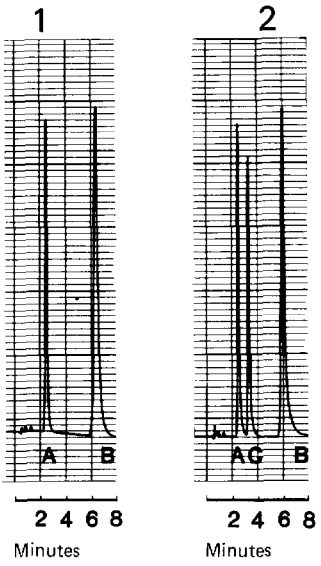

Fig. 1. (1) Chromatogram of etoposide (A) with teniposide (B) as internal standard. (2) Chromatogram of etoposide (A) and teniposide (B) with DPH (C) as internal standard.

MATERIALS AND METHODS

Materials

Etoposide was provided by Bristol-Myers U.K. Chloroform (AnalaR grade) and methanol (liquid chromatography grade) were obtained from BDH, and DPH and MPPH from Sigma. The HPLC separation was carried out using an Applied Chromatography Systems Series 300 pump with a Rheodyne 7125 injector and a Laboratory Data Control 1204D variable-wavelength UV detector. Separation was achieved with an isocratic solvent mixture using an ODS Hypersil column (see below).

Methods

Standards and control samples. Standards and control samples were prepared from a stock solution of 1000 μg/ml etoposide in methanol—water (51:49) which was added to drug-free pooled plasma or urine to give final concentrations of 0, 0.5, 1.0, 5.0, 10.0, 15.0 and 25.0 μg/ml of plasma and 0, 10.0, 25.0, 50.0, 75.0 and 100.0 μg/ml of urine. Quality-control samples to monitor within-run and between-run imprecision were prepared in the same way but from a different stock standard. Standards and controls were aliquoted into 1.5-ml capped tubes and stored at −20° C prior to use.

Sample extraction. A 50-μl volume of internal standard (200 μg/ml DPH, or MPPH in those patients receiving therapeutic DPH) was added to 1 ml plasma or 200 μl urine (urine was buffered with 1 ml phosphate-buffered saline, pH 7.3). Following the addition of 5 ml chloroform the tubes were mixed by rotation and centrifuged at 400 *g*, each for 10 min. The organic layer was then filtered into a clean glass tube, evaporated to dryness at 50° C and reconstituted in 200 μl mobile phase. Extraction efficiency was determined by addition of internal standard after extraction.

Chromatography. Chromatography was carried out using a 5-μm ODS Hypersil column (100 × 5 mm) with methanol—water (51:49) solvent at a flow-rate of 2 ml/min. Sample introduction was by means of a 7125 Rheodyne injection valve with a 50-μl sample loop and detection was by UV absorbance at 229 nm. This system typically gave retention times of 2.1 min for etoposide, 2.9 min for DPH and 4.5 min for MPPH. Flow-rate and mobile phase methanol content were adjusted as necessary to optimise resolution and retention on different columns. Calibration was achieved by running standards samples and using peak height ratios of etoposide to internal standard.

RESULTS

Chromatographic separation of etoposide and internal standards is shown in Fig. 2. Extraction efficiency of etoposide in plasma was > 80% at levels of 1.0, 5.0 and 15.0 μg/ml and in urine was > 90% at levels of 25.0 and 50.0 μg/ml. Extraction efficiency of DPH and MPPH determined using etoposide as internal standard was > 75% for both plasma and urine. Linear regression of peak height ratios against etoposide concentrations typically gave correlation coefficients of > 0.99. Within-run imprecision was < 4% in plasma at levels of 0.80, 4.71 and 15.9 μg/ml (n = 10) and < 3% in urine at a level of 50.0 μg/ml (n = 9).

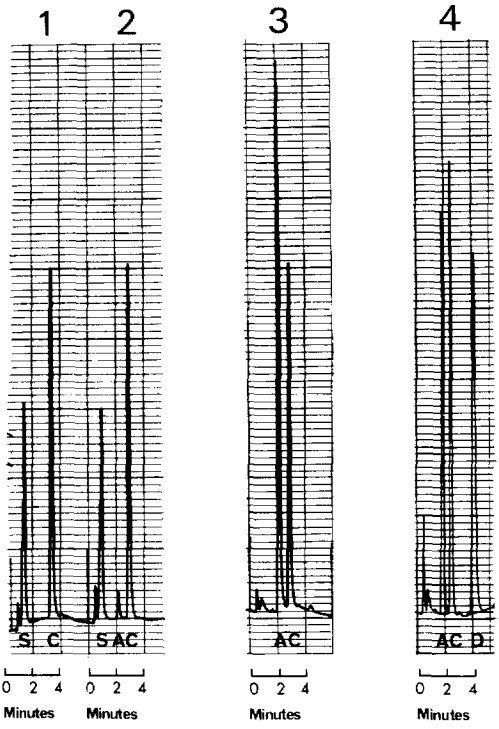

Fig. 2. Chromatograms of human plasma extracts (0.2 a.u.f.s. throughout). (1) Human plasma extract before etoposide administration showing solvent front (S) with DPH (C) as internal standard. (2) Etoposide peak (A) equivalent to 0.55 μg/ml. (3) Etoposide peak (A) equivalent to 10.27 μg/ml. (4) Patient sample containing both etoposide (A) and DPH (C) with MPPH (D) as internal standard.

Between-run imprecision found by running control samples twice daily was < 6.7% in plasma at levels of 0.82, 4.49 and 16.02 μg/ml (n = 24), and < 5% in urine at levels of 10.0, 25.0 and 50.0 μg/ml (n = 12).

There was no interference from metabolites of the drug or other UV-absorbing substances. The major metabolite, the picrohydroxy acid, is chloroform-insoluble [7] and another metabolite [8], the picro isomer, was not found. Drugs tested for possible interference included cytotoxics (cyclophosphamide, adriamycin, vincristine, methotrexate and procarbazine) and other commonly co-administered drugs (aspirin, paracetamol, dextropropoxyphene, dihydrocodeine, diamorphine, morphine, prednisone, metoclopramide, prochlorperazine and phenobarbitone). None of these compounds interfered in the assay either in simple solution or in the plasma and urine of patients to whom they had been administered.

DISCUSSION

The method described allows for the rapid measurement of etoposide in multiple samples with a run time of less than 3.5 min. This is a considerable improvement over the run times encountered with teniposide as internal standard and avoids handling a second cytotoxic agent on a regular basis. In patients taking DPH for therapeutic reasons MPPH was used as internal standard. Despite the longer retention time of 4.5 min this was still an improvement over teniposide. The limit of detection in this assay of 100 ng/ml is adequate for sample measurement up to 24 h following doses within the clinical range commonly used (> 100 mg orally or > 50 mg intravenously) but may not be sufficiently sensitive to monitor drug levels over more prolonged periods. Under these conditions alternative means of detection such as fluorescence [7, 13] or electrochemical activity [11] may be required.

There was no interference from the major metabolite, the picrohydroxy acid, which is chloroform-insoluble [7]. The picro isomer of etoposide was poorly resolved from the parent drug in this system, but was still detectable. Although this metabolite has been found in some children [8] it was not present in any of the patient samples. It has been suggested that co-administered drugs may interfere with the UV detection of etoposide [13] but this was not noted for a variety of drugs given with etoposide, including cytotoxics, analgesics and anti-emetics.

The method can also be used for the determination of teniposide and may be particularly useful if etoposide and teniposide are given concurrently, as has been suggested by Allen et al. [14].

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