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Short Communication

Determination of etoposide (VP16-213) and teniposide (VM-26) in serum by high-performance liquid chromatography with electrochemical detection

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Introduction

Etoposide (VP16-213) and teniposide (VM-26) are important anti-cancer agents derived from the lignan demethylepipodophyllotoxin. These drugs (Fig. 1) are usually given in large doses by intravenous infusion. Since they are known to have toxic side-effects, therapeutic monitoring is essential to ensure effective treatment with minimum damage to the patient.

The determination of VP16-213 and VM-26 by high-performance liquid chromatography (HPLC) has been described [1–5], most of these methods being based on an

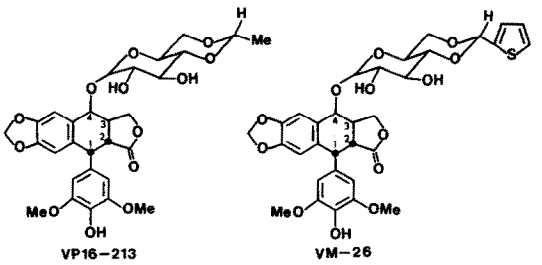

Figure 1
Structure of etoposide (VP16-213) and teniposide (VM-26).

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ODS column for the separation. Strife *et al.* [4], unable to resolve VP16-213 and picro-VP16-213 on C_{18} columns, used a phenyl-bonded column for their separation. These workers also suggested that the C_2-epimer picro-VP16-213 may be a metabolite of VP16-213.

The present paper describes a highly efficient, specific and sensitive HPLC system, with electrochemical detection, for the simultaneous determination of VP16-213 and VM-26 in serum. The system is also capable of resolving VP16-213 and VM-26 from their epimers. The results provide evidence that C_2-epimers of the drugs are formed artefactually rather than as true metabolites.

Materials and Methods

Chemicals and reagents

VP16-213 and VM-26 were gifts from Bristol–Myers Company, Slough, Berkshire, UK, and were prepared by Bristol Laboratory, Syracuse, NY, USA. Picro-VP16-213 and picro-VM-26 were prepared by treating 1 mg of VP16-213 and VM-26, respectively, with 1 drop of triethylamine. The internal standard α-peltatin was isolated from the root of *Podophyllum peltatum* [6].

Acetic acid, ammonium acetate and EDTA were AnalaR grade from BDH (Poole, Dorset, UK). Acetonitrile was HPLC grade from Rathburn Chemicals Ltd. (Walkerburn, Peebleshire, UK).

Sample preparation

Serum (500 μl) was mixed with the internal standard α-peltatin (10 μl, 10 μg/ml) and loaded onto a C_{18} 'Bond-Elut' cartridge (Waters Associates, Milford, MA, USA). The cartridge was rinsed with methanol (2 × 5 ml) followed by water 2 × 5 ml) before use. The serum was forced slowly through the cartridge by vacuum suction. After washing the column with water (5 ml), the drugs and the internal standard were eluted from the column with acetonitrile (2 × 500 μl). The combined eluates were dried by evaporation with nitrogen and redissolved in 500 μl of acetonitrile. Depending on the concentration of the drugs, a sample of 10–100 μl was injected onto the column.

Calibration and recovery

The standard curves were prepared by plotting the peak height ratio of each drug to that of the internal standard for the following concentrations: 0.5, 1.0, 5.0, 10.0, 15.0, 20.0, 25.0, 30.0 μg/ml (20 μl injection). Linear regression analysis of calibration curve data indicated that there was no significant deviation from linearity ($r = 0.9995$–0.9998), and that intercept values did not differ significantly from zero.

The recovery, determined by 10 repetitive analyses at 15 μg/ml for drug added to a serum sample, was 95 ± 3.5%.

The on-column detection limits for VP16-213 and VM-26 were 500 and 750 pg injected respectively, based on a signal-to-noise ratio of 3. For replicate injections of each drug at 10 μg/ml, the relative standard deviations were: VP16-213, 2% ($n = 10$); VM-26, 3% ($n = 10$).

HPLC

A Pye–Unicam (Cambridge, UK) LC3-XP solvent delivery system was used with a model LCA electrochemical detector (EDT Research, London, UK). The detector was

used in oxidation mode at a potential of +0.90 V, with acetonitrile–0.5 M ammonium acetate buffer (pH 6) (37.5: 62.5, v/v), containing 100 mg/l of EDTA, as the eluent. The mobile phase flow rate was 1 ml/min.

Results and Discussion

The separation of a standard mixture VP16-213, VM-26 and α-peltatin (internal standard) is shown in Fig. 2. Previous methods [1–5] for the determination of VP16-213 used VM-26 as the internal standard. These two drugs, however, are sometimes given in combination [7]. α-Peltatin was therefore chosen as the internal standard to allow the simultaneous assay of all the drugs. These lignans contain phenolic groups which can be easily oxidized and therefore an electrochemical detector is ideal for the sensitive and specific detection of these compounds. They can be detected at the low ng/ml concentration ranges which is at least 10–30 times more sensitive than the UV-detector and well below the concentrations likely to be found in the sera of patients undergoing treatment. A slightly acidic mobile phase was used to prevent the possibility of base-catalysed isomerization of the drugs to the biologically-inactive picro series.

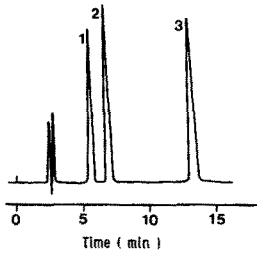

Figure 2
Separation of VP16-213 (1), α-peltatin (2) and VM-26 (3) on 5-μm ODS-Hypersil; HPLC conditions as in text. Electrochemical detector sensitivity: 10 nA f.s.d.

The picro isomers of VP16-213 and VM-26 can be readily resolved from the parent compounds (Fig. 3). It was reported [4] that C_{18}-reversed-phase columns were unsuitable for separating VP16-213 from picro-VP16-213, so that a μ-Bondapak phenyl column had to be employed for the separation. The inclusion of ammonium acetate in the present mobile phase system greatly improved the column efficiency leading to superior resolution. Ammonium acetate is a good masking agent for the residual silanol groups on the reversed-phase column and may account for the improved resolution achieved. In addition, ammonium acetate is compatible with both the UV and the electrochemical detector. EDTA, essential for protecting the glassy carbon electrode of the electrochemical detector from metallic contamination, can be omitted from the mobile phase if a UV-detector is used.

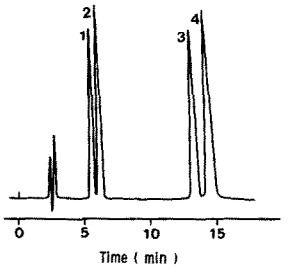

Figure 3
Separation of VP16-213 (1), picro-VP16-213 (2), VM-26 (3) and picro-VM-26 (4); HPLC conditions as in text, Electrochemical detector sensitivity: 10 nA f.s.d.

Figures 4a and 4b show the separation of VP16-213, VM-26 and α-peltatin in a patient serum sample and in a drug-free serum respectively. Contrary to previous observations [4] the present authors have never detected the picro-isomers in fresh serum. In stored sera, however, isomerization of the drugs sometimes takes place and the picro-isomers were detected.

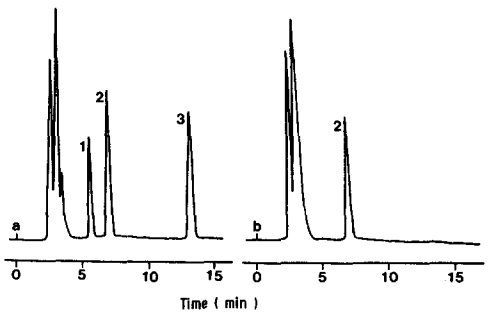

Figure 4
Separation of VP16-213 (1), α-peltatin (internal standard) (2), and VM-26 (3) in: (a) patient serum; and (b) drug-free serum. HPLC conditions as in text. Electrochemical detector sensitivity: 10 nA f.s.d.

It is well known that lignans of the podophyllotoxin series are susceptible to base-catalysed isomerization, even by traces of a weak base in the atmosphere. The precipitation of the drugs (probably due to isomerization to the less soluble picro compounds) during infusion has been observed. The picro-VP16-213 detected in the sera of patients treated with VP16-213 [4] is most likely to be formed during the extraction of the sera, or due to the patients being given the drugs in a partially isomerized form, rather than a true metabolite. It is therefore recommended that the mobile phase for the separation of these compounds should be slightly acidic and that serum should be analysed fresh with the extraction carried out in an atmosphere free of bases.

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