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STABILITY-INDICATING HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY OF ETOPOSIDE AT VARIOUS pH CONDITIONS USING A REVERSED-PHASE OCTYL COLUMN

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SUMMARY

A stability-indicating reversed-phase high-performance liquid chromatographic assay of etoposide is described. Baseline resolution was achieved with an octyl column for etoposide and its four degradation products, including picrolactone. Peak homogeneity of etoposide was confirmed by quantitating etoposide in degraded samples at 230, 254 and 286 nm, respectively. The assay was reproducible with low within-day and between-day variations. Application of the assay for stability kinetic study was demonstrated. Etoposide in 0.1 *M* hydrochloric acid (pH 1.29) degraded with a half-life of 2.85 h, which may be responsible for the low oral bioavailability of etoposide.

INTRODUCTION

Etoposide is a semisynthetic epipodophyllotoxin derivative, active against a variety of malignancies[1]. Etoposide is the most active single agent for small-cell lung cancer[2], and also approved by the Food and Drug Administration for treating testicular carcinoma[3]. The currently available dosage form is non-aqueous parenteral solution[1]. The oral dosage form is highly desired for long-term out-patient administration, but has not yet been satisfactorily formulated. Several investigational oral formulations, namely: (i) hydrophilic, soft gelatin capsule containing etoposide solution[4]; (ii) lipophilic capsule of etoposide suspension[5]; and (iii) drinking ampules[6], have been evaluated, and found that all had the drawback of low bioavailability.

In order to improve effectively the oral bioavailability with formulation approaches, thorough preformulation studies are necessary to identify the factors responsible for the low bioavailability. To conduct the preformulation studies, a prerequisite is to develop a stability-indicating high-performance liquid chromatography (HPLC) method of etoposide at various pH conditions.

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The paper herein describes the assay developed, using a reversed-phase octyl column for stability-indicating analysis.

EXPERIMENTAL

Materials

Etoposide was supplied by Bristol-Myers (lot No. 319661). Methoxypsoralen was purchased from Sigma. Acetonitrile (HPLC grade) was from Fisher. All other chemicals were from Baker, except that potassium chloride and dibassic potassium phosphate were from MCB.

High-performance liquid chromatography

A liquid chromatograph (Consta-Metric I, Laboratories Data Control, Riviera Beach, FL, U.S.A.), equipped with a 50-μl sample loop (Rheodyne, Berkeley, CA, U.S.A.), a variable-wavelength UV detector (Spectro Monitor III, Laboratories Data Control), a reversed-phase octyl column (5 μm, 15 cm $\times$ 4.6 mm I.D., Custom LC, Houston, TX, U.S.A.) and a chart recorder (Linear Instrument, Irvine CA, U.S.A.) was used. The isocratic mobile phase consisted of acetonitrile–acetic acid–water (27:1:72), pH 4.0. The acidic mobile phase was selected to prevent epimerization of etoposide during the assay[7–10]. The flow-rate was 1.5 ml/min, and eluents were monitored at 230, 254 and 286 nm unless otherwise specified. Methoxypsoralen was used as the internal standard.

Calibration curve

Calibration curves were constructed at concentration ranges of 5–30 μg/ml and 0.5–5 μg/ml, respectively. Stock methanol solutions of etoposide at 100 μg/ml and 10 μg/ml, and of methoxypsoralen at 10 μg/ml were prepared. A series dilution was made to prepare the standard solutions of desired concentrations.

Validation of the assay

The assay was validated by establishing the within-day and between-day variations. Five sets of samples were prepared on the same day to establish the within-day variation. The assay was repeated monthly for five months to establish the between-day variation.

Buffers and solutions for various pH conditions

The buffers and solutions for various pH conditions in the range of pH 1.29–10 were prepared as given in Table I. Buffer concentrations were all of 0.1 *M*, and the ionic strength of each solution was adjusted to 0.5 *M* with potassium chloride.

Peak homogeneity of etoposide

The calibration curves of undegraded etoposide at 5–30 μg/ml were constructed at three wavelengths, 230, 254 and 286 nm. The homogeneity of the etoposide peak in degraded samples was confirmed by quantitating the drug at the three wavelengths using the corresponding calibration curves.

TABLE I

BUFFERS AND SOLUTIONS FOR VARIOUS pH CONDITIONS

pH	*Buffer composition**
1.29	Hydrochloric acid
2.03	Hydrochloric acid–potassium chloride
3.05	Sodium citrate–citric acid
4.50	Distilled water
5.00	Sodium acetate–acetic acid
6.15	K_2HPO_4–KH_2PO_4 (0.062:1)
7.30	K_2HPO_4–KH_2PO_4(1.250:1)
8.00	K_2HPO_4–KH_2PO_4(6.170:1)
10.00	Sodium borate–boric acid

* Buffer concentrations were all 0.1 *M*, and the ionic strengths were adjusted to 0.5 *M* with potassium chloride.

Samples with forced degradation

Etoposide solution was boiled for 3 min in concentrated hydrochloric acid for the extremely acidic condition and in 0.1 *M* sodium hydroxide for the alkaline condition.

Degradation kinetic study

Etoposide, 100 μg/ml in 0.1 *M* hydrochloric acid , pH 1.29, was maintained in a water bath of 25°C. The degradation profile of etoposide was established by monitoring the remaining etoposide in 500-μl sample solution at various time intervals up to 12 h. The pH was selected to simulate the condition for the drug in stomach fluid.

RESULTS

Etoposide was resolved with a baseline separation from the internal standard, methoxypsoralen, under the developed conditions, as shown in Fig. 1a. The retention times of etoposide and methoxypsoralen were 6 and 11 min, respectively.

Etoposide was extensively degraded at pH < 2 and pH > 8, as illustrated in Fig. 1b and 1c, respectively. Degradation was negligible at pH of 3.05–7.30 within 48 h. The degradation products, D1 and D2 under acidic condition had retention times of 1.8 and 4.8 min, respectively, and those under alkaline condition, D3 and D4, had retention times of 2.7 and 7.2 min, respectively. No degradation products were eluted with the same retention time of etoposide. The homogeneity of the etoposide peak in degraded samples was confirmed by quantitating the drug at the three wavelengths. The quantities determined from different wavelengths were all in agreement with each other.

Three calibration curves of 5–30 μg/ml of etoposide, with absorbance measurements at 230, 254 and 286 nm, respectively, are shown in Fig. 2. The monitoring at 230 nm yielded the greatest sensitivity of the assay as reflected by the highest value of the slope (Table II), and was thus selected for future studies. The calibration curve of etoposide at 0.5–5 μg/ml was constructed with absorbance at 230 nm (Fig. 3).

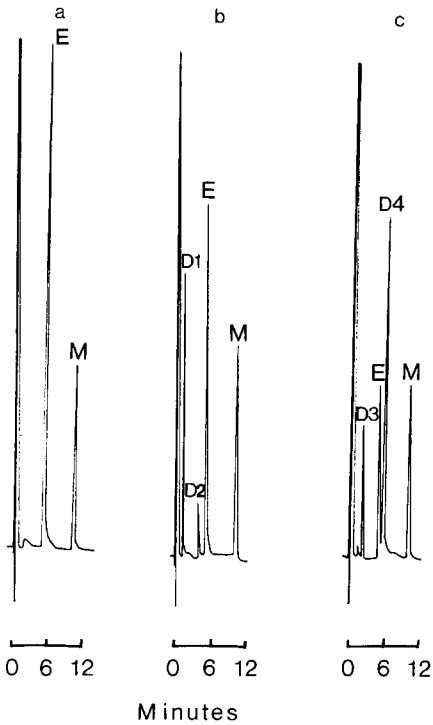

Fig. 1. Authentic chromatograms of (a) etoposide (E) undegraded with methoxypsoralen (M); (b) etoposide sample degraded at pH < 2; (c) etoposide sample degraded at pH > 8. D1, D2, D3 and D4 are degradation products.

The assay was reproducible. The within-day and between-day variations were 2.5–5.8% and 4.4–6.2%, respectively (Table II).

The typical degradation profile of etoposide at pH 1.29 is shown in Fig. 4. Etoposide degraded with an apparent first-order kinetics. The degradation half-life and rate constant were 2.85 h and 5.84 ± 0.09 day^{-1}, respectively.

DISCUSSION

The assay developed with the reversed-phase octyl column enabled us to monitor etoposide at the level of 0.5 μg/ml, comparable to the assay sensitivity with UV detection reported in the literature[9,11–13]. In literature, UV detection has been employed at various wavelengths, 229[13], 252[11], 254[7,8,10,12,14], 280[8,10] and 284 nm[9]. In this study, we established the assay at 230, 254 and 286 nm, and achieved the highest sensitivity with 230 nm monitoring.

The assay with the octyl column was stability-indicating. It well resolved the degradation products of etoposide in both acidic (D1 and D2) and alkaline (D3 and D4) conditions from the parent compound. One of the alkaline degradation products, D4, was confirmed to be the picrolactone isomer with the artifactually formed epimerized isomer, obtained by treating 1 mg of etoposide with 1 drop of triethylamine

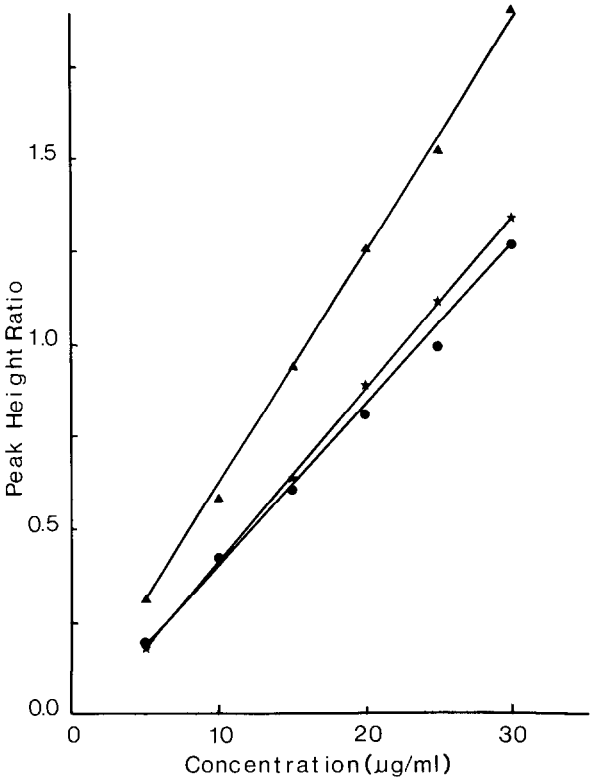

Fig. 2. Calibration curves of 5–30 μg/ml of etoposide with detection wavelength at 230 (▲), 254 (★) and 286 (●) nm.

at room temperature for 10–30 min[15]. The resolution of picrolactone isomers was not achieved with a C_{18} column[14], but achievable with a phenyl column[7,9,15] and a CN column[7]. This is the first report to demonstrate such a resolution of picrolactone isomers with an octyl column. The octyl column (LiChrosorb RP-8) was once used

TABLE II

VALIDATION OF HPLC ASSAY OF ETOPOSIDE

	Concentration range (μg/ml)			
	0.5–5	*5–30*		
	UV detection wavelength (nm)			
	230	*230*	*254*	*286*
Slope	0.386	0.096	0.047	0.042
Intercept	−0.055	−0.038	−0.054	−0.013
Within-day variation*	5.78	2.49		
Between-day variation*	6.20	4.40		
Coefficient of correlation	0.994	0.998	0.999	0.996

* $n = 5$.

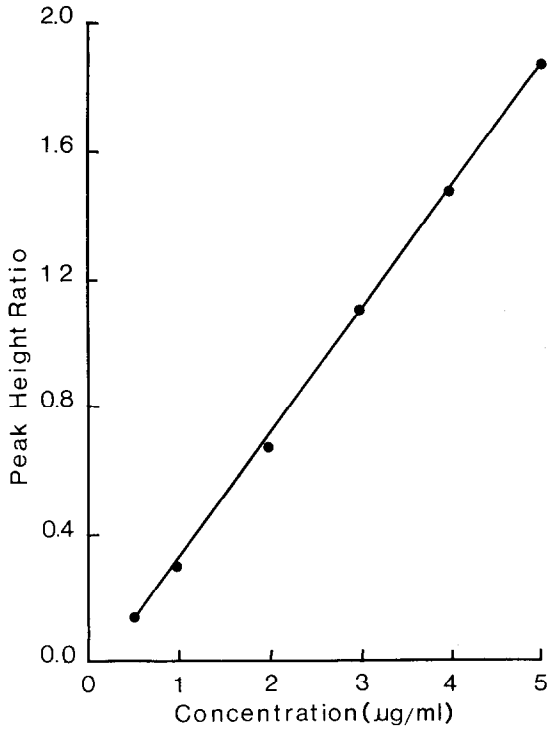

Fig. 3. Calibration curve of 0.5–5 μg/ml of etoposide at 230 nm.

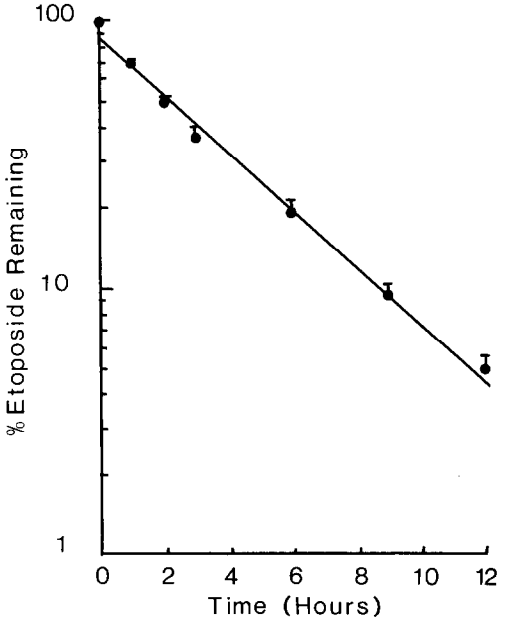

Fig. 4. Degradation profile of etoposide at pH 1.29. Points represent the mean values of data from three separate runs.

to monitor only etoposide parent compound[12]. Moreover, the developed assay, with the resolution of etoposide and its four degradation products, is useful for the stability kinetic study of the drug under various pH conditions.

The application of the assay on the etoposide degradation kinetic study, under acidic conditions, pH 1.29, was demonstrated. The rapid and significant degradation of etoposide under this condition may be, at least in part, accounted for the low bioavailability when the drug was orally administered and subsequently exposed in acidic gastric fluid.

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