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A rapid reversed phase high performance liquid chromatographic method for determination of etoposide (VP-16) in human plasma

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Abstract

A rapid, simple and sensitive isocratic High Performance Liquid Chromatography (HPLC) method was developed to measure the concentration of etoposide in plasma samples with UV detection at 220 nm. The method uses a Bondapac C18 column at 60°C. The mobile phase consists of Methanol: water (45:55 v/v) at a flow rate of 2.8 ml/min. Phenacetin was used as an internal standard. The plasma samples were extracted using ether with the organic layer evaporated under nitrogen. The residue was dissolved in 200 μl methanol with 20 μl injected into the HPLC column. The extraction method showed a recovery of 91.5 ± 3% for etoposide. In this system, the retention time of phenacetin and etoposide were 3.3 and 4.4 min, respectively. The limit of detection of etoposide in plasma is 20 ng/ml and the limit of quantitation is 40 ng/ml. This analytical method has very good reproducibility (8.1% between-day variability at a concentration of 50 ng/ml). It is a fast, sensitive and economic method applicable for clinical and pharmacokinetic studies.

Keywords: Reversed phase chromatography; HPLC; Etoposide; Plasma

1. Introduction

Etoposide, or VP-16-213, is a semisynthetic derivative of podophyllotoxin, a naturally occurring compound extracted from the roots and rhizomes of the plants *Podophyllum peltatum* and *P. emodi*. Aqueous extracts from these plants were recognized as having medicinal properties by the American Indians and in 1946, its antimitotic properties were recognized [1]. Its mechanism of action is believed to be the inhibition of topoisomerase II enzyme and/or induction of direct DNA

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breaks [2]. Etoposide is widely used in the treatment of patients with small cell lung cancer, testicular tumors, Kaposi's sarcoma, lymphomas, and leukemia [3].

The clinical pharmacokinetics of etoposide have been reported. Most of published studies have confirmed a biexponential plasma decay of the etoposide with a half-life of $\sim$6–8 h [4], however, some investigators have reported that, in some patients, a third exponential phase of the plasma decay could be distinguished with a terminal half-life of 20–46 h [5]. Because of its multi-compartmental behaviour and large individual differences, a sensitive analytical method is of great importance. Different high performance liquid chromatographic (HPLC) methods for analysis etoposide have been described [6]. In general, these methods use a reverse-phase column with a methanol or acetonitrile-containing mobile phase with isocratic elution [7]. Many investigators have used of electrochemical detectors for the detection of etoposide in plasma [8], however, electrochemical detectors are difficult to adjust and unavailable in many laboratories. Also, the exposure of and the long running times due to the presence of late eluting peaks in biological fluids are among the problems in using the previously described methods. This paper describes a practical, specific, sensitive and inexpensive HPLC assay method for etoposide in clinical concentrations using methanol and water in an isocratic HPLC technique.

2. Materials and methods

2.1. Materials

Etoposide was donated by Rhône-Poulenc Rorer (Montreal, Canada). Phenacetin (from Sigma-Aldrich Canada, Oakville) was used as internal standard. Methanol was HPLC grade from Fisher Scientific, Canada. Distilled deionized water were used throughout the study. The C18 Bondapac HPLC column was purchased from Varian, Mississauga, Canada.

2.2. Methods

2.2.1. Sample preparation

A 50 μg/ml stock solution of etoposide was prepared for the calibration curve. Concentrations of 0.01–20 μg/ml of etoposide in blood bank human plasma were prepared from this stock. To each of a 0.5 ml etoposide blended plasma sample, 5 ml of ether and 50 μl of 100 ng/ml Phenacetin (as internal standard) were added. This mixture was vortexted for 30 s and the ether layer was transferred to a clean glass tube. Ether was evaporated at 40°C. The residue was dissolved in 200 μl methanol from which 20 μl was injected into the HPLC column.

2.2.2. Chromatography system

The HPLC system consisted of two Shimadzu LC-6A pumps (Shimadzu, Tokyo, Japan), a SPD-6AV Shimadzu UV–VIS Detector at 220 nm, a C-R5A Shimadzu integrator, a SIL-6A Shimadzu autoinjector, a Shimadzu SCL-6A system controller and a Shimadzu FCV-2AH flow channel selection valve. A Bondapac C18 column was used as the analytical column. The column was heated to 60°C. Mobile phase consisted of Methanol:water (45:55 v/v) at a flow rate of 2.8 ml/min.

3. Results and discussion

Fig. 1 shows a typical chromatogram of blank plasma and plasma spiked with etoposide and phenacetin. This figure shows very good resolution between these two substances. There are small peaks related to endogenous compounds prior to the etoposide peak that do not interfere with the analytical assay. These peaks could be eliminated if a column switching technique is used, however, we did not included that apparatus in order to keep the practicality and simplicity of the method for the clinical pharmacology laboratory.

The extraction procedure involved an initial volume of 0.5 ml plasma and ended with $\sim$200 μl of final extraction solvent which has enough volume for $\sim$10 repetitive injections. Volumes $<$

200 ml of methanol could be used to increase the concentration and therefore increase the sensitivity of assay for etoposide; however, this attempt has resulted in the decrease in recovery of etoposide from 91.5 ± 3 to $82 \pm 2\%$. Using this method, the concentration of etoposide in the final extract is suitable for the clinical pharmacology purposes.

The recovery of internal standard of a final plasma concentration of 10 ng/ml was $95 \pm 3\%$, which is comparable to the recovery of etoposide. The retention times of phenacetin and etoposide were 3.3 and 4.4 min, respectively. These retention times are shorter than the 8 min reported by Kiya et al. [9] for detection of etoposide in plasma using a fluoroscence detector and the 6 min reported by Strife et al. [10] for the detection of teniposide.

There is a good resolution between phenacetin, etoposide and various endogenous interfering compounds. There were no problem of late eluting peaks after the etoposide peak, which enable us to have the injection of next sample 8 min after the previous one.

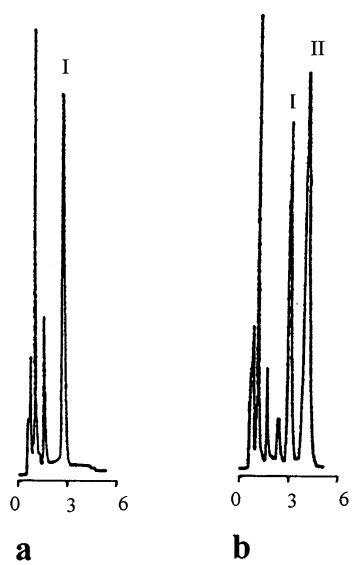

Fig. 1. Chromatograms of etoposide extracted from plasma samples. (a) plasma sample of a patient before drug injection; and (b) plasma sample of the same patient 1 h after injection. The retention time for phenacetin (I) and etoposide (II) are 3.3 and 4.4 min, respectively.

The effect of column temperature on the retention time and peak resolution was investigated in the range of 30–70°C (data not shown). Controls have shown that an increase in column temperature did not have an adverse affect on either the sensitivity or the accuracy of analytical procedure, thus allowing for the use of a higher flow rate (2.8 ml/min). This arrangement of increased flow rate and column temperature provided reasonable back pressure with better peak resolution and shorter retention time.

Acetonitrile of HPLC grade is a relatively expensive solvent. The elimination of this solvent what has generally been used in other publications provides a safe and economic procedure useful for almost any clinical pharmacology laboratory.

Zimm et al. has explained that lower detection limit of 200 ng/ml for etoposide is not practical [11]. Our limit of detection of 20 ng/ml and limit of quantification of 40 ng/ml (with a signal to noise ratio of 2.5) is among the best for an etoposide assay in plasma. The majority of other investigators have been forced to use either electrochemical or fluoroscence detectors to reach a limit of detection of ~ 50 ng/ml [12].

Table 1 shows the reproducibility of our method in aqueous solution and plasma samples. The calibration curve obtained for day-to-day experiments (data obtained over 1 month), showed a correlation coefficient of 0.99. The CV% for lowest and highest concentrations tested were 8.1 and 4.3%, respectively. Fig. 2 shows the concentration–time profile of plasma from a patient who received 75 mg/m^2 etoposide as a 1 h intravenous infusion.

Patient's plasma samples were tested for etoposide peak on the day of injection and also 2 and 3 months later. No decrease in peak area of etoposide was detected over 3 months of storage at -20°C (ANOVA $P < 0.005$, $df = 3$). Calibration of data obtained from aqueous and plasma samples in 1 day experiments in the range of 20–2000 ng/ml have equations of $y = 0.1341x + 0.0205$, with $r = 0.99$, and $y = 0.0924x + 0.0199$, with $r = 0.99$, respectively.

In conclusion, the method described provides a rapid, simple, sensitive, reproducible and economic procedure for the determination of

Table 1
Reproducibility of the assay method in one and different days (aqueous and plasma samples)

Concentration (ng/ml)	*n*	Aqueous (W-d[a] CV%)	Samples (B-d[b] CV%)	Plasma (W-d CV%)	Samples (B-d CV%)
20	6	4.2	8.1	3.5	8.0
200	6	2.3	5.1	3.1	7.1
500	6	1.5	5.8	2.9	5.2
1000	6	1.5	4.9	3.8	6.4
1500	6	1.3	7.1	2.4	5.0
2000	6	2	4.3	4.8	7.8

[a] W-d, within-day.
[b] B-d, between-days.

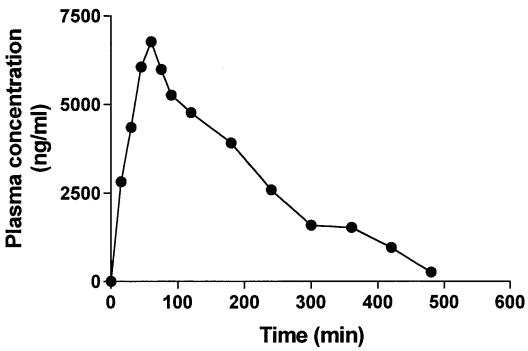

Fig. 2. Plasma–concentration time profile of a patient who received 75 mg/m^2 etoposide over 1 h as an intravenous infusion.

etoposide in human plasma for clinical and pharmacokinetic studies.

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References

[1] B.F. Issell, A.R. Rudolph, A.C. Louie, in: B.F. Issell, F.M. Muggia, S.K. Carter (Eds.), Etoposide (VP-16); Current Status and New Development, 1983, pp. 1–13.
[2] A.J. Wozniak, W.E. Ross, Cancer Res. 43 (1983) 120–124.
[3] S. Kaul, N.R. Srinivas, L.N. Igwemezie, R.H. Barbhaiya, Sem. Oncol. 23 (1996) 15–22.
[4] P.J Creaven, in: B.F. Issell, F.M. Muggia, S.K. Carter (Eds.), Etoposide (VP-16); Current Status and New Development, 1983, pp. 103–115.
[5] S. Lawrie, M. Dodson, A. Arnold, J.M.A. Whitehouse, Cancer Chemother. Pharmacol. 7 (1982) 236–237.
[6] J.J.M. Holthus, W.J. Van Oort, H.M. Pinedo, Anal. Chim. Acta 130 (1981) 23–30.
[7] G. Idzu, Y. Yazawa, M. Tachibana, Clin. Rep. 23 (1989) 479–483.
[8] K. Mross, et al., J. Clin. Oncol. 12 (1994) 1468–1474.
[9] K. Kiya, et al., Cancer Chemother. Pharmacol. 29 (1992) 339–342.
[10] R.J. Strife, I. Jardine, J. Chromatogr. 224 (1981) 168–174.
[11] S. Zimm, et al., Cancer Res. 47 (1987) 1712–1716.
[12] T.A.W. Splinter, et al., Sem. Oncol. 19 (1992) 28–34.