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VALIDATION AND ESTIMATION OF ETOPOSIDE IN BULK AND INJECTABLE DOSAGE FORMS USING RP-HPLC

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ABSTRACT

A simple, sensitive precise and specified reverse phase high performance liquid chromatographic method was developed and validated for determination of Etoposide in bulk and injectable dosages form. It is found that the excipient in the formulation does not interfere in the quantification of active drug by proposed method. The HPLC separation was carried out by reverse phase chromatography on NH_2-(250mm×4.6mm) 5 µm with mobile phase composed of 0.030 M Sodium acetate Buffer pH 4.0: Acetonitrile (70:30) in isocratic mode at flow rate of 1.2 ml/min. The detection was monitored at 254 nm at ambient temperature. The calibration curve of Etoposide is linear from 100 ppm to 300 ppm. The accuracy (recovery 98-102.4%) and reproducibility is satisfactory.

KEYWORDS: Etoposide, RP-HPLC Method, Acetonitrile, Validation.

INTRODUCTION

Etoposide 4-demethyl epipodophyllotoxin 9 (4,6-oethylidine –β-D-glucopyranoside) is a very active drug having pharmacological property to inhibit DNA synthesis and active against cell in G2 phase. And cause DNA break by affecting DNA topoisomerase II function. It is the semi-synthetic derivative of podophyllotoxin with molecular formula: $C_{29}H_{32}O_{13}$. [1-3]

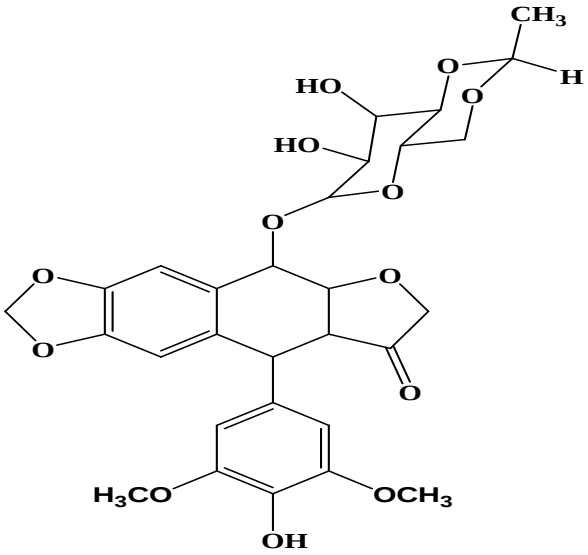

Fig 1: Semi-synthetic derivative of podophyllotoxin

The proposed RP-HPLC method was simple precise and accurate method for determination of Etoposide in bulk and its pharmaceutical dosages form (injection).

MATERIAL AND METHODS

Chemicals and Reagents: Working standard and sample was obtained from V.H.B. Medi Sciences Rudrapur, Uttarakhand, India. Acetonitrile and water of HPLC Grade were used and other reagents used in study were of analytical reagent grade.

Instrumentation: The determination was carried out on Waters HPLC model 2695 equipped with 2489 UV Visible detector (Dual λ absorbance detector) and waters HPLC model 2695 equipped with 2998 photo diode array detector using the empower software. The column used in development in is Zorbex NH_2 (250mm×4.6mm) 5µm. The detector wavelength set as 254 nm with flow rate 1.2 ml/min. The mobile phase composition was Sodium acetate buffer (0.030M) pH 4.0: Acetonitrile (70:30). The pH was adjusted with Glacial acetic acid. The sample and standard was diluted with mobile phase and 20 µl sample was injected in to loop with ambient temperature. The mobile phase was filtered through 0.45 µ filter and sonicate.[1-4]

Table 1-Chromatographic condition

Parameter	Optimized Condition
Chromatograph	Waters HPLC model 2695 equipped with 2489 UV Visible detector
Column	Zorbex NH_2 (250mm×4.6mm) 5µm
Mobile phase	0.030 M Sodium acetate buffer pH 4.0: Acetonitrile (70:30) isocratic.
Flow rate	1.2 ml/min
Detection	254 nm
Injection volume	20µl
Column temperature	Ambient

Preparation of Solutions

Standard Solution: 2000 ppm solution of working standard was prepared in Acetonitrile and further diluted with mobile phase to prepare 200 ppm standard solution.

Sample Solution: Each vial of sample contains 100mg/5ml of Etoposide. Take 5 ml of sample solution and diluted with mobile phase to prepare solution of 200 ppm with diluent.

Method Validation: Once the method was developed, it is validated according to the I.C.H guidelines (ICH Guidelines; 2006).[5-6]

Linearity: The linearity was evaluated by different concentration of standard solution. The concentration was found 100-300 ppm. The calibration curve was plotted between the average area of peak against concentration. The correlation coefficient, slope and intercept found to be 0.9911, 15223.75862, and 212039.4979.[5-6]

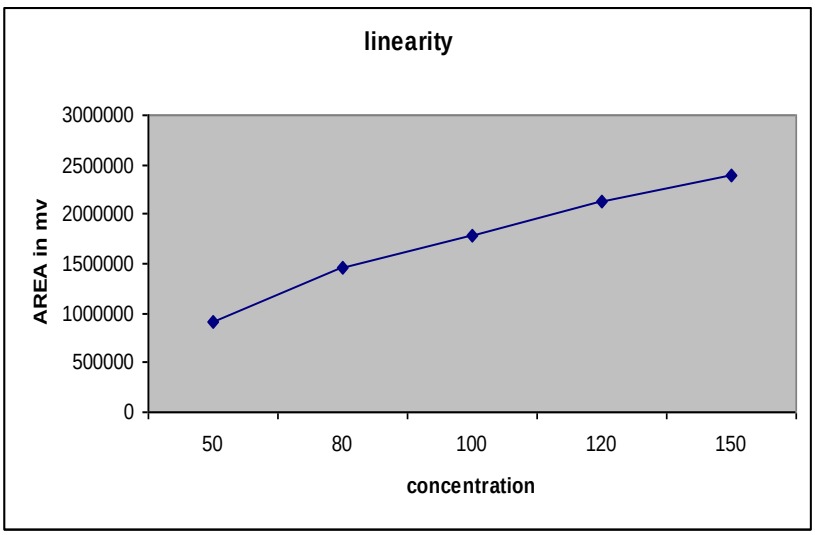

Fig. no. 2.0

Table -2 Etoposide linearity parameter

Parameters	Etoposide
Linearity Range	100-300 ppm
Correlation Coefficient	0.9911
Slope	15223.75862
Intercept	212039.4979

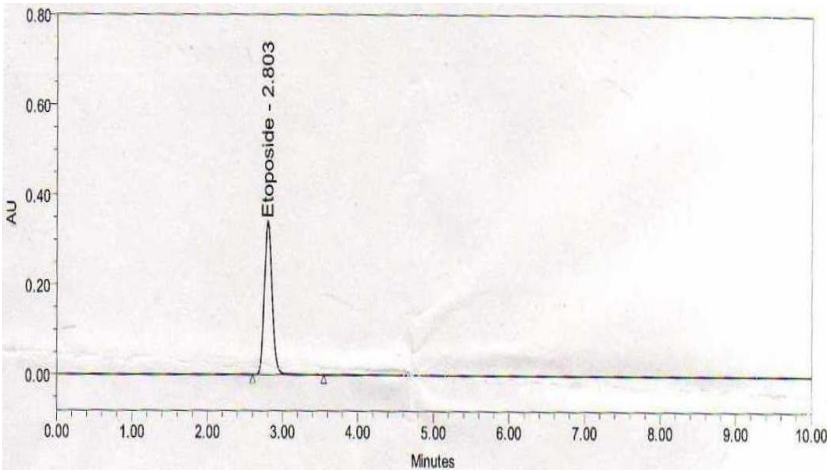

Fig. No. 3.0 Chromatogram of Etoposide

System suitability parameter

To check the system suitability standard solution was injected and the tailing factor and theoretical plates were checked.

Precision

Precision was evaluated by carrying out independent sample preparation of a single lot of formulation. The sample was prepared in a same manner. The percentage relative standard deviation was found to be less than 0.5% within a day and day to day variation and cumulative RSD of 18 injections not more than 2.0%.[5-6]

Table-3 Precision of etoposide ppm[a]-part per million

Conc.(ppm)	Area Day-1	Area Day-2	Area Day-3
200	1838778.0	1864458-0	1826840.0
	1847082.0	1870474.5	1837540.0
	1841897.5	1869419.0	1834624.9
	1845867.0	1867015.5	1839778.2
	1846959.0	1874755.5	1824988.0
	1842149.0	1869459.5	1835837.3
%RSD	0.16	0.16	0.29

Accuracy and Recovery

To study the suitability and method accuracy recovery were carried out. A known quantity of pure drug add to the pre analyzed sample formulation at 50%, 100%, 150% and further diluted to make 100-300 ppm solution and recovery solution was prepared as same but treated with placebo and then check the assay of the recovery solution.[5-6]

Table-4 Recovery studies

Level	% Recovery	%RSD
50%	101.2	0.23
100%	98.1	0.01
150%	102.3	0.17

Limit of Detection and Limit of Quantification

The LOD is the smallest concentration of analyte that give a measurable response (signal to noise ratio 3.3). The LOD of Etoposide found to be 2 ppm. The LOQ is the smallest concentration of analyte (signal to noise ratio 10.1). The LOQ was 6 ppm. It was concluded that the developed method is sensitive. [5-6]

Ruggedness and Robustness

The ruggedness of the method was determined by performing the method in different instrument like waters HPLC model 2695 equipped with 2489 UV Visible detector (Dual λ absorbance detector) and waters HPLC model 2695 equipped with 2998 photo diode array detector using the empower software by different column and different operators. So the % RSD values between the two different instrument, analyst, and column were 0.18, 0.18, and 0.31 respectively.

Robustness was determined by making slightly changes in the chromatographic conditions such as change in mobile phase, flow rate, column temperature. It obtained that no change in chromatogram. So that method is having good system suitability and precision under set condition and acceptance criteria not more than 2%. [5-6]

RESULT AND DISCUSSION

UV spectrum of Etoposide was recorded 254nm selected as wavelength .Flow rate 1.2 ml/min was selected. 0.030M Sodium acetate buffer maintaining pH 4.0 with Glacial acetic acid: Acetonitrile (70:30) was selected as mobile phase. The retention time was found to be 2.8 min. The linearity in the range of 100-300 ppm found 0.9911. Recovery studies were

performed at 50%, 100%, 150% found 101.2%, 98.1%, 102.3%. The stability at ambient temperature found that standard is stable up to 12 hr and sample is stable up to 24 hr at ambient temperature. Thus the method is simple accurate and rapid can employed for routine analysis.

Table-5 System suitability Parameter

Parameter	Etoposide
Calibration Range	100-300 ppm
Theoritical plates	3623.55
Tailing Factor	1.16
LOD	2 ppm
LOQ	6 ppm

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