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RP-HPLC Method development and validation for the Estimation of Etoposide in Bulk Drug and its Pharmaceutical Dosage Form.

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ABSTRACT

A specific and accurate HPLC method is developed for the determination of etoposidein bulk drugs and in solid capsule dosage form. Best symmetric peak shape obtained with Inertsil ODS C-18 column (250 X 4.6 mm, 5µ) column in an isocratic mode, with retention time 5 min.The mobile phase used was Water : Acetonitrile 60:40(v/v)with flow rate 1.0 ml/min and effluent was monitored at 263 nm. As per ICH guidelines method has validated. Method has found linear in the range of 5-45 µg/ml. The LOD and LOQ were found to be 0.02 and 0.06 µg/ml respectively. Method was found specific with respective of diluents, excipients and degradants.

Keywords: Etoposide, RP-HPLC, ICH guidelines, Method validation.

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INTRODUCTION

Etoposide is an anti-cancer (antineoplastic or cytotoxic) chemotherapy drug. Itis a semisynthetic derivative of podophyllotoxin that exhibits antitumor activity. It was synthesized from a toxin found in the American Mayapple in 1966 and approved in 1983 by U.S FDA[1]. The drug used to diagnose different types of cancers like testicular cancer, ovarian cancer, prostate cancer. It can be given to the patients at the time of bone marrow transplant arrangement[2-4], to diagnose refractory testicular tumors and also to treat the patients who suffers from small cell lung cancer.

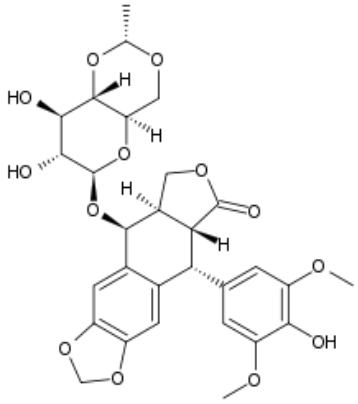

Figure 1: Structure of etoposide

Etoposide It is also commonly known as VP-16 works by slowing cancer cell growth. It inhibits DNA synthesis by forming a complex with topoisomerase II and DNA which breaks in double stranded DNA and prevents repair by topoisomerase II binding [2. 5-12]. Accumulated breaks in DNA lead to cell death. The drug is also capable of inhibiting the beta isoform but inhibition of this target is not associated with the anti-tumour activity. The drug is administered intravenously or orally in capsule form. Side effects with etoposide include alopecia, constipation, diarrhea, nausea and vomiting and secondary malignancies (leukemia)[8]. Many analytical methods reported for estimation of etoposide in human plasma, but very few methods are reported for estimation of drug in pharmaceutical dosage forms[13-20]. So the present study is aimed to develop a RP-HPLC method for determination and estimation etoposide in marketed dosage forms.

MATERIALS AND METHOD

HPLC instrumentation and chromatographic conditions

Chromatographic separation was performed on a PEAK chromatographic system equipped with LC-P7000 isocratic pump; variable wavelength programmable UV detector UV7000 and integrated by PEAK Chromatographic Software version 1.06. Rheodyne injector with 20µl fixed volume loop. Sonicator (1.5L) Ultrasonicator was used to sonicating the mobile phase and

samples. Denver electronic analytical balance (SI-234) is used to weigh the standard and sample drugs.

Chemicals

Water and Acetonitrile used were HPLC grade and were purchased from Merck Specialties Private Limited, Mumbai, India. Etoposide was obtained as a gift from Orchid Health Care Pvt. Ltd, Chennai. All the chemicals and reagents used for optimization studies were of AR grade and purchased from S.D. Fine Chemicals (Mumbai, India).

Preparation of stock standard solution

A stock solution of etoposide (1mg/ml) was prepared in methanol. Standard stock solution of etoposide pure drug (1mg/ml) was prepared by accurately weighing about 100 mg of drug dissolved with 25ml of methanol, and sonicated to dissolve it completely and made up to the mark with the same solvent up to 100 ml in volumetric flask. The contents were mixed well and filtered through Nylon membrane sample filter paper.

Calibration Solutions

Calibration solutions for etoposide were prepared by diluting the stock solution to furnish concentrations in the range 5-45 µg mL-1.

Preparation of sample solutions

Ten capsules (E50-50mg) were taken and weighed. A portion of the powder equivalent to about 10mg of etoposide hydrochloride was weighed accurately and transferred into10mL volumetric flask and sonicated for 20minutes for complete dissolution of etoposide hydrochloride and then the sample solution was filtered and diluted to 100mL with methanol to get concentration of 25 µg/mL and used for analysis. The samples were filtered through 0.45µm nylon membrane filter paper.

Preparation of Bulk drug sample

The bulk drug sample 10mg was weighed accurately in a 10ml volumetric flask. Then it was sonicated until the sample dissolves completely in the solvent. Then make the solution up to the mark with the same solvent. The insoluble particles were removed by filter the solution using membrane filter paper. A concentration of 1mg/ml was obtained. Then it was serially diluted to prepare 25µg/ml concentrated sample and injected.

Method development and validation

Preliminary studies like solubility, polarity, UV absorbtivity etc are study for initial development of method conditions. Various solvents with different ratios has been tried with different reverse phase columns. Later optimization of methods conditions has been carried out to evaluated system suitable chromatogram.

RESULTS AND DISCUSSION

HPLC method development and optimization

To optimize the chromatographic conditions, different combinations of water and acetonitrile compositions were tested. The effect of the flow rate was studied in the range 0.8 to 1.2 mL.min−1. With methanol content as a mobile phase prolonged analysis time was observed. Mobile phase with water: Acetonitrile: 60:40 (v/v) composition was therefore used at a flow rate of 1.0mL min−1, for further studies. Under these conditions, the analyte peak obtained was well-defined and free from tailing (Figure.2). The retention time (RT) was 5 min. Other advantages of this mobile phase included its low cost and simplicity. The short retention time achieved implied that many samples can be run using a small quantity of mobile phase, thus minimizing analysis time and cost per analysis. The optimized chromatographic conditions for the determin1ation of etoposide are represented in Table 1. After completion of method development and optimization method was validated as per ICH guideline such as linearity, precision, specificity and accuracy, limit of detection (LOD), limit of quantitation (LOQ) and robustness.

Table 1: optimized chromatographic conditions of Etoposide

Standard Concentration	25µg/ml
Pump mode	Isocratic
Mobile phase	Water :acetonitrile 60:40 (v/v)
Wavelength	263nm
Column	Inertsil C18 column (250 X 4.6 mm, 5µ)
Column Temp	Ambient
Diluent	Methanol
Injector	Rheodyne
Injection Volume	20µl
Flow rate	1 ml/min
Retention Time	5 minutes
Run time	10 minutes
Peak Area	199182
Pump Pressure	5.9±5MPa

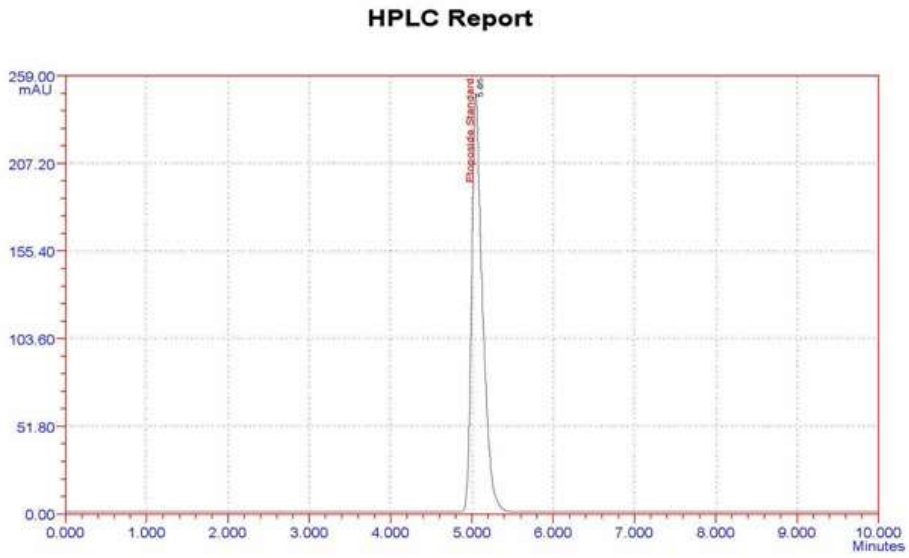

Figure 2: Chromatogram of Etoposide

VALIDATION

Linearity and Range

Peak areas were recorded for each injected concentration of drugs and the calibration curves, concentration vs. peak area were constructed for the drugs. The calibration curve showed good linearity in the range of 5-45 µg/ml, for etoposide with correlation coefficient (r2) of 0.9989. A typical calibration curve has the regression equation of y = 6172.6 x – 77861. Results of linearity studies are given in Table 2 and calibration curve was shown in figure 3.

Table 2: Linearity results of Etoposide

S.NO	Concentration in µg/ml	Peak Area
1	5	105945
2	10	140207
3	15	170529
4	20	203353
5	25	230231
6	30	264976
7	35	298100
8	40	325542
9	45	350695
	Slope: 6172.6	
	Intercept: 77861	
	Correlation Coefficient:0.9989	

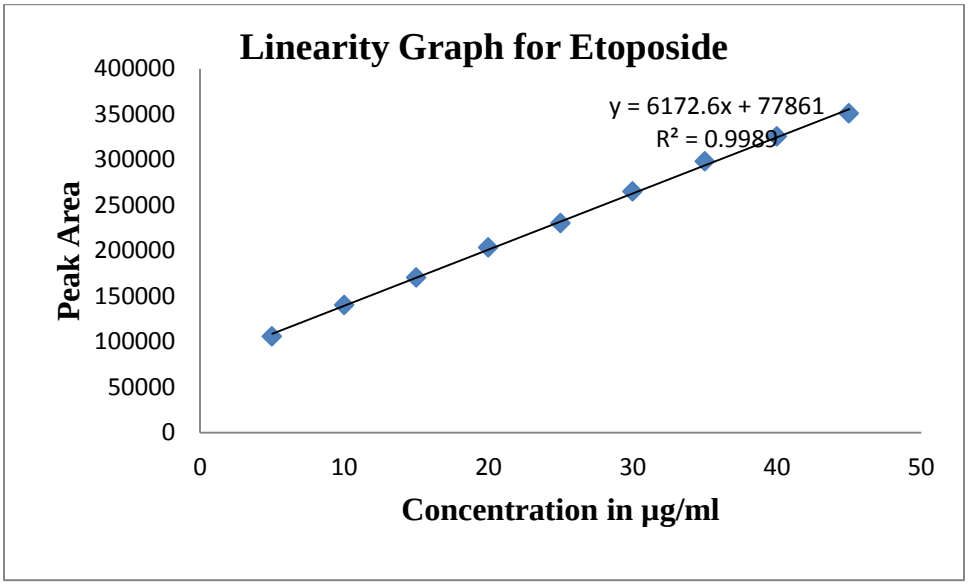

Figure 3: Linearity graph for etoposide

Accuracy

Accuracy of proposed method was ascertained on the basis of recovery studies performed by standard addition at different level of labeled claim (50%, 75% and 100%) of standard 25µg/ml. Percentage of recovery for each case was calculated and was found to be 98.14 to 101.70.This was found to be well within the acceptance criteria of 98-102%.

Limit of Detection (LOD) and Limit of Quantitation (LOQ)

The LOD and LOQ of etoposide were calculated by mathematical equation. LOD= 3.3×standard deviation ÷ slope and LOQ=10×standard deviation ÷ slope. LOD value for etoposide is found to be 0.02µg/ml and LOQ value for etoposide is found to be 0.06µg/ml.

Precision

The precision of the method was demonstrated by inter day and intraday variation studies by injecting six repeated injections of standard solution (25 µg/mL) of etoposide and the response factor of peaks obtained were recorded . From the peaks obtained the %RSD was calculated**.** The percentage of RSD found to be within the limit i.e0.22for interday and 0.30for intraday precision.

Robustness

25 µg /mL standard solution was studied for robustness change in mobile phase ratio and pH, wavelength. Percentage change in the results was calculated and was found to be within the acceptance criteria of bellow 2. Other validation parameters like ruggedness and system suitability were also studied. The percent relative standard deviation (% RSD) was calculated and it was found to be 0.37. The results of suitability test for representative chromatograms obtained were within acceptable limits of tailing factor ≤2.0 and theoretical plates >2000.Thus, the system meets

suitable criteria. Summary of validation studies were shown in table 3. To study the application of proposed method it was applied for assay of commercial tablets of etoposide and bulk drug sample. The results 98.76% for formulation presented good agreement with the labeled content and was found to be 98.05% results were observed. This confirms that the proposed method can be applicable for the estimation of etoposide in bulk drug also. The chromatogram of bulk drug sample was shown in figure 4.(inserted chromatogram)

Table 3: Linearity results of etoposide

S. NO	Parameter	Conditions and result
1	Retention Time	5 min
2	Tailing factor	1.68
3	Theoretical plate	8284
4	Slope	6172.6
5	Linearity range	5-45 g/ml
6	Intercept	77861
7	r^2	0.9989
8	Intraday Precision	0.22
9	Interday Precision	0.30
10	Ruggedness	0.37
11	% Recovery	98.14-101.7
12	Robustness difference	0.214-1.5
13	Limit of Quantification	0.06µg/ml
14	Limit of Detection	0.02µg/ml
15	Formulation assay	98.76%

CONCLUSION

In the present work, an attempt has been made to develop the method using RP HPLC method for estimation of etoposide bulk and pharmaceutical dosage form with simple, accurate, specified with less retention times. The mobile phase is simple to prepare and economical. The proposed method was found to be rapid, accurate, precise, specific, robust and economical and complies system suitability limits. The calibration was linear in the concentration range of 5-45 µg/ml for etoposide. The RSD indicates the method is precise and accurate, the mean recoveries were found in the range of 98-102%. The sample recoveries in all formulations were in good agreement with their respective label claims and they suggested non-interference of formulation excipients in the estimation. Thus the method is not time consuming and can be used in laboratories for the routine analysis. The method was also specific, stable and robust. The proposed method was validated in accordance with ICH parameters and the results of all methods were very close to each other as well as to the label value of commercial pharmaceutical dosage form and bulk drug.

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