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Analytical method development and validation for estimation of etoposide using reverse phase high performance liquid chromatography

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ABSTRACT

A simple and reliable reverse phase high-performance liquid chromatographic (RP-HPLC) method was described for the determination of Etoposide dosage forms. Chromatographic separation was achieved on a Zodiac C_{18} column using mobile phase consisting of a mixture of Triethylamine buffer (4.5): Acetonitrile (60:40 v/v), at 25 °C, with detection of 260 nm. Linearity was observed in the range 20-100 µg /ml for Etoposide (r^2 =0.9955) for the amount of drugs estimated by the proposed methods was in good agreement with the label claim. For intermediate and precision percentage, the RSD values were found to be 1.49% and 0.88% respectively, that falls within acceptance criteria, that is RSD NMT 2%. The accuracy limit is the percentage recovery should be in the range of 98.0% - 102.0%. The total recovery was found to be 98.78%. The solutions were chromatographed at a constant flow rate of 1.0 ml/min. The retention time was found to be 2.750 mins. The assay was performed and the % purity was found to be 99.56%. The validation of developed method shows that the accuracy is well within the limits and showed good accuracy and reproducibility. The robustness limit for wavelength variation and flow rate variation are well within the limits. It was concluded that, the method described for the determination of Etoposide dosage forms was found to be simple, precise, accurate and high resolution and shorter retention time, makes this method more acceptable and cost effective.

Keywords: Etoposide, recovery, Validation, RP-HPLC.

INTRODUCTION

Etoposide inhibits DNA synthesis by forming a complex with topoisomerase II and DNA. This complex induces breaks in double stranded DNA and prevents repair by topoisomerase II binding. Accumulated breaks in DNA prevent entry into the mitotic phase of cell division, and lead to cell death. Etoposide acts primarily in the G2 and S phases of the cell cycle.[1-4]

Etoposide inhibits DNA topoisomerase II, thereby inhibiting DNA re-ligation. This causes critical errors in DNA synthesis at the premitotic stage of cell division and can lead to apoptosis of the cancer cell. Etoposide is cell cycle dependent and phase specific, affecting mainly the S and G2 phases of cell division. Inhibition of the topoisomerase II alpha isoform results in the anti-tumor activity of etoposide. The drug is also capable of inhibiting the beta isoform but inhibition of this target is not associated with the anti-tumor activity. It is instead associated with the carcinogenic effect.[2] Absorbed well, time to peak plasma concentration is 1-1.5 hrs. Mean bioavailability is 50% (range of 25% - 75%). Cmax and AUC values for orally administered etoposide capsules display intra- and inter-subject variability. There is no evidence of first-pass effect for etoposide with 97% protein bound.[3] Primarily hepatic (through O-demethylation via the CYP450 3A4 isoenzyme pathway) with 40% excreted unchanged in the urine. Etoposide also undergoes glutathione and glucuronide conjugation which are catalyzed by GSTT1/GSTP1 and UGT1A1, respectively. Prostaglandin synthases are also responsible for the conversion of etoposide to O-demethylated metabolites (quinone).[5,6]

Etoposide is an antineoplastic agent and an epipodophyllotoxin (a semisynthetic derivative of the podophyllotoxins). It inhibits DNA topoisomerase II, thereby ultimately inhibiting DNA synthesis. Etoposide is cell cycle dependent and phase specific, affecting mainly the S and G2

phases. Two different dose-dependent responses are seen. At high concentrations (10 µg/mL or more), lysis of cells entering mitosis is observed. At low concentrations (0.3 to 10 µg/mL), cells are inhibited from entering prophase. It does not interfere with micro tubular assembly. The predominant macromolecular effect of etoposide appears to be the induction of DNA strand breaks by an interaction with DNA-topoisomerase II or the formation of free radicals.[7-9]

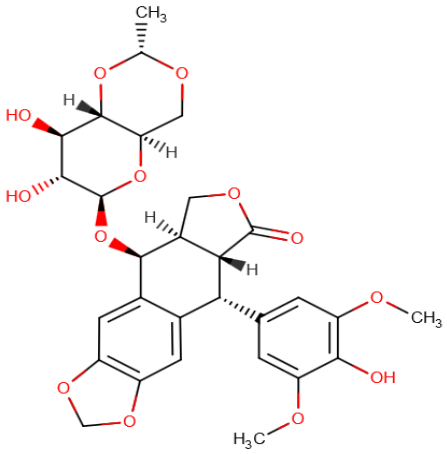

Fig 1: Structure of etoposide

Only few methods were reported for the estimation of etoposide by HPLC.[10-13] Hence we had made an attempt to develop a simple, accurate and precise RP-HPLC method for the estimation of etoposide.

METHODOLOGY

Gift sample of etoposide was received from Chandra labs, Prashnathi nagar, kukatpally, Hyderabad, whereas water, methanol for HPLC, acetonitrile for HPLC, Ammonium acetate, Triethyl amine, Ortho phosphoric acid, Potassium Dihydrogen ortho Phosphat, Sodium dihydrogen phosphate were purchased from Merck.

Instrumentation

Shimadzu (LC 20 AT VP) was used for the separation of etoposide. UV/VIS spectrophotometer (LABINDIA UV 12.500^{+}) was used for detection. Instruments such as; pH meter used was of Adwa- AD 10100 and weighing machine was of Afcoset ER-1000A.

Preparation of buffer

Pipette out 8.5 ml of triethylamine in 1000 ml of water by adjusting the solution with orthophosphoric acid to pH 4.5.

Preparation of mobile phase

A mixture of 60 volumes of Triethylamine buffer and 40 volumes of Acetonitrile Ph 4.5 were prepared. The mobile phase was sonicated for 10min to remove gases.

Standard Solution Preparation

Weigh accurately 10 mg of etoposide in 10 ml of volumetric flask and dissolve in 10ml of mobile phase and make up the volume with mobile phase. From above stock solution 10µg/ml of etoposide is prepared by diluting 1 ml of etoposide to 10ml with mobile phase. This solution is used for recording chromatogram.

Sample Solution Preparation

5 Capsules (each Capsule contains 100 mg of etoposide) contents were weighed and uniformly mixed. Stock solutions of 100µg/ml were prepared by dissolving weight equivalent to 100 mg of etoposide dissolved in sufficient mobile phase. After that filtered the solution using 0.45-micron syringe filter and Sonicated for 5 min and dilute to 10 ml with mobile phase. Further dilutions are prepared in 5 replicates of 10 µg/ml of etoposide was made by adding 1ml of stock solution to 10 ml of mobile phase.

Method development and optimization

The suitability of the column and the mobile phase used in the optimized method have been decided based upon the basis of the selectivity, sensitivity as well as acceptable chromatographic parameters of the produced peaks in terms of peak sharpness, peak symmetry, tailing factor and resolution. We used the mobile phase as a solvent for all samples to ensure minimum noise and to eliminate any unwanted solvent peaks.

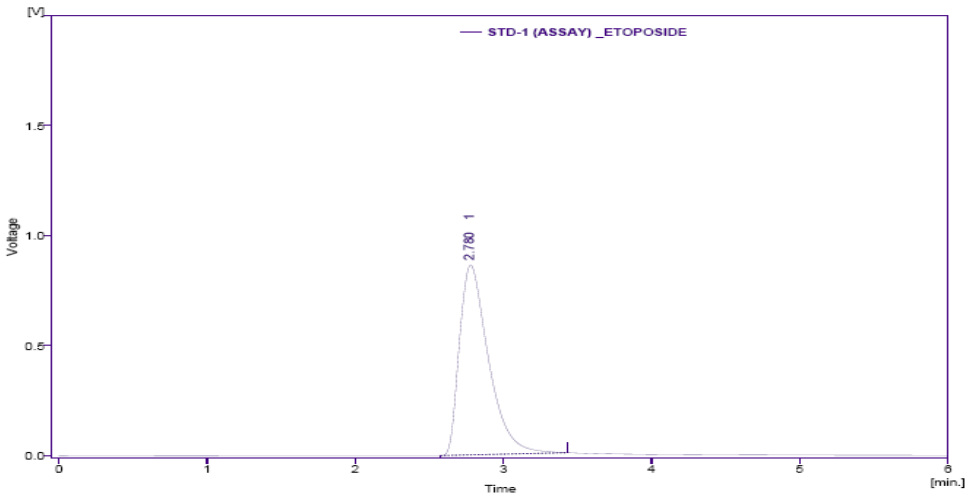

Fig 2: Standard Chromatogram of etoposide

RESULTS AND DISCUSSION

Method Validation

The optimized method for determination of etoposide has been validated as per International Conference of Harmonisation (ICH) guidelines Q2 (R1) for evaluating system suitability, specificity, precision, accuracy, linearity, limit of detection (LOD), limit of quantitation (LOQ) and robustness.[13]

OPTIMIZED CHROMATOGRAPHIC CONDITIONS

Mobile phase	Triethyl amine buffer pH 4.5:Acetonitrile
pH	4.5
Column	INERTSIL column,C18(150x4.6 ID) 5µm
Flow rate	1.0 ml/min
Column temperature	Room temperature(20-25ºC)
Sample temperature	Room temperature(20-25ºC)
Wavelength	260 nm
Injection volume	20 µl
Run time	6min
Retention time	About 2.750 min for etoposide

Linearity and range

Weigh accurately 10 mg of Etoposide in 10 ml of volumetric flask and from this, 1ml dissolve in 10ml of mobile phase and make up the volume with mobile phase. Slope, intercept and correlation coefficient of the calibration curves (peak area versus concentration) were determined to ensure linearity of the analytical method.

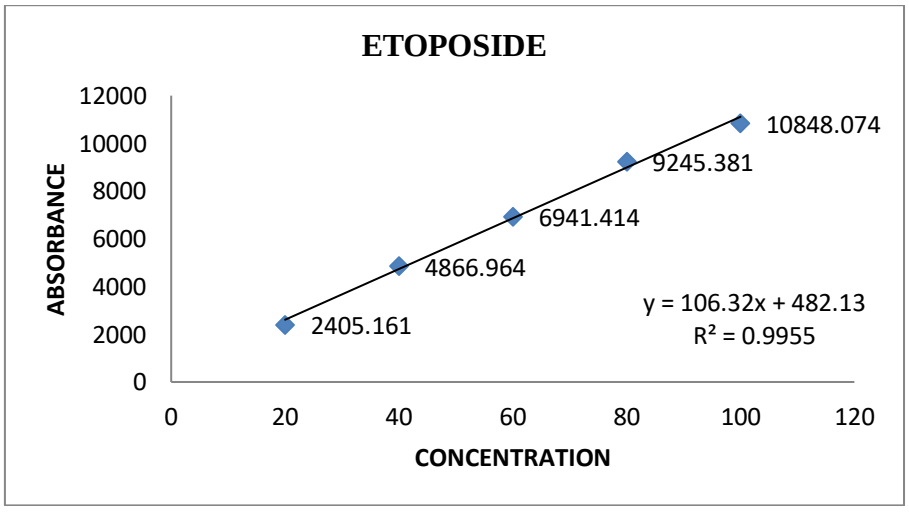

Fig 3: Linearity calibration graph

Accuracy

Accuracy of the method was determined by Recovery studies. To the formulation (pre analyzed sample), the reference standards of the drugs were added at the level of 50%, 100%, 150%. The recovery studies were carried out three times and the percentage recovery and percentage mean recovery were calculated for drug is shown in table. To check the accuracy of the method, recovery studies were carried out by addition of standard drug solution to pre-analyzed sample solution at three different levels 50%, 100% & 150%. (Table 1)

Table 1: Recovery results for Etoposide

Recovery level	Accuracy Etoposide				Average % Recovery
	Amount taken (mcg/ml)	Area	Average area	% Recovery	
50%	60	4724.885	4722.516	98.69	
	60	4663.014			
	60	4779.649			
100%	80	11784.578	11731.638	98.07	
	80	11800.464			**98.78%**
	80	11609.872			
150%	100	10734.111	10722.23	99.59	
	100	10689.433			
	100	10743.154			

Method precision

Prepared sample preparations of Etoposide as per test method and injected 5 times in to the column.

Table 2: Results for Method precision of Etoposide

	Etoposide	
S.No.	**Rt**	**Area**
1	2.743	10158.72
2	2.743	10260.01
3	2.74	10075.3
4	2.743	10304.42
5	2.74	10255.73
6	2.740	10313.540
avg	2.7415	10227.95
Stdev.	0.001643	92.80981
%RSD	0.058738	0.889265

Robustness

Chromatographic conditions variation

To demonstrate the robustness of the method, prepared solution as per test method and injected at different variable conditions like using different conditions like Temperature and wavelength. System suitability parameters were compared with that of method precision.

Table 3: Result of Robustness study

	Etoposide	
Parameter	**Retention time(min)**	**Tailing factor**
Flow		
0.8ml/min	3.450	2.044
1.0 ml/min	2.767	1,795
1.2ml/min	2.327	1.676
Wavelength		
258nm	2.757	2.027
260nm	2.757	2.00
262nm	2.757	2.083

Ruggedness

The ruggedness of the method was studied by the determining the analyst to analyst variation by performing the Assay by two different analysts.

Table 6: Results for Ruggedness

Etoposide	%Assay
Analyst 01	98.72
Analyst 02	97.23

CONCLUSION

From the above experimental results and parameters it was concluded that, this newly developed method for the estimation of etoposide was found to be simple, precise, accurate and high resolution and shorter retention time makes this method more acceptable and cost effective and it can be effectively applied for routine analysis in research institutions, quality control department in industries, approved testing laboratories, bio-pharmaceutical and bio-equivalence studies and in clinical pharmacokinetic studies in near future.

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