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Development and Validation of HPLC Method for the Estimation of Etoposiide in Pharmaceutical Formulations

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Abstract:

The development and perhaps validation of a novel, quick, precise, yet efficient Reverse Phase HighPerformance Liquid Chromatographic technique for quantification of etoposide in bulk and pharmaceutical formulations. The mode of this process was isocratic employing analytical Kromasil C_{18}-column(250×4.6mm,5µm), at 25ºC with methanol:water:orthophosphoric acid(45:45:10) as mobile phase, at 6.10 pH with 1mL/min rate of flow and UV detection in 282nm.The chromatogram produced as a consequence does have a high resolution and then a lower tailing factor (1.05). For such a wide array of drug doses of 1-5 ppm, the linearity curve exhibited a correlation coefficient -r^2 of 0.999. The precision, accuracy, and specificity of the technique were also assessed. The method's usability was further evaluated with Posid 100mg commercial sample.

Keywords: Etoposide, Posid tablets, HPLC, Method development, Validation

Introduction:

Etoposide (MF: C_{29} H_{32} O_{13}; MW: 588.56) is chemically termed as 4'-demethyl-epipodophyllotoxin 9-(4,6-O-(*R*)-ethylidene)- beta-D-glucopyranoside alsovery marginallywater soluble; and its solubility in alcohol, dichloromethane, chloroform, and ethyl acetate is slight; alsoin methyl alcohol it is sparingly soluble[1].Etoposide is a semi - synthetic component of podophyllotoxin, which is derived out from roots & rhizomes of Podophyllum peltatum and Podophyllum emodi.It is a potent antineoplastic drug that is been used to treat small cell lung cancer, testicular cancer, and lymphomas [2,3]. The suppression of enzyme topoisomerase II is thought to be its mode of action.

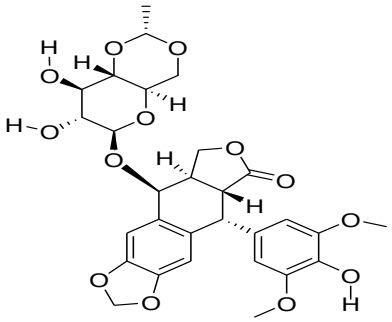


Fig. 1: Structure of Etoposide

Literature survey revealed that several HPLC methods were reported for etoposide with UV detection [4-7], fluorescence detection [8-12], and electrochemical detection [13-20] and scanty HPLC techniques for the etoposide estimation in forms that are injectable [21-22]. It has also been determined by LC-MS [23-24] and stability-indicating LC [25]. The majority of these techniques utilized phenyl and cyano columns, necessitated an internal baseline for etoposide measurement, and had long retention periods. The previously published methods [16, 23-24] use mixture of expensive organic solvents or demand of costly equipment. The goal of this study was to create an alternative RP-HPLC technique for assessing etoposide in pharmaceutical formulations that was simple, precise, yet efficient.

Materials and Methods:

Equipment

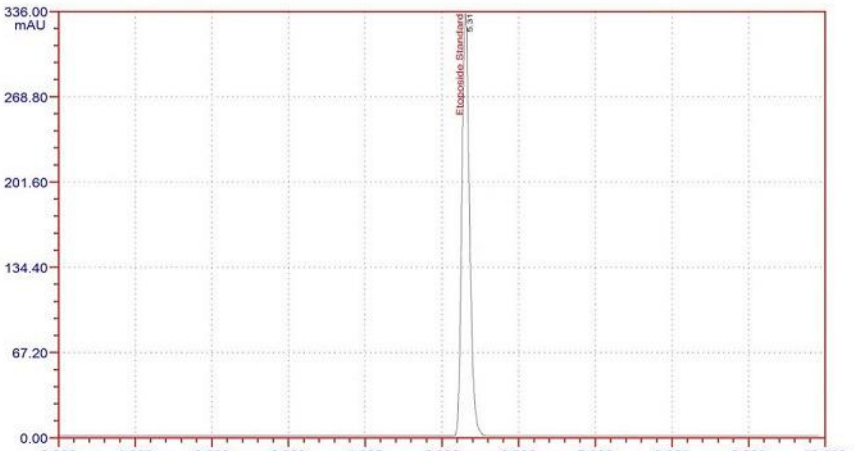
Drug sampleanalysis was worked out with PEAK 7000 isocratic HPLC usingrheodyne manual sample injector having switch (77251) and kromosil C18 (250x4.6mm, 5µm) analytical column.ELB 300 was the electronic balance, DIGISUN pH meter was employed for every pH measurements.

Chemicals and reagents

Cipla pharma, Hyderabad, generously provided the etoposide reference standard, and also the tablet formulation of Posid 100 mg utilised to test the technique was acquired on the local market. The solvents utilised were HPLC grade methanol, Merck orthophosphoric acid, and triple distilled water produced with a Borosil Glass Distillation Unit.

Optimized Chromatographic Conditions

Utilizing a Kromasil C18 (250x4.6mm, 5 µm) column, etoposide was analysed chromatographically. The mobile phase was made up of 45% MeOH of HPLC grade, 45% triple distilled water, and 10% orthophosphoric acid, which was strained over a 0.5µ nylon membrane filter prior to application, with a pH of 6.10.The experiment was run in isocratic mode with a flow rate of 1mL/min. At room temperature, 282 nm is the detector wavelength, and 18-19.5 MPa of working pressure. The injection volume had been 20 µlitres, the 5.310 minutes of retention time, and the whole run duration was 10 minutes. Figure 2 shows the chromatogram that resulted.



ID	Name	Retain.T	Height	Area	Conc	Tail.Factor	Theo.Plate
1	Etoposide Standard	5.310	18956	80313.7	100.000	1.05	31305
	Sum:		18956	80313.7	100.0000		

Fig 2: Standard chromatogram of the Etoposide

Preparation of standard solutions

In the study, pure etoposde standards were utilised as external standards. Depending on the range necessary to construct an acceptable calibration curve, various concentration of such standards were employed. Approximately 10 mg of etoposide drug was put into a 10 mL volumetric flask and brought up to the required concentration by adding enough mobile phase.To degas the flask holding the standard stock solution, this one was sonicated over 10 minutes. After that, filter paper of 0.45 µm membrane was used to filter the standard solution.By using aforementioned stock solution with mobile phase (Methanol, water, and orthophosphoric acid in the ratio 50:40:10 (v/v/v)), a sequence of various dilutions (1-5 ppm) were produced.

Sample preparation

By precisely weighing the appropriate amount of the drug and putting it into a 100 mL volumetric flask, 1 ppm of sample solution was produced. After that, a 0.45µm membrane sample filter was used to filter the sample solution.

Procedure for analysis

A research base line was recorded then stabilised for around 30 minutes using the optimal chromatographic conditions of etoposide. Following the stabilisation of base line, aliquots of the sample solution were administered individually and chromatograms were documented until the peak areas' repeatability was sufficient.The commercial sample utilized to assess the method's accuracy went through the same technique. At 1mL/min flow rate, the sample was loaded into column.

Results and Discussion:

Method Validation

Following the HPLC technique development was completed, the technique was validated in respect of precision, linearity, accuracy, LOQ, LOD, ruggedness, and robustness.

Evaluation of linearity

Different concentrations of the standard solutions were analyzed for evaluating the linearity of the method.Etoposide solutions of 1 ppm, 2 ppm, 3 ppm, 4 ppm, and 5 ppm were produced and evaluated in the mobile phase using standard 100 percent pure etoposide. Following the study, the area of peaks was noted and given in Table-1. For a wide range of drug concentrations of 1-5 ppm, there were no noticeable changes in the chromatograms due to flow rate alteration, column temperature fluctuations, or mobile phase variance.The concentration was plotted on the X-axis, and even the peak area was plotted on the Y-axis. The correlation coefficient r2=0.999, from Fig. 3 of regression was observed to be practically equivalent to 1 when a straight line meeting the linearity requirement was obtained.

Table 1: Etoposde- Linearity data

S.No	Concentration (ppm)	Peak Area	
1	1	80521.4	INTERCEPT= -466.15 SLOPE=778738.19 C.C= 0.999. r^2=0.999
2	2	156326.4	
3	3	234461.8	
4	4	310113.9	
5	5	397318.6	

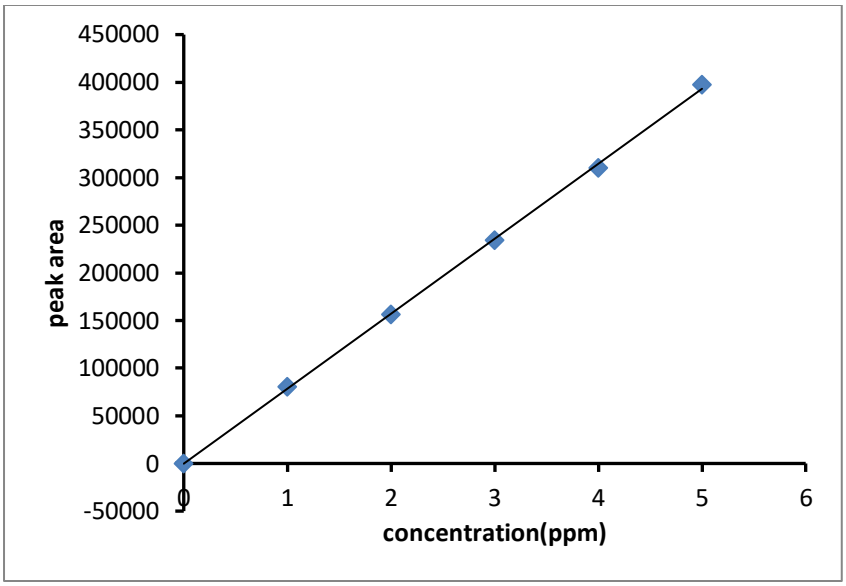


Fig 3: Etoposide Linearity

Precision

6 times a standard solution of drug material (1 ppm) was administered, and the matching peak regions were recorded. The percent RSD discovered has been less than 1%. The intraday precision percent RSD achieved is 0.4086 (Table 2&3), whereas 0.4521 is the interday precision. The percent RSD within a day, day to day fluctuation (<1%) demonstrates the precision of the technique.

Table 2: Precision for Intraday assay

TEST.1	**PRECISSION**			
	CONC 1ppm			
Intraday	INJECTION	AREA	T.P	% R.S.D = 0.4086
	1	80586.2	9675.72	
	2	80048.5	9738.94	
	3	80798.5	9650.64	
	4	80899.5	9639.40	
	5	80944.0	9634.24	
	6	80746.4	9657.58	

Table 3: Inter-day precision

TEST.1	**PRECISSION**			
	CONC 1ppm			
Interday	INJECTION	AREA	T.P	
	1	80904.7	9638.15	

	2	80950.1	9631.01	% R.S.D = 0.4521
	3	80069.2	9735.78	
	4	80981.0	9627.31	
	5	80969.6	9626.36	
	6	80555.1	9679.49	

Accuracy (% Recovery)

The method's accuracy was evaluated by completing recovery tests by the standard addition method, in which a measured quantity of pure drug was put to the pre-analysed working standard solution of drug over three different concentration levels. The sample solutions were evaluated in triplicate at every level using the suggested method, and the percentage recovery for each stage was determined. This approach yielded a recovery rate ranging from 98.65 to 98.92 percent (Table 4).

Table 4: Data of Accuracy

Concentration taken (ppm)	Concentration found (ppm)	% Recovery	Mean % recovery	% RSD
3	2.9565	98.55	98.65	0.1266
3	2.9637	98.79		
3	2.9583	98.61		
4	3.9476	98.69	98.92	0.3767
4	3.974	99.35		
4	3.9488	98.72		
5	4.9395	98.79	98.83	0.0404
5	4.9435	98.87		
5	4.9415	98.83		

Specificity of the method

The specificity of technique was evaluated by monitoring any interference that the components in the formulations caused. The test findings were likened to those obtained for a standard medication. It was discovered in this investigation that such components do not interfere with the established technique.

Robustness

The method's robustness was investigated by changing only one parameter in the optimal chromatographic settings at a time, such as mobile phase composition, pH, and wavelength, while leaving all other parameters fixed. Flow rate change, column temperature fluctuations, and mobile phase modulation all resulted in no discernible changes in the chromatograms.The robustness threshold for above parameter changes has been less to 2%, which is substantially within the acceptable range. This demonstrates that the approach is suitable for the provided collection of conditions.

Table 5: Robustness Results

Parameter changed	Variation	Peak area	% Assay

Standard	-	80521	-
Mobile phase	55:40:05 50:45:05	79110 79084	98.25 98.22
Wave length	279 nm 285 nm	79112 79189	98.26 98.35
pH	5.9 6.3	79202 79757	98.37 99.06

Ruggedness

Six replicate injections comprising standard and sample solutions with concentrations had been produced and evaluated by separate analysts on two different days over a one-week period to accomplish inter-day variations.

Limit of Detection and limit of Quantification (LOD and LOQ)

Following the established HPLC technique, the created method's limit of detection (LOD) and limit of quantification (LOQ) (Table 6) were evaluated by infusing increasingly low quantities of standard solutions.The LOD is the analyte concentration at which a detectable response may be obtained. Etoposide's LOD was discovered to be 20 ng/mL. The LOQ is the analyte's lowest concentration that produces an unambiguously quantifiable reaction. Etoposide's LOQ was discovered to be 65 ng/mL. The sensitivity of the proposed technique was validated by the LOD and LOQ results.

Table 6: LOD and LOQ

LOD	20ng/mL
LOQ	65ng/mL

Different chromatographic conditions were attempted in order to achieve simultaneous elution of etoposide. Experiments indicated that the kromosil C18 (250x4.6mm, 5 µm) column was the best choice, as it generated symmetrical peaks with excellent resolution and sensitivity. Various combinations of methanol, water, and 1% orthophosphoric acid were tried to create an appropriate mobile phase for the investigation of the chosen drug combo.After a series of tests, it was discovered that a mobile phase consisting of methanol, water, and 1% orthophosphoric acid (50:40:10(*v/v/v*)) produced a symmetric peak at 282nm in a short amount of time (10 min). The pH was determined to be 6.10, and the mobile phase chromatogram revealed excellent affinity with the retention duration of 5.332 minutes.

For different parameters, a system suitability analysis was performed on a reference chromatogram[26-28]. The calibration curve created in the range of 1-5 ppm was determined to be linear, and a five-point graph encompassing a range of concentrations of 1-5 ppm was generated.Table 1 displays the results of the regression analysis of calibration graph. Lower standard deviation results indicated that the measurement was extremely repeatable[29]. As a result, it was demonstrated that the study's equipment and the proposed analytical procedure were both consistent.A research was conducted for intermediate precision, which revealed an RSD little less than 2. The above-mentioned technique for estimating etoposide was statistically evaluated and shown to have high linearity, reproducibility, and validity for various parameters[29,30].

Conclusion:

For the assessment of etoposide in pharmaceutical preparations, a validated RP-HPLC technique has been established. The developed approach was straightforward, quick, accurate, and precise.For both medicines, it

generates a similar peak shape, high resolution, and a tolerable retention duration. As a result, this approach may be used to estimate lornoxicam and thiocolchicoside simultaneously in quality control tests for regular analysis.

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