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A new validated RP-HPLC method for estimation of eflornithine hydrochloride in tablet dosage form

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ABSTRACT

A rapid, sensitive and specific RP-HPLC method involving UV detection was developed and validated for determination and quantification of Eflornithine hydrochloride in tablet dosage form. Chromatography was carried out on a pre-packed Thermo Hypersil C18 (5µm, 250x4.6mm) column using filtered and degassed mixture of Acetonitrile:Buffer (900:100) as mobile phase at a flow rate of 0.8ml/min and effluent was monitored at 210nm. The buffer was prepared by dissolving 6.8gm of potassium dihydrogen orthophosphate in 100ml of water and the pH was adjusted to 5.0 by using O-phosphoric acid. The method was validated in terms of linearity, precision, accuracy, and specificity, limit of quantification and limit of detection. The assay was linear over the concentration range of 125mcg-750mcg/ml. Accuracy of the method was determined through recovery studies by adding known quantities of standard drug to the pre analysed test solution and was found to be 100.30%-101.48% within precision RSD of 0.32 for Eflornithine hydrochloride. The system suitability parameters such as theoritical plates, resolution and tailintg factor were found to be 9093.00, 1.4 and 1.08 respectively and the LOD and LOQ of Efflornithine hydrochloride were found within the limit(less than 2%). The method does require only 10 minutes as run time for analysis which prove the adoptability of the method for the routine quality control of the drug.

Keywords: Eflornithine hydrochloride, Analysis, RP-HPLC, Validation.

INTRODUCTION

Eflornithine hydrochloride is chemically 2,5-diamino-2-(difluoromethyl)pentanoicacid hydrochloride and it is an antineoplastic and antiprotozoal orphan drug used in the treatment of Pneunocystis carinii pneumonia in AIDS. In this paper we describe a simple, inexpensive, sensitive and validated HPLC method for the determination of Eflornithine hydrochloride in bulk and pharmaceutical formulation.

EXPERIMENTAL WORK:

Working standard of eflornithine hydrochloride was obtained from well reputed research laboratories. The purity of this standard was 99.61%. HPLC grade acetonitrile, Merck grade KH_2PO_4 and Milli-Q water were procured from the market. The separation was carried out on isocratic HPLC system (Shimadzu) with Class-VP software with pre-packed Thermo(Hypersil-C18(5µm,250x4.6mm)) column using filtered and degassed mixture of Acetonitrile:Buffer (900:100) as mobile phase.

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Standard preparation: About 50mg of Eflornithine hydrochloride was accurately weighed and transferred to a 200ml volumetric flask and dissolved in the diluent by sonication to give standard stock solution of 25mg/ml.

Chromatographic conditions: Flow rate 0.8ml/min; detection wavelength 210nm; injection volume 20µl; column used Thermo Hypersil C18 (5µm, 250x4.6mm); column temperature: 25°C; mobile phase: Acetonitrile:Buffer (900:100).

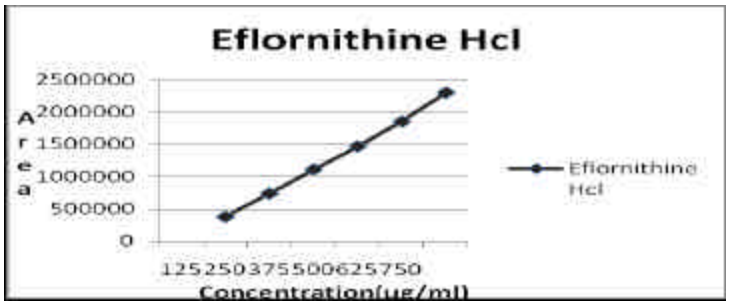
Method development: Working standard of various concentrations was prepared by taking aliquots of standard solution and diluted to get required concentration for calibration plot and which was injected.

Assay preparation for commercial formulation: Twenty tablets were weighed accurately and finely powdered. Powder equivalent to 50mg of eflornithine hydrochloride was transferred into 200ml volumetric flask and dissolved in sufficient amount of diluent and sonicated to dissolve. Solution was filtered through 0.45µ membrane filter and then the filtrate was further diluted to get the required concentrations.

Procedure: 20µl of the standard preparation and assay preparation were separately injected and chromatographed.

Method validation

Linearity: Linearity was demonstrated by analysing six different concentrations of active compound. Peak areas were recorded for all the peaks and calibration plot was constructed by plotting peak area



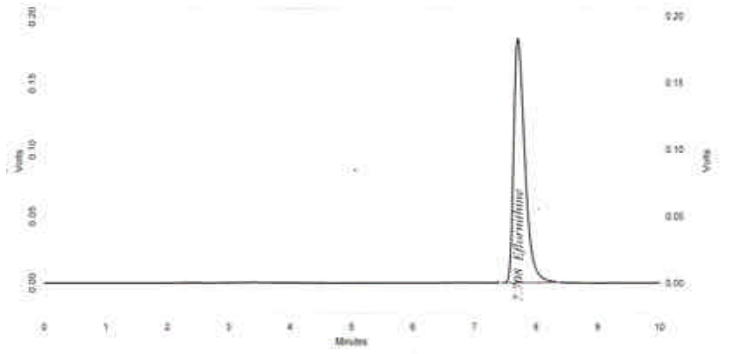


Fig.1. & 2. Calibration graph of Eflornithine hydrochloride & RP-HPLC estimation of Eflornithine hydrochloride

vs concentrations of eflornithine hydrochloride which were found to be linear in the range of 125mcg/ml-755mcg/ml. Coefficient of correlation was 0.9999(Fig-1&2).

Accuracy: accuracy was done by recovery study using standard addition method, known amount of standard eflornithine hydrochloride in to pre-analysed samples and subjected to proposed HPLC method. The results of recovery studies are shown in Table-1.

Table-1Analysis of tablet containing eflornthine hydrochloride

Formu -lation	Drug	Label claim	Amount found (mg)	Found (%)	Amount std. added	Amount recovered	Recovery (%)
Tablet	Eflornithine hcl	50mg	50.10	100.2	1	50.43	100.86

Precision:

To demonstrate agreement among results, a series of measurements are done with eflornithine hydrochloride six replicate injections of the specific standard at various time intervals on the same day were injected into the chromatograph and the value of %RSD was found to be 0.32 (Table-2).

Table-2. Percentage recovery of eflornthine hydrochloride

Amount found on	Mean (n=6)	RSD (%)
Eflornithine hydrochloride (250 µg/ml)	51.105	0.32

RESULTS AND DISCUSSION:

The regression value was found to be 0.9999 for eflornithine hydrochloride, which shows the response is linear from 125µg/ml to 750µg/ml. Selectivity experiment showed that there is no interference or overlapping of the peaks either due to excipients or diluents with the main peak of eflornithine hydrochloride. The percentage RSD for precision is <2 which confirms that method is sufficiently precise and the total run time required for the method is only 10mins for eluting eflornithine hydrochloride. The proposed method is simple, fast, accurate, and precise and can be used for routine analysis in quality control of eflornithine hydrochloride.

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