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New Validated RP-HPLC Method for the Determination of Eflornithine Hydrochloride

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Abstract : Objective: A Newer highly sensitive, accurate, precise and specific RP-HPLC method of Eflornithine hydrochloride (2-difluoromethyl-DL-ornithine; DFMO) was developed and validated as per ICH guidelines.
Method: The developed method is not only eligible of identifying and quantifying the impurities but also can be capable for the assay of Eflornithine hydrochloride in marketed parenteral formulations. The separation was performed using BDS Hypersil 5µ C18 (150 × 4.6 mm) column at room temperature by using methanol and water, 60:40 v/v as mobile phase at the flow rate 1 ml/min with UV detection at 254 nm.
Results: The retention time of DFMO was 4.8 min. The method was linear over concentration range of 5-15 ng/ml for DFMO. The accuracy of the proposed method was determined by recovery studies and was found 98.4% for DFMO. The developed method was validated as per ICH guidelines for linearity, accuracy, precision, limit of detection, limit of quantification, ruggedness, robustness and system suitability for Eflornithine hydrochloride and its impurities. This method can be successfully used for quantitative analysis of DFMO in parenteral formulation.
Keywords : RP-HPLC, Eflornithine hydrochloride (DFMO), Validation, ICH guidelines.

Introduction

Eflornithine hydrochloride (DMFO) DL-α-Difluoromethylornithine hydrochloride is a suicide inhibitor of the polyamine biosynthesis enzyme ornithine decarboxylase (ODC). The chemical structure of Eflornithine is shown in Figure 1.

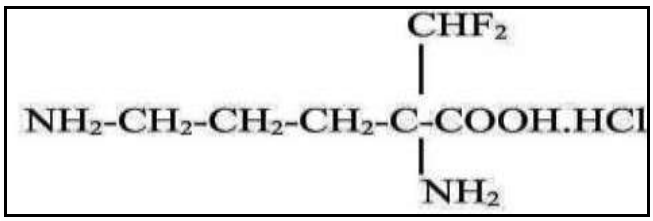

Fig.1: Structure of Eflornithine hydrochloride (DFMO)

One of the key enzymes in the polyamine biosynthetic pathway.[1-2] The drug was originally developed for use in cancer and is in phase III clinical trials for its use in preventing recurrence of superficial bladder cancer. It has been used as antiprotozoal agent in the treatment of meningoencephalic stage of trypanosomiasis caused by *Trypanosoma brucei gambienze* (African trypanosomiasis).[3-4] DFMO currently is in development and testing for its anti inflammatory activity. DFMO 13.9% cream is used to inhibit growth and reduce the amount of facial hair in women.[5]

A number of analytical methods have been reported for measuring DFMO in biological fluids and tissue extracts. These methods involve HPLC techniques.[6-8] The HPLC techniques currently available for the quantification of DFMO in biological fluids involve either pre or post column derivatization with UV or fluorescence detection[9-10] and LC carried out by evaporative light scattering detection.[11] Few methods have been reported in the literature for the analysis of DFMO including spectrophotometry.[12-13]

Regarding all the above mentioned, we decided to develop a newer RP-HPLC method suitable for determination of DFMO for commercial parenteral formulations.

Since there is no RP-HPLC analytical method for the determination of DFMO in the pharmaceutical formulations described in the literature. Therefore, the aim of this work was to develop and validate such a method that was newer, simpler, precise, sensitive, selective, economic, rapid and accurate.

In pharmaceutical industry the analysis of pharmaceuticals is an integral and increasingly important part of an overall drug development process. Therefore, a rapid and simple method for routine analysis and quality control of commercial formulations is desirable. Hence, in the present study, new, simple and selective reverse phase high performance liquid chromatography (RP-HPLC) method used for the determination of DFMO drug in commercially available pharmaceutical preparation was developed.

Materials and Methods

Equipment

The HPLC (Shimadzu class LC-10A, including pump LC-10AT, SPD-10A UV–VIS detector, Japan) and Hamilton syringe (all from Shimadzu, Kyoto Japan) were used. The separation was achieved on a BDS Hypersil 5µ C18 (150 × 4.6mm) with UV detection at 254 nm. Analytical weighing balance (Shimadzu AUX 200), sonicator (SONICA 2200MH), vacuum pump (model XI 5522050 of Millipore), millipore filtration kit for solvents and sample filtration were used throughout the experiment. The LC solution software -multiple channels was used for acquisition, evaluation and storage of chromatographic data. DFMO obtained as a gift sample (Wintac Pvt. Ltd. Bengaluru, India) and certified to contain 99.6% purity. All the solvents used in analysis were of HPLC grade.

Preparation of standard stock solution

Stock solution of DFMO was prepared at a concentration of (1 mg/ml) by dissolving the accurate weighed amount in a definite volume of Eflornithine to get the required concentration. Dilute solutions were prepared by accurate dilution from stock solution to get the desired concentrations.

Preparation of mobile Phase

The mobile phase was prepared by mixing of methanol and water in the ratio 60:40 v/v.

Preparation of sample solution

Sample Ornidyl (1 mg/ml) solution (label claimed, each fill volume of the vial contains 100 ml and each ml contains 200 mg) was prepared by pipetting out 10 ml of the contents of the vial and geometrically diluted with methanol to get the desired concentration.

The solutions were sonicated for 10 min and filtered into a 100 ml volumetric flask through Whatman filter paper (no. 44). The residue was washed 3 times with 10 ml of methanol and then the volume was made up

to 100 ml with the same solvent. The proposed RP-HPLC method was applied and the concentration of each component in the formulation was determined. The results obtained were shown in Figure 2.

Chromatography condition

The mobile phase methanol and water in the ratio 60:40 v/v was selected, because it was found that it ideally resolved the peak with retention time 4.8 min (Figure 2) for DFMO. Wavelength was selected by scanning standard drug over a wide range of wavelength from 200 nm to 400 nm. The component showed reasonably good response at 254 nm.

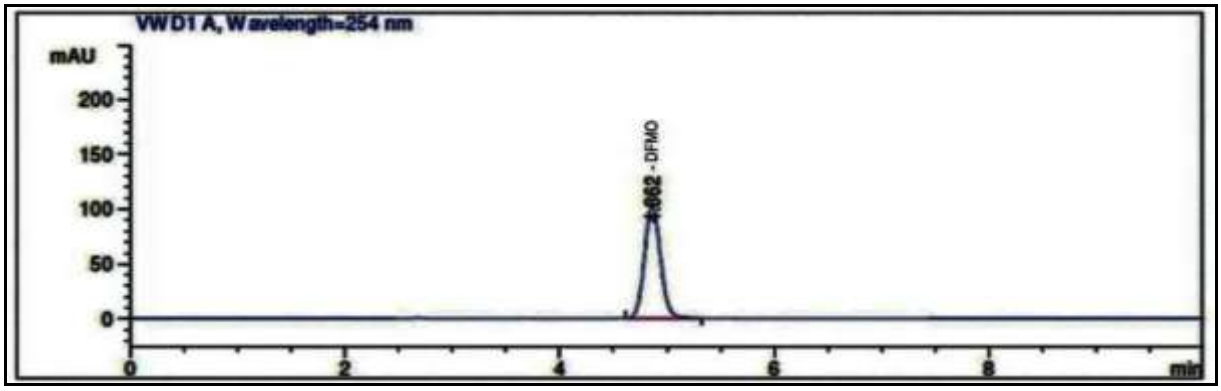

Fig.2: Chromatogram of DFMO 10 ng/ml

Validation

The method was validated for linearity, accuracy, precision, limit of detection, limit of quantification and robustness as per the ICH guidelines.[14] All analytical parameters are shown in Table 1.

Table 1: Analytical parameters of method

Parameters	**DFMO-HPLC**
Calibration range (ng/ml)	5-15
Detection limit (ng/ml)	0.006438
Quantitation limit (ng/ml)	0.022126
slop (b)	10.76
Intercept (a)	21.57
Correlation coefficient	0.999
Retention time	4.898
Theoretical plate#	5169
Tailing factor	0.89

Linearity

In this study five concentrations were prepared, ranging between 5-15 ng/ml of DFMO. Each concentration was repeated three times and obtained information on the variation in the peak area response ratio of the internal standard to pure analytes is presented in Figure 3. A linear relationship was obtained for DFMO in the range of 5-15 ng/ml.

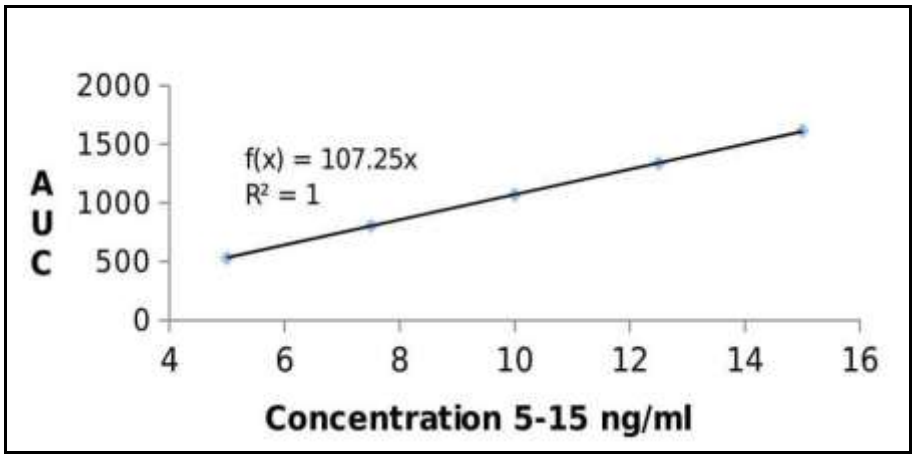

Fig.3: Linearity curve of DFMO

Accuracy

The resulting mixtures were analyzed by the proposed HPLC method and the response obtained was plotted against the initial unknown concentration set at 0. The results obtained are compared with expected results. The excellent mean recoveries and standard deviation as shown in Table 2 suggested good accuracy of the proposed method and no interference from formulation exicipients.

Table 2: Accuracy study of the method

Level	Area Response	% Recovery	Mean	RSD	Average % recovery	Average % RSD
50%	531.57126	97.5	97.7	0.54	98.40	0.33
	535.90051	98.3				
	530.84039	97.3				
100%	1070.8374	98.2	98.4	0.29		
	1075.95142	98.7				
	1070.49359	98.2				
150%	1617.52747	98.9	99.1	0.15		
	1622.60541	99.2				
	1621.47412	99.1				

Precision

The repeatability (within-day in triplicates) and intermediate precision (for 3 days) was carried out at four concentration levels for each compound. The obtained results within and between days trials are in acceptable range indicating good precision of the proposed method. The precision expressed as % RSD is given in Table 3.

Table 3: Precision study results of method

Parameters	% Recovery ± RSD*
Method Precision	97.46±0.41
Intermediate Precision	97.1±0.142
Mean of six observations	97.3
RSD of six observations	0.37

Limit of detection (LOD) and limit of quantification (LOQ)

The limit of detection (LOD) and limit of quantization (LOQ) were calculated according to ICH.[14] The LOD and LOQ were calculated from the standard calibration curves based on standard deviation formula with equations LOD=3.3σ/S and LOQ = 10σ/S; where, σ is the standard deviation of the response and S is the slope of the calibration curve. The data is represented into Table 1.

Robustness

The robustness of the method was determined by making slight at column temperature ± 5°C, mobile phase flow rate ± 0.2 ml/min and change in organic phase ± 2%. The robustness of the method shows that there were no marked changes in the chromatographic parameters. The data was represented into Table 4 and graphically shown into Figure 4.

Table 4: Robustness evaluation of method

Acceptance criteria	**The % RSD for area response of Eflornithine obtained from five replicate injections of standard preparation should be not more than 2%.**	**Tailing factor for the Eflornithine peak from the standard preparation should be not more than 2**	**Theoretical plates of Eflornithine peak should be not less than 2000 in standard preparation.**
Original Condition	0.2%	1.09	5165
Decrease in Flow	0.1%	1.21	4821
Increase in Flow	0.2%	1.05	5376
Decrease in Temperature	0.5%	1.02	4712
Increase in Temperature	0.4%	1.51	5564
Decrease in Organic Phase	0.5%	1.51	5631
Increase in Organic Phase	0.4%	1.12	5021

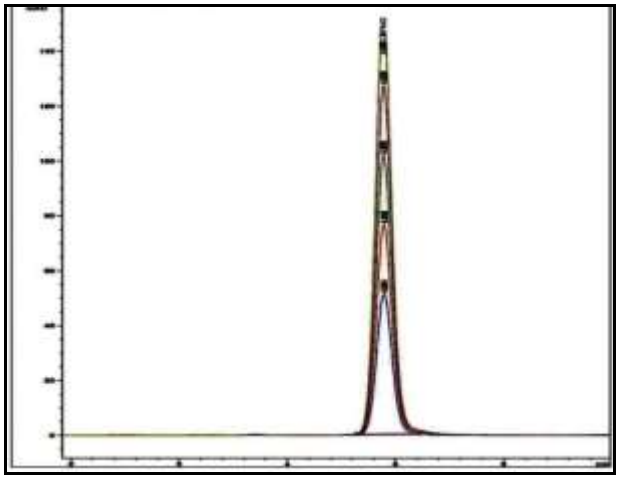

Fig.4: Robustness study of method.

Estimation of percentage purity of Eflornithine (Ornidyl)

The values of % recovery from formulation are found to be very close to the label value of commercial pharmaceutical formulation. It shows that the method is applicable for determination of DFMO from the

formulation. Applicability of the method is tested by analyzing the commercially available formulation containing sample ornidyl. The percentage recovery of Ornidyl was found 98.22%.

Results and Discussion

The objective of this RP-HPLC method was to estimate Eflornithine hydrochloride in parenteral formulation and validate it in accordance with the ICH guidelines.

In this method, the conditions were optimized to obtain good peak of eluted compound. Initially, various mobile phase compositions were tried. Mobile phase and flow rate selection was based on peak parameters (height, tailing, theoretical plates, capacity or symmetry factor, run time, resolution). The system with a ratio of 60:40 v/v methanol: water with 01 ml/min flow rate was quite robust. The optimum wavelength for detection was 254 nm at which better detector response for the drug was obtained. The average retention time for DFMO was found to be 4.8 min. System suitability tests were an integral part of chromatographic method. They were used to verify the reproducibility of the chromatographic system. To ascertain its effectiveness, system suitability tests were carried out on freshly prepared stock solutions. The calibration was linear in concentration range of 5-15 ng/ml, with regression 0.999 intercept 21.57 and slope 10.76 for DFMO. The low values of % RSD indicated that method was precise and accurate. Mean recoveries were found in the range of 97.7-99.1%. Sample to sample precision and accuracy were evaluated using five samples of five different concentrations, which were prepared and analyzed on same day. Day to day variability was assessed using five concentrations analyzed on three different days. These results showed the accuracy and reproducibility of the assay.

Ruggedness of the proposed method was determined by analysis of aliquots from homogeneous slot in different laboratories, by different analysts, using similar operational and environmental conditions. The % RSD reported was found to be less than 2 %. The proposed method was validated in accordance with ICH parameters and applied for analysis of the same in marketed formulations.

Conclusion

A newer RP-HPLC method was developed and validated for estimation of Eflornithine hydrochloride and its impurities using methanol and water. The entire compound eluted within 5 min and thus required shorter time of analysis. The method is simple, accurate, precise and robust and can be used for routine analysis of Eflornithine in its marketed formulation.

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