December 2009 Volume 8 Issue 4

Trade Science Inc.

Analytical CHEMISTRY

An Indian Journal

Full Paper

ACAIJ, 8(4) 2009 [450-454]

Estimation of eflornithine hydrochloride by UV spectroscopy

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Received: 4th August, 2009 ; Accepted: 14th August, 2009

ABSTRACT

A simple highly sensitive spectrophotometric method was developed for the quantification of Eflornithine hydrochloride (2-difluoromethyl-DL-ornithine; DFMO). U.V. Spectrophotometric estimation of Eflornithine hydrochloride (DFMO) in bulk and in Large Volume Parental (L.V.P.) formulation using 0.1M Sodium hydroxide (NaOH) solution as solvent. The absorption maxima was measured at 215.0 nm. The linearity was found in the concentration range 50.0-400.0 mcg/ml. The proposed method can be utilized as a stability indicating assay. Under the described conditions the proposed method is linear over the concentration range of 50-400 mcg/ml and the coefficient of determination were >0.999 with a relative standard deviation of 0.001 %. The average recovery of the target compound is 99.99% with a limit of quantification (LOQ) of 8.41mcg/ml and the limit of detection (LOD) 2.77mcg/ml.

KEYWORDS

Eflornithine hydrochloride (DFMO);
NaOH.

INTRODUCTION

Eflornithine is not official in any of the pharmacopoeias, the literarture survey did not reveal any analytical method for quantitative estimation of eflornithine. Thus there is a need for the development of newer effective, sensitive, accurate and economical methods of analysis for quantitative estimations of eflornithine as an active pharmaceutical ingredient.

The aim of this work was to develop a sensitive and simple spectrophotometric method for the quantification of DFMO is finding new dimensions of clinical importance. To support its investigation an appropriate analytical method (sensitive, selective, reproducible and simple) for quantification of DFMO is essential.

Eflornithine (2-fluoromethyl-DL-ornithine; MDL 71782A; DFMO) is a selective, irreversible inhibitor of ornithine decarboxylase enzyme, 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)[3-5]. It is now licensed for use in sleeping sickness in the USA, Europe and twelve African countries[6]. In African trypanosomiasis, DFMO has been approved by the FDA, USA for the treatment of the meningoencephalic stage[7,8]. DFMO currently is in development and testing for its anti inflammatory activity[3]. DFMO 13.9% cream is used to inhibit growth and reduce the amount of facial hair in women[6]. The drug development process of DFMO in these diseases is currently at a relatively early stage, and therefore the full pharmacokinetic characterization in patients, in con-

junction with pharmacodynamics (clinical efficacy/safety) is essential for optimization of drug therapy.

For the drugs that obey the beer Lambert's law, spectrophometric methods of analysis of single component in solution are usually rapid, sensitive and economical[13].

EXPERIMENTAL

Apparatus

A model Shimadzu UV-1601 double beam spectrophotometer with a fixed slit width of 2nm using a pair of 1cm matched quartz cells was used for spectrophotometric analysis.

Materials

All the chemicals were of analytical reagent grade, and the solvents were of spectroscopic grades. A eflornithine hydrochloride (2-difluoro methyl- DL-ornithine; DFMO) (Wintac Limited, Bangalore, Karnataka State, India), Distilled water and 0.1M NaOH solution.

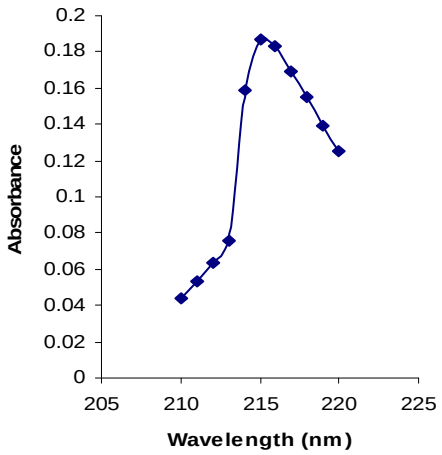
Spectrophometric method

Prepartion of standrad eflornithine hydrochloride (DFMO) solution

An accurately weighed 100.0 mg of pure drug Eflornithine hydrochloride (DFMO) was taken in clean, dry 100 ml volumetric flask and dissolved in small volume of 0.1M sodium hydroxide solution (10.0 – 20.0 ml). The solution is diluted to 100.0 ml with 0.1M sodium hydroxide solution, resulting in 1000.0 mcg/ml of drug concentration.

Determination of absorption maxima of eflornithine hydrochloride in 0.1M NaOH solution

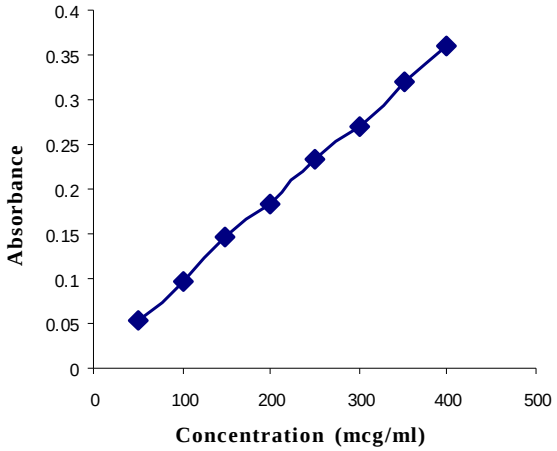
2.0 ml aliquot of Eflornithine hydrochloride (DFMO) solution of 1000 mcg/ml in 0.1 M sodium hydroxide was pipetted into 10 ml volumetric flask and volume was made up to the mark with 0.1 M sodium hydroxide solution. The final concentration of drug was 200.0 mcg/ml. The solution was then scanned in UV range between 200.0 to 400.0 nm to get absorption maxima using 0.1 M sodium hydroxide solution as blank.On scanning the absorption maxima of Eflornithine hydrochloride (DFMO) 200 mcg/ml in 0.1M sodium hydroxide was found out to be 215.0 nm. and represented graphically in Graph 1.



Graph 1 : DFMO: 200 mcg/ml in 0.1M NaOH solution.

Determination of concentration range of eflornithine hydrochloride in 0.1M NaOH solution

Aliquots of 0.5 ml, 1.0 ml, 1.5 ml, 2.0 ml, 2.5 ml, 3.0 ml, 3.5 ml and 4.0 ml of 1000.0 mcg/ml solution of Eflonithine hydrochloride (DFMO) was pipetted into each of eight 10 ml volumetric flasks. The volume was made up to 10.0 ml with 0.1M sodium hydroxide solution. The absorbance of solution was measured at 215.0 nm against 0.1M sodium hydroxide solution as blank. The abs. Vs conc. curve was found to be linear in the concentration range 50.0-400.0 mcg/ml of Eflornithine hydrochloride (DFMO) in 0.1M sodium hydroxide solution, and represented graphically in Graph 2.



Graph 2 : DFMO: 50.0 - 400.0 mcg/ml in 0.1M NaOH solution.

Preparation of standard curve of eflornithine hydrochloride in 0.1M NaOH solution

Aliquots of 0.5 ml, 1.0 ml, 1.5 ml, 2.0 ml, 2.5

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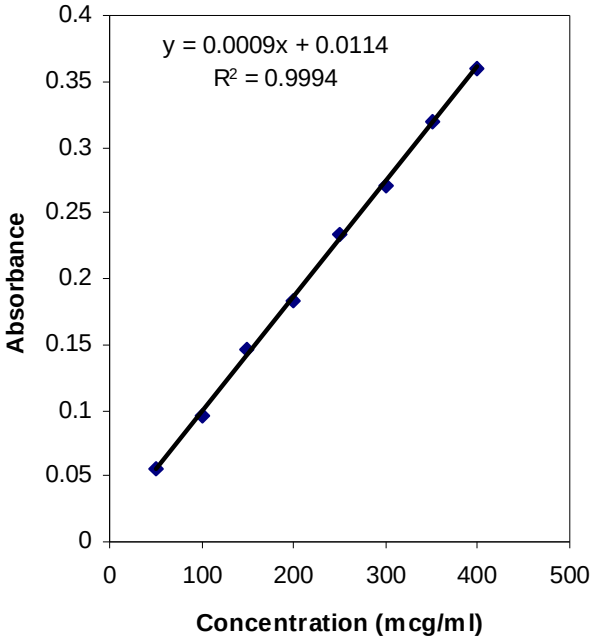
ml, 3.0 ml, 3.5 ml and 4.0 ml of 1000.0 mcg/ml solution of Eflonithine hydrochloride (DFMO) was pipetted into each of eight 10 ml volumetric flasks. The volume was made up to 10.0 ml with 0.1 M sodium hydroxide solution. The absorbance of the solution was measured at 215.0 nm against 0.1M sodium hydroxide solution as blank. The concentration C of DFMO is obtained in the range of 50-400 mcg/ml. The linear regression equation obtained from the calibration graph is A = 0.0114 + 0.0009 C (mg l-1) with a correlation coefficient of 0.9994 and a molar absorption coefficient of 9.25×10^4 $mole^{-1}$ cm^{-1}. The absorbance has been plotted as a function of the concentration of DFMO (Figure 3). The linear range of DFMO is 50-400 mcg/ml.The calibration curve shows that Beer's law was obeyed in the concentration range 50.0-400.0 mcg/ml of Eflornithine hydrochloride (DFMO) in 0.1M sodium hydroxide solution and represented graphically in Graph 3.



Graph 3 : DFMO: 50.0 - 400.0 mcg/ml in 0.1M NaOH solution.

Optical characteristics

The optical characteristics of the proposed method have been calculated. The values are given in TABLE 1.

Validation parameters

Recovery studies

Aliquots of 2.0 ml of sample drug (DFMO) solution of 1000.0 mcg/ml were pipetted into each of four 10 ml volumetric flasks. To the first three volumetric flasks 0.5 ml, 01 ml and 1.5 ml of standard drug (DFMO) solution of 500.0 mcg/ml was added respectively. The volume was made up to 10.0 ml with 0.1M sodium hydroxide solution and the absorbance was measured at 215.0 nm against reagent blank. The percentage recovery by the proposed method was ranging from 100.34 to 100.46 % indicating no interference of excipients present in the Large Volume Parental (L.V.P.) formulation.

SI.NO.	Parameters	Results
1.	Absorption maxima (nm)	215.0
2.	Beer's law limits (mcg/ml)	50.0-400.0
3.	Molar extinction coefficient ($mole^{-1}$ cm^{-1})	9.25×10^4
4.	Sandall's sensitivity (mcg/cm^2/0.001 absorbance units)	1.0414
5.	Regression equation (y) Slope (b) Intercept (a)	0.9994 0.0114 0.0009
6.	Coefficient of variance	5.3020625
7.	Standard deviation	0.0095125
8.	Limit of detection (mcg/ml)	2.7743363
9.	Limit of quantitation (mcg/ml)	8.4070796

Precision

Repeatability

100.0 mg of Eflornithine hydrochloride (DFMO) was weighed in three replicates and transferred into three clean and dry 100 ml volumetric flasks. The compound was first dissolved in small volume (10.0 – 20.0 ml) of 0.1M sodium hydroxide solution and volume was made up to 100.0 ml with 0.1M sodium hydroxide solution. 2.0 ml aliquots of the above stock solution were pipetted into three different 10.0 ml volumetric flasks and volume was made up to 10.0 ml with 0.1M sodium hydroxide solution. The absorbance of each of these solutions was recorded at 215.0 nm against reagent blank. The average percentage recovery by the proposed method was ranging from 99.99 and standard standard 0.001 % indicating good repeatability.

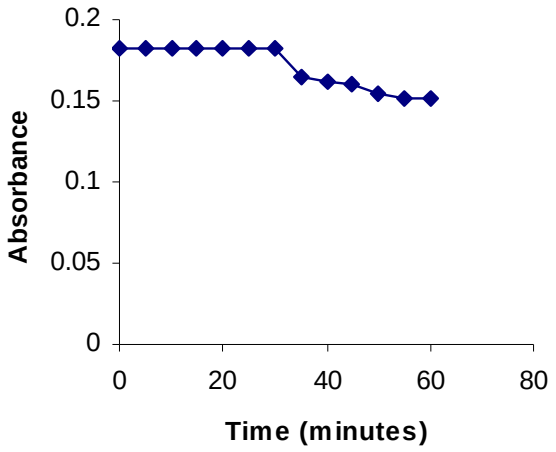
Intermediate precision

100.0 mg of Eflornithine hydrochloride (DFMO) was weighed in three replicates and transferred into three clean and dry 100 ml volumetric flasks. The compound

was first dissolved in small volume of 0.1M sodium hydroxide solution and volume was made up to 100.0 ml with 0.1M sodium hydroxide solution. 2.0 ml aliquots of each of the stock solution were pipetted into three different 10.0 ml volumetric flasks and volume was made up to 10.0 ml with 0.1M sodium hydroxide. The absorbance of each of test solutions was recorded at 215.0 nm against reagent blank. The average percentage recovery by the proposed method was ranging from 100.62 and standard standard 0.0043 % indicating good repeatability.

Stability

An aliquot of 2.0 ml standard Eflornithine hydrochloride (DFMO) solution of 1000.0 mcg/ml was pipetted into 10 ml volumetric flask. The volume was made up to 10 ml with 0.1 M sodium hydroxide solution. The absorbance was measured at 215.0 nm against reagent blank at different time intervals. The results are represents in Graph 4. The absorbance of Eflornithine hydrochloride (DFMO) solution 200 mcg/ml in 0.1M NaOH was found to be stable for 30.0 minutes after which the absorbance decreases and represented graphically in Graph 4.



Graph 4 : DFMO: 200.0 mcg/ml in 0.1M NaOH solution.

RESULT AND DISCUSSIONS

U.V. Spectrophotometric estimation of Eflornithine hydrochloride (DFMO) in bulk and in Large Volume Parental (L.V.P.) formulation using 0.1M Sodium hydroxide (NaOH) solution as solvent. The absorption maxima was measured at 215.0 nm. The linearity was found in the concentration range 50.0-400.0 mcg/ml. The Sandell's sensitivity was found to be 1.0414 mcg/cm^2 0.001 absorbance units and Molar absortivity 9.25×10^4 mol^{-1}cm^{-1}. The total concentration of DFMO can be calculated using the corresponding correlation equation with a correlation coefficient (r) = 0.999 for n=6 with LOD and LOQ 2.7743863 mcg/ml and 8.4070796 (mcg/ml), indicated accuracy and reproducibility of the method. The Standard deviation of 0.0095125.The method was extended for the determination in Large Volume Parental (L.V.P.) formulation. It was observed that the results obtained were comparable to that of Lable claim. The recovery studies of the standard drug when performed in the preanalysed formulation gave percentage recovery of 100.34% to 100.46% indicating practically no interference of formulation excipients with the proposed method.The precision of the proposed method was studied by determination of the drug in six replicates, individually at concentration of 200 mcg/ ml obtaining relative standard deviations of 0.001% (TABLE 2). The absorbance of Eflornithine hydrochloride (DFMO) solution 200 mcg/ml in 0.1M NaOH was found to be stable for 30.0 minutes after which the absorbance decreases.

CONCLUSION

It was found that no spectrophotometric methods were available for the estimation of Eflornithine hydrochloride (DFMO) in bulk and in formulations. Eflornithine hydrochloride is available as large volume parental (L.V.P.) formulation.

The new method developed was New Simple, Accurate, Sensitivity, Economical and Reproducible which could provide satisfactory results. The methods can be used for routine analysis of Eflornithine hydrochloride in bulk and in formulation. The methods are practical and valuable.

The described method has many advantages: it does not need expensive apparatus; it is simple and quick; its linear range is relatively wide, it has good selectivity. Furthermore, the proposed method may be successfully used to determine Eflornithine in bulk and in pharmaceutical formulations. Accordingly, the method is practical and valuable.

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