

Original Article

Spectrophotometric determination of Eflornithine hydrochloride through schiff's base system using PDAB reagent in pharmaceutical preparation

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ABSTRACT

A rapid, economic and highly sensitive spectrophotometric method was developed for the quantification of eflornithine hydrochloride (DFMO). The method involves the reaction DFMO with para-dimethylamino benzaldehyde (PDAB) in acidic medium (pH 5.4) which results in an orange-coloured product exhibiting maximum absorbance λ_{\max} at 563. The proposed method can be utilized as a stability indicating assay. The different experimental parameters affecting the derivatization reaction were carefully studied and incorporated into the procedure. Under the described conditions the apparent molar absorption coefficient (ϵ_{563}) is 4.9×10^3 L/mol cm. The linear equation is $Y = 1.60 + 0.038X$ ($\mu\text{g/mL}$) in the range of 5–40 $\mu\text{g/mL}$ of DFMO with a correlation coefficient $r = 0.9992$, and the detection limit (LOD) is 11.842 $\mu\text{g/mL}$. The average recovery of the target compound is 100.07% with a limit of quantification (LOQ) of 39.47 $\mu\text{g/mL}$. The mechanism of the derivatization reaction is proposed and advantages of the proposed method are discussed. The method was validated in terms of accuracy and precision and was successfully applied to the determination of DFMO in its pharmaceutical dosage form. The proposed method is useful for routine analysis of DFMO in quality control laboratories.

1. INTRODUCTION

Eflornithine hydrochloride belongs to a class of antiprotozoal. Its chemical designation is (RS)-2,5-diamino-2-(difluoromethyl) pentanoic acid (Fig.1). Eflornithine hydrochloride (difluoromethylornithine, 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 gambiense* (African trypanosomiasis) [3, 4, 5].

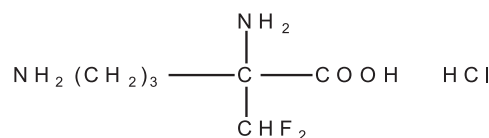


Fig. 1. Chemical structure of Eflornithine hydrochloride.

A number of analytical methods have been reported for measuring DFMO in biological fluids and tissue extracts. These methods involved 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 [9-11] and LC carried out by evaporative light scattering detection. A reverse HPLC method utilizing pre-

column dansylation was described for the analysis of DFMO in serum [11]. Derivatization for at least 04 hrs was necessary for maximum derivative formation. All the above mentioned methods are either long procedures or require sophisticated sample preparation or chromatographic procedures [6-11]. The quality control of active pharmaceutical ingredients (APIs) in the formulation is always a thrust area for the pharmaceutical industries. So the development of reproducible, sensitive, simple and extremely inexpensive methods for the determination of APIs in the formulation is always challenging.

Spectrophotometry is considered the most convenient analytical technique, because of its inherent simplicity, low cost and wide availability in most quality control laboratories. In this paper, we report new novel spectrophotometric method for the determination of DFMO in pharmaceutical formulations in accordance with ICH recommendations [12, 13].

2. EXPERIMENTAL

Material and Method

All the solvents and chemicals were of analytical reagent grade and were supplied by Sigma-Aldrich and Qualigens fine chemicals, India. Eflornithine hydrochloride is marketed under the trade name Ornidyl. Each sample vial (SVP) is containing 200 mg mL⁻¹. The pure drug (DFMO) was gifted by Wintac Limited, Bangalore, India. HPLC grade water was used through this study. A stock solution of 0.5% w/v para-dimethylaminobenzaldehyde (PDAB) was prepared by dissolving 500 mg in ethanol and diluting it to 100 mL. Buffer solution of pH 5.4 was prepared by mixing 25 mL solution of NaH₂PO₄ (0.2M) and 66 mL solution of NaOH (0.2M) in 100 mL volumetric flask, pH was adjusted by pH meter model of Digisun D1-707 (HANNA, Italy). Other buffer solutions of different pH values were also prepared.

Apparatus

Spectrophotometric measurements were carried out on a UV-1700 Shimadzu double beam spectrophotometer (Japan) with a fixed slit width of 2 nm using a pair of 1cm matched quartz cells. All pH measurements were made with Digisun D1-707 digital pH meter.

Preparation of Stock Solutions of DFMO

The stock solution of eflornithine hydrochloride (DFMO) 1 mg/mL was prepared in ethanol and further diluted with ethanol to prepare working standard solution 5-40 µg/mL.

Preparation of sample solution

Sample Ornidyl (1 mg/mL) solution (Label claim, each fill volume of the vial contains 100 ml and each ml contains 200 mg) was prepared by pipetting out 0.5 ml of the contents of the vial into a 100 mL volumetric flask, containing 50 mL ethanol, shaken for 15 min, and filled up to the volume with ethanol. The prepared solution was further diluted with ethanol to obtain a suitable concentration for the analysis.

Determination of eflornithine (DFMO) using PDAB

Accurate aliquots containing (5-40 µg/mL) of DFMO standard solution were transferred from the stock standard solution into 25 mL calibrated flasks, followed by addition of 10 mL of buffer solution (pH 5.4), 3.5 mL of PDAB solution. After mixing the flasks were kept on water bath for 30 min at 90 °C, filling up to the mark with ethanol and mixed. The absorbance of each solution was measured at 563 nm against the reagent blank. UV-vis linear spectra were recorded in 1 cm path length quartz cuvettes on a shimadzu UV-1700 uv/vis double beam spectrophotometer (Kyoto, Japan). The (Figure 2 (2a,2b) shows the calibration curves. pH value was measured by model of Digisun D1-707 (HANNA, Italy). Data processing was performed with Sigmaplot software.

3. RESULTS AND DISCUSSION

The absorption spectrum of DFMO recorded against ethanol maximum showed absorption peak (λ_{\max})

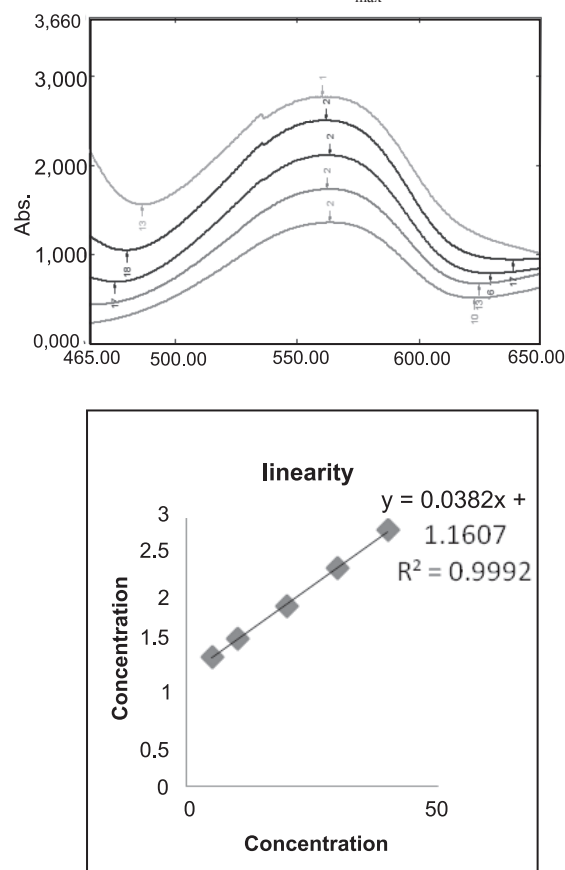


Fig. 2. Zero-order calibration spectra for DFMO showing λ_{\max} at 563 nm (2a) and calibration curve of DFMO (2b).

at 563 nm (Fig. 3), which means that its determination in the dosage forms based on the direct measurement of its absorption in the uv/vis spectral range. Therefore, derivatization of DFMO with PDAB (λ_{\max} =563 nm) was performed under the described optimal experimental conditions which was used for all the measurements. The relative absorption intensity was found to be linearly correlated to DFMO concentration.

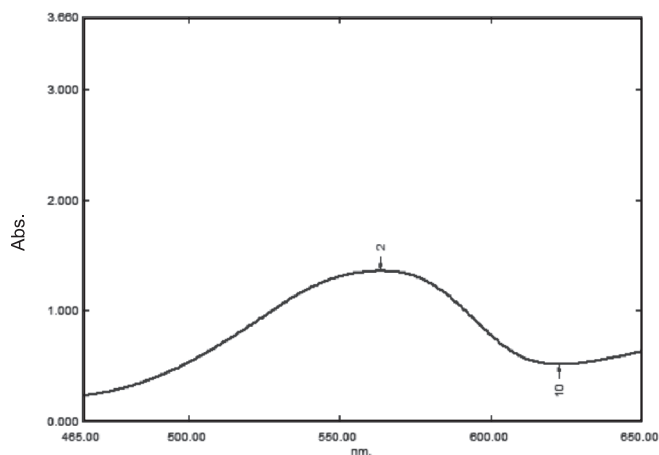


Fig. 3. Absorption spectra of DFMO (10 µg/mL).

Results indicate that the reaction was dependent on the PDAB concentration with the highest absorption at PDAB concentration of 0.5% (w/v), while higher concentrations of PDAB up to 1% had no effect on the absorption (Fig. 4). Therefore, a concentration of 0.5% PDAB was considered optimum.

To generate the nucleophile from DFMO and activate the nucleophilic substitution reaction, alkaline medium was required. The influence of pH on the absorbance of DFMO–PDAB product was investigated. The results revealed that the absorbance at pH < 6 were close to 0, indicating that in acidic media DFMO has

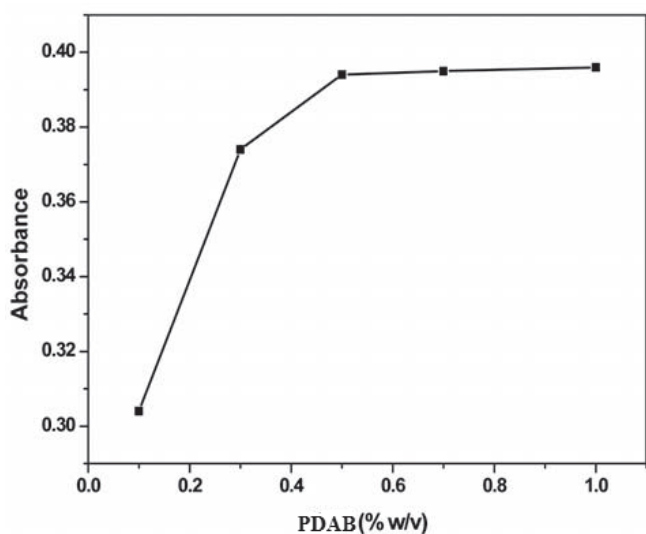


Fig. 4. Effect of PDAB concentration on the reaction of DFMO with PDAB (1 mL DFMO solution 10 µg/mL, 10 mL buffer solution pH 5.4, 3.5 mL PDAB) at T = 90°C and reaction time 30 min.

difficulty to react with PDAB (Fig. 5). The effect of reaction time on the formation of the reaction product at room temperature was investigated in the range of 5–55 min (Fig. 5). The experimental results show that DFMO reacts immediately with PDAB. Subsequently, the absorbance begins to decrease after 30 min and this time was selected as the optimal. Heating of the solution is necessary for DFMO–PDAB complex formation, which can clearly be seen by change from yellow to orange. Colour of the

DFMO–PDAB complex develops during 30 min of heating on a water bath at 90°C. The influence of heating time on DFMO–PDAB complex formation is shown in (Fig. 6).

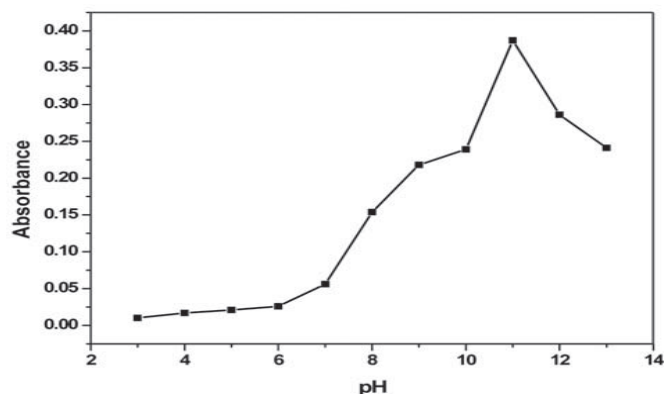


Fig. 5. Effect of pH on the reaction of DFMO with PDAB (DFMO 10 µg/mL, 1 mL buffer solution, 1 mL PDAB) at T = 25°C and reaction time 30 min.

It was reported [14], that PDAB could react with the amino group of primary amino derivative. Alpha amino group of DFMO displays nucleophilicity due to the fact that its lone pairs of electrons of nitrogen can attack the electron deficient center. Hence DFMO can react with PDAB in a condensation reaction. PDAB to form Schiff's base, the possible reaction equation is as

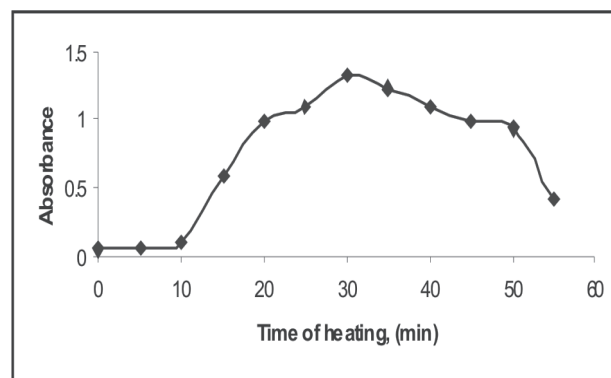


Fig. 6. Influence of heating time on DFMO–PDAB complex formation, (DFMO 10 µg/mL, 10 mL buffer solution, 3.5 mL PDAB) at T = 90 °C and reaction time 30 min.

follows (Fig. 7), indicating that the degree of the nucleophilic substitution reaction is maximal. The reason behind this change is that the protonated amine salt of DFMO turns back into amino group when the pH is increased. The higher the pH, the more the protonated amino group becomes free amino group, and the more easily the nucleophilic substitution reaction proceeds. However, when pH is higher than 6 the absorbance of the system of DFMO–PDAB decrease. Hydroxide ion has good nucleophilic ability and can hold back the nucleophilic substitution reaction between DFMO and PDAB, resulting in the descent of the absorbance of each system. Therefore, pH 5.4 was selected for the optimal experimental conditions.

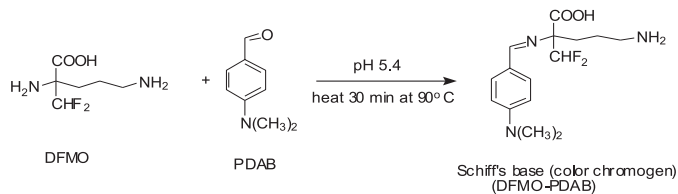


Fig. 7. The proposed reactions involved in formation of colored Schiff's bases (DFMO-PDAB) in acidic medium.

Reaction mechanism, PDAB is a highly sensitive fluorogenic and chromogenic reagent widely used for derivatization of amines. DFMO contains a primary amino group, which is a suitable candidate for derivatization by PDAB. So DFMO can react with PDAB in a nucleophilic substitution reaction. At the same time, it has been proved by the Job's method that, the composition of the product is 1:1. So it is concluded that one amino group of DFMO substitutes the 4C of one PDAB molecule to form orange compound. Validation of the Method, Calibration curves for the method was linear in the ranges 5–40 µg/mL with regression equations $Y = 1.160 + 0.038X$ ($r = 0.9992$). The molar absorptivity (ε563) at 563 nm for Method was 4.9×10^3 L/mol cm. LOD, LOQ values were found to be 11.842 and 39.47 µg/mL, respectively. All analytical parameters are shown in (Table 1).

Table 1. Analytical parameters of proposed method.

Analytical parameters	DFMO-PDAB complex
λ _{max} (nm)	563
Beer's law limit (µg/mL)	5-40
Molar absorptivity (L/mol/cm)	4.9×10 ⁴
Sandell's sensitivity (µg/cm)	0.1518
Regression equation* $Y = mC + b$	
Intercept (b)	1.160
Slope (m)	0.038
Correlation coefficient** (r)	0.9992
LOD (µg/mL)	11.842
LOQ (µg/mL)	39.47
SD	0.001-0.006
RSD (%)	1.30-1.42
Percentage recovery (%)	99.80-100.28

* $Y = mC + X$, where Y is the absorbance and X is the concentration of the drug in µg/mL.

** Average of six determinations.

Accuracy was within 1.76% for method respectively, with corresponding intra-day precision expressed as RSD was < 1.6% for the method. The results are compiled in (Table 2). The inter-day precision expressed as RSD was < 3.7% reflecting the validity of the method for routine analysis in quality control laboratories.

Table 2. Evaluation of accuracy and precision.

Amount of DFMO standard (µg/mL)	Amount of DFMO found (µg/mL)	Range	Relative error %	SD	RSD (%) (n=5)*
1.7	1.67	0.012	1.76	0.006	1.30
2.2	2.19	0.020	0.45	0.008	1.50
2.7	2.69	0.020	0.37	0.001	1.42

* Average of five determinations.

Recovery studies of the proposed method to be studied. To a known amount of the drug in the dosage form, pure drug (the standard) was added at three different levels and the total was found by the proposed method. Each test was performed in triplicate. The percent recoveries of DFMO were in the ranges of 99.80–100.28 for method, respectively (Table 3). This shows the absence of interference from vials excipients.

Table 3. Results of recovery study by standard-addition methods, using Ornidyl vials (200 mg).

Amount of DFMO in formulation, (µg)	Amount of standard DFMO added, (µg)	Total found, (µg)	% recovery of pure drug ± SD (n=3)*
1.0	0.5	1.497	99.80 ± 0.008
1.0	1.0	2.003	100.15 ± 0.002
1.0	1.5	2.507	100.28 ± 0.002

* Average of three determinations.

Analysis of DFMO in Dosage Forms is performed. It is evident from the above mentioned results that the proposed method gave satisfactory results for DFMO. Thus its pharmaceutical dosage forms (vials) were subjected to the analysis of their DFMO contents by the proposed method. The label claim percentage was $99.50 \pm 0.96\%$ for method respectively (Table 4).

4. CONCLUSION

In this study, we have successfully developed a new novel spectrophotometric method for the determination of DFMO in its dosage form. The method is sensitive, selective, rapid and economical for routine analysis. Furthermore, the entire analytical reagents are inexpensive, have excellent

Table 4. Results of analysis of dosage forms in Ornidyl vials containing DFMO.

Label claim, mg/vial	Amount found mg/vial	% found ± RSD (n=5)
200	199.01	99.50 ± 0.96

shelf life, and are available in any analytical laboratory. The method is practically and valuable for routine application in quality control laboratories for analysis of DFMO.

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