

Original Article

Quantitative estimation of Eflornithine by Nitrite (Diazotization) titration

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ABSTRACT

Eflornithine hydrochloride (DFMO) being a primary aliphatic amine reacts with the nitrous acid (in situ liberation by the reaction of ice cold sodium nitrite and hydrochloric acid) and gets converted to diazonium salt. At the end point an excess drop of titrant sodium nitrite from the burette liberates nitrous acid; this free acid is determined by the starch-mucilage paper, used as an external indicator. The free nitrous acid reacts with potassium iodide and reduces the iodide to free iodine, which produces intense blue color with the alpha-amylase portion of the starch indicating the end point. The Eflornithine hydrochloride (DFMO, Difluoromethylornithine) determined by Diazotization Titration (DAT M-01). Here the amino group of Eflornithine is diazotized with sodium nitrite solution in cold acid solution. The nitrous acid formed diazotizes the compound. The iodine formed reacts with starch mucilage to give the blue color. The end point can be determined by using starch iodide paper as external indicator. The percentage of purity in DFMO is found 99.74%.

1. INTRODUCTION

Eflornithine, DL-alpha-difluoromethylornithine (DFMO; Ornidyl) is a selective, irreversible inhibitor of ornithine decarboxylase enzyme and one of the key enzymes in the polyamine biosynthetic pathway [1,2]. The drug is originally developed to use in cancer and in phase III clinical trials for 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]. It is now licensed for use in sleeping sickness in USA, Europe and twelve African countries [6,7]. In African trypanosomiasis, DFMO has been approved by the FDA, USA for the treatment of the meningoencephalic stage [8, 9]. DFMO currently used in development and testing for its anti-inflammatory activity [10]. DFMO 13.9% cream is used to inhibit growth and reduce the amount of facial hair in women [11, 12]. 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 conjunction with pharmacodynamics (clinical efficacy/safety) is essential for optimization of drug therapy.

A number of analytical methods have been reported for measuring DFMO in biological fluids and tissue extracts. These methods involved HPLC techniques [13,14,15]. 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 [16] and LC carried out by evaporative light scattering detection.

A reverse HPLC method utilizing pre-column dansylation is described for the analysis of DFMO in serum. Derivatization is necessary at least 04 hrs is necessary for maximum derivative formation. All the above mentioned methods are either long procedures or require sophisticated sample preparation or chromatographic procedures [13,14,15,17,18].

2. EXPERIMENTAL

2.1 Material and Methods

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 (Ornidyl) vial (SVP) is containing 200 mg/ml. The pure drug (DFMO) was gifted by Wintac Limited, Bangalore, India. Distilled water was used through this study.

2.2 Preparation and standardization of 0.1 M Sodium Nitrite solution

7.5 gm of sodium nitrite is dissolved in sufficient water to produce 1000 ml and is standardized. About 0.5 gm of sulphanilamide, previously dried at 105°C for three hours is transferred to a suitable beaker. 50 ml of water and 20 ml of hydrochloric acid is added, stirred until it dissolves and cooled to 5°C. The contents of the beaker are titrated against 0.1M sodium nitrite solution [19]. The standardization reaction of nitrite titration is shown in Fig. 1.

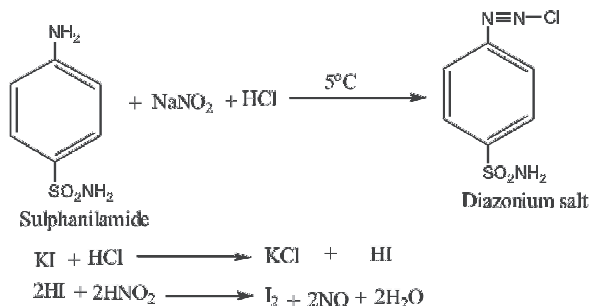


Fig. 1. Standardization reaction of nitrite titration

2.3 Estimation of % purity of Eflornithine (DFMO)

Weigh accurately 0.5234 g DFMO and transferred into a conical flask, dissolved in about 25 ml of water and 15 ml of 2 M hydrochloric acid and cool to about 5°C. The mixture is titrated against 0.1M sodium nitrite solution by using external indicator as starch iodide solution until blue color appears. Each ml of 0.1M sodium nitrite solution is equivalent to 0.021865 g of DFMO. The volume of 0.1M sodium nitrite solution 26.53 ml (average volume of three readings) is consumed by 0.5234 g DFMO (Average weight of three weighing). The % Purity of Eflornithine is found 99.74%, indicated the robustness of the method.

2.4 Assay Reaction of Nitrite Titration

DFMO being a primary aliphatic amine reacts with the nitrous acid (in situ liberation by the reaction of ice cold sodium nitrite and hydrochloric acid) and gets converted to diazonium salt. At the end point an excess drop of titrant sodium nitrite from the burette liberates nitrous acid; this free acid is determined by the starch-mucilage paper, used as an external indicator. The free nitrous acid reacts with potassium iodide and reduces the iodide

to free iodine, which produces intense blue color with the alpha-amylase portion of the starch indicating the end point. The assay reaction of nitrite titration is shown in Fig. 2.

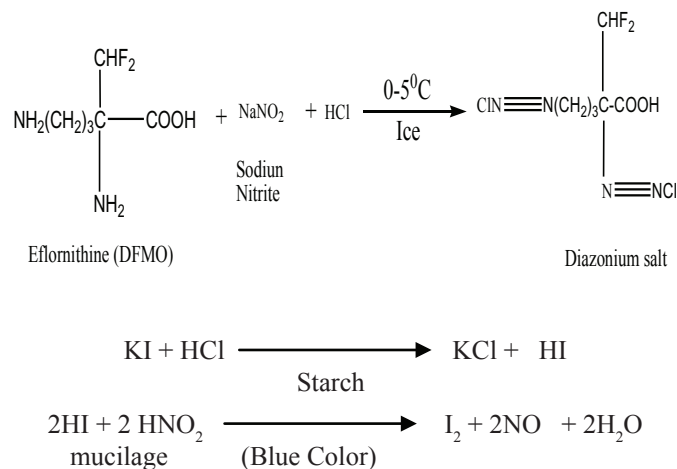


Fig. 2. Assay reaction of the nitrite titration.

3. RESULTS AND DISCUSSION

Reaction mechanism of diazotization titration

Titrant: standardized 0.1N NaNO2

Conical Flask: acidified solution of DFMO

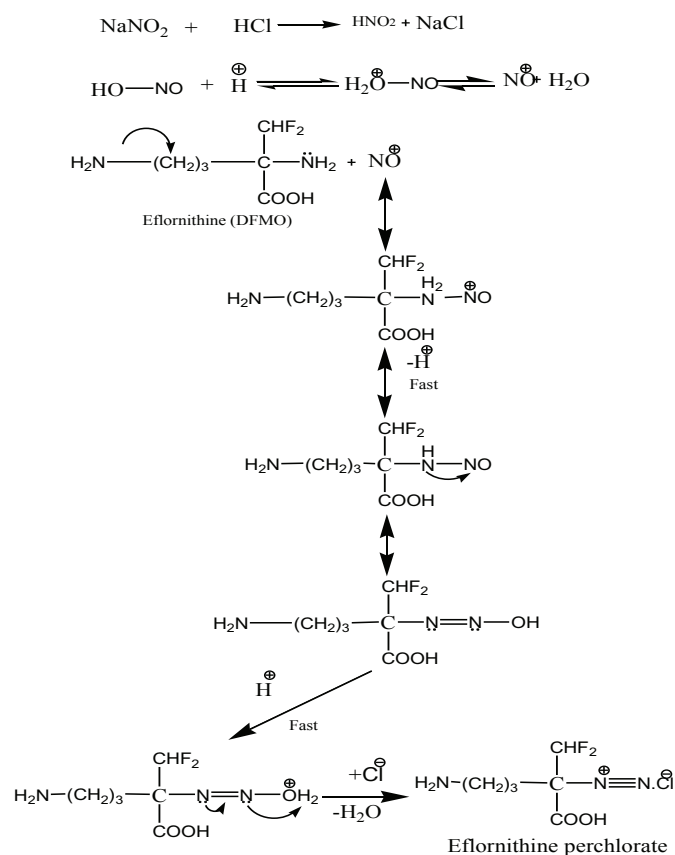


Fig. 3. Assay reaction mechanism of Eflornithine.

Sodium nitrate reacts with the hydrochloric acid to produce the nitrous acid (in situ), which in turn generates the nitrosyl ion. This nitrosyl ion being an electrophile, it attaches to the electron rich primary amino group of the DFMO (nitrogen of the primary amino group contains the lone pair electrons) to form the N-nitrosyl derivative, which rearranges to form resonance stabilized diazonium salt after the elimination of water molecule. (Inductive effect coupled with the electro-negativity of the both oxygen of the carboxylic acid group and the fluorine of the difluoro methyne group influences the attack of the nitrosyl group with the amino group, thus the amino group of the carbon bearing both amino and carboxylic acid group is preferably attacked). At the end point an excess drop of sodium nitrite produces the nitrous acid; this will react with the potassium iodide to produce the iodine which is detected by the starch mucilage paper used as the external indicator. The assay reaction mechanism of Eflornithine (DFMO) is shown in Fig. 3.

4. CONCLUSION

In this study, we have successfully developed a new novel nitrite 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 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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