Quantitatively Determination of Eflornithine HCl by Non-aqueous Titration
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ABSTRACT
A simple volumetric method for the estimation of weak base (Eflornithine Hydrochloride, DFMO), using non-aqueous titration with non-aqueous solvent is described. Weak base such as DFMO can also be titrated with sharp end point in the same media. This method consists of dissolving the DFMO in non–aqueous solvent such as glacial acetic acid and titrated with perchloric acid in the same solvent, the end point being determined with the help of crystal-violet.

DFMO being a weak base, its basicity is potentiated using the glacial acetic acid, which acts as an amphoteric solvent. Under these conditions the DFMO is titrated with the standardized acetic perchloric acid using crystal violet solution as an indicator. DFMO reacts with the acetic perchloric acid to form perchlorate salt of DFMO. At the end point the pH change changes the color of the indicator to emerald green.

Key Words: Carbonitrile, Antimicrobial activity, benzaldehydes

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 meningoencephalitic stage of trypanosomiasis caused by Trypanosoma brucei gambiensae (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 meningoencephalitic 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\textsuperscript{[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.\textsuperscript{[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.\textsuperscript{[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.\textsuperscript{[13,14,15,17,18]}

2. EXPERIMENTAL

Material and Method

All the solvents and chemicals are of analytical reagent grade and are 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) is gifted by Wintac Limited, Bangalore, India. Distilled is used through this study.

**Preparation of 0.1 M Perchloric acid solution.**

Mix 8.5 ml of perchloric acid (72\%) with 500 ml of glacial acetic acid and 21ml of acetic anhydride, cool and add glacial acetic acid to make 1000 ml and allow to stand for 24 hours before use\textsuperscript{19}.

**Standardization of 0.1 M perchloric acid solution.**

Weigh accurately 0.7g of potassium hydrogen phthalate, previously powdered lightly and dried for 2 hours and dissolve it in 50 ml of glacial acetic acid. Add few drops of crystal violet solution as indicator and titrate with 0.1M perchloric acid solution until the violet color changes to emerald green.\textsuperscript{19}

**Standardization reaction:**
Estimation of percentage purity of Eflornithine (DFMO)

Weigh accurately about 0.3242 g DFMO (average weight of three weighing) and dissolve in 30 ml glacial acetic acid. Add 2-3 drops of crystal violet solution as indicator and titrated with 0.1N perchloric acid until the solution become yellowish green. Each ml of 0.1N perchloric acid is equivalent to 0.021865 g of DFMO. The volume of 0.1N perchloric acid 16.43 ml (average volume of three readings) is consumed by 16.43 g DFMO (Average weight of three weighing). The percentage purity of Eflornithine is found 99.72%, indicated the robustness and precise of the method.

ASSAY REACTION of non-aqueous TITRATION

DFMO reacts with the acetous perchloric acid to form perchlorate salt of DFMO. At the end point the pH change changes the color of the indicator to emerald green. The assay reaction of non-aqueous titration is shown in Figure NAT M-01.01.

Fig. NAT M-01.01. Assay reaction of the non-aqueous titration.
generate the acetoniun ion, which reacts with the leuco-base form of crystal violet (violet in color) and produces the quinoidal form of the crystal violet (emerald green in color) after the abstraction of water molecule from leuco-base form of crystal violet. The assay reaction mechanism of Eflornithine (DFMO) is shown in Figure NAT M-01.02.

**Figure NAT M-01.02. Assay reaction mechanism of Eflornithine.**

[Chemical structures and reaction mechanisms as shown in the image]

**REFERENCES**


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