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METHOD DEVELOPMENT & VALIDATION OF EFLORNITHINE FROM PARENTERAL FORMULATION BY USING UV SPECTROPHOTOMETRIC TECHNOLOGY

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ABSTRACT

The work details the creation and validation of a new UV spectroscopy procedure for analyzing eflornithine hydrochloride (DFMO) in parenteral forms. Key aspects of the method include: - **Wavelength and Solvent**: Measurements were conducted at a wavelength of 283 nm using ethanol. -**Beer's Law**: The method follows Beer's law in the concentration range of 4to 32 µg/mL with a correlation coefficient of 1. - **Detection Limits**: The limit of detection (LOD) was 0.0675 µg/mL and the limit of quantification (LOQ) was 0.2045 µg/mL. - **Molar Absorptivity and Sensitivity**: Molar absorptivity was 0.3918 × 10^4 L/mol·cm and Sandell's sensitivity was 0.1633 µg/cm^2. -**Validation**: The method was validated following International Conference on Harmonisation (ICH) guidelines for specificity, linearity, accuracy, precision, and robustness. The method showed 100.23% accuracy and less than 1%relative standard deviation in precision. - **Applications**: The method has been successfully applied to both commercial formulations and laboratory-prepared mixtures, demonstrating its suitability for routine quality control in the pharmaceutical industry due to its precision, accuracy, simplicity.

Keywords: Eflornithine Hydrochloride, Uv Spectrophotometric, Pharmaceutical Preparation.

I. INTRODUCTION

Eflornithine Hydrochloride, also known as DL-alpha-Difluoromethylornithine (DFMO), has the IUPAC name 2,5-diamino-2-(difluoromethyl)pentanoic acid.[1,2] Eflornithine is a specific, irreversible inhibitor of ornithine decarboxylase, a key enzyme in the polyamine biosynthetic pathway.[3,4]The drug was originally developed for cancer treatment and is currently in phase III clinical trials to prevent the recurrence of superficial bladder cancer. Additionally, it has been used as an antiprotozoal agent for treating the meningoencephalitis stage of trypanosomiasis caused by *Trypanosoma brucei gambiense* (African Trypanosomiasis).[5,6]The HPLC techniques available for quantifying DFMO in biological fluids typically involve either pre- or post-column derivatization with UV or fluorescence detection.[7,8]The drug was initially developed for cancer treatment and is currently in phase III clinical trials for preventing the recurrence of superficial bladder cancer. Additionally, it is used as an antiprotozoal agent for treating the meningoencephalitis stage of trypanosomiasis caused by *Trypanosoma brucei gambiense* (African trypanosomiasis).[9,10,11]Quality control of active pharmaceutical ingredients(APIs) in formulations is a critical focus for the pharmaceutical industry.Developing reproducible, sensitive, simple, and cost-effective methods for APIdetermination remains challenging. Spectrophotometry is favored for its simplicity, low cost, and widespread availability in quality control labs. Thispaper presents a novel spectrophotometric method for determining DFMO pharmaceutical formulations, adhering to ICH recommendation.[12,13]

INDICATIONS

Eflornithine is indicated to reduce relapse risk in adult and pediatric patients with high-risk neuroblastoma (HRNB) who have shown at least a partial response to prior multi-agent therapies, including anti-GD2 immunotherapy. Previously, it was also used for female hirsutism and Africantrypanosomiasis, but those indications have since been discontinued.[14]

PHARMACODYNAMICS

Eflornithine's inhibition of polyamine synthesis restores the balance of theLIN28/Let-7 pathway, crucial for regulating cancer stem cells and glycolytic metabolism. This leads to decreased expression of oncogenic drivers MYCNand LIN28B in MYCN-amplified neuroblastoma. In vitro, eflornithine induces senescence and inhibits

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neurosphere formation in both MYCN-amplified and non-amplified neuroblastoma cells, demonstrating a cytostatic effect. In vivo,it prevents or delays tumor formation in mice injected with MYCN-amplified neuroblastoma cells, highlighting its potential as a therapeutic agent.[15]Polyamines play a crucial role in keratin synthesis, and their inhibition can reduce the proliferation of hair matrix cells. This suppression impacts the anagen phase of hair production, effectively inhibiting hair growth.[16]

MECHANISM OF ACTION

Eflornithine is an irreversible inhibitor of ornithine decarboxylase (ODC), the first and rate-limiting enzyme in polyamine biosynthesis and a transcriptional target of MYCN. By inhibiting ODC, eflornithine disrupts polyamine synthesis,which is crucial for cell differentiation, proliferation, and neoplastic transformation. This mechanism is particularly relevant in contexts like MYCN-amplified neuroblastoma, where polyamines contribute to tumor growth and survival.[17]

ABSORPTION

After oral administration, eflornithine reaches peak plasma concentrations(Cmax) at approximately 3.5 hours (Tmax). Food, including high-fat and high-calorie meals, does not impact the Cmax or AUC (area under the concentration-time curve) of eflornithine. Additionally, administering crushed tablets mixed in standard pudding does not alter eflornithine exposure(Cmax and AUC over 6 hours).[18] In women with unwanted facial hair, the mean percutaneous absorption of eflornithine from a 13.9% w/w cream is less than 1% of the radioactive dose, even after shaving and other hair removal methods. Steady state is achieved within four days of twice-daily application.When 0.5 g of the cream is applied twice daily (total 1.0 g/day), the steady-state concentrations are approximately 10 ng/mL (Cmax), 5 ng/mL (Ctrough),and 92 ng hr/mL (AUC12hr). Compared to a once-daily oral dose of 370 mg,the dose-normalized peak concentrations (Cmax) and systemic exposure(AUC) are roughly 100-fold and 60-fold lower, respectively.[19]

TOXICITY

Eflornithine can pose risks during pregnancy, as animal studies indicate that oral administration to pregnant rats and rabbits during organogenesis leads to embryo lethality at doses equivalent to the recommended human dose.There are no data on its use in pregnant women, so it is essential to advise

pregnant women and those of reproductive potential about potential fetal

harm.In a 2-year carcinogenicity study, daily oral administration of eflornithine to female rats did not lead to drug-related tumors at doses up to 600 mg/kg/day, which is about 10.5 times the human Cmax at the recommended clinical dose. Additionally, eflornithine was not found to be mutagenic in the in vitro Ames assay. However, dedicated fertility studies have not been conducted.[20]

SYNTHESIS

The scalable telescoped continuous flow procedure developed for the difluoromethylation of a protected amino acid using fluoroform (CHF3) gasleads to the efficient synthesis of eflornithine, an essential medicine for treating sleeping sickness and hirsutism. Fluoroform, a non-toxic byproduct of polytetrafluoroethylene (PTFE) production, offers a sustainable alternative to chlorodifluoromethane (CHClF2), which is being phased out due to environmental concerns. The laboratory-optimized process enhances product yield and scalability, achieving an impressive 86% isolated yield over two steps in just 4 hours, with a throughput of 24 mmol/h. This method not only aligns with regulatory requirements but also minimizes environmental impact,making it a promising route for eflornithine production.

II. CONCLUSION

Purity, and are readily available, making the method accessible for routine laboratory use. The developed spectrophotometric technique offers a reliable alternative to existing methods, with minimal sample preparation required.Validation studies demonstrate its accuracy and precision, ensuring that it meets the necessary regulatory standards for pharmaceutical analysis.Overall, this method represents a significant advancement in the determination of DFMO in dosage forms, facilitating better quality control in pharmaceutical settings.

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[18] FDA Approved Drug Products: IWILFIN™ (eflornithine) tablets, for oral use

[19] FDA Approved Drug Products: VANIQA™ (eflornithine hydrochloride) Cream 13.9%, for topical use

[20] FDA Approved Drug Products: VANIQA™ (eflornithine hydrochloride) Cream 13.9%, for topical use