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DEVELOPMENT AND VALIDATION OF UV-SPECTROPHOTOMETER ANALYTICAL METHOD OF EFLORNITHINE HYDROCHLORIDE

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Abstract

The objective of present analysis is development and validation of UV-spectrophotometer analytical technique for quantitative analysis of Eflornithine hydrochloride (EFH). The standard curve was plotted for EFH concentration range of 0.5-4.5 µg/ml. The analytical method was validated for linearity, accuracy, robustness and intra-day as well as inter-day precision. Limit of detection (LOD) and limit of quantification (LOQ) was determined for sensitivity determination. Regression equation obtained from calibration curve was y = 0.2221 × -0.016. Developed analytical method for EFH was found linear in concentration range of 0.5-4.5 µg/ml with high correlation coefficient of 0.9992. Mean percentage recovery was in range of 98-102% which validated the accuracy of the technique. Method exhibited robustness, intra-day and inter-day precision as demonstrated by relative standard deviation <2%. LOD and LOQ of EFH were found 0.01716 and 0.052 µg/ml, respectively. It was concluded that developed UV spectrophotometer method was accurate, precise, linear, robust and sensitive; therefore, it was concluded that developed UV-spectrophotometer methodology using dansyl chloride as derivatizing agent could be employed for regular assessment and quantifiable evaluation of the EFH.

Key words: Eflornithine hydrochloride, Accuracy, Robustness, Limit of Detection, Limit of Quantification.

Introduction

UV-spectrophotometer is typically used to obtain specific information on the chromophoric portion of the molecules for structural details of different drugs which on subjected to ultraviolet light absorbs specific wavelength based on the type of electronic shift involved with the absorption [Jordaln, C., R. Ohen, K. Icharjd, L.L. Alicet, D. Mark, N.D. ShieldsA and G. Thomams (1989) 'High-pressure Liquid Chromatographic Analysis of Eflornithine hydrochloride in Serum', *Journal of Pharmaceutical Sciences*, 78(2): 114-116]. The analytical procedure validation was typically implemented in attempt to validate that the experimental procedure used during such tests satisfies the required criteria and could be considered to determine the intensity, specifications, consistency, potency and quality of pharmaceutical products together with the accuracy of analysis findings (Carr *et al*., 1990). Eflornithine hydrochloride (EFH) is chemically 2, 5-diamino-2- (difluoromethyl) pentanoic acid hydrate hydrochloride (Fig. 1). Its molecular formula is $C_6H_{12}F_2N_2O_2.HCl.H_2O$. It is drug of preference for management of excessive hair growth (facial hirsutism). It is white to off-white crystalline powder with molecular weight and melting point values 236.65 g/mol and 245-251°C, respectively (Balfour *et al*., 2001; Goldberg *et al*., 1997).

The literature discovered that reverse phase high performance liquid chromatography technique was established and validated for analysis of EFH (Kumar *et al*., 2018; Kumar *et al*., 2018; Saravanan *et al*., 2009). Cohen *et al*., 1989 carried out high pressure liquid chromatographic analysis of eflornithine in serum using dansyl chloride as derivatizing. Kumar *et al*., 2013 established spectrophotometer method for assessment of EFH in parenterals. Kumar *et al*., 2014 carried out UV-spectrophotometer determination of EFH with vanillin as chromogenic reagent. In present investigation, UV-spectrophotometer analytical method was established for analysis of EFH using dansyl chloride as derivatizing agent (Cohen *et al*., 1989; Kumar *et al*., 2013). This analytical methodology has been validated to validate relevant parameters *e.g*. linearity, accuracy, intra-day and inter-

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Table 1: Linearity data of UV spectrophotometer analytical procedure for EFH.

Concentration (µg/ml)	Absorbance 1	Absorbance 1	Absorbance 1	Average Absorbance	% RSD
0.5	0.089	0.09	0.092	0.0903	1.69
1	0.204	0.205	0.206	0.205	0.49
1.5	0.325	0.322	0.324	0.3236	0.47
2	0.421	0.423	0.423	0.4233	0.27
2.5	0.555	0.552	0.551	0.5526	0.38
3	0.657	0.656	0.656	0.6563	0.09
3.5	0.742	0.742	0.751	0.745	0.70
4	0.875	0.875	0.878	0.876	0.20
4.5	0.976	0.986	0.991	0.9843	0.78

day precision, limit of detection (LOD) and limit of quantification (LOQ) as per the international conference on harmonization guidelines Q2 (R1) (ICH Guideline, 2005; Sharma *et al*., 2017; Singh *et al*., 2016). This analytical method was developed and validated for evaluation of EFH in solid lipid micro-or nanoparticle synthesized by solvent evaporation method (Singh *et al*., 2012; Singh *et al*., 2016a; Singh *et al*., 2016b).

Materials and Methods

Instruments

Double beam scanning UV-Spectrophotometer (Systronics 2202 and Systronics AU-2701, Ahmedabad, India) were utilized for measuring absorbance. Digital pH meters (Deluxe model 101, Ambala, India).

Reagents and Chemicals

Eflornithine hydrochloride (CAS NO: 96020-91-6) was purchased from Rusan Pharma Ltd., Mumbai, India. All ingredients used were of analytical grade.

Preparation of Reagents and Standards Stock Solution of EFH

Dansyl chloride as derivatizing reagent was prepared by dissolving 15 mg of Dansyl chloride in 2.5 ml of acetone. The EFH aqueous solution was prepared by dissolving 5 mg of EFH in 2.5 ml of distilled water. Both solutions were stored in the dark. A 2.5 ml aliquot of the aqueous solution of EFH was reacted with 2.5 ml of Dansyl chloride solution and 2-3 drops of methanol were added. Subsequently, the solution was kept at room temperature in dark for at least 4h. followed by addition of 20 mL of distilled water and vortexing of mixture for 15 seconds to produce 200 µg/ml EFH solutions. Then, 0.5 ml of EFH solution was transferred to 10 ml volumetric flask and diluted with distilled water up to the mark to obtain 10 µg/ml standard stock solution of EFH. Similar derivatization procedure was carried out during UV spectrophotometer method validation of EFH for linearity, accuracy, intra-day and inter-day precision, limit of detection (LOD) and limit of quantification (LOQ).

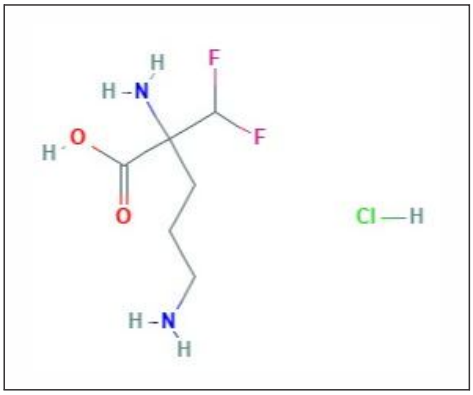

Fig. 1: Chemical structure of Eflornithine hydrochloride.

Absorption Maxima (λ_{max}) and Standard Curve of EFH

Accurately measured 10 µg/ml standard stock of EFH (0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4 and 4.5 ml) were transferred to 10 ml volumetric flasks and diluted up to the mark with distilled water to obtain EFH dilutions from 0.5-4.5 µg/ml. EFH solution (3 µg/ml) was scanned over an UV-spectroscopic scanning range (200-400 nm) to determine λ_{max} of EFH using distilled water as blank. EFH dilutions (0.5-4.5 µg/ml) were analyzed for absorbance (n=3) at λ_{max} (243 nm) against distilled water as blank using UV spectrophotometer and standard curve was plotted by taking concentration on x-axis and absorbance on y-axis to obtain intercept, slope, straight line equation and correlation coefficient.

Analytical Method Validation Parameters

• Linearity: The linearity of analytical procedure was determined using standard concentration of EFH ranging from 0.5-4.5 µg/ml in distilled water. Standard solutions of EFH were prepared in triplicate and subjected to determination of absorbance at 243 nm. A standard curve was prepared by plotting actual concentration (µg/ml) vs. absorbance and correlation coefficient was calculated. The correlation coefficient was used for evaluation of linearity of analytical procedure (Marakkarakath *et al*., 2019; Uyar *et al*., 2007).

Table 2: Accuracy determination of UV-spectrophotometer analytical method for EFH.

Spiked amount (µg/ml)	Recovered amount (µg/ml)	% Mean recovery	Statistical analysis
2	1.997	99.87	Mean = 101.34%
2.5	2.567	102.7	SD = 1.418%%
3	3.043	101.46	RSD = 1.4%

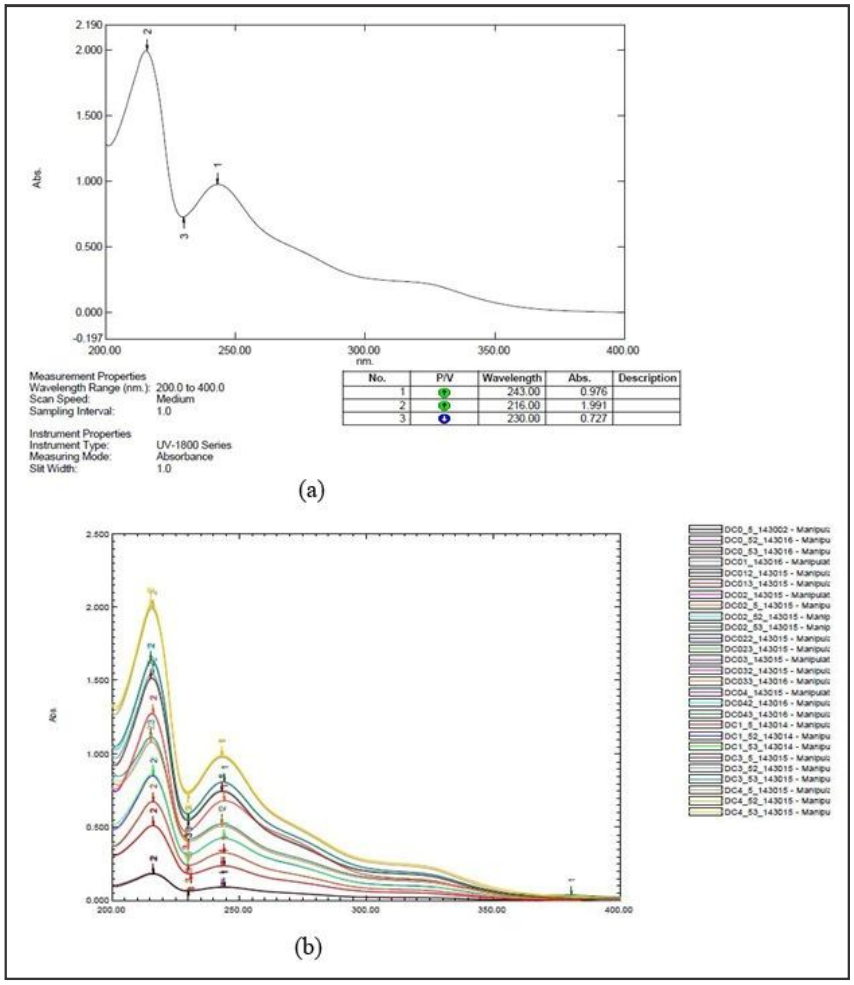

Fig. 2: (a) UV spectrum and (b) overlay UV spectrum graph of EFH in distilled water (λ_{max} = 243 nm)

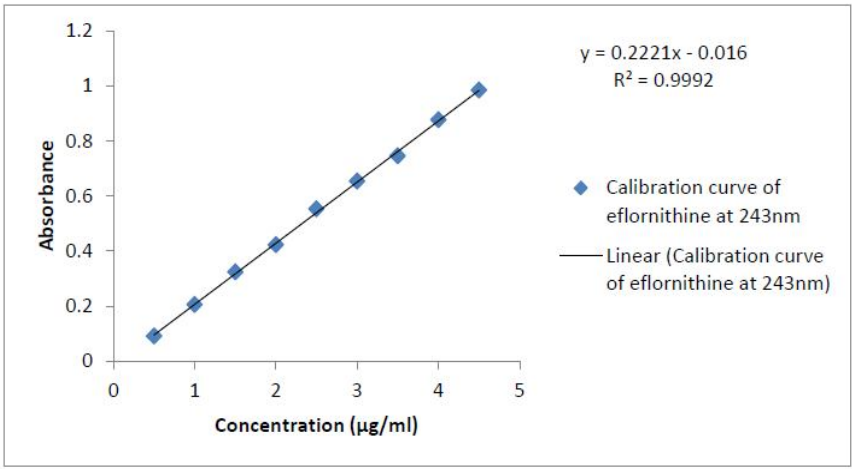

Fig. 3: Standard curve of EFH in distilled water using UV-spectroscopy (*n*=3).

Table 3: Intra-day precision of analytical method for EFH.

Concentration (µg/ml)	Absorbance 1	Absorbance 2	Absorbance 3	Mean	S.D.	% RSD
2	0.423	0.421	0.424	0.4226	0.00152	0.36%
2.5	0.551	0.552	0.555	0.5526	0.00208	0.38%
3	0.657	0.656	0.652	0.6546	0.00321	0.49%

• Accuracy: Accuracy was checked by spiking technique. Absorbance of 2, 2.5 and 3 µg/ml dilution was determined at 243 nm. The accuracy was calculated as the mean percentage drug recovery from each dilution. The accepted limits of mean percentage recovery are 98%-102% (Abdelwahab *et al.*, 2012; Almasri *et al.*, 2019; Belal *et al.*, 2013).

• Intra-day and inter-day Precision: 2, 2.5 and 3 µg/ml concentrations of EFH were analyzed at three different times within a day (intra-day precision) and on three different days (inter-day precision) (Breier *et al.*, 2007; Jain *et al.*, 2013; Patil *et al.*, 2015; Alamri *et al.*, 2016; Divya *et al.*, 2013; Prashant *et al.*, 2013).

• Robustness: Robustness of UV spectrophotometer analytical method was determined by analyzing the 2.5 ìg/ml EFH solutions at different wavelengths (λ) *i.e.* 243 ± 15 nm (Christian *et al.*, 2017).

• LOD and LOQ: LOD and LOQ of EFH were assessed from slope (S) of calibration curve and standard deviation of y-intercept of regression equation using subsequent equations:

$$LOD = 3.\frac{3\sigma}{S} \quad (1)$$

$$LOQ = \frac{10\sigma}{S} \quad (2)$$

LOD is least quantity of analyte which can be detected in sample, but not necessarily quantities as an accurate value while LOQ is minimum quantity that can be quantified by the instrument (Divya *et al.*, 2013; Divya and Narayana, 2014).

Results and Discussion

Absorption Maxima (λ_{max}) and Standard Curve of EFH

Absorption Maxima (λ_{max}) of EFH acquired through UV scan of EFH solution *i.e.* 3 µg/ml was found to be 243 nm (Fig. 2). Regression coefficient and regression equation estimated through calibration curve of EFH was

Table 4: Inter-day precision of analytical method for EFH.

Concentration (µg/ml)	Absorbance 1	Absorbance 2	Absorbance 3	Mean	S.D.	% RSD
2	0.423	0.431	0.428	0.4273	0.0040	0.95%
2.5	0.551	0.561	0.554	0.5553	0.0051	0.92%
3	0.657	0.652	0.669	0.6593	0.00873	1.33%

Table 5: Robustness studies of analytical method for EFH.

Condition	Parameter	Absorbance	Mean	SD	% RSD
Change	228 nm	0.566	0.5646	0.00709	1.26
in	243 nm	0.557			
Wavelength	258 nm	0.571			

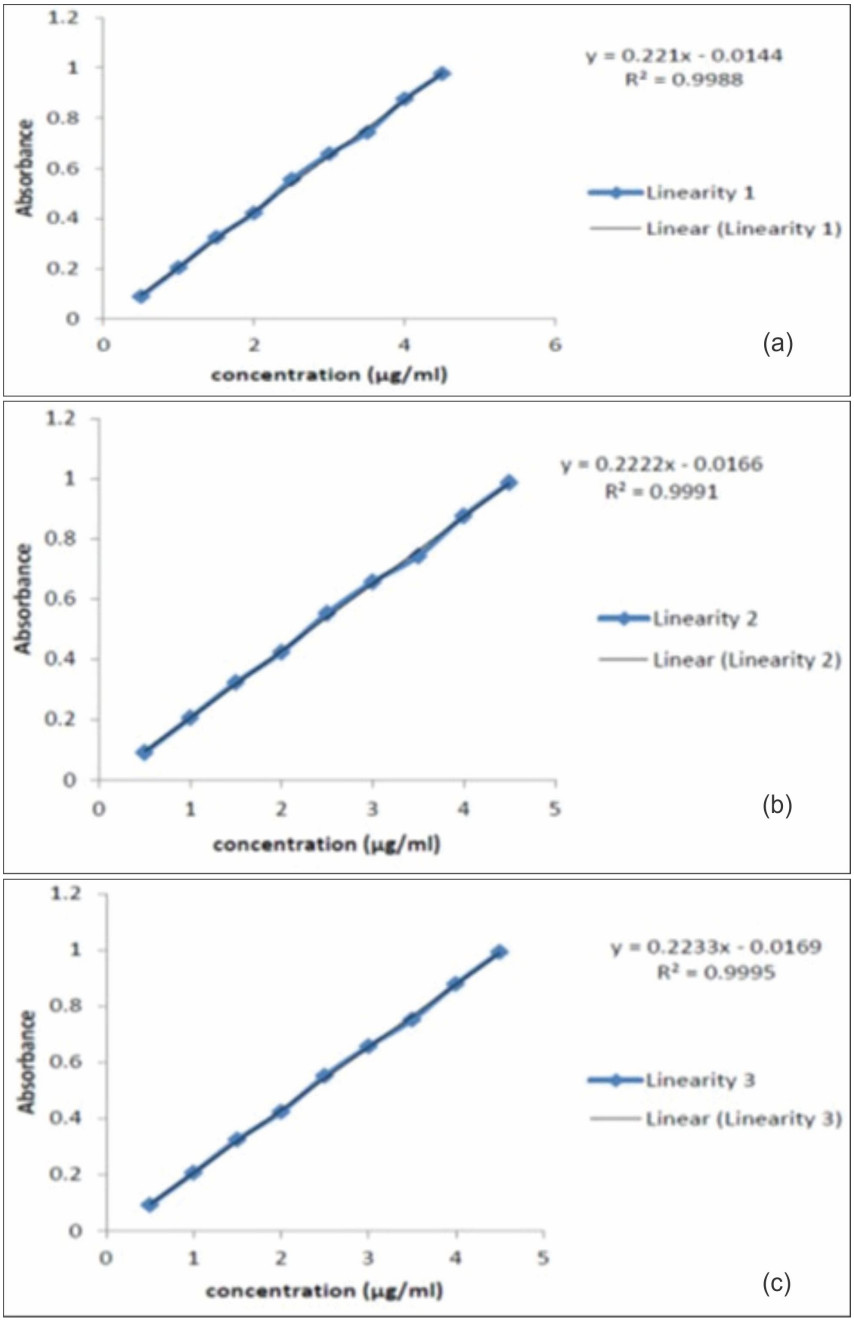

Fig. 4: Standard Curve of EFH at 243 nm for linearity determination.

found to be 0.9992 and y = 0.2221 × - 0.016 respectively (Fig. 3).

Linearity

The linearity range for EFH at 243 nm was found 0.5-4.5 µg/ml which has been confirmed by correlation coefficient value of 0.9988, 0.9991 and 0.9995 which are within the limit (not less than 0.99) and confirms linearity of the method (Table 1 and Fig. 4) (Marakkarakath *et al*., 2019; Uyar *et al*., 2007).

Accuracy

The % mean recovery of EFH was found to be 99.87, 102.7 and 101.46%, respectively for 2, 2.5 and 3 µg/ml solutions (Table 2). Average % recovery of EFH was 101.34% which lies in acceptable limits of mean percentage recovery are 98%-102% with % RSD value 1.4% which indicated good accuracy (Almasri *et al*., 2019; Belal *et al*., 2013).

Intra-day and inter-day Precision

The % RSD for absorbance values of 2, 2.5 and 3 µg/ml EFH at three different time periods within a day was found to be 0.36, 0.38 and 0.49 % (Table 3) while on three different days (inter-day) was found 0.95, 0.92 and 1.33 % (Table 4) which were found to be within the specified limits (< 2%) as specified in the ICH guidelines (Alamri *et al*., 2016; Breier *et al*., 2007; Divya *et al*., 2013; Jain *et al*., 2013; Patil *et al*., 2015; Prashant *et al*., 2013).

Robustness

% RSD of absorbance values of sample solutions analyzed at different wavelengths was found 1.26 (< 2%) which validated the robustness of analytical method (Table 5) (Christian *et al*., 2017).

Limit of Detection (LOD) and Limit of Quantitation (LOQ)

The LOD and LOQ of EFH were found to be 0.01716 and 0.052 µg/ml, respectively, which showed high sensitivity of analytical method. Results

Table 6: Validation parameters of UV-spectrophotometer analytical method for EFH.

Parameter	Result
λ_{max} (nm)	243
Regression equation (y= mx + c)	y= 0.2221x-0.016
Regression coefficient (r^2)	0.9992
Linearity (r^2)	0.9988, 0.9991, 0.9995
Accuracy (% mean drug recovery)	
2 µg/ml	99.87
2.5 µg/ml	102.7
3 µg/ml	101.46
Intra-day precision indicated by % RSD for EFH	0.36, 0.38, 0.49
Inter-day precision indicated by % RSD for EFH	0.95, 0.92, 1.33
Robustness indicated by % RSD (λ_{max}, 243 ± 15 nm)	1.26
Limit of detection (LOD)	0.01716 µg/ml
Limit of quantitation (LOQ)	0.052 µg/ml

of numerous validation parameters of UV-spectrophotometer analytical technique for EFH have been recapitulated in table 6.

Conclusion

The developed UV-spectrophotometer method was found accurate, precise, linear and sensitive. Minor variations in wavelength did not affect the method which validated robustness of developed analytical technique. It was concluded that developed UV-spectrophotometer methodology using dansyl chloride as derivatizing agent could be employed for regular assessment and quantifiable evaluation of the EFH.

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Conflict of Interests

The authors report no conflicts of interest in this work.

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