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PREFORMULATION AND FORCED DEGRADATION STUDIES OF EFLORNITHINE HYDROCHLORIDE

Ikmeet Kaur Grewal[1,2], Sukhbir Singh[1*] and Sandeep Arora[1]

[1*]Chitkara College of Pharmacy, Chitkara University, Punjab, India.
[2]Department of Pharmacy, Government Medical College, Patiala (Punjab) India.

Abstract

The rationale of this pre-formulation research was to carry out physicochemical characterization and forced degradation stability study of eflornithine hydrochloride (EFH) and to evaluate compatibility of eflornithine hydrochloride (EFH) with softemul 165 and monecol PC. Melting point, log P and percentage loss on drying of EFH was found 246±0.5°C, -2.1 and 0.084% (≤ 0.5%), respectively. Abundant spiky crystalline peaks in x-ray diffraction pattern of EFH confirmed extremely crystalline nature of drug. It was found that EFH has higher solubility in water in comparison to organic solvents. Differential scanning calorimetry demonstrated compatibility of EFH with monecol PC and softemul 165. Forced degradation study of EFH concluded that drug has better heat stability but slightly prone to oxidative and photo-degradation.

Key words: Pre-formulation, Eflornithine hydrochloride, Forced degradation stability, Softemul 165, Monecol PC.

Introduction

Eflornithine hydrochloride (EFH) is irreversible enzyme inhibitor of decarboxylase ornithine. Chemically, EFH is 2,5-diamino-2-(difluoromethyl) pentanoic acid hydrate hydrochloride. It is white to off-white crystalline powder with molecular formula and weight $C_6H_{12}F_2N_2O_2.HCl.H_2O$ and 236.65 g/mol, respectively. It is acidic salt of basic drug with pKa 10.2. It is drug of choice for treatment of facial hirsutism (excessive hair growth) (Balfour *et al*., 2001; Goldberg *et al*., 1997). Pre-formulation is the main phase for stable, logical, effective and safe product design of an active pharmaceutical ingredient (API). The goal was to investigate the physicochemical properties of EFH and the compatibility analysis of EFH with softemul 165 and monecol PC for conducting further research involving development of solid lipid micro-or nanoparticle of EFH with Softemul 165 and Monecol PC by solvent evaporation process (Censi *et al*., 2014; Krupa *et al*., 2014; Penumetcha *et al*., 2016; Sanghvi *et al*., 2009; Singh *et al*., 2012; Singh *et al*., 2016a; Singh *et al*., 2016b; Sharma *et al*., 2019).

Materials and Methods

Materials

Eflornithine hydrochloride (CAS NO: 96020-91-6) was purchased from Rusan Pharma Ltd., Mumbai, India. Softemul 165 and Monecol PC were procured from Mohini Organics Private Limited, Mumbai, India.

Determination of Physicochemical Characteristics of EFH

• Melting Point Determination Using Capillary Method: Small quantity of drug was placed into a sealed capillary tube. The tube was placed in the melting point apparatus. The temperature in the apparatus was gradually increased and the observation of temperature was noted at which drug started to melt and the temperature when the entire drug gets melted (Killedar *et al*., 2014; Sharma *et al*., 2017a; Sharma *et al*., 2019).

Loss on Drying (% LOD)

Place approx. 1.0 g of drug sample into the crucible and tap carefully, record the weight (W_{wet}) to ± 1 mg. Place the drug sample into the drying oven with lid in tilted position at 60 ±2°C. After the 24 hour time period, take the drug sample out of the oven, being careful not to create turbulence. Replace lid to closed position. Place the drug sample in the desiccator and allow cooling for at least 30 minutes. Reweigh the crucible with closed lid (W_{dry}) to ± 1 mg (Chablani *et al*., 2011; Mohamed *et al*., 2012; Sharma *et al*., 2017a).

***Author for correspondence :** E-mail: sukhbir.singh@chitkara.edu.in; singh.sukhbir12@gmail.com

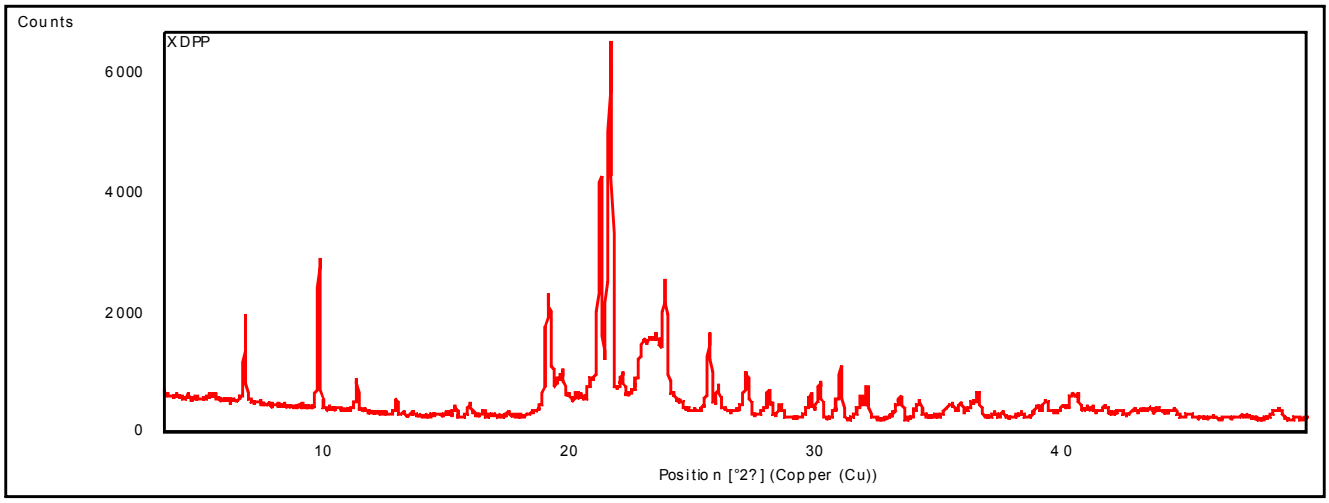

Fig. 1: X-ray Diffraction Pattern of EFH.

$$\% \text{ Loss on drying (\% LOD)} = \frac{W_{wet} - W_{dry}}{W_{wet} - W_{empty}} \times 100 \quad (1)$$

Partition Coefficient (n-Octanol/Water) by Shake Flask Method

100 mg of EFH has been transferred to a separation funnel containing 1:1 n-octanol/water and put on a 4-hour mechanical shaker (Baka *et al*., 2008; Bharate *et al*., 2016; Sharma *et al*., 2017a; Sharma *et al*., 2019). Samples were collected and examined using UV spectroscopy. The EFH partitioning coefficient was calculated using the following equation:

$$\text{Partition coefficient (P)} = \frac{\text{amount of EFH in n - octanol}}{\text{amount of EFH in water}} \quad (2)$$

Solid Form Identification Using X-Ray Diffraction Study

Table 1: Observed peaks of EFH after different types of forced degradation.

Degradation study	Time (hr)	Observed peak	Reported peak
Acid hydrolysis	1	242	243
	2	254	243
	4	253	243
Base hydrolysis	1	238	243
	2	246	243
	4	244	243
Oxidation hydrolysis	1	243	243
	2	214	243
	4	215	243
Photo degradation	1	244	243
	2	244	243
	4	-	243
Heat-induced degradation	1	242	243
	2	242	243
	4	244	243

Solid form verification was performed through powder x-ray diffraction (PXRD) pattern of EFH obtained using x-ray diffractometer (Xpert-Pro diffractometer) employing 1.54 A° CuKα and 1.39 A° CuKβ radiations. Data was mounted in continuous scanning mode over an angular range from 5-50° at 2θ scale at 2° per min scan mode (Sharma *et al*., 2019; Vippagunta *et al*., 2002).

Solubility Study by Equilibrium Solubility Method

The solubility of EFH was determined in various solvents *i.e.* methanol, acetone, distilled water, ethanol, 0.1N hydrochloric acid, phosphate buffer pH 7.4, chloroform and dichloromethane. Excess quantity of EFH was dissolved in 10 ml solvent in screw-capped glass vials and placed in orbital shaker (Remi, India) for 24 hours at 37°C. Samples were withdrawn and absorbance

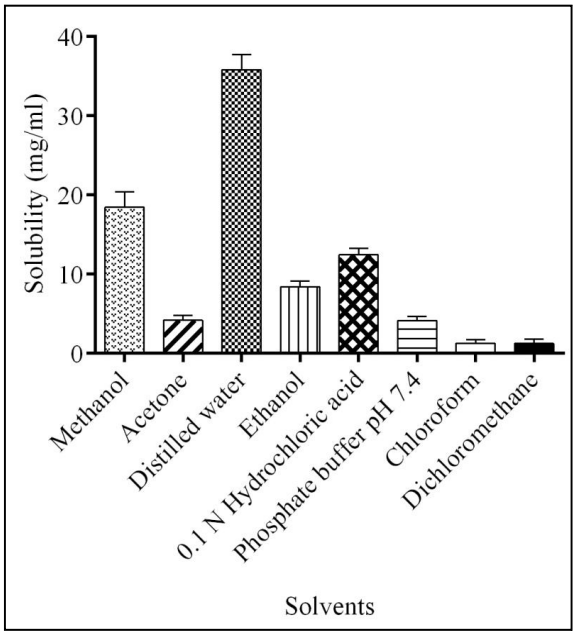

Fig. 2: Solubility of EFH in different solvents.

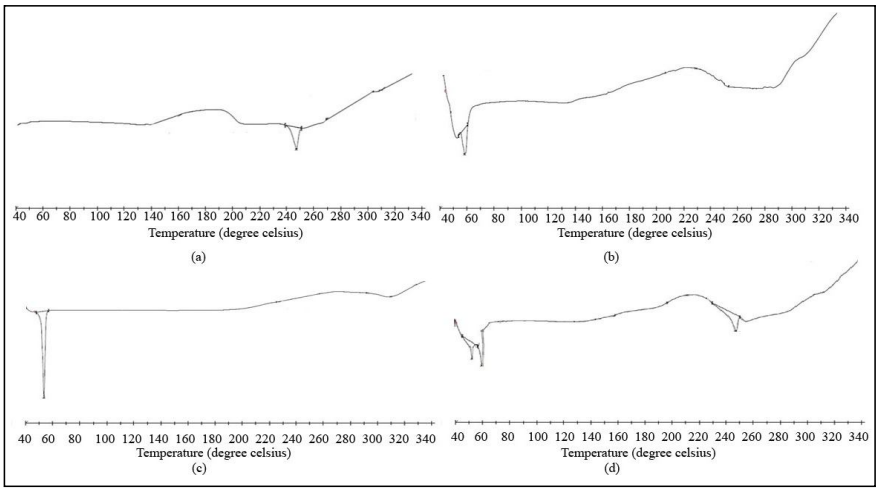

Fig. 3: DSC Curves of (a) EFH, (b) monecol PC, (c) softemul 165, (d) physical mixture of EFH, softemul 165 and monecol PC.

was recorded using UV-spectrophotometer for quantification of EFH (Table 1) (Dezani *et al*., 2013; Sharma *et al*., 2017a; Sharma *et al*., 2019; Shete, *et al*., 2013).

Force Degradation Stability Studies *via* UV Spectrophotometer

Stress degradation study like alkaline/acidic stress, oxidative, photolytic and heat induced degradation of drug solution was carried out for 1, 2 and 4 hr. After stress degradation, the resultant solution was diluted with distilled water up to 10 ml and spectra's were

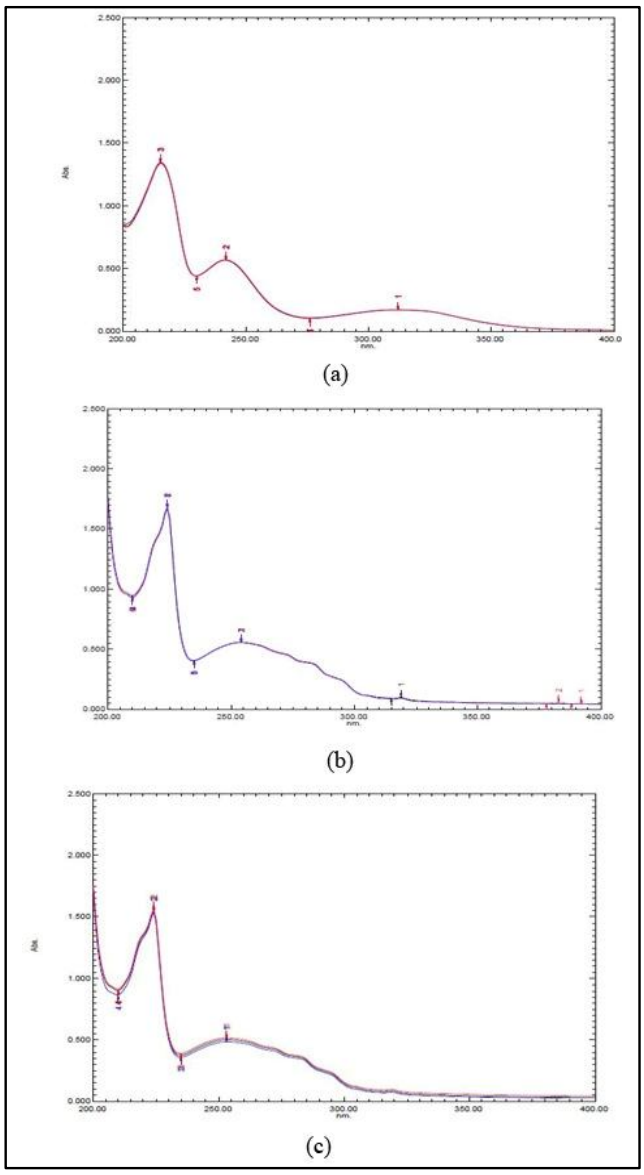

Fig. 4: Overlay graph of acidic degradation of EFH at (a) 1 hr (b) 2 hr and (c) 4 hr.

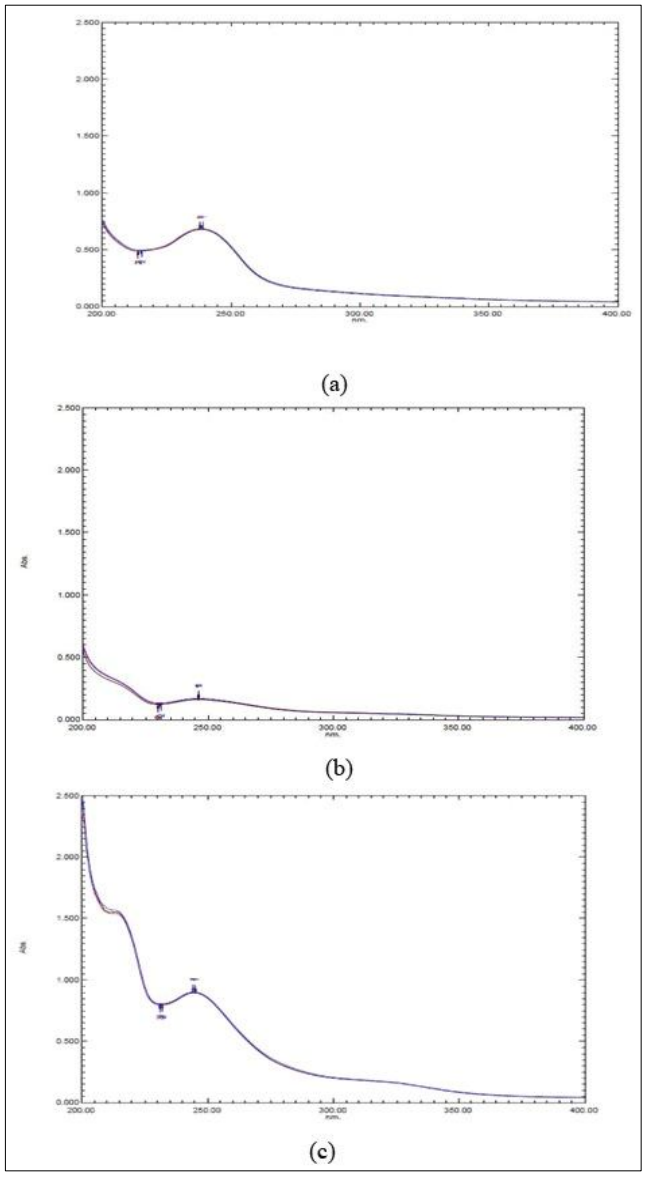

Fig. 5: Overlay graph of basic degradation of EFH at (a) 1 hr (b) 2 hr and (c) 4 hr.

recorded (Sharma *et al*., 2017b; Singh *et al*., 2016c). The spectra's of the stressed samples were compared with those of control samples that were freshly prepared from the standard stock solution and without stress. All samples were analyzed in triplicate (Blessy *et al*., 2014). For acid and base hydrolysis, sample solution containing 1 ml aliquot of drug was transferred into 10 ml of volumetric flask, mixed with 1 ml of 0.1M hydrochloric acid as well as 0.1M sodium hydroxide independently and allowed to stand for 1, 2 and 4 hr. at 60±2°C. Subsequently, samples were neutralized with 1 ml of 0.1M sodium hydroxide and 0.1M hydrochloric acid, respectively. For oxidative stress, sample solution containing 1 ml aliquot of drug was transferred into a 10 ml volumetric flask, mixed with 1 ml of 1% v/v hydrogen peroxide and kept aside for 1, 2 and 4 hr at 60±2°C. Photolytic degradation was studied by placing drug solution in a clear volumetric flask and exposing it to direct UV light for 1, 2 and 4 hr. for heat-induced degradation, one milliliter aliquot of sample solution containing drug was transferred to 10 ml amber volumetric flask and heated for 1, 2 and 4 hr. at 60±2°C.

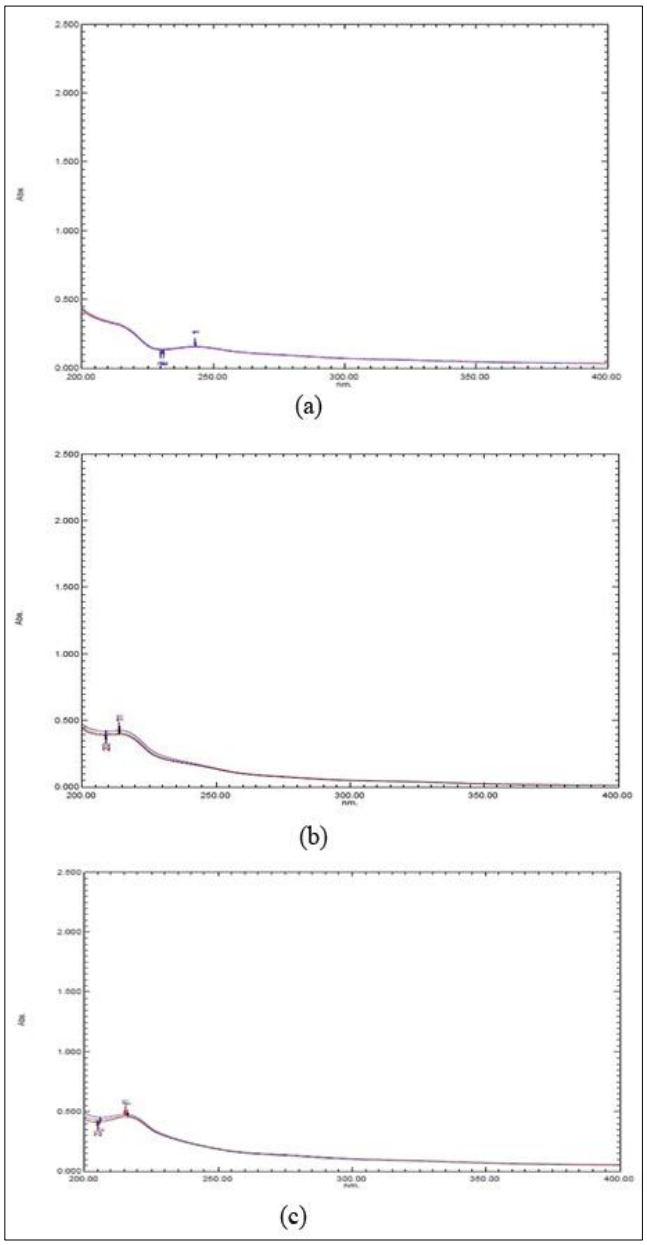

Fig. 6: Overlay graph of oxidative degradation of EFH at (a) 1 hr (b) 2 hr and (c) 4 hr.

Drug-Polymer Compatibility investigation by Differential Scanning Calorimetric (DSC)

The thermal analysis of EFH, softemul 165, monecol PC and physical mixture (EFH: softemul 165: monecol PC in 1:1:1) was performed on DSC 4000 Perkin Almer, Germany using Pyris Software. 5 mg samples were taken in an aluminum pan and heated over 25-350°C at 20°C/

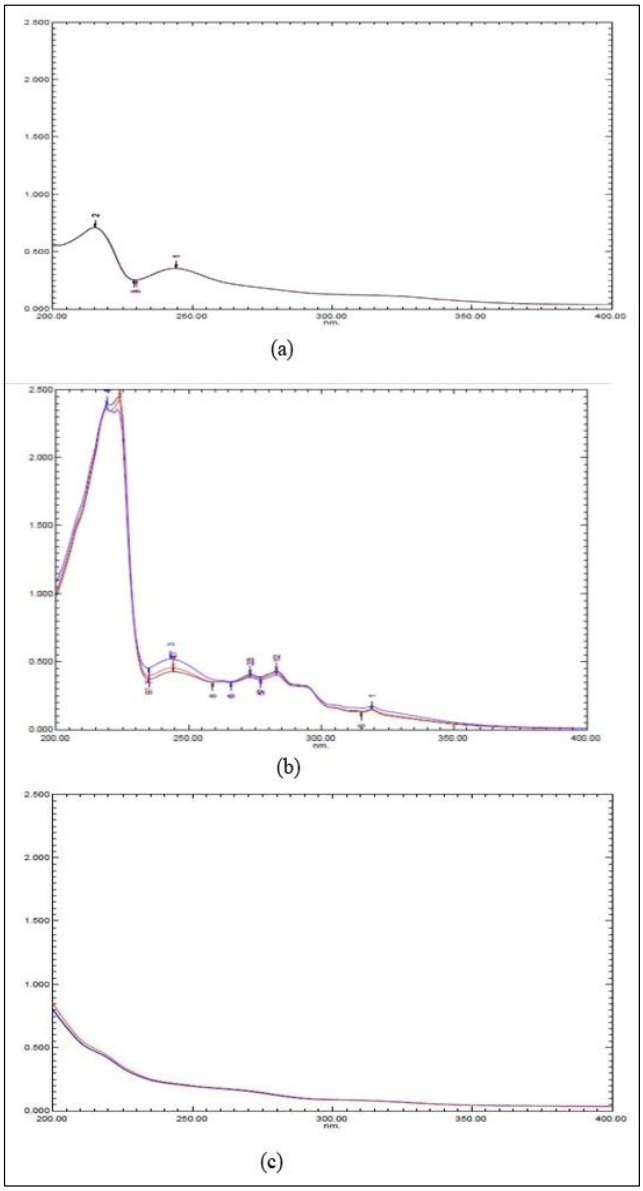

Fig. 7: Overlay graph of photolytic degradation of EFH at (a) 1 hr (b) 2 hr and (c) 4 hr.

min with nitrogen purging at 100 mL/min (Choudhary *et al*., 2012; Dong *et al*., 2018).

Results and Discussion

Physicochemical Characteristics of EFH

• Melting Point, % LOD and Partition Coefficient: Melting point of EFH was found 246±0.5°C which was in compliance with theoretical value. % LOD of EFH was found 0.084% of its weight after being dried at 105°C for three hours which was in compliance with the reported value (% LOD $\leq$ 0.5%). Log P of EFH as estimated by shake flask method was found -2.1.

Solid Form Identification

X-ray diffractogram of EFH showed spiky crystalline peaks at 2θ=5.6°, 10.2°, 19.8°, 21.3°, 22.7°, 24.8°, 25.4°, 26.1°, 27.3°, 28.7°, 29.6°, 30.4°, 31.1°, 32.4°, 33.2°, 33.9° and 42.9° which confirmed hig crystallinity of EFH (Fig. 1).

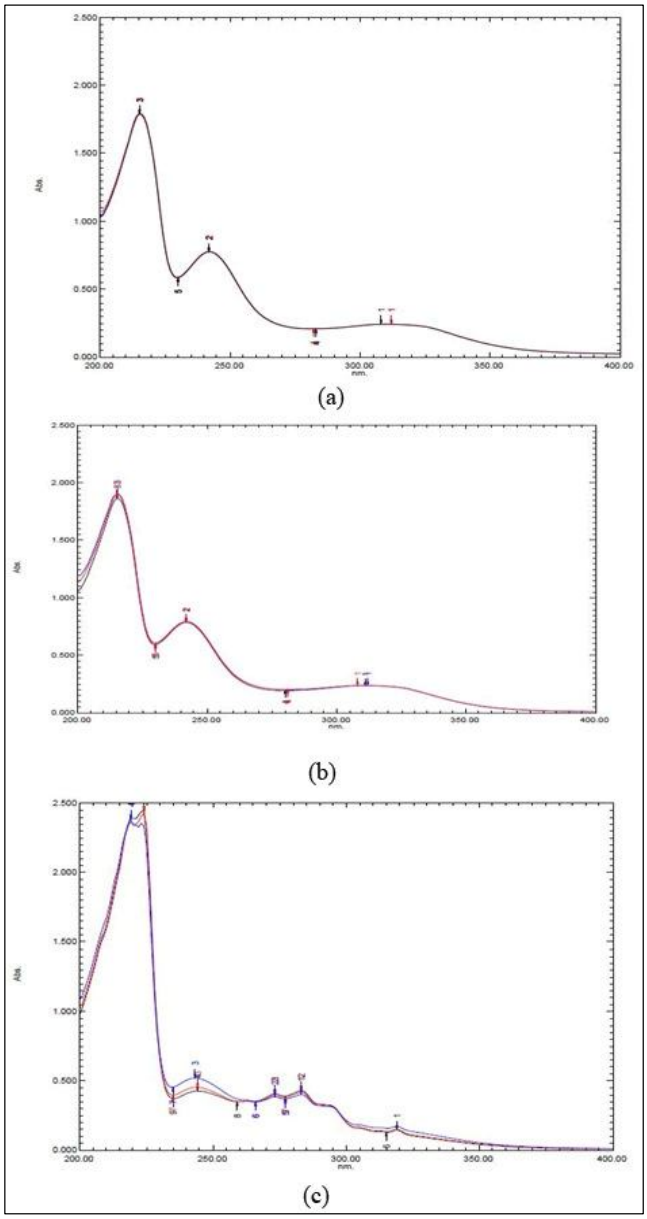

Fig. 8: Overlay graph of heat-induced degradation of EFH at (a) 1 hr (b) 2 hr, and (c) 4 hr.

Solubility Study

Solubility of EFH estimated in various solvents by equilibrium solubility method has been represented in fig. 2. It was found that EFH has higher solubility in water in comparison to organic solvents.

Drug-Polymer Compatibility Study

Fig. 3, embodied the DSC thermograms of (a) EFH, (b) softemul 165, (c) monecol PC and (d) physical mixture of EFH, softemul 165 and monecol PC. DSC thermograms of EFH revealed distinctive endothermic peak at 246.8°C analogous to its melting point (T_m) which indicated significantly crystalline characteristics of drug (Fig. 3a). No characteristic peak was observed in DSC thermogram of softemul 165 indicating its amorphous nature (Fig. 3b). Monecol PC showed typical peak at 65.44°C (Fig. 3c). The distinctive endothermic peaks of EFH and monecol PC were remarkably observed in physical mixtures which illustrated drug-polymer compatibility (Fig. 3d) (Choudhary *et al*., 2012; Dong *et al*., 2018).

Force Degradation Stability Studies *via* UV Spectrophotometer

The UV spectra of EFH did not show any significant acidic degradation (Fig. 4) while small alkaline degradation was observed in comparison to unexposed EFH (Fig. 5). EFH did not demonstrate any noteworthy oxidative degradation at 1 hr but show significant degradation at 2 and 4 hr. at 60±2°C (Fig. 6). EFH did not illustrate any considerable photo-degradation at 1 and 2 hr but reveal noteworthy degradation at 4 hr at 60±2°C (Fig. 7). EFH did not show any significant heat-induced degradation at 1, 2 and 4 hr. at 60±2°C (Fig. 8). Table 1 depicts the comparison of reported and observed peaks of EFH achieved after different types of forced degradation of drug for 1, 2 and 4 hr.

Conclusion

This study demonstrated that melting point, % LOD, residue on ignition and log P of EFH were 246±0.5°C, 0.084% ($\leq$ 0.5%) and -2.11687±0.011, respectively. It was researched that EFH has highly crystalline nature. EFH was found highly soluble in water. Differential scanning calorimetry concluded compatibility of EFH with monecol PC and softemul 165. Forced degradation study of EFH concluded that drug has better heat stability but slightly prone to oxidative and photo-degradation.

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Conflict of Interests

Conflict of interest declared none.

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