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PREFORMULATION CHARACTERIZATION, DEVELOPMENTAND EVALUATION OF HARD GELATIN CAPSULES OF DACLATASVIR DIHYDROCHLORIDE

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ABSTRACT

Daclatasvirdihydrochloride (DCLD) is a new drug gained its FDA approval on July 24, 2015 for treatment of hepatitis C.There are no reports on preformulation characters and formulations. Hence present research work was assessment of preformulation characters, development of hard gelatin capsules and ***in-vitro*** evaluation. DCLD is hydrophilic with aqueous solubility. XRD indicated amorphous nature. DSC showed melting peak at 273.60^0C. FTIR revealed amide C=O stretch at 1641.46 cm^{-1}, aromatic C=C bending.at 1726.67 cm^{-1}. Hard gelatin capsules containing 60 mg of DCLD, 320 mg of Lactose and 4mg magnesium stearate evidenced optimum disintegration and drug release.

KEY WORDS: Daclatasvir dihydrochloride, New drug, Preformulation, XRD, DSC, FTIR, hard gelatin capsules***in-vitro***drug release.

1. INTRODUCTION

Daclatasvir dihydrochloride (DCLD) is a new drug useful for treatment of hepatitis C (HCV)[1]was approved in Europe on 22 August 2014 and gained its FDA approval on July 24, 2015 in United States.[2]

It has wide scope for formulations to be developed for effective treatment of HCV. There is no report on its preformulation characteristics and its formulations. Hence present research work was aimed for investigation of its preformulation characteristics such as solubility,partition coefficient,diffusion Studies**,** particle surface morphology,differential Scanning Colorimeter (DSC),Fourier Transform infrared analysis, crystal properties by x-ray powder diffraction. Further it was prepared in the form of hard gelatin capsule and subjected to ***in-vitro*** evaluation.

2. MATERIALS

Daclatasvir dihydrochloride was obtained as a gift sample from Mylan laboratories Ltd., Hyderabad, **INDIA.** Lactose anhydrous, Magnesium steratewere purchased from SD Fine chemicals Ltd and all other chemicals are of analytical grade.

3. METHODS

3.1. Assessment of Preformulation properties:

3.1.1. Solubility analysis

Solubility analysis was carried out for DCLD by placing5 mg in 10 ml of distilled water in sealed glass tubes. The tubewas mixed occasionally and observed for dissolution and results are given in **Table 2**.

3.1.2. Partition Coefficient

Thoroughly cleaned and well dried separating funnel was taken and into it .25 ml of n-octanol and 25 ml of distilled water were taken. 60mg of DCLD was added to mixture and shaked for 30 min. using incubator shaker. Then it was allowed to stand for 24 hours to separate aqueous and organic layers. There after aqueous and organic layers were separated out in different beakers.Then the were analyzed for the drug content by UV-visible spectrophotometer (Systronics, India) $\lambda_{max}$of 214 nm.

3.1.3. Particle Surface Morphology by SEM

The morphological characteristics and particle size analysis of DCLD were determined by scanning electronic microscopic method (SEM, Zeiss Ultra-60 FE SEM)). Specified quantity of pure drug was taken and mounted directly on the SEM stab, using double sided, sticking tape and scanned in a high vacuum chamber with a focused electron beam.

3.1.4. FTIR analysis of drug-PEG conjugate

FTIR spectra of pure DCLD was obtained on a FTIR Spectrophotometer (Bruker, JAPAN) equipped with a DTSG detector. Samples were prepared by KBr pressed pellet technique. The scanning range was 400 to 4000^{-1} and the resolution was $4cm^{-1}$.

3.1.5. XRD analysis of drug- PEG conjugates

Powder X-Ray diffraction pattern of pure was recorded using Philips PW 17291 powder X-ray diffractometer. The cross sections of the samples were held in place on

quartz plate and subjected to CuKα radiations. The samples were analyzed at room temperature over a range of 0-5^0 at an angle of 2θ with sampling interval of 0.02^0. The scanning rate was 2^0 /min. The diffractogram of the samples are shown in **Fig. 7.**

3.1.6. Differential scanning colorimeter (DSC)

DSC studies were performed using TA Q1000 on 2to3mg sample. Sample were heated in an open aluminum crimp pans at a rate of 10^{o}C/min in a 30 to 300^{o}C temperature range under a nitrogen flow of 50ml/mi.n. The relevant spectrum is presented in Fig.5

3.1.7. *Ex-vivo* absorption studies

Ex-vivo absorption studies were carried out for DCLD by using modified Franz diffusion cell (Fig.3) with a receiver compartment of 25 ml volume and effective diffusion area of 2.5 cm^2 was used for the study by using goat intestinal membrane in ph 7.4 phosphate buffer solution. Fresh intestinal membrane of goat was collected from slaughter house and used in absorption experiments. The receptor compartment was filled with 25 ml of ph 7.4 phosphate buffer maintained at 37±0.5^{0}C and stirred by a magnetic bar at 100 rpm. 30 mg of DCLD was placed on membrane. The top of the cell was covered. At appropriate time intervals 3 ml aliquots of the receptor medium were withdrawn and immediately replaced by an equal volume of buffer to maintain sink conditions. Correction factors for each aliquot were considered in calculation of release profile. the were analyzed for the drug content by UV-visible spectrophotometer (Systronics, India) λ_{max} of 214 nm.

3.2. Development and evaluationof hard gelatin capsules of DCLD

3.2.1. Developmentof hard gelatin capsules of DCLD

Five formulation DCLD capsules were prepared by using capsule filling equipment (Shreeja chemicals, INDIA) the composition shown in **Table 1**. DCLD powder was admixed to the capsule excipients: Lactose (15mg); Magnesium stearate (1mg) by a tumbler. The mixed powder was filled into hard gelatin capsule No. 1 using a simple filling capsule equipment (Shreejachemicals, India) for lab scale. The final product of the capsules were collected and immediately transferred into dry plastic containers and tightly sealed.

Table 1: Compositionof hard gelatin capsules of Daclatasvir dihydrochloride.

INGREDIENT	FORMULATION CODE				
	F1	F2	F3	F4	F5
DCLD	60mg	60mg	60mg	60mg	60mg
Lactose	320mg	316mg	330mg	340mg	330mg
Magnesium stearate	4mg	4mg	2mg	2mg	4mg

3.2.2. Evaluation of hard gelatin capsules of DCLD

3.2.2.1. Disintegration test

The disintegration time[4] of capsules was determined for 6 capsules by using Thermionic tablet disintegration test apparatus. Distilled water maintained in 1 litre beaker at constant temperature of 37±0.5^0 C in isothermal water bath was used as medium. The medium level was maintained such that the tablets remain 2.5 cm below the surface of liquid on their upward movement and descend not closer than 2.5 cm from the bottom of the beaker. One tablet was placed in each tube of basket rack and set to make up and down movements at frequency of 28-32 cycles per minute. The time required for tablets to disintegrate and pass through 10-mesh screen was denoted.

3.2.2.2. Estimation of drug content

Drug content estimation was carried out by collecting ten capsules from each batch at random and ransferred to a 100 ml volumetric flask and dissolved in water.The solution were made up to the volume, filtered, suitably diluted and drug contents were estimated using UV-visible spectrophotometer (Systronics, India) according

3.2.2.*3: In-vitro* dissolution studies

The dissolution studies were performed for pure drug and prepared capsules using USP XXI dissolution testing apparatus basket type I, (Electrolab, India). Dissolution medium was pH 7.4 phosphate buffer at 37±5^{o}C and a rotation speed of 50 rpm.At appropriate time intervals 10ml of solution was withdrawn and filtered through wattmen filter. The initial volume was maintained by adding 10ml of fresh dissolution medium. The amount of drug released in each sample was estimated by using UV-visible spectrophotometer (Systronics, India) at $\lambda_{max}$of 214nm.

4. RESULT AND CONCLUSION

Solubility analysis evidenced free aqueous solubility of DCLD.The partition coefficient of daclatasvirdihydrochloride between n-octanol and distilled water by spectrophotometric analysis was found to be k = 0.495 indicating DCLD as highly hydrophilic.

The particle surface morphology of pure DCLD is shown in **Fig.1.** The particles are non spherical, irregularlyshapedand lying in size range of micrometers with average size of 8.55µm.

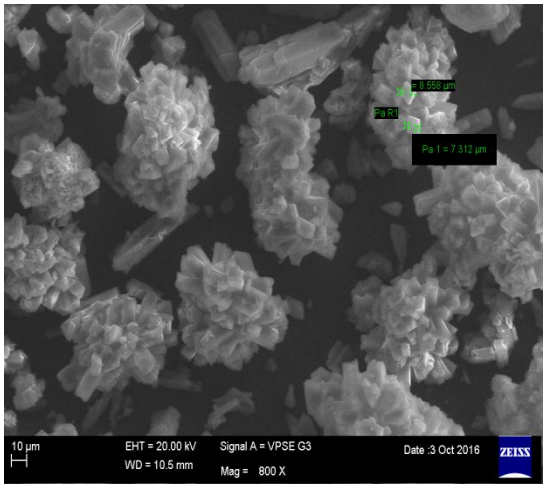

Fig. 1: SEM picture of daclatasvirdihtydrochloride

Diffractogram by XRD analysis of DCLD in **Fig.2** evidencedreduced complexity with less intensified peaks indicating DCLD as amorphous whichsupports aqueous solubility property of DCLD.

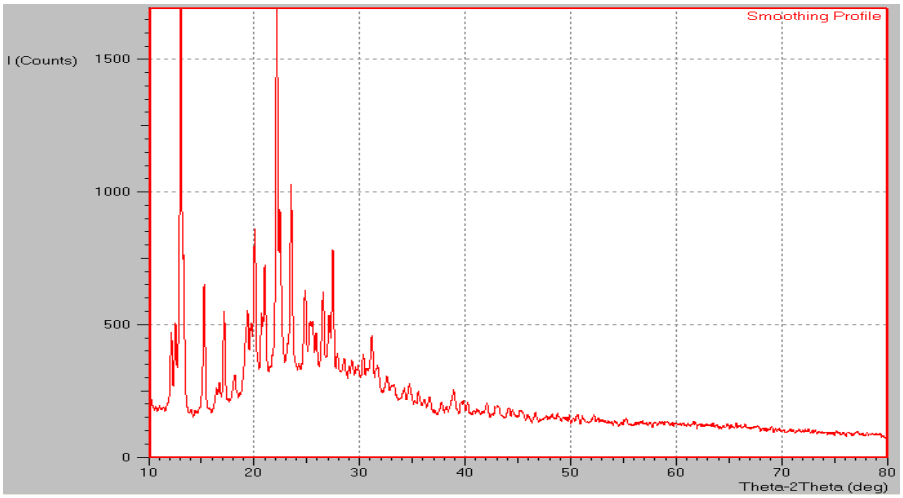

Fig.2: XRD spectrum of DCLD

FTIR spectrum of DCLD is given in **Fig.3.** The characteristic peaks were observed due to aromatic C-H bending at 666.37cm^{-1}, amide C=O stretch at 1641.46cm^{-1}, aromatic C=C bending at 1726.67 cm^{-1} and alkyl C-H stretch at 2964. 62cm^{-1}.

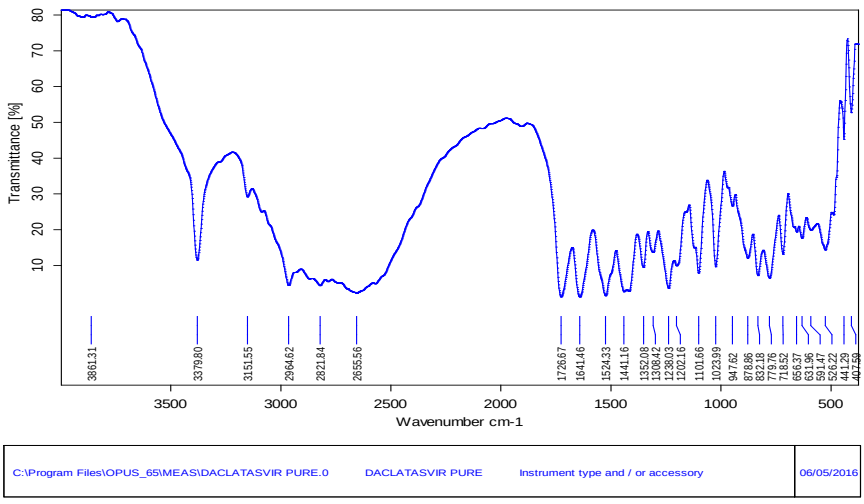

Fig.3: IRspectrum of DCLD

DSC t**hermogram o**f daclatasvirdihydrochlorideraled drug melting point at **273.60^{0}C as in Fig.4**

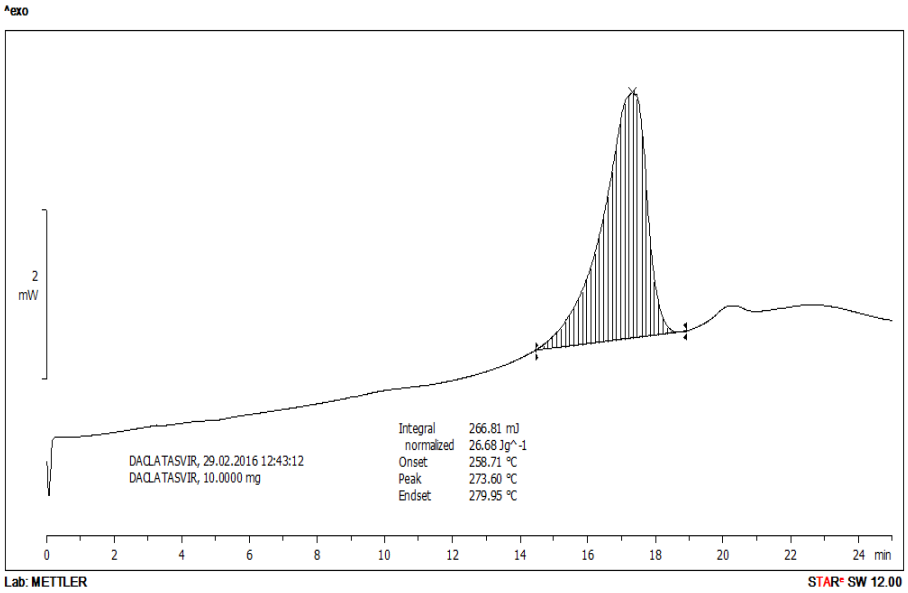

Fig.4: DSC Thermogram ofdaclatasvirdihydrochloride

The results of ***ex-vivo***permeation studies of daclatasvirdihydrochlorides are shown in Table 2. It is observed from the results there is 79.2 percent drug released after 15 min and 96.7 percent drug released after 2 hrsindicatig min at fair GI absorption of DCLD from intestinal membrane.

Table.2: ex-vivo permeation studies of daclatasvir pH 7.4 phosphate buffer

Time(hrs)	Percentage drug released
0.25	79.2±0.61
0.5	81.5±0.32
1	84.3±0.55
1.5	84.9±0.68
2	91.6±0.65
3	92.4±0.54
4	95.4±0.11
5	96.7±0.42
6	97.3±0.62
12	97.8±0.22
24	98.6±0.28

Results of disintegration time in the range of 3.56min ±0.9to 6.8min±0.61 and drug content in the range of 98.87% ±0.89 to 99.08%±0.56 were found acceptable as shown in **Table 3.**

Table 3: Evaluation parameters of hard gelatin capsules of DCLD

Parameter	Result
Disintegration time (min) (n=6)	3.56±0.9 to 6.8±0.61
Drug content (%) (n=10)	98.87 ±0.89to99.08±0.56

Cumulativepercent drug release was reasonable from all prepared formulations F1 to F5 as represented in **Table 4 and Fig 5**. However F2 showing 80.91% in 60 min was considered as on optimum formulation of DCLD.

Table 4: Cumulative percent of DCLD frompure drug andhard gelatin capsules of DCLD

Time (min)	Pure DCLD	F1	F2	F3	F4	F5
10	27.7±0.6	24.93±0.19	48.76±0.28	50.24±0.31	38.42±0.52	53.75±0.43
20	3.40±0.65	41.37±0.21	52.46±0.31	59.66±0.14	41±0.23	54.31±0.9
30	40.45±0.66	42.3±0.15	57.45±0.27	68.90±.46	43. ±0.32	54.31±0.57
40	41.56±0.57	48±0.22	65.20±0.12	71.12±.052	47.47±0.59	66.13±0.56
50	43.04±0.56	73.52±08	65.76±0.15	77.95±0.54	48.39±0.7	70.75±0.32

60	48.95±0.55	80.91±0.3	81.28±0.67	81.65±0.19	50.06±0.34	72.22±0.45
90	64.10±0.5	92.73±0.22	83.49±0.64	97.35±0.21	61.33±0.36	75.73±0.29
120	75.91±0.3	92.73±0.81	48.76±0.65	97.6±0.22	63.8±0.27	75.88±0.35

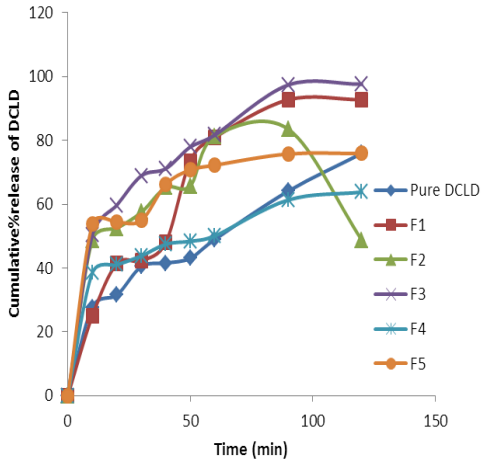

Fig 5: Cumulative percent of DCLD from pure drug andhard gelatin capsules of DCLD

CONCLSION

Daclatasvir Dihydrochloride is freely soluble, hydrophilic amorphous drug with good GI absorption. Hard gelatin capsules prepared to contain. 60 mg of DCLD, 320 mg of Lactose, and 4 mg of magnesium stearate elicit optimum disintegration, percent drug content and drug release characteristics.

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