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Formulation and Evaluation of Delayed Release Pellets of Duloxetine HCl

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ABSTRACT

Duloxetine HCl is an Anti-Depressant drug belonging to the class of Dual Monoamine Reuptake inhibitor. Duloxetine HCl is a potent inhibitor of neuronal serotonin and nor-epinephrine reuptake. Duloxetine hydrochloride is acid labile drug so Duloxetine hydrochloride enteric coated pellets were formulated using fluidized bed process and different enteric coating polymers. Three separate layers, the drug layer, the barrier layer and the enteric layer, were coated on to the inert core pellets. The pellets were optimized with the acid resistance and drug release in simulated intestinal fluid as the process parameters. Various other properties, such as bulk and tapped density, Hausner's ratio, abrasion resistance, yield of pellets, moisture content, and particle size distribution were also studied in the optimized pellets. The concentration of the enteric polymer played a vital role in acid resistance, while the type of enteric polymer affected the drug release in simulated intestinal fluid. In both cases, it was determined that binder polymer concentration was not affected much. The comparisons between the optimized pellets and innovator formulation yielded Similarity (f2) and Dis-similarity (f1) values within a range of 72 and 4 respectively. One month stability studies, conducted at accelerated conditions showed optimized pellets to be stable. So the optimized formula can be utilized to perform scale up trials. It is also expected to be bioequivalent to the innovator product.

KEYWORDS

Duloxetine, Pellet, Enteric polymer, Barrier layer, Similarity factor, Dissimilarity factor, Bio equivalent, Innovator.

INTRODUCTION

Pellets, multi-unit dosage forms, have both therapeutic and pharmaceutical advantages. Therapeutic advantages include modification of drug release, division of dose strength, and free dispersion in the gastro intestinal tract when administered orally.[1,2] The pharmaceutical advantages include a high degree of flexibility in design and development during delivery of incompatible bioactive agents due to the low surface area to volume ratio compared to powders and granules. Therefore, pellets serve as an excellent coating substrate and can be incorporated in to high amount so actives without producing an excessively large particle.[3,4]

Duloxetine belongs to the class of Seratonin and Nor-adrenaline reuptake inhibitor.[5,6] Preclinical studies have shown that Duloxetine HCl is a potent inhibitor of neuronal serotonin and nor-epinephrine reuptake and a less potent inhibitor of dopamine reuptake and so used to treat Major Depressive Disorders.[7] Duloxetine also produces therapeutic advantages in case of fibromyalgia, diabetic peripheral neuropathic pain, premenstrual disphoretic disorder, phantom limb pain, and male and female stress incontinence. It is desirable to formulate this multi-purpose drug in a more patient-compliant pellet form.[8,9]

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Duloxetine hydrochloride is acid labile drug; it degrades to highly toxic compound 1-naphthol in acidic pH. Its degradation in acidic pH leads to sub therapeutic level of drug in the body which is not desirable.[10] So, in order to avoid this degradation and to bypass the acidic pH of the stomach, delayed release pellets of Duloxetine HCl was developed. The pellets were coated with Drug layer, Barrier layer and Enteric layer.[11,12] Core pellets were coated with three different layers using fluid bed coating system. Enteric coating was performed using HPMC Phthalate and Eudragit L30 D55. Drug release from the pellets was optimized using concentration and type of enteric polymers as process parameters. Barrier coating was performed to prevent drug and enteric coating polymer. HPMC AS reacts with duloxetine to form toxic impurities. Coating with sufficient amount of barrier layer can prevent drug interaction with polymer.[13,14] Dissolution rate of pellets was compared with innovator product by similarity and dis-similarity factor. Other processing parameters such as rate of fluidization of product bed, spray rate of solution, atomization, curing time and temperature etc were optimized. Optimized pellets were evaluated for bulk and tapped density, Hausner's ratio, abrasion resistance, yield of pellets, moisture content, and particle size distribution. Optimized pellets were utilized to conduct scale up trials.

MATERIALS AND METHODS

Materials

Duloxetine HCl was obtained from Cadila Health Care Ltd. Sugar sphere #25-30 sieve size was purchased from Hann G. Werner GmbH & CO. HPMC 6 cps and HPMC 15 cps were received from Colorcon India Pvt Ltd. C 15 cps, HPMC Phthalate 55 were gifted by Shinetsu Chemical Co Ltd. Japan. Eudragit L30 D 55 was purchased from Evonic Industries. Talc (Luzenac) was purchased from Imerys Talc Industries, France. Triethyl citrate was purchased from Morflex, Inc. Sucrose was obtained from M. B. Sugars & Pharmaceuticals Ltd. Dehydrated Alcohol and Methylene dichloride was purchased from Merck Chemicals Pvt. Ltd.

Method

Drug and Excipient compatibility study

Drug and Excipient compatibility study was performed by FTIR and DSC mentioned as below.

Drug and Excipient compatibility study by FTIR[14]

Fourier-transform infrared **(**FTIR) spectra were obtained using an FTIR spectrometer (Shimadzu 8400S, Japan). The pure drug and excipients were mixed to prepare binary mixtures. The mixtures were mixed thoroughly with potassium bromide, an infrared transparent matrix, KBr pellets were prepared so as to contain approximately 2% (2:100) of drug and excipient mixture at a pressure of 30.7 MPa and a dwell time of 3 minutes. The spectrum for drug was recorded over the range of 4000 to 400 cm^{-1}.

Drug and Excipient compatibility study by DSC[14]

The DSC study was carried out using DSC-60 (Shimadzu, Tokyo, Japan). The instrument comprises of calorimeter, flow controller, thermal analyzer and operating software. The drug were heated in sealed aluminum pans under nitrogen flow (30 ml/min) at a scanning rate of 20°C/min from 50 to 300°C. Empty aluminum pan was used as a reference. The heat flow as a function of temperature was measured for the samples. The results are shown in figure.

Preparation of Pellets[15, 16]

The reported Pellets were prepared by Solution/Suspension layering technique of pelletization.

Core pellets of 25 to 30 # sieve were coated with subsequent layers mentioned below

Drug layer: Drug was loaded on sugar spheres of 25 to 30 # sieve using binder polymer HPMC 6 cps/ HPMC 15 cps

Barrier layer: Barrier layer was formed to separate drug layer and enteric layer. Barrier

layer forms hindrance between drug and enteric polymer so that it reduces chances of interaction and enhances stability of product.

Enteric layer: Enteric coating was performed to target drug release to the intestine. Drug undergoes degradation at acidic pH so pellets were coated with enteric polymer to bypass drug release in to the stomach. Enteric coating was performed using HPMC Phthalate 55 and Eudragit L30 D55.

Drug coating

Preparation of Duloxetine HCl solution for drug loading of pellets

- 16 % W/W of coating solution was prepared.
- Required quantity of ingredients was weighed using Digital weighing Balance.
- Almost half of the Purified water was heated up to 50oC using Electrical heater. To the heated water required quantity of HPMC 6 cps / HPMC 15 cps was dispersed for 20 minutes using stirrer.
- Duloxetine HCl was dissolved in remaining quantity of water with continuous stirring up to 15 minutes.
- Then Duloxetine solution was added to HPMC solution with continuous stirring. And the mixture was stirred for 20 minutes, mean while talc was dispersed and stirred to obtain homogenous dispersion.

During the preparation of coating solution the 10% of excess was prepared to recover the loss during practical work. And the coating solution was sprayed over sugar spheres using Fluid bed coater until weight gain was achieved and % yield was calculated.

Barrier Coating

Preparation of barrier coating solution

- 15 % w/w of coating solution was prepared.
- Required ingredients were weighed using Digital weighing Balance.
- Purified water was heated up to 60oC using Electrical heater. To the heated water required quantity of HPMC 15 cps was dispersed for 20 minutes using stirrer.
- Sucrose was added to the above solution with continuous stirring for 15 minutes. Mean while Talc was dispersed and continuously stirred still homogenous dispersion was obtained.

During the preparation of coating solution the 20% of excess was prepared to recover the loss during practical work.

Formula for Drug loading

Table: 1 Formula compositions of Drug coating

Drug Coating	D1	D2	D3	D4
Sugar spheres 25 - 30 #	64	150	150	150
Duloxetine HCl	67.36	67.36	67.36	67.36
HPMC6cps	12.7	8.63	10	-
HPMC15cps	-	-	-	10
Talc	-	-	-	10
Purified Water (16 % Soln)	q.s	q.s	q.s	q.s
Capsule fill weight	**144.06**	**225.99**	**227.36**	**237.36**

Enteric Coating

Preparation of HPMC P 55 coating solution

- 20% W/W of coating solution was prepared.
- Excipients were weighed accurately in required amount using Digital weighing balance.
- Denatured Alcohol was mixed with Methylene dichloride in 1:1 ratio in stainless steel container.

- To above solvent mixture HPMC P 55 was dissolved with continuous stirring for 15 minutes. During stirring the container was kept closed.
- Triethyl citrate was added to HPMC Phthalate solution with continuous stirring

Formula for Barrier coating

- Triethyl citrate was added to the above solution and stirred for 10 minutes. Then talc was added to the solution with continuous stirring until homogenous dispersion was obtained.
- During the preparation of coating solution the 20% of excess was prepared to recover the lost during practical work.

Table 2: Formula compositions of Barrier coating

Barrier coating (% w/w)	**B1 30%**	**B2 25%**	**B3 35%**	**B4 45%**	**B5 60%**	**B6 32%**	**B7 10%**	**B8 13%**	**B9 9%**
Drug coated Pellets (D4)	237.36	237.36	237.36	237.36	237.36	237.36	237.36	237.36	237.36
HPMC15cps	51	32.5	44.4	57	76	40	13.73	13.73	8.5
Sucrose	7.74	21.5	29.6	38	50.5	27	11	11	7
Talc	10.2	6.5	9	11.4	15.5	8	-	7	6
Purified Water	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s
Capsule Fill	306.2	297.86	320.36	343.76	379.36	312.36	262.09	269.09	258.86

Formula for enteric coating with HPMC phthalate

Table: 3 Formula compositions of trial Batches of HPMC Phthalate polymer

Enteric coating (% w/w)	**E1 25%**	**E2 15%**	**E3 11%**	**E4 11%**	**E5 11%**	**E6 11%**	**E7 10%**	**E8 10%**
Barrier coated pellets	B2	B2	B2	B3	B4	B5	B3	B6
HPMC P-55	55	33	24.5	24.5	24.5	24.5	21.9	21.9
Triethyl citrate	8.25	5	3.7	3.7	3.7	3.7	3.4	3.4
Talc	11	6.5	4.5	4.5	4.5	4.5	4.4	4.4
Denatured Alcohol	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s
Methylene Dichloride	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s
Capsule fill weight	**372**	**342.5**	**330.5**	**352**	**375.5**	**411**	**349**	**341.5**

Preparation Eudragit L30 D-55 coating solution

- 12% W/W of coating solution was prepared.
- All required excipients were weighed accurately using digital weighing balance.
- Eudragit L30 D-55 solution was added to remaining quantity of water with continuous stirring for 10 minutes.
- Triethyl citrate was added to the above solution and stirred for 10 minutes. Then talc was added to the solution with continuous stirring until homogenous dispersion was obtained.
- During the preparation of coating solution the 20% of excess was prepared to recover the lost during practical work.

Formula for enteric coating with Eudragit L30 D-55

Table: 4 Formula compositions of trial Batches of Eudragit L30 D-55polymer

Enteric coating % w/w	**E9 30%**	**E10 25%**	**E11 25%**	**E12 25%**	**E13 25%**	**E14 20%**
Barrier coated pellets	B7	B7	B7	B8	B9	B9
Eudrgit L30 D-55	59	50	48	48	48	38.5
Triethyl citrate	11.5	5	7.3	7.3	7.3	5.78
Talc	8	10	10	10	10	7.5
Purified Water	Qs	q.s	q.s	q.s	q.s	q.s
Capsule Fill weight	**340**	**326.5**	**326.5**	**333.5**	**324**	**310.5**

Processing Parameters

Table: 5 Processing parameters for various steps

Processing Parameters	**Drug Coating**	**Barrier coating**	**Enteric coating with HPMC P 55**	**Enteric coating with Eudragit L30 D55**
Inlet temperature^{o}C	45 – 55	40 - 55	35 - 50	30 – 50
Bed temperature^{o}C	35- 42	35 - 42	25- 30	25 – 30
Outlet temperature^{o}C	35-45	35 - 45	35-45	25 – 40
Spray pump speed(rpm)	2-8	2 - 8	2-13	2 - 6
Spray Rate (gm/ min)	2-7	2 – 7	2-10	2 - 6
Atomization (bar)	1.2- 1.4	1.2- 1.4	1.2- 1.4	1.2- 1.4
Fluidization (CFM)	40- 65	40 – 65	40- 65	40- 65
Spray gun diameter (mm)	1.0	1.0	1.0	1.0

EVALUATION OF ENTERIC COATED PELLETS

Drug Content[17,18]

Pellets from the capsule were dispersed in to 190 ml of pH 6.8 phosphate buffers by ultrasonication for 30 minutes followed by 10 minutes stirring using magnetic stirrer. The solution was then filtered and the residues over filter paper were washed with 10 ml phosphate buffer. The solution was then diluted up to suitable concentration and absorbance was measured using double beam UV-VIS Spectro photometer at 288.5 nm.

Acid Resistance test[18]

Principle: Residual Assay

Apparatus: USP Dissolution apparatus type I (basket)

Simulated Gastric fluid: 0.1N HCl (pH 1.2)

Volume of media: 1000 ml

Capsules were placed in the Basket and were rotated at 100 rpm at 37 ± 0.5°C for 2 (Two) hours. After two hours drug content left in the pellets was assayed. Pellets left in the Basket after two hours were dissolved in 190ml 6.8 pH Phosphate buffer for 30 minute by Ultra sonicator followed by 10 min stirring using magnetic stirrer until pellets disintegrates completely. The solution was filtered and the residues over filter paper were washed with 10 ml phosphate buffer. The solution was then diluted up to suitable concentration and absorbance was measured using double beam UV-VIS Spectro photometer at 288.5 nm. Drug release in 0.1 N HCl was calculated using following equation.

Drug released in Gastric Fluid = Drug content of Capsule – Residual Assay…... (I)

In-vitro drug release study[19]

Capsules were evaluated for *in-vitro* release study in 0.1 N HCl and phosphate buffer 6.8 pH. The drug dissolution test of Capsule was carried out using USP Dissolution apparatus type I (basket). Capsules were placed into the baskets and 1000 ml of 0.1 N HCl (pH 1.2) solution was filled in to the beaker. The baskets were rotated at 100 rpm. Buffer temperature was maintained at 37 ± 0.5 ºC for two hours. Then 0.1 N HCl solution was replaced with 1000 ml of pH 6.8 phosphate buffer and the baskets were rotated at 100 rpm and 37 ± 0.5 ºC buffer temperature. The 10 ml of sample aliquots were collected at 5, 10, 15, 30, 45, 60, 120 minutes replaced with 10 ml of fresh media. The absorbance of sample was then measured using Double beam UV Visible spectrophotometer at 288.5 nm.

Similarity Factor[20]

The similarity factor (f_2) is defined by CDER, FDA and EMEA as the "logarithmic reciprocal square root transformation of one plus the mean squared difference in percent dissolved between the test and the reference products". The similarity factor (F_2) given by SUPAC guidelines for modified release dosage form was used as a basis to compare dissolution profile. The dissolution profiles of products were compared using F_2. The similarity factor is calculated by following formula,

$$f2 = 50 \times \log \{[1 + (1/n) \Sigma_{t=1}^{n} (R_t - T_t)^2]^{-0.5} \times 100\} \ldots\ldots\ldots\ldots\ldots \text{(II)}$$

Where, n is the number of dissolution time points

R_t - The reference profile at the time point.

T_t - The test profile at the same point.

Table: 6 Significance of similarity factor

Similarity factor (F_2)	Significance
< 50	Test and reference profiles are dissimilar
50 – 99.99	Test and reference release profiles are similar
100	Test and reference release profiles are identical
> 100	The equation yields a negative value

Dissimilarity Factor[21]

Difference factor focuses on the difference in percent dissolved between reference and test at various time intervals. It can be mathematically computed by using following equation.

$f1 = \{[\Sigma_{t=1}^{n} |R_t - T_t|] / [\Sigma_{t=1}^{n} R_t]\} \times 100$…..... (III)

Where, n is the number of dissolution time points

R_t- The reference profile at the time point t

T_t- The test profile at the same point

As per US FDA guidelines Difference factor of 0-15 ensures minor difference between two products.

Pellets Size distribution[22]

Size distribution is an important parameter because it has significant influence on the release kinetics. Pellet size depends upon coating thickness. Uniformity of size indicates uniformity of coating thickness. Particle size determination carried out by simple sieve analysis using sieve shaker.

Abrasion resistance[23]

The resistance to abrasion was analyzed using friability tester. A pre weighed sample taken from the usable yield fraction was placed in a friabilator along with 15 steel spheres, each 2mm in diameter. After 100 revolutions at 25 rpm, the mass retained on the sieve was weighed and the abrasion resistance was calculated as the percentage loss of mass between initial and final weights pellet.

Flow Property Characterization[22]

Flow of pellet depends upon roundness of pellet shape. So flow measurement of pellet can indirectly give an idea about shape of the pellets. More the circularity more will be the flow of pellets. Flow of pellets is very important parameter for capsule filling.

Angle of Repose

This is the maximum angle possible between the surface of a pile of powder and the horizontal plane. 100 gm of pellets were allowed to flow by funnel from 4 cm of height from the base. The height of pile and diameter of base was measured and calculate the angle of repose by following formula

$$\theta = \tan^{-1} \frac{h}{r} \quad \text{..... (IV)}$$

Where, θ = angle of repose,

h = Height of the heap,

r = Radius of the heap.

Table 7: The relationship between Angle of repose and powder flow

Angle of repose	Powder flow
< 25	Excellent
25-30	Good
30-40	Passable
40 >	Very poor

Density

Density measurement of pellets determines the fill volume of pellets so bulk and tap density of pellets was determined. An accurately weighed 100gm of drug pellets transferred to a 200ml measuring cylinder and the volume occupied by the powder in terms of ml was recorded. Bulk density of drug was calculated using following equation (V).

The loosely packed 50 gm powder of drug in the measuring cylinder was tapped 100 times on a plane hard surface and volume occupied in ml was noted. Tapped density of drug was calculated using following equation (VI).

Bulk Density (ρ) = Mass (m) / Volume (v)..... (V)

Tapped Density (ρ_t) = Mass (m) / Volume (v)..... (VI)

Hausner's Ratio

Hausner found that the ratio TBD/LBD was related to interparticle friction and, as such, could be used to predict powder flow properties.

$$\text{Hausner's Ratio} = \frac{\text{Tapped bulk density (TBD)}}{\text{Loose bulk density (LBD)}} \quad \text{.... (VII)}$$

Carr's compressibility index

The flow ability of powder can be evaluated by comparing LBD and TBD of powder and the rate at which it packed down. Compressibility index is calculated by following equation.

Compressibility Index= (TBD-LBD) × 100

$$\text{Compressibility index} = \frac{\text{TBD}-\text{LBD}}{\text{TBD}} \times 100 \quad \text{... (VIII)}$$

Table 8: Effect of Carr's Index and Hausner's Ratio on flow property

Carr's Index (%)	Flow Character	Hausner's Ratio
< 10	Excellent	1.00–1.11
11–15	Good	1.12–1.18
16-20	Fair	1.19-1.25
21-25	Passable	1.26-1.34
26-31	Poor	1.35-1.45
32-37	Very poor	1.46-1,59
>38	Very very Poor	>1.60

Stability study[25]

Stability testing of drug products begins as a part of drug discovery and ends with the demise of the compound or commercial product. FDA and ICH specifies the guidelines for stability testing of new drug products, as a technical requirement for the registration of pharmaceuticals for human use.

The ICH Guidelines have established different temperatures and period of stability testing. Depending on the time duration for which stability study performed temperature and humidity conditions are listed in the table given below.

Adopted process for stability Testing

In the present study 30 capsules from F11 and F17 batch each were filled in different HDPE

Table 9: ICH guidelines for Stability Study

Study	Storage condition	Time period
Long term	25°C±2°C / 60% RH±5% RH or 30°C±2°C / 65% RH±5% RH	12 month
Intermediate	30°C±2°C / 65% RH±5% RH	6 month
Accelerated	40°C±2°C / 75% RH±5% RH	6 month

Bottle with molecular sieve desiccant. Closure was sealed. Filled Bottles were labeled and stored in Stability Chamber at 40°C± 2°C and 75% ±5% RH for 1 month. At the end of one month capsules were evaluated for Physical changes, Assay, Gastric Resistance and Dissolution test.

RESULTS & DISCUSSION

Drug and Excipients compatibility study by FTIR Spectroscopy

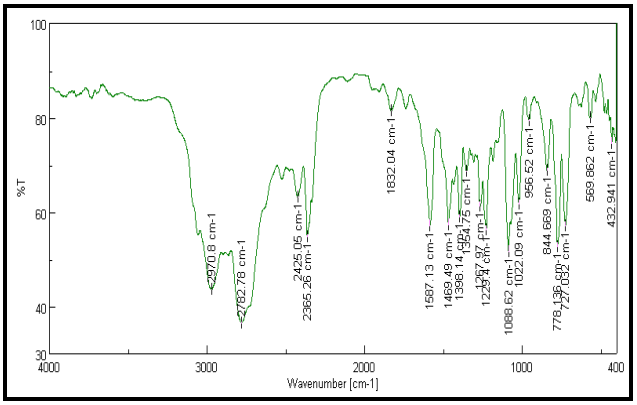

Figure 1: FTIR Spectra of Duloxetine HCl Pure Drug

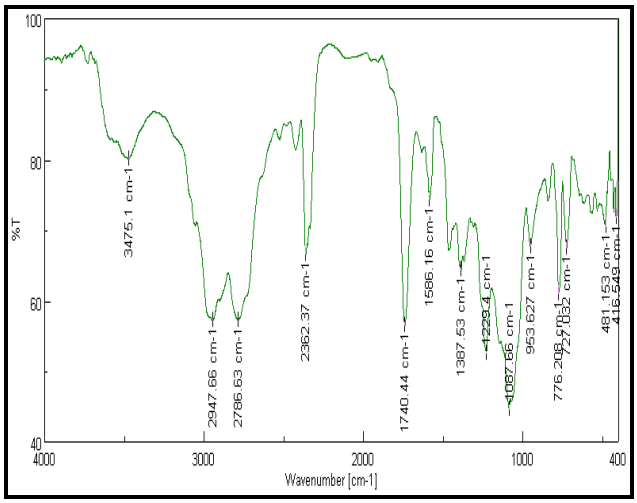

Figure 2: FTIR Spectra of Drug and HPMC Mixture

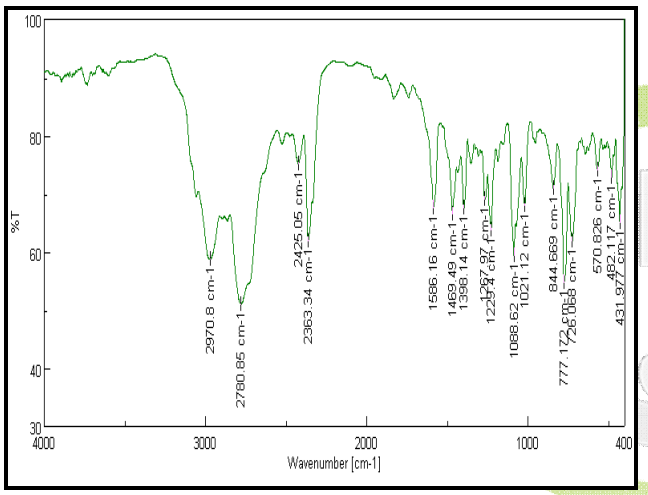

Figure 3: FTIR Spectra of Drug and HPMC P 55 mixture

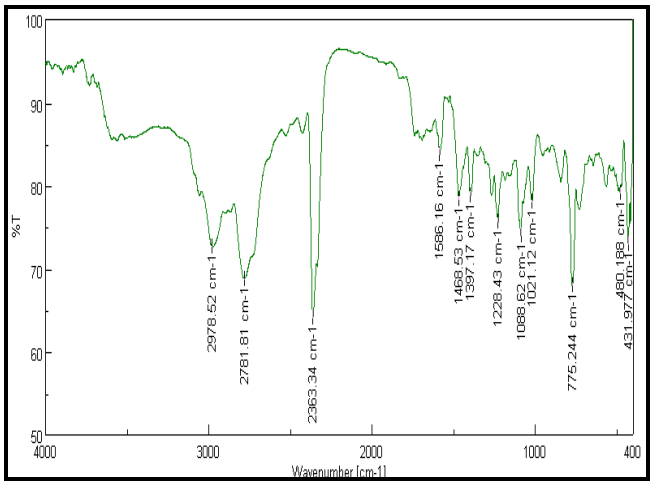

Figure 4: FTIR Spectra of Drug and Eudragit L30 D 55 mixture

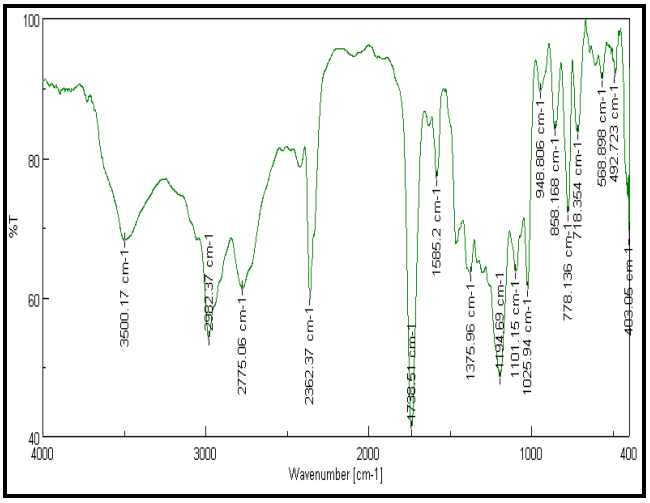

Figure 5: FTIR Spectra of Drug and TEC mixture

Drug and Excipients compatibility by Differential scanning calorimetry

The thermal behavior of Duloxetine and Excipients mixture was investigated by heating the respective samples at 20°C/min. For this sample an endothermic peak was observed at 162.9°C and 183.91°C. The pure Drug (Figure) had endothermic peaks at 171.57°C. So from DSC and FTIR spectroscopy excipients compatibility with drug was confirmed.

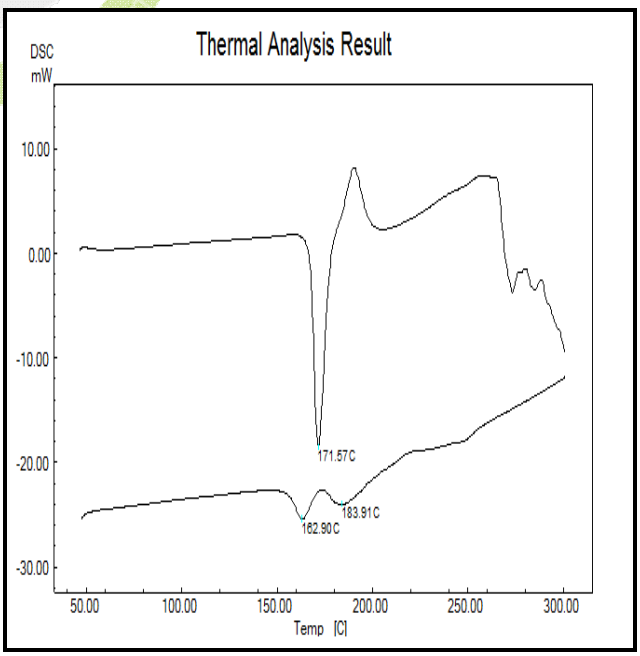

Figure: 6 DSC curves of Drug and Excipients mixture overlapped

Table: 10 Interpretations of FTIR Spectra of drug and excipients mixture

Functional group	Wave numbers cm^{-1}					
	Drug	Mixtures (Drug+Excipients)				
		HPMC	HPMC P 55	Eudragit L 30D55	HPMC AS LF	TEC
C-H Stretching	2970.8	2947.66	2970.8	2978.52	2947.66	2982.37
C=C Stretching	1587.13	1586.16	1586.16	1586.16	1586.16	1585.2
C-H Bending	1398.14	1387.53	1398.14	1397.17	1387.53	1375.96
C-O Stretching	1088.62	1087.66	1088.62	1088.62	1087.66	1025.94
C-N Stretching	1229.4	1229.4	1228.43	1228.43	1229.4	1228.43
N-H Bending	760	776.208	775.24	775.244	776.208	775.244

From the results it was observed that there are no significant changes in the vibrational frequencies of functional groups of pure drug and mixture. This indicates excipients are compatible with the drug.

Drug content

Table 11: Result of Drug content

Batch No.	Drug Content* (% w/v)
Innovator	100.1±2.8
E1	101.7±2.95
E2	100.6±1.89
E3	99.3±2.54
E4	100.2±2.75
E5	101.4±2.33
E6	98.7±2.46
E7	100.2±2.17
E8	100.3±2.2
E9	97.6±2.55
E10	99.5±1.25
E11	98.6±1.33
E12	100.5±3.34
E13	102.3±2.68
E14	100.7±2.12

*Listed value indicates mean value of results and Standard deviation (where n=6)

Acid Resiatance Test

Results indicated that Batches E1 to E13 produced good gastric resistance and E14 failed to provide gastric resistance which was coated with 20% w/w of Eudragit L30 D55. From results it was concluded that minimum concentration of HPMC P55 and Eudragit l30 D55 required to achieve gastric resistance was 10% w/w and 25% w/w respectively.

Table 12: Result for Acid resistance Test

Batch No.	Residual Assay (% w/v)	% Drug Release in 0.1N HCl*
Innovator	100±2.12	0.1
E1	101.3±2.25	0.4
E2	99.9±2.3	0.7
E3	97.9±2.56	1.4
E4	98.7±2.3	1.5
E5	100.0±2.65	1.4
E6	97.37±2.23	1.33
E7	98.7±2.67	1.5
E8	98.9±1.56	1.4
E9	96.1±2.49	1.5
E10	99.1±1.35	0.4
E11	98.24±1.3	0.36
E12	100.4±1.54	0.1
E13	102.1±1.48	0.2
E14	83.4±1.67	17.3

*Listed value indicates mean value of results and Standard deviation (Where n=6)

In-Vitro Drug release study

In-vitro drug release study was performed using USP apparatus-I (Basket type) in 0.1 N HCl for first two hrs and then in phosphate buffer 6.8 pH for other 2 hrs and the graphs was plotted between Time Vs % Cumulative Drug Release. % Cumulative Drug Release from batch E1 to E13 is shown in figure 7 to 9.

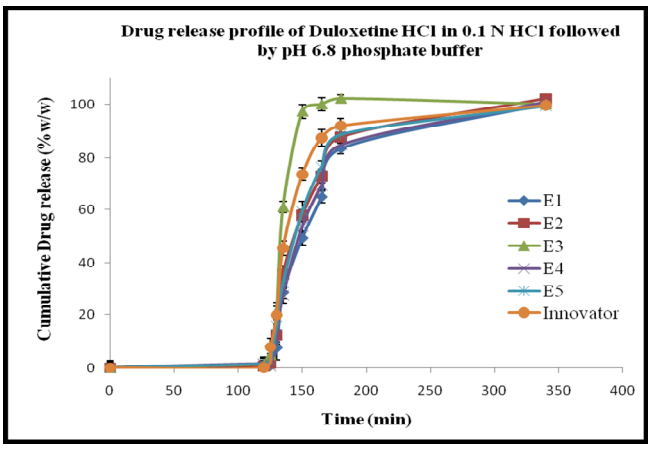

Figure: 7 Comparative Disssolution study of Batch E1 to E5 with Innovator

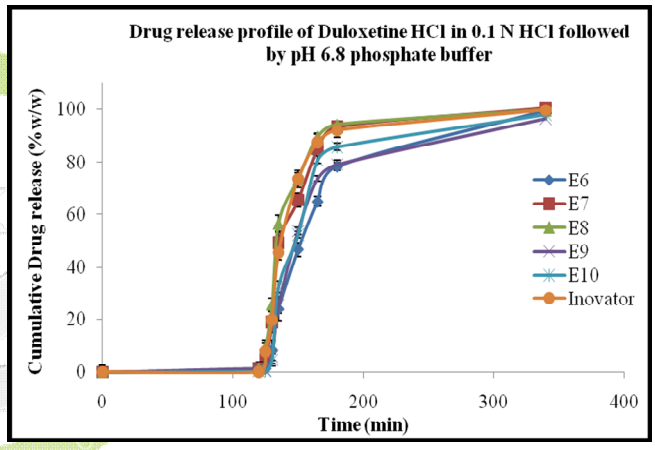

Figure 8: Comparative Dissolution Study of Batch E6 to E10 with Innovator

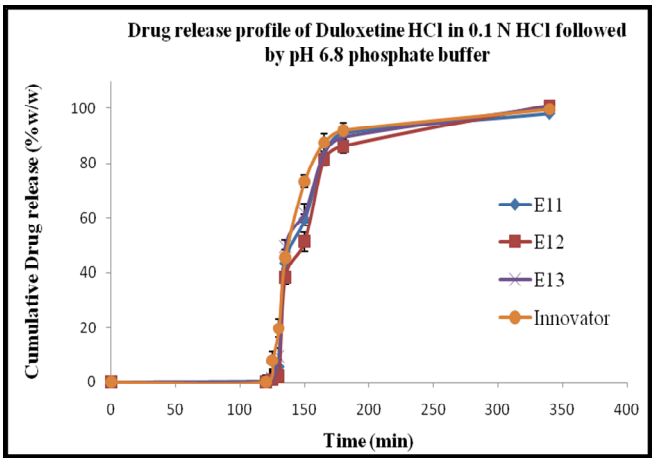

Figure 9: Comparative Dissolution Study of Batch E11, E12 and E13 with Innovator

Similarity & Dissimilarity factor

From the dissolution data developed formula was compared with the dissolution rate of innovator product. Results obtained are listed in the table 13.

Table: 13 Similarity and Dissimilarity factors

Batch No	Similarity (f_2)	Dissimilarity (f_1)
Innovator	**100**	**0**
E1	42	18
E2	51	12
E3	47	13
E4	45	15
E5	54	10
E6	40	20
E7	72	4
E8	67	4
E9	44	17
E10	48	14
E11	55	9
E12	48	13
E13	60	8

From results it was observed that Batches E2, E5, E7, E8, E11 and E13 have Similarity and Dissimilarity factors within the standards limits among them batch no E7 and E13 respectively enteric coated with HPMC P 55 and Eudragit L30 D55 produces much betters Similarity and Dissimilarity factor values. So they can be used for further studies and scale up trials.

Pellets Size Distribution

Table: 14 Result for Pellets size distribution

Sieve size	E7(%w/w)	E13 (%w/w)
#14 Retained	0	0
#16 Retained	3	4
#20 Retained	95	93.5
#30 Retained	1	1.5
#45 Retained	0.5	0.5

Sieve analysis revealed that almost 95% of pellets were distributed between 16 to 20 # sieves so pellets size distribution was found uniform.

Abrasion resistance

Test was performed using friability tester. Usable yield fraction was placed in a friabilator along with 25 steel spheres, each 2mm in diameter. After 100 revolutions at 25 rpm initial and final weight was measured. The results obtained are listed as below.

Results observed that loss of pellets mass was lesser than 1%w/w so pellets passes the abrasion resistance.

Table: 15 Result of Abrasion resistance test

Observation	E7	E13
Initial weight	6.50	6.50
Final Weight	6.49	6.48
Loss of mass (% w/w)	0.15 %	0.30 %

Flow Property Characterization

Flow of pellets is very important during capsule filling stage to assure weight uniformity of capsules so flow property of pellet was

evaluated and (Avg. of 3) results obtained are listed as below in the table.

Table: 16 Result of Flow Property Characterization

Test	Result		Flow
	E7	E13	
Angle of repose	20.3	21.2	Excellent
Bulk Density (gm/ml)	0.86	0.87	Excellent
Tapped density (gm/ml)	0.88	0.91	Excellent
Hausner's Ratio	1.02	1.04	Excellent
Carr's Index	2.29	4.6	Excellent

From the above observations it was noted that flow property of pellets was excellent. That indicates uniformity of size and sphericity of pellet was good.

Stability Study

Capsules from E7 and E13 batches each were filled in different HDPE Bottle with molecular sieve desiccant. Closure was sealed. Filled Bottles were labeled and stored in Humidity chamber at 40°C± 2°C and 75% ±5% RH for 1 month. After one month capsules were evaluated. Results obtained are recorded in the table 17.

After one month treatment in humidity chamber no physical changes were observed. Assay and Acid resistance test of capsule complies with specifications and the drug release in pH 6.8 phosphate buffer meets Q Point requirement that is drug release is not less than 75% w/w in 60 minutes. So both the batches pass stability study. E7 and E13 batches are stable.

Table 17: Results of stability testing

Test	Observation after one month		Inference
	E7	E13	
Description	Whit to off white pellets filled in size '1' Hard gelatin capsule	Whit to off white pellets filled in size '1' Hard gelatin capsule	Satisfactory
Drug content %w/w (90% - 110%)	99.8 ± 2.23	101.12 ± 1.61	Satisfactory
Drug release in 0.1 N HCl in 2 hours (NMT 10%)	1.2 ± 2.4	1.4 ± 2.1	Satisfactory

CONCLUSION

It is observed from the results that the type and concentration of enteric polymer plays a primary role in acid-resistant pellets. Pellets with good release at the end of 2 h in SIF were mostly affected by the type of enteric polymer since polymers dissolve at different pH. The barrier polymer is another factor affecting drug release patterns since it is the next layer that obstructs the drug release. Therefore, an increase in the concentration of the barrier polymer increases the extent of obstruction of drug release. Also the composition of barrier layer affects drug release from the pellet. Sucrose present in the barrier layer dissolves rapidly in the water and promotes drug release so more the amount of sucrose to HPMC, the drug release was faster. The concentration of the binding polymer did not affect acid resistance or the release in phosphate buffer. Processing with HPMC P 55 was easier compared to processing with Eudragit L30 D55.

Enteric coated pellets evaluated for Gastric resistance test and In-Vitro drug release study. Results revealed that among the trails performed using pellets coated using HPMC P 55 and

Eudragit L30 D55 produced sufficient gastric resistance. Drug release from prepared pellets was compared with Drug release profile of innovator product using Similarity and Dissimilarity factor. Based on f_1 and f_2 data pellets were optimized to achieve drug release pattern similar to the innovator product. Results showed that Trial batch E7 is the best match with innovator product. For E7 Batch f_2 value is 72 and f_1 value is 4. The pellets consisting of 35 % of barrier coating and 10 % of enteric coating and 4.60 % of binder polymer. Barrier layer composed of HPMC 15 cps: Sucrose: Talc in the ratio 1.5: 1: 0.3 and enteric layer composed of HPMC P 55: TEC: Talc in the ratio 6.44: 1: 1.30. Trial no E13 also produces satisfactory Similarity and Dis-similarity values consequently 60 and 8. So E7 can be used for scale up trials and expected to be Bio-equivalent to the innovator product but *In- Vivo* Bio equivalence study is required to be performed as *In- Vitro* results doesn't always resemble the *In-Vivo* performance.

Table 18: Results for Dissolution study of stability batches

	Batch No. / Sample time (min)	**Dissolution media: pH 6.8 phosphate buffer**		
		Innovator	**E7**	**E13**
% CDR* **(NLT 75% of the labeled amount of Duloxtine in 60 min)**	5	7.8 ± 2.5	5.3 ± 2.56	1.3 ± 1.45
	10	19.8 ± 3.35	17.5 ± 2.68	10.9 ± 2.55
	15	45.5 ± 3.3	47.3 ± 2.59	43.7 ± 2.45
	30	73.5 ± 2.85	64.9 ± 2.95	59.9 ± 2.62
	45	87.5 ± 2.35	84.7 ± 3.56	79.5 ± 2.34
	60	92 ± 3.14	95.4 ± 1.39	91.2 ± 1.49
	120	99.7± 2.67	99.5 ± 1.23	99.1 ± 1.79
Similarity factor		100	71	58
Dissimilarity factor		0	4	8

* Listed value indicates mean value of results and Standard deviation (Where n=6)

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