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Original Article

FORMULATION DESIGN AND *IN VITRO* EVALUATION OF BILAYER SUSTAINED RELEASE MATRIX TABLETS OF DOXOFYLLINE

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ABSTRACT

Objective: To develop bilayer matrix tablet of Doxofylline by providing a loading dose followed by the maintenance dose that suppose to enhance the therapeutic efficacy the drug for acute and sustainable asthma.

Methods: Both immediate release layer and sustained release layer were prepared by wet granulation methods. Different Pre compression and post compression characterization of the tablet were carried out. Swelling studies were carried out for all the formulation. To optimise the immediate release layer, similarity (f_2) and difference factor (f_1) were calculated and optimised IR formulation was used for all formulations of bilayer tablet. In-vitro release studies were carried out in USP II paddle type dissolution apparatus for different formulations and release kinetic studies were carried out different kinetic model. FTIR and DSC studies were carried out for pure drug Doxofylline, IR layer and SR layer of optimised formulation to know the physical and chemical compatibility of drug and excipients. Accelerated stability studies were carried out to confirm the stability of dosage forms.

Results: Pre compression and post compression parameters satisfied with pharmacopeia specifications. The formulation that contained highest percent of HPMC had highest swelling index. Formulation $DBMF_6$ showed an initial release of 44% of drug within one hour as the loading dose and remaining drug were sustained release up to 12 h. Release kinetic followed Hixon-Crowell kinetic model with drug release mechanism quasi-fickian diffusion. From accelerated stability studies no significant changes in physicochemical properties were noticed.

Conclusion: Doxofylline bilayer matrix tablets were successfully developed and can be used as an alternative to the conventional dosage form because it can be therapeutically beneficial for management of asthma.

Keywords: Bilayer tablet, Sustained release, Doxofylline, Immediate release, HPMC, Eudragit.

INTRODUCTION

Bilayer tableting technology has gained popularity in recent times, as bilayer tablets offer several advantages over conventional tablets. Usually conventional dosage form produce wide ranging fluctuation in drug concentration in the blood stream and tissues with consequent undesirable toxicity and poor efficiency. This factor such as repetitive dosing and unpredictable absorption led to the concept of controlled drug delivery systems.

The goal in designing sustained or controlled delivery systems is to reduce the frequency of the dosing or to increase effectiveness of the drug by localization at the site of action, reducing the dose required or providing uniform drug delivery. The primary objective of sustained release drug delivery is to ensure safety and to improve efficacy of drugs as well as patient compliance. Bilayer tablet is suitable for sequential release of two drugs in combination, separate two incompatible substances and also for sustained release tablet in which one layer is immediate release as an initial dose and the second layer is maintenance dose [1].

The bilayer tablet concept has long been utilized to formulate biphasic release of drugs [2]. Such a bilayer tablet contains a first release layer and a sustain release layer. The first releasing layer leads to rapid release of the drug, so as to reach high serum concentration in a short period of time that is called as loading dose. The sustain release layer of the bilayer tablet releases the drug for the prolonged period of time to maintain the effective concentration of drug within the therapeutic index [3].

This release pattern is required for successful treatment in many therapies, primarily when maximum relief needs to be achieved as soon as possible, and is followed by a sustained release phase to avoid repeated drug administration. So bilayer matrix tablet containing a single drug having one layer as fast release layer and another as sustained release layer will be beneficial for the chronic disease like asthma, diabetes, hypertension and inflammation that require immediate effect as well as maintenance therapy [4].

Asthma and COPD (Chronic Obstructive Pulmonary Disease) are the most common life threatening pulmonary disease that requires constant monitoring. Doxofylline, a methyl xanthine derivative that works by inhibition of phosphodiesterase IV activities, has recently drawn attention because of its better safety profile and similar efficacy over the most widely prescribed analogue, theophylline, indicated for asthma and chronic obstructive pulmonary disease due to decreased affinities towards adenosine A1 and A2 receptors. Doxofylline is chemically designated as 7-(1, 3 dioxolone-2-yl methyl) theophylline. Presence of a dioxolane group in position C-7 differentiates it from theophylline. Doxofylline is an anti-tussive and bronchodilator used for maintenance therapy in patients suffering with asthma and chronic obstructive pulmonary disease (COPD) and is extensively metabolized in the liver by demethylation and oxidation to an extent of 80-90% and 48% plasma protein bound. Elimination half life ($t_{½}$) is around 6-7 h and<4% of an administered dose of Doxofylline is excreted unchanged in the urine. The daily dose is 200-400 mg two to three times in a day. Doxofylline is coming under class III of BCS classification and oral absorption is 62.2%. It is having solubility of 12 mg/ml in water and having P^{Ka} 9.87 [5].

The objective of the present study was to develop bilayer tablets of Doxofylline with a fast-release layer using cross carmellose as superdisintegrant and a sustaining layer using hydroxyl propyl methylcellulose (HPMC K4M, K15M) and Eudragit RSPO, RLPO as polymeric retardant materials. This formulation is suppose to be superior to available Doxofylline conventional and sustained release tablet marketed formulation as the former required frequent dosing and later having low initial dose that may leads to slow unset of action. The formulation under study is suppose to deliver an initial loading dose from immediate release layer followed by maintenance dose from sustained release layer that may be useful for better therapeutic management of acute and sustainable asthmatic conditions. Different proportion of HPMC K4M, K15M and Eudragit

RSPO, RLPO were selected to form sustained release layer for different bilayer formulation to optimise drug release profile [6].

MATERIALS AND METHODS

Materials

Doxofylline was procured as a gift sample from Dr. Reddy's Laboratories Hyderabad, India. The super disintegrant cross carmellose sodium was also obtained as a gift sample from Dr. Reddy's laboratories Pvt. Ltd. HPMC K4M, HPMC K15M and Eudragit RSPO, RLPO polymers were received as gift sample from Glenmark Pharma, Nasik, India. The diluent Micro crystalline cellulose (MCC) and lactose was purchased from Otto Manufacturers. PVP K30, Talc and magnesium Stearate were purchased from S. D. fine chemicals Pvt. Ltd' Mumbai, India. All the ingredients were of laboratory grade. The distilled water used in the process of research work was prepared by double distillation process in the laboratory.

Methods

Calculation of dose of drug in a bilayer sustained release tablets containing single drug

The total dose of Doxofylline for a bilayer sustained release tablet formulation containing an immediate release layer and sustained release layer was calculated by the following four equations using available pharmaco kinetic data from a design of one compartment model with simultaneous release of loading dose and maintenance dose with a zero-order release as described by Robison and Eriksen: [6, 7]

$$K_0 = D_I K_E \ldots\ldots\ldots\ldots\ldots (1)$$

$$D_M = K_0 T \ldots\ldots\ldots\ldots\ldots (2)$$

$$D_L = D_I - K_0 T_{max} \ldots\ldots\ldots\ldots\ldots (3)$$

$$D_T = D_L + D_M \ldots\ldots\ldots\ldots\ldots (4)$$

D_M = maintenance dose; T = time for sustained action; T_{max} = Time to reach peak plasma concentration; elimination half-life ($t_{1/2}$) of Doxofylline is 7–10 h (average 8.5); time to reach peak plasma concentration (T_{max}) = 1.19 h; initial dose (D_I) = 400 mg.

Elimination rate constant $K_E = {}^{0.693}/_{t_{1/2}}$

= 0.693/8.5h

= 0.082 h^{-1}

Zero-order release constant $K_0 = D_I \times K_E$

= 400 mg × 0.082 h^{-1}

= 32.8 mg/h

Loading dose $D_L = D_I - (K_0 \times T_{max})$

= 400–(32.8 × 1.19 h)

= 400–39.032

= 360.97 mg

So, maintenance dose = Total dose–loading dose

= 800 mg–360.97 mg

= 439.03 mg.

Hence, the matrix tablet should contain a total dose of 800 mg for 12 h SR dosage form and it should release 400–39.032 = 360.97 (45.12%) mg in the 1st hour like conventional dosage form and the remaining dose (800– 360.97) in remaining 11 h, i.e. 439.03 (54.88%) mg or 39.91 (4.98%) mg per hour up to 12 h.

Formulation of bilayer matrix tablets of doxofylline

Bilayer matrix tablets of Doxofylline consist of two types of granules *i.e.* the first layer consists of the optimized immediate release layer granules and for second layer, different proportion of polymers were used for sustained release layer to optimise drug release profile according the need. For preparation of both immediate release layer and sustained release layer granules; wet granulation methods were adopted. Accurate quantities of all ingredients were weighed and passed through sieve no #80 before their use in formulations. A lump wet mass was produced by adding the required quantity of distilled water as granulating agent. The aggregates formed were initially dried for 5-10 min to reduce moisture level and to prevent sticking with sieve. The aggregates were passed through sieve # 20 to get wet granules. The granules were then dried at 40° C for 20 min to reduce moisture content upto 2-5 %. After lubrication with magnesium stearate and talc; the granules of both the layer were evaluated for an angle of repose, bulk density, compressibility index and Hausner's ratio; prior to compression. The evaluated granules along with the immediate release layer granule were compressed into bilayer tablets on a 10-station rotary bilayer tablet punching machine using 12 mm concave punches. Each tablet contains 361 mg of Doxofylline in an immediate release layer and 439 mg of Doxofylline in sustained release layer. The composition for different formulations of an immediate release layer and sustained release layer was shown in table 1 **and** table 2 respectively**.** Same methods were followed for all the formulations. The prepared bilayer tablet formulations were evaluated for various post compression parameters like average thickness, weight variation, hardness, friability, drug content study and *in vitro* dissolution studies [8].

Table 1: Compositions of immediate release layer for Doxofylline bilayer matrix tablets

F. No.	Doxofylline (mg)	Avicel 101 (mg)	Lactose (mg)	PVP K30 (mg)	Starch (Soluble) (mg)	AC-Di-Sol (mg)	Polyplasdone-XL (mg)	Mg. stearate (mg)	Talc (mg)	Total wt. (mg)
$DIRF_1$	361	25	39	50	10	-	-	5	10	500
$DIRF_2$	361	25	29	50	20	-	-	5	10	500
$DIRF_3$	361	25	19	50	30	-	-	5	10	500
$DIRF_4$	361	25	39	50	-	10	-	5	10	500
$DIRF_5$	361	25	29	50	-	20	-	5	10	500
$DIRF_6$	361	25	19	50	-	30	-	5	10	500
$DIRF_7$	361	25	39	50	-	-	10	5	10	500
$DIRF_8$	**361**	**25**	**29**	**50**	**-**	**-**	**20**	**5**	**10**	**500**
$DIRF_9$	361	25	19	50	-	-	30	5	10	500
$DIRF_{10}$	361	25	29	50	-	10	10	5	10	500
$DIRF_{11}$	361	25	19	40	-	10	20	5	10	500
$DIRF_{12}$	361	25	19	40	-	20	10	5	10	500

Evaluation

Drug excipients compatibility studies

Drug excipients compatibility studies were done by FTIR and DSC

Fourier transforms infrared (FTIR) spectroscopy [9]

Fourier transform infrared (FTIR) study was performed to verify any physical or chemical interaction between the pure drug and the excipients used for formulation. In the present investigation FTIR

spectrum of pure drug Doxofylline, physical mixture of drug and excipients used for IR layer and SR layer of optimised formulation was carried out. These were performed by potassium bromide (KBr) pellet method.

The samples were triturated with KBr and pellet was prepared by setting the pressure to 100 kg/cm² for 2 min. The obtained pellet was analyzed in FTIR 8400S, Shimadzu, Japan. KBr background was obtained initially before analysis of test samples.

Differential scanning calorimetric (DSC) analysis [10]

The DSC analysis of pure drug Doxofylline, physical mixture of drug and excipients used for IR layer and SR layer of optimised formulation was carried out using a Shimadzu DSC 60, Japan; to evaluate any possible drug-excipients thermal interaction. Exactly weighed 5 to 6 mg samples were hermetically sealed in aluminium crucible and heated at the constant rate of 10 °C/min over a temperature range of 40 to 300 °C. Inert atmosphere was maintained by purging nitrogen gas at a flow rate of 50 ml/min.

Table 2: Composition of sustained release layer for Doxofylline bilayer matrix tablets

F. No.	Doxofylline (mg)	HPMC K4M (mg)	HPMC K15M (mg	Eudragit RSPO (mg)	Eudragit RLPO (mg)	Lactose (mg)	Starch (Insoluble) (mg)	Mg. stearate (mg)	Talc (mg)	Total wt. (mg)
$DSRF_1$	439	100	-	30	-	16	45	7	13	650
$DSRF_2$	439	-	100	30	-	16	45	7	13	650
$DSRF_3$	439	100	-	-	30	16	45	7	13	650
$DSRF_4$	439	-	100	-	30	16	45	7	13	650
$DSRF_5$	439	80	-	50	-	16	45	7	13	650
$DSRF_6$	439	-	80	50	-	16	45	7	13	650
$DSRF_7$	439	80	-	-	50	16	45	7	13	650
$DSRF_8$	439	-	80	-	50	16	45	7	13	650
$DSRF_9$	439	50	50	30	-	16	45	7	13	650
$DSRF_{10}$	439	50	50	-	30	16	45	7	13	650

Evaluation of pre compression parameters [10, 11]

The prepared dry granules of both the layers of Doxofylline bilayer matrix tablets were taken and evaluated for different pre compression parameters like angle of repose, bulk density, tapped density, Carr's index, and Hausner's ratio to ensure good flow properties of the same during tablet compressions. All the pre compression parameters are outlined below

Angle of repose (θ)

Angle of repose is indicated as maximum angle possible between the surface of a pile of granules and the horizontal plane. The granules were allowed to flow through the funnel fixed to a stand at the definite height (h). The angle of repose was then calculated by measuring the height and radius of the heap of granules formed.

$$\theta = \tan^{-1}\frac{h}{r}$$

Where θ was called as an angle of repose, h and r were height and radius of the granule heap respectably. According to the specifications the angle of repose value less than 25 ° indicates excellent flow whereas angle "between" 25 °-30 ° indicates good flow. The angle "between" 30 °-40 ° indicates passable flow and angle greater than 40 ° indicates very poor flow.

Bulk density and tapped density [12]

Both the loose bulk density (LBD) and tapped bulk density (TBD) of prepared granules of an immediate release layer and sustained release layer of all the formulations were determined. The quantity of 2 g of granules from each formula, previously lightly shaken to break any agglomerates formed; were introduced into a 10 ml measuring cylinder. After the initial volume was observed, the cylinder was allowed to fall under its own weight on to a hard surface from the height of 2.5 cm at second interval.

The tapings were continued until no further changes in volume were noted. The process was carried out thrice for each formulation and average was taken. Standard deviation was calculated to know the variation in the formulation. LBD and TBD of prepared granules were calculated using the following formulas.

$$LBD = \frac{\text{weight of the granule}}{\text{volume of the packing}}$$

$$TBD = \frac{\text{weight of the granule}}{\text{tapped volume of the packing}}$$

Compressibility index (Carr's index)

Compressibility index (Carr's index) of prepared Doxofylline granules for immediate release and sustained release layer were calculated by following formula

$$Carr's\ index\ (\%) = \frac{TBD - LBD}{TBD} \times 100$$

According to the specification, the Carr's index values "between" 5-15 indicates excellent flow whereas between 12-16 indicates good flow. Values "between" 18-21 indicate fare-passable whereas between 23-25 indicates poor flow whereas values between 33-38 indicates very poor flow and greater than 40 indicates extremely poor flow [12].

Hausner's ratio

The Hausner's ratios of prepared Doxofylline granules for immediate release and sustained release layer were determined by following formula.

$$Hausner's\ ratio = \frac{TBD}{LBD}$$

According to specifications values less than 1.25 indicate good flow (=20% of Carr's index), where as greater than 1.25 indicates poor flow (=33% of Carr's index). Between 1.25 and 1.5, added glidant normally improves flow [13].

Evaluation of doxofylline bilayer matrix tablets

Thickness

Thickness of each tablet was measured by using digital Vernier Callipers (Mitutoyo digital Thickness Gauge, Mitutoyo, Japan). Ten tablets of Doxofylline bilayer matrix tablets from each formulation were randomly selected and used for thickness determination. The results were expressed as mean values of ten readings, with standard deviations. According to specification tablet thickness should be controlled within a±5% variation of standard value [14].

Tablet hardness

All the formulations of Doxofylline bilayer matrix tablets were subjected to hardness measurement by using Monsanto hardness tester (Cad Mach). From each formulation the crushing strength of ten tablets with known weights was recorded in kg/cm² and average, were calculated and presented with standard deviation. According to specifications of USP hardness values of 5-7 kg/cm² for bilayer matrix tablet is considered as acceptable limit [14].

Friability

Previously weighed ten bilayer matrix tablets of Doxofylline from each batch were taken in Roche friabilator (Secor India). After100 revolutions of friabilator, tablets were recovered. The tablets were then made free from dust and the total remaining weight was recorded. Friability was calculated from the following formula.

$$\%F = \frac{(Wi - Wf)}{Wi} \times 100$$

Where W_i and W_f were the initial and final weight of the tablets before and after friability test. For compress tablet, lose between 0.1 to 0.5 % and maximum up to 1% of the tablet weigh are consider acceptable [14].

Weight variation test

According to USP monograph, the weight variation tolerance limit for the uncoated tablet having average weight 130 mg or less is 10% whereas for average weight between 130-324 mg is 7.5% and for average weight more than 324 mg is 5%. For the tablet to be accepted, the weight of not more than two tablets deviate from the average weight by not more than 7.5% and no tablet deviates by more than 15%. All formulated Doxofylline bilayer matrix tablets were evaluated for weight variation as per USP monograph. Twenty tablets were weighed collectively and individually using an electronic balance (Citizen CTG-302). The average weight and percent variation of each tablet was calculated [15].

Content uniformity

Twenty Doxofylline bilayer matrix tablets were taken and triturated to form powder and powder equivalent to one tablet was taken and dissolved in 100 ml of phosphate buffer P^H 6.8 and heated at 37 °C for 60 min with stirring. The solution was filtered, suitably diluted and the Doxofylline content was measured by using UV Spectrophotometer (Analytical Technologies Ltd. Spectro 2080) at λmax of 274 nm. Each measurement was carried out in triplicate and the average drug content in the Doxofylline bilayer matrix tablets was calculated [15].

Swelling index (*SI*)

The swelling behaviour of all formulations of Doxofylline bilayer tablet was measured by studying its weight gain in the dissolution medium under study. The swelling index of selected bilayer matrix tablets were determined by placing the tablets in the basket of dissolution apparatus maintaining dissolution medium at 37±0.5 °C. After every one hour interval and up to 12 h, each dissolution basket containing tablet was withdrawn and blotted with tissue paper to remove the excess water and weighed on the analytical balance (Shimadzu, Ax 120). The experiment was performed in triplicate for each time point. Swelling index was calculated by using the following formula [16].

$$\text{Swelling Index (SI)} = \frac{Wf - Wi}{Wi} \times 100$$

Where W_f and W_i is called as wet and dry weight of the tablet respectively.

***In-vitro* drug release study**

The *in vitro* release studies was conducted for all Doxofylline bilayer matrix tablet formulations using eight station USP dissolution rate test apparatus type-II (LABINDIA DS 8000, Mumbai, India.) at 37±0.5 °C. A proper simulation of gastrointestinal (GIT) condition was maintained by altering the P^H of dissolution medium at different time intervals following two step dissolution conditions. To simulate the physiological conditions of GIT, first 2 h of dissolution was carried out in 900 ml of simulated gastric fluid (SGF, 3.2 mg/ml pepsin in 0.05 M HCl, P^H 1.2) and the rest of the time in 900 ml of simulated intestinal fluid (SIF, 10 mg/ml pancreatic fluid in Sorensen's phosphate buffer, P^H 7.4) at regular intervals of time, the aliquots were withdrawn and analyzed for drug using the UV-Visible spectrophotometer (Analytical Technologies Ltd. Spectro 2080) at λmax of 273 nm and 274 nm for SGF and SIF respectively. After each sampling an equal volume of fresh dissolution fluid was added to the dissolution medium. All the dissolution studies were repeated three times [17].

Calculation of similarity and difference factors for Doxofylline immediate release layer

The optimized formulation for immediate release layer was chosen according to comparative dissolution study with a reference marketed product of DOXOBRON TAB (Invision) containing Doxofylline 400 mg, employing the similarity factor (f_2) and difference factor (f_1) equation introduced by Moore and Flanner [18].

The similarity factor (f_2) adopted by the U. S. Food and Drug Administration (FDA) was used to evaluate the similarity in release profiles between the two pharmaceutical preparations. The similarity factor, which is a logarithmic transformation of the sum squared error of differences between the test preparation and reference preparation, was calculated by the following equation:

$$f2 = 50 \times \log\left\{\left[1 + \frac{1}{n}\sum_{t=1}^{n}(Rt - Tt)^2\right]^{-0.5} \times 100\right\}$$

Where R_t and T_t are the accumulated release rates of the reference preparation and test preparation at the predetermined time points, respectively, and n represents the number of the time points. The value of the similarity factor is between 0 and 100. The value 100 indicates that the test and reference profiles are identical; the more it approaches 0, the more dissimilarity of the two preparations occurs. Generally, if f_2>50, the release profiles are considered to be similar, and the larger the f_2 value, the higher the similarity.

Difference factor (f_1) measures the percent error between two drug release curves over all time points.

$$f1 = \frac{\sum_{t=1}^{n}|Rt - Tt|}{\sum_{t=1}^{n} Rt} \times 100$$

Dissolution profile was considered satisfactory if $f1$ values lies below 15 (nearing zero, more it approaches towards zero more similarity is the product [18, 19].

Determination of the release kinetics from the *in vitro* drug release profile [19, 20]

The rate and mechanism of release of Doxofylline from prepared bilayer tablets were analyzed by fitting the dissolution data into following exponential equations.

Zero order release equation is expressed as

$$Q = K_0 t$$

Where Q is the amount of drug released at time t and K_0 is the zero order release rate constant.

The first order equation is expressed as

$$\log(100 - Q) = \log 100 - \frac{K_1 t}{2.303}$$

Where, K_1 is the first order release rate constant.

The dissolution data was fitted to the Higuchi's equation as follows

$$Q = K_2 t^{1/2}$$

Where, K_2 is the diffusion rate constant.

The dissolution data was also fitted to the Korsmeyer-Peppas equation, which is often used to describe the drug release behaviour from polymeric systems as follows

$$\log\left(M_t/M_\infty\right) = \log K + n \log t$$

Where M_t is the amount of drug released at time *t*, $M\infty$ is the amount of drug release after infinite time, K is a release rate constant and n is the diffusion exponent indicative of the mechanism of drug release.

For matrix tablets, if the exponent n<0.5, then the drug release mechanism is quasi-fickian diffusion (If n = 0.5 then fickian diffusion and if the value is 0.5<n<1, then it is anomalous diffusion coupled with erosion. An exponent value of 1 is indicative of Case-II transport or typical zero-order and n>1 non-fickian super Case II). The diffusion exponent was based on Korsmeyer-Peppas equation.

Hixson-Crowell recognized that area of the particle is proportional to the cubic root of its volume, and derived an equation as follows.

$$W_0^{1/3} - W_t^{1/3} = K_s t$$

Where W_o is the initial amount of drug, W_t is the remaining amount of drug in dosage form at time t, and K_S is a constant incorporating the surface volume relation. The graphs are plotted as cube root of percent drug remaining versus time [21, 22].

Stability studies of optimised formulation

The tablets of optimized batch ($DBMF_6$) were packed in air tight bottles and subjected to accelerated stability studies according to ICH guidelines. The accelerated condition that was chosen for stability study at 40 °C±2 °C/75%±5% RH using humidity control oven NEC 210R10 (Newtronic Instruments, India) for 90 d. After that period the product was evaluated for friability, hardness, weight variation, thickness, drug content and *in vitro* release study [23, 24].

RESULTS AND DISCUSSION

Compatibility studies by FTIR and DSC

By comparing the spectra of Doxofylline, IR and SR layer of optimised formulation ($DBMF_6$), the sharp peaks that appear in spectra of Doxofylline at~ 3110 cm^{-1} due to presence of aliphatic N-H stretching functional group also appears in IR layer and SR layer at~ 2916 $cm^{-1}$and 3110 cm^{-1}respectively.

The characteristic infrared absorption peaks of Doxofylline at ~ 1700 cm^{-1} (C=O stretch), at ~ 1656 cm^{-1} (C=C stretch), at ~ 1547 cm^{-1} (C=N stretch), at ~ 1477 cm^{-1} (C-H bend) and at ~ 1190 cm^{-1} (C-N vibration) were also present in the physical mixture (drug and excipients of both IR and SR layer) with no shifting in the major peaks that indicate there were no interaction occurred between the Doxofylline and excipients used in the preparation of different bilayer formulations. Therefore the drug and excipients are compatible to form stable formulations under study. The FTIR spectra of pure drug Doxofylline and physical mixture used for IR layer and SR layer in optimized formulation $DBMF_6$ were obtained and shown in fig. 1.

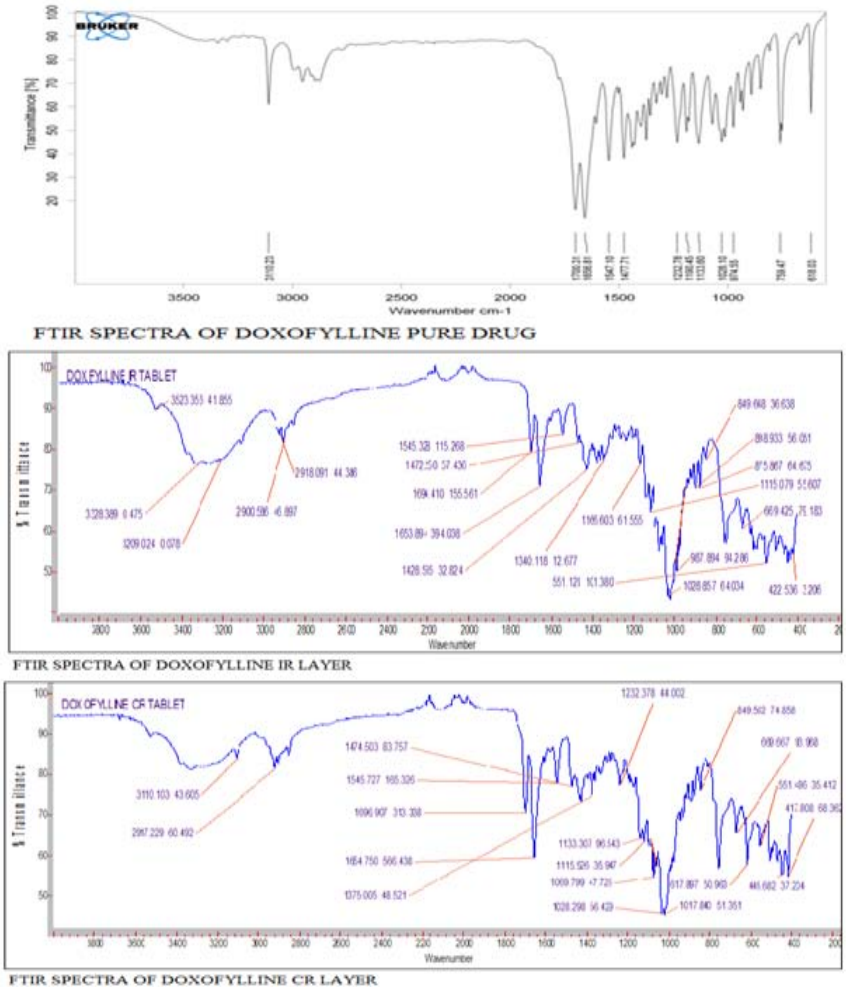


Fig. 1: Compatibility studies through FTIR analysis

DSC thermogram of pure drug Doxofylline and physical mixture used for IR layer and SR layer in optimized formulation $DBMF_6$ were obtained and shown in fig. 2**.** It was observed that the endothermic peak appeared at 146 °C for pure drug Doxofylline also appeared at 148 °C and 151 °C in optimised formulation of IR and SR layer respectively which indicate that the formulation is thermodynamically stable because the formulation required slightly more heat than pure drug due to presence of various excipients. Appearance of other peaks due to presence of other excipients in the formulations. The DSC thermogram of pure drug and the formulations showed there was no major shifting i. e from endothermic to exothermic with appearance of major thermal peaks in the optimised formulation.

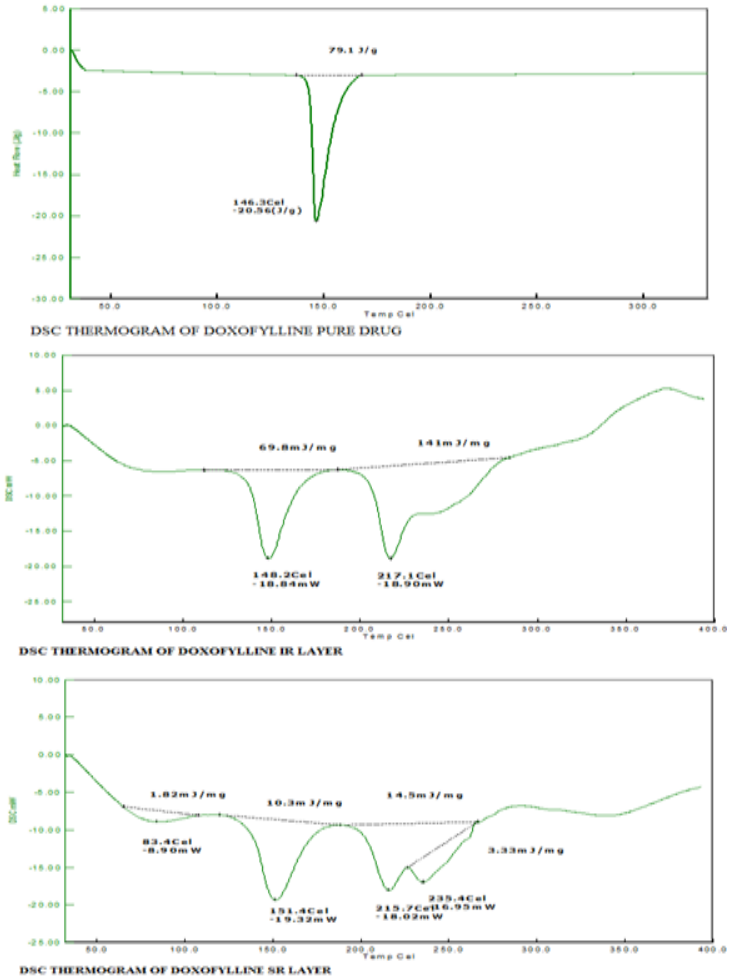


Fig. 2: Compatibility studies through DSC analysis

The loose bulk densities of Doxofylline immediate release layer and sustained release layer granules of all formulations were found to be in the range of 0.399 to 0.541 g/cm³ and 0.487±0.04 to 0.532±0.02 g/cm³ respectively. The tapped densities were found to be in between 0.475 to 0.589 g/cm³ and 0.512±0.08 to 0.593±0.06 g/cm³ for IR and SR layer respectively.

This indicates good packing capacity of granules. Bulk density and tapped density measurements found that the density of granules depends on particle packing and that density changes as the granules consolidates.

Values of Carr's index for all the formulations and for both the layer were found below 16% that usually indicates good flow characteristics. In all formulations, the Hausner's ratios values were found "between" 1.07 to 1.21 for IR layer and "between" 1.05 to 1.15 that indicates good flow characteristics.

Angle of repose is suited for particle>150 µm. The angle of repose of all formulations fell within the range of 18.62 ° ± 0.12 to 22.65 ° ± 0.12 for IR layer and 18.51 °±0.16 to 23.92 °±0.13 for SR layer *i.e.* dry granules of Doxofylline immediate release and sustained release layer showed good flow properties.

Table 3: Evaluation of doxofylline immediate release layer granules ($DIRF_1$–$DIRF_{10}$)

F. No.	Bulk density (g/cm³)	Tapped density (g/cm³)	Angle of repose (θ)	Carr's Index (%)	Hausner's ratio
$DIRF_1$	0.432±0.05	0.521±0.06	22.41±0.11	17.08	1.21
$DIRF_2$	0.490±0.06	0.584±0.05	21.53±0.10	16.09	1.19
$DIRF_3$	0.465±0.08	0.502±0.06	20.91±0.14	7.37	1.07
$DIRF_4$	0.501±0.06	0.573±0.08	19.62±0.16	12.57	1.14
$DIRF_5$	0.423±0.05	0.492±0.07	22.84±0.18	14.02	1.16
$DIRF_6$	0.399±0.08	0.475±0.05	18.62±0.12	16.01	1.19
$DIRF_7$	0.482±0.09	0.521±0.08	21.52±0.11	7.48	1.08
$DIRF_8$	0.541±0.07	0.589±0.07	20.63±0.13	8.15	1.09
$DIRF_9$	0.477±0.05	0.524±0.06	19.49±0.14	8.97	1.10
$DIRF_{10}$	0.472±0.06	0.525±0.08	22.28±0.15	10.10	1.11
$DIRF_{11}$	0.482±0.03	0.521±0.06	19.46±0.14	7.48	1.08
$DIRF_{12}$	0.490±0.08	0.584±0.09	22.65±0.12	16.09	1.19

All values are expressed as mean±SD; (n=3)

Table 4: Evaluation of doxofylline sustained release layer granules ($DSRF_1$–$DSRF_{10}$)

F. No.	Bulk density (g/ml)	Tapped density (g/ml)	Angle of repose (θ)	Carr's Index (%)	Hausner's ratio
$DSRF_1$	0.532±0.02	0.564±0.05	20.41±0.19	05.67	1.06
$DSRF_2$	0.520±0.03	0.593±0.06	21.54±0.14	12.31	1.14
$DSRF_3$	0.501±0.02	0.578±0.06	23.92±0.13	13.32	1.15
$DSRF_4$	0.508±0.06	0.582±0.07	20.66±0.12	12.71	1.15
$DSRF_5$	0.498±0.04	0.556±0.04	21.88±0.14	10.43	1.12
$DSRF_6$	0.496±0.05	0.532±0.07	19.69±0.15	06.77	1.07
$DSRF_7$	0.487±0.04	0.512±0.08	18.51±0.16	04.88	1.05
$DSRF_8$	0.512±0.06	0.568±0.08	22.62±0.15	09.86	1.11
$DSRF_9$	0.518±0.05	0.549±0.05	19.08±0.14	05.65	1.06
$DSRF_{10}$	0.519±0.07	0.562±0.06	20.17±0.15	07.65	1.08

All values are expressed as mean±SD; (n=3)

The physical parameters of the all the formulations of Doxofylline bilayer sustained released matrix tablets were found to be satisfactory. The tablets from all factorial batches were white, circular. The surface texture was smooth. Typical tablet defects, such as capping, chipping and picking, were not observed. The average thicknesses of the tablets was ranged between 5.82±0.11 to 5.90±0.14 mm and it was found to be within limit of deviation from an average value (not more than 5%).

Weight variations for different formulations were ranged between 1147±3.56 to 1154±4.24 mg. The weight variation for all the formulations within limits indicates uniformity in tablet compression and consequently content of drug in a unit. The average percentage deviation of all tablet formulations was found to be within the limit, and hence all formulations passed the test for uniformity of weight as per official requirement.

The hardness of all the Doxofylline bilayer sustained released matrix tablets formulations were ranged from 7.54±0.7 to 8.98±0.6 kg/cm^2 which indicated good handling and transportation characteristics of tablets under study.

The percentage friability of all the formulations was ranged from 0.63±0.06% to 0.41±0.05 %. In the present study, the percentage friability for all for formulations was within the prescribed limits that indicated the product is resistant to wear and tear during handling and transportation. The percentages of drug content for Doxofylline bilayer sustained released matrix tablet formulations ($DBMF_1$ to $DBMF_{10}$) were found to be in between 98.39±1.4 % to 102.42±1.2% (i.e. variation of±4%) which were within the acceptable limits. The value ensures good uniformity of the drug content in the tablet.

The physicochemical characterizations of different batches of bilayer sustained released tablets are given in table 3.

Swelling study was performed on all the formulations ($DBMF_1$ to $DBMF_{10}$) upto 12 h. The formulation containing more concentration of HPMC K4M, HPMC K15M showed higher swelling indices due to higher hydrophilicity and more water uptake of the polymers. But reverse is observed with the formulations containing higher percentage of Eudragit RSPO and RLPO, as it is a hydrophobic polymer. The formulations ($DBMF_9$ and $DBMF_{10}$) containing the combination of both the grade of HPMC in equal proportion, showed higher swelling index. Among all the formulations, $DBMF_{10}$ that contained around 8% of HPMCK4M, 8% of HPMCK15M and 5% of Eudragit RLPO showed highest swelling index than other formulations. Formulations $DBMF_5$, $DBMF_6$, $DBMF_7$, and $DBMF_8$ that contained more % of Eudragit had lower swelling index as compare to other formulations as Eudragit is a hydrophobic polymer. The result of swelling index is shown in fig. 2.

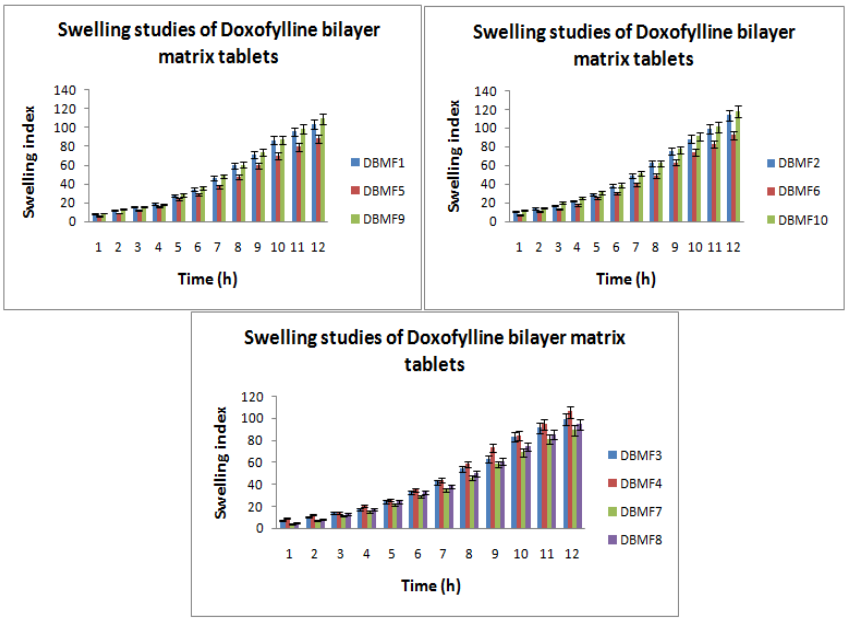
Table 5: Evaluation of postcompression parameters of doxofylline bilayer matrix tablets

F. No.	Average hardness (kg/cm^2)	Average weight variation (%)	Average friability (% w/w)	Average thickness (mm)	Drug content uniformity (%)
$DBMF_1$	7.97±0.6	1152±4.32	0.63±0.05	5.89±0.12	102.42±1.2
$DBMF_2$	7.54±0.7	1151±3.54	0.64±0.06	5.90±0.14	98.39±1.4
$DBMF_3$	7.89±0.9	1153±4.27	0.51±0.07	5.89±0.18	99.75±1.6
$DBMF_4$	7.95±0.8	1148±3.61	0.47±0.08	5.82±0.16	100.95±1.2
$DBMF_5$	8.02±0.6	1147±4.82	0.63±0.06	5.84±0.12	101.48±1.1
$DBMF_6$	8.01±0.8	1152±3.43	0.48±0.04	5.82±0.11	101.54±1.5
$DBMF_7$	8.00±0.7	1147±3.56	0.41±0.05	5.84±0.15	98.84±1.4
$DBMF_8$	8.91±0.9	1148±3.72	0.46±0.06	5.83±0.14	99.36±1.6
$DBMF_9$	8.98±0.6	1153±3.93	0.42±0.08	5.85±0.15	99.68±1.2
$DBMF_{10}$	8.97±0.6	1154±4.24	0.44±0.08	5.84±0.16	100.19±1.6

All values are expressed as mean±SD; (n=3)

Table 6: Similarity (f_2) and difference factor (f_1) with dissolution profile of all formulations

F. No.	f_1	f_2	Dissolution profiles
$DIRF_1$	33.55	35.67	Dissimilar
$DIRF_2$	27.95	39.23	Dissimilar
$DIRF_3$	20.17	45.5	Dissimilar
$DIRF_4$	18.39	48.14	Dissimilar
$DIRF_5$	6.46	68.74	Similar
$DIRF_6$	3.44	81.5	Similar
$DIRF_7$	13.33	54.46	Similar
$DIRF_8$	2.4	82.78	Similar
$DIRF_9$	9.29	63.58	Similar
$DIRF_{10}$	3.36	79.50	Similar
$DIRF_{11}$	15.49	53.48	Similar
$DIRF_{12}$	10.18	61.79	Similar



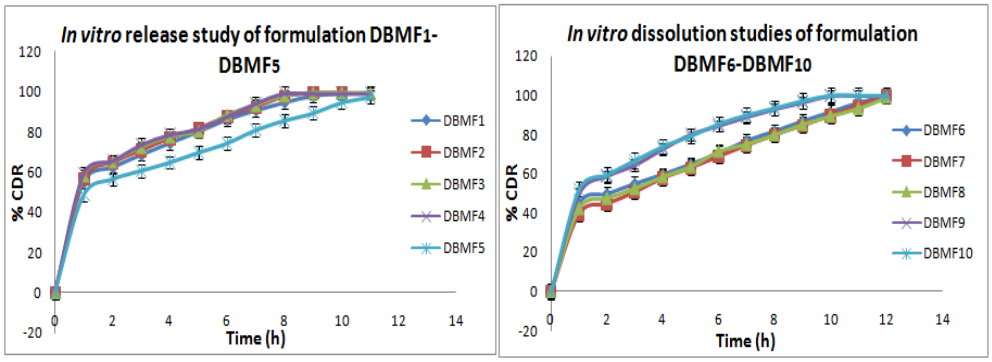
All values are expressed as mean±SD; (n=3)

Fig. 3: Comparative swelling studies of all the formulations of Doxofylline bilayer matrix tablets

To optimise the immediate release layer for use in bilayer matrix tablet, the similarity and difference factors were calculated from the *in vitro* dissolution data of all the immediate release formulations by comparing with standard marketed formulation. Formulation $DIRF_1$ to $DIRF_4$ showed dissimilarity in the dissolution profile whereas formulation $DIRF_5$ to $DIRF_{12}$ showed similarity in the dissolution profile. Among all the formulations, $DIRF_8$ showed highest f_2 value (82.78) and lowest f_1 value (2.4) was considered as the best formulation. The dissolution profiles of all the batches of immediate release layer formulations calculated in the present investigation were presented in table 6.

Based upon the highest f_2 and lowest f_1 values; $DIRF_8$ formulation was identified as the optimised formulation and used as immediate release layer for the different formulations of Doxofylline bilayer sustained released matrix tablets. In order to optimise the *in vitro* drug release profile of Doxofylline bilayer sustained released matrix tablets; different hydrophilic matrix polymers *viz.*, HPMC K4M, HPMC K15M and hydrophobic matrix polymer *viz.*, Eudragit RSPO and Eudragit RLPO were used and ten different formulations were prepared. Between the two grades of HPMC used, HPMC K15M having better controlled release profile than HPMC K4M as it is having higher viscosity grade. It was observed that using HPMC polymer alone causes initial burst release because drug is hydrophilic in nature and maximum of the drug was released up to 8 h. Because of that one more hydrophobic polymer *i. e* Eudragit was added to reduce the initial burst release of sustained release layer. Among the two grade of Eudragit used, Eudragit RSPO showed better sustained release effect than Eudragit RLPO. $DBMF_6$ formulation that contained 12.5% of HPMC K15M and 8% of Eudragit RSPO was considered as optimised formulation as the initial release was 44% as a loading dose within the first hour and further as sustained release upto 12 h.

Further increase in the concentration of Eudragit, the initial release rate was much slower which was not desirable. So 8% of Eudragit was considered as optimum. The available marketed sustained release tablet containing 800 mg of Doxofylline had low initial release to elicit pharmacological action because drug is highly metabolised (80-90%) whereas the present formulation under study released around 44% of the drug within first hour that can be useful as loading dose. The loading dose can be used to elicit pharmacological action for acute asthma attack and the sustained release dose can be utilised as maintenance therapy so that it is not necessary to take repeated dose. So by using the present optimised formulation once daily medication is possible with proper management of disease conditions. The drug release profiles of different formulations were shown in fig. 4.



All values are expressed as mean±SD; (n=3)

Fig. 4: *In vitro* release studies of all the formulations of Doxofylline bilayer tablets

The *in vitro* dissolution data of optimised formulation $DBMF_6$ were fitted in different kinetic models viz. zero order, first order, Higuchi, Hixon-Crowell and Korse-Meyer Peppa's kinetic model equation and the graphs were plotted those were shown fig. **5**. The Hixon-Crowell plots was found to be fairly linear as indicated by it's highest regression (0.989) values.

The release exponent 'n' for optimised formulation $DBMF_6$ was found to be 0.334 (n<0.5), which appears to indicate a quasi-fickian diffusion. So in present study *in vitro* drug release kinetic of optimised formulation of Doxofylline bilayer matrix tablets ($DBMF_6$) followed Hixon-Crowell release kinetic models and drug release mechanism is quasi-fickian diffusion.

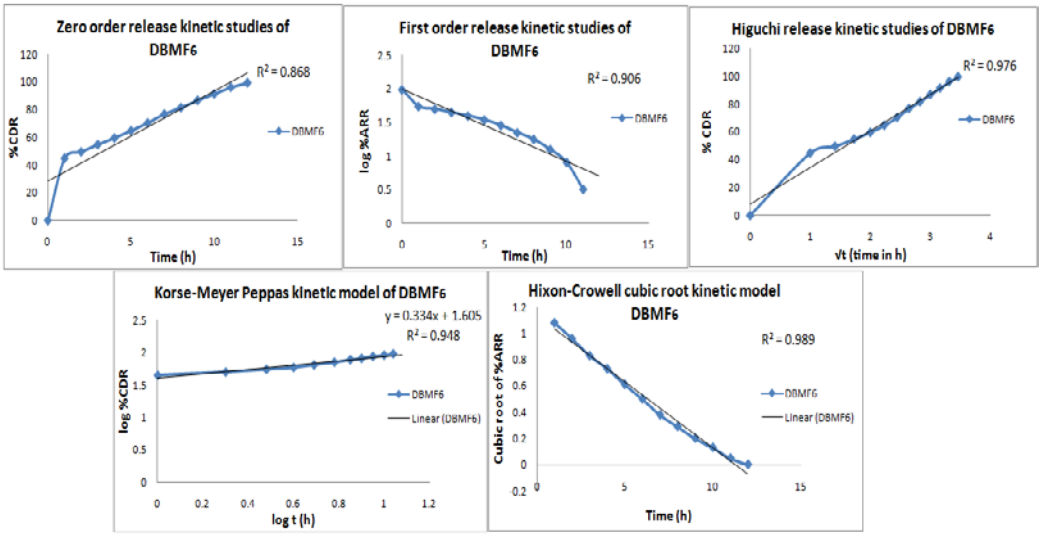


Fig. 5: *In vitro* release kinetic studies of optimised formulation ($DBMF_6$) Doxofylline bilayer matrix tablet

Table 6: Regression values of *in-vitro* release kinetic study optimized Doxofylline bilayer matrix tablet ($DBMF_6$)

Formulation	R^2value of zero order	R^2 value of 1st order	R^2value of Higuchi model	R^2value of Hixon-Crowell model	R^2value of Peppa's model	'n' value of Peppa's model
$DBMF_6$	0.868	0.906	0.976	0.989	0.948	0.334

Table 7: Comparison of *in vitro* dissolution data after stability studies at accelerated conditions of optimized batch ($DBMF_6$)

Time (h)	Initial	After 15 d	After 30 d	After 45 d	After 60 d	After 90 d
1	44.81±1.58	42.62±1.29	41.46±1.19	40.38±1.20	39.68±1.35	37.84±1.54
2	49.57±1.81	47.19±1.89	46.28±1.77	44.56±1.36	42.66±1.24	40.52±1.28
3	54.81±1.84	53.95±1.45	52.47±1.74	50.45±1.69	47.70±1.92	44.19±1.65
4	59.52±1.73	57.78±1.59	58.62±1.61	57.19±1.53	55.81±1.84	52.86±1.47
5	64.75±1.67	62.26±1.92	61.92±1.57	60.94±1.48	58.83±1.45	56.19±2.18
6	70.42±1.57	67.22±1.71	66.58±1.92	64.18±1.84	62.94±1.54	59.55±1.36
7	76.82±1.66	74.84±1.85	73.28±1.16	71.74±1.85	70.10±1.66	68.19±1.57
8	81.60±1.45	79.69±1.94	78.51±1.78	76.59±1.74	74.95±1.76	73.59±2.30
9	86.72±2.71	85.14±2.10	84.28±2.65	82.64±2.45	80.27±2.84	78.25±2.14
10	91.38±2.84	89.87±2.41	88.51±2.62	85.38±2.52	83.19±2.52	80.85±2.25
11	96.16±3.20	95.49±3.09	94.42±3.14	92.67±3.12	90.44±3.38	89.59±3.05
12	99.42±3.56	98.58±3.17	97.19±3.46	94.19±3.44	92.68±3.51	90.08±3.10

All values are expressed as mean±SD; (n=3)

The optimised formulation of Doxofylline bilayer matrix tablets ($DBMF_6$) did not show any significant change in physicochemical parameters like hardness, weight variation, friability, content uniformity and *in vitro* drug release characteristics. More than 90% of the drug had been retained that was confirmed through *in vitro* dissolution studies for 90 d after it had been stored under stressed condition. The results of *in vitro* release profile of optimised formulation at different time interval after storing it at accelerated stressed conditions were shown in table 7.

CONCLUSION

In the present investigation Doxofylline bilayer matrix tablet was successfully developed. The major challenge in these studies was to design a bilayer matrix tablet of Doxofylline that can provide an initial loading dose followed by sustained release dose. Here cross carmellose and cross povidone were used as superdisintegrants for immediate release layer and different grade HPMC and Eudragit for sustained release layer of Doxofylline. The main objective of using hydrophobic polymer Eudragit with HPMC was to prevent the burst release effect the hydrophilic drug under study which was successfully developed. Formulation $DBMF_6$ that contained 12.5% of HPMC K15M and 8% of Eudragit RSPO in SR layer with 4% of Polyplasdone-XL in immediate release layer showed 44% of drug release within first hour as a loading dose and sustained release upto 12 h with almost complete release (99%) emerged as optimised formulation. Increase in proportion of hydrophilic polymer caused initial burst release effect. Kinetic of *in vitro* drug release of optimized formulation $DBMF_6$ found to be a mixed order kinetic as it obey almost first order within first one hour then it followed zero order release kinetic. The drug release mechanism

followed Hixon-Crowell cube root law with quasi-fickian diffusion. FTIR and DSC studies revelled that there is no chemical and thermal interaction between drug Doxofylline and excipients used for formulations. The accelerated stability studies were carried out for the optimised formulation and were found to be stable without any remarkable physicochemical changes. Thus from the results of the current study clearly indicate, a promising potential of the Doxofylline bilayer matrix tablets system as an alternative to the conventional dosage form as it enhance bioavailability of the Doxofylline by producing an initial loading dose followed by sustained release effect and can be therapeutically beneficial for acute and sustainable asthma. However, further clinical studies are needed to assess the utility of this system for patients suffering from asthma.

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CONFLICT OF INTERESTS

Declared None

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