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MATRIX RELEASE FOR CHRONIC INFLAMMATORY DISEASE

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ABSTRACT

Asthma is a common chronic inflammatory disease of the airways characterized by variable and recurring symptoms, reversible airflow obstruction and bronchospasm.[1] Asthma is the result of chronic inflammation of the airways which subsequently results in increased contractability of the surrounding smooth muscles. This among other factors leads to bouts of narrowing of the airway. The narrowing is typically reversible with or without treatment. Occasionally the airways themselves change. Common symptoms include wheezing, coughing, chest tightness.

KEYWORDS: Asthma, chronic inflammatory disease.

1. INTRODUCTION

1.1 Asthma

1.1.1 Definition[1,2]

Asthma is a common chronic inflammatory disease of the airways characterized by variable and recurring symptoms, reversible airflow obstruction and bronchospasm.[1] Asthma is the result of chronic inflammation of the airways which subsequently results in increased contractability of the surrounding smooth muscles. This among other factors leads to bouts of narrowing of the airway. The narrowing is typically reversible with or without treatment. Occasionally the airways themselves change. Common symptoms include wheezing, coughing, chest tightness, and shortness of breath.[2]

II. Optimization by using 3^2 full factorial Experimental Design: III. Stability study of matrix tablet

5.3.1 Analysis of drug candidate

1. Melting point

It was one of the parameters to judge the purity of crude drugs. In case of pure chemicals or photochemical, melting points are very sharp and constant. Since the crude drugs contain the mixed chemicals, they are described with certain range of melting point.

Procedure

A small quantity of powder was placed into a Capillary. That was placed in the melting point determining apparatus containing castor oil. The temperature of the castor oil was gradual increased automatically and read the temperature at which powder started to melt and the temperature when all the powder gets melted.

2. Drug Identification

Drug Identification can be found by UV spectroscopy, IR Spectroscopy.

UV Spectroscopy

A stock solution of Doxofylline 5 µg/ml was prepared separately in Water. The UV spectrum of Doxofylline was recorded using double beam UV-Visible Spectrophotometer (Shimadzu, UV-2450) at 1.0 cm slit width using 0.1 N HCl pH 1.2 as solvent in the range of 200-400nm. The wavelength of maximum absorption at 274 nm was found to be sharp and satisfactory.

FTIR of pure drug

Identification of Doxofylline was carried out using FTIR study. For this the FTIR spectra of plain drug was recorded in FTIR 8400 S Shimadzu spectrophotometer. The pure Doxofylline drug was mixed thoroughly with potassium bromide. For the scans were obtained at a resolution of 4000-400cm^{-1}.

3. Calibration curve of Doxofylline in 0.1 N HCl[23]

Solvent:- 0.1 N HCl pH 1.2

Concentration:- 5 µg/ml(λmax=274.20 nm)

Stock solution was prepared by dissolving 50 mg drug in 100 ml simulated 0.1N HCL pH 1.2(500µg/ml).

From this solution withdraw 4 ml and make up simulated 0.1 N HCl buffer pH 1.2 up to 100 ml

(20µg/ml).

Withdraw 2.5,5,7.5,10,12.5 ml from stock solution and make upto 10 ml with simulated 0.1 N HCl pH 1.2 to produce solution of concentration 5,10,15,20 and 25µg/ml respectively.

4. Calibration curve of Doxofylline in Phosphate buffer pH 6.8[32]

Solvent:- Phosphate buffer pH 6.8

Concentration:- 5 µg/ml (λmax=273.20 nm)

Stock solution was prepared by dissolving 50 mg drug in 100 ml simulated Phoshate Buffer pH 6.8(500µg/ml).

From this solution withdraw 4 ml and make up simulated Phosphate buffer pH 6.8 up to 100 ml (20µg/ml).

Withdraw 2.5,5,7.5,10,12.5 ml from stock solution and make upto 10 ml with simulated Phosphate Buffer pH 6.8 to produce solution of concentration 5,10,15,20 and 25 µg/ml respectively.

5. Drug excipients compatibility study

By FTIR

Compatibility of Doxofylline with the respective Polymers that is Hydroxypropyl Methyl Cellulose, Xanthan Gum, Guar Gum. Individual excipients was established by Infrared Absorption Spectral Analysis (FTIR). Any changes in the chemical composition after combining with the excipients were investigated with IR spectral analysis.

DSC (Differential scanning calorimetry)

The DSC spectrum of the Doxofylline and selected formulation (F7) were recorded using DSC with TDA trend line software. The thermal traces were obtained by heating from 20 ºC to 900 ºC at heating rate of 20 ºC under atmospheric condition in open crucibles.

Formulation

Table 5.3: Formulations Composition of Sustained Release Matrix Tablets of Trial Batches.

Sr. No.	Ingredients (mg)	T1	T2	T3	T4	T5	T6
1	**Doxofylline**	400	400	400	400	400	400
2	**HPMC K100M**	200	---	---	100	100	---
3	**Xanthan Gum**	---	200	---	100	---	100
4	**Guar Gum**	---	---	200	---	100	100
5	**Avicel 101**	34	34	34	34	34	34
6	**PVP K90D**	6	6	6	6	6	6
7	**Magnesium Stearate**	5	5	5	5	5	5
8	**Talc**	5	5	5	5	5	5
9	**IPA**	q.s	q.s	q.s	q.s	q.s	q.s

5.3.3 Evaluation of Matrix Tablets[44]

1. Pre compressional parameters a. Angle of repose

A funnel was kept vertically in a stand at a specified height above a paper placed on a horizontal surface. The funnel bottom was closed and 10 gm of sample powder was filled in funnel. Then funnel was opened to release the powder on the paper to form a smooth conical heap, was found by measuring in different direction. The height of the heap was measured by using scale. The values of angle of repose are calculated by using the following formula

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

Where, h: height of the heap r: radius of the heap

Table 5.4: Standard value of angle of repose.

Flow property	Angle of repose(θ)
Excellent	25-30
Good	31-35
Fair	36-40
Passable	41-45
Poor	46-55
Very poor	56-65
Very very poor	>66

b. Bulk density

A known quantity of powder was poured into the measuring cylinder carefully leave the powder without compacting, if necessary and read the unsettled apparent volume, to the nearest graduated unit. Calculate the bulk density, in gm per ml, by the formula

Bulk density = Bulk Mass/ Bulk Volume

c. Tapped density

Tapped density was achieved by mechanically tapping a measuring cylinder containing a powder sample. After observing the initial volume, the cylinder was mechanically tapped and volume readings are taken until

little further volume changes were observed.

d. Carr's Index
The compressibility index of all ingredients was determined by following equation.

Carr's index = (Tapped density- Bulk density/ Tapped density) ×100

Table 5.5: Standard value of Carr's index.

Flow property	Carr's Index
Excellent	≤10
Good	11-15
Fair	16-20
Passable	21-25
Poor	26-31
Very poor	32-37
Very very poor	>38

e. Hausner Ratio
Hausner predict the flow properties of powder by using inter particle friction.

Hausner ratio = tapped density /poured density

Table 5.6: Standard value of Hausner ratio.

Flow property	Carr's Index
Free flowing	1-1.2
Cohesive powder	1.2-1.6

2. Post compressional parameters a. Thickness and Diameter
Tablet thickness and Diameter was measured by Vernier caliper.

b. Hardness
The hardness is expressed as Kg/ cm2. The tablet crushing load, which is the force required to break a tablet into halves by compression .It was measured using a tablet hardness tester (Pfizer Hardness Tester).

c. Friability
Friability test is performed to assess the effect of friction and shocks, which may often cause tablet to chip, cap or break. Roche friabilator was used for the purpose. This device subjects a number of tablets to the combined effect of abrasion and shock by utilizing a plastic chamber that revolves at 25 rpm dropping the tablets at a distance of six inches with each revolution. Pre-weighed sample of tablets were placed in the friabilator, which was then operated for 100 revolutions. Tablets were dusted and reweighed. Compressed tablets should not lose more than 1% of their weight.

d. Weight variation
USP weight variation test is done by weighing 20 tablets individually; calculating the average weight and comparing the individual tablet weight to the average weight variation tolerance.

Table 5.7: Variation Tolerance.

Average weight of Tablet (mg)	Maximum % deviation allowed
130mg or less	10%
130mg to 324mg	7.5%
More than 324mg	5%

e. *In vitro* dissolution study of matrix tablet.[39]
The release rate of Doxofylline sustained release matrix tablets was determined using USP type II dissolution apparatus. *In-vitro* dissolution study was carried out in 0.1 N HCl for 2 hours & in Phosphate buffer (pH 6.8) mimicking passage of dosage form from stomach to ileum. In order to simulate pH changes along the GI tract two dissolution media with pH 1.2 & 6.8 were sequentially used referred to as sequential pH change method.

When performing experiments, the pH 1.2 medium was first used for 2 h (since the average gastric emptying time is 2 h), then removed and the fresh pH 6.8 Phosphate buffer was added. 900 ml of the dissolution medium was used each time. Rotation speed was 100 rpm and temperature was maintained at 37 ± 0.5^0C. The sample were filtered through 0.45µm nylon filter and spectrophotometrically analysed at 274 nm.

f. Tablet Dosage Form Assay (% Drug Content Uniformity)[39]
Ten randomly selected tablets of each batch were weighed & powdered in a pestle & mortar. The quantity of powder equivalent to 10 mg of drug was transferred to a 100 ml volumetric flask & dissolved in 40ml of distilled water in a bath sonicator for 2 hr .Solution was filtered through Whatmann paper (no.41) .Filter paper was washed with water. Washings were added to the filtrate & final volume made up to 100 ml. After suitable dilution corresponding to 20µg /ml, absorbance of final sample was recorded at 274 nm taking distilled water as blank.

5.3.4 Optimization by using 3^2 full factorial Experimental Design[32]
It is desirable to develop an acceptable pharmaceutical formulation in shortest possible time using minimum number of man, hours and raw materials. Traditionally pharmaceutical formulations after developed by changing one variable at a time by trial and error method which is time consuming in nature and requires a lot of imaginative efforts. In addition to the art of formulation, the technique of factorial design is an effective method of indicating the relative significance of a number of variables and their interaction. The number of experiments required for these studies is dependent on the number of independent variables selected.

3^2 factorial design
Consider a simple example of a 3^2 factorial design. Each of the k factors is assigned at three levels. The

levels are usually High = 1, Medium = 0 and Low = -1. Such a scheme is useful as a preliminary experimental program before a more ambitious study is undertaken. The outcome of the 3^2 factorial experiment will help identify the relative importance of factors and also will offer some knowledge about the interaction effects. Let us take a simple case where the number of factors is 2. Let these factors be X1 and X2. The number of experiments that may be performed is 9 corresponding to the following combinations:

Table 5.8: Full factorial design matrix layout.

Experiment Trials.	X1	X2
F1	-1	-1
F2	0	-1
F3	+1	-1
F4	-1	0
F5	0	0
F6	+1	0
F7	-1	+1
F8	0	+1
F9	+1	+1

The response (Y) is measured for each trial.

Y= b0 + b1X1 + b2X2 + b12X1X2 + b11X11 + b22X22

Where,
Y is the dependent variable,
b0 is the arithmetic mean response of the total runs,
b1 is the estimated coefficient for factor **X1**,
b2 is the estimated coefficient for factor **X2**,

The main effect (**X1** and **X2**) represent the average result of changing one factor at a time from its low to high value.

The interaction terms (**X1X2**) show how the response changes when two factors are simultaneously changed.

The Polynomial terms (**X11 and X22)** are included to investigate nonlinearity.
In the present study, a 3^2 full factorial design was employed to study the effect of independent variables, i.e. concentration of HPMC K100M (**X1**) and concentration of Xanthan Gum (**X2**) on dependent variable i.e. T50% and T80%.

Table 5.9: Selection of independent and dependent variables.

Translation of coded value in actual units			
Independent variables	Variable level		
	Low(-1)	Medium(0)	High(1)
Conc. Of HPMC K100M	40	80	120
Conc. Of Xanthan Gum	40	80	120
Dependent Variables			
1.	T50%		
2.	T80%		

Table 5.10: Formulation of sustained release matrix tablets of factorial batches.

Sr. No.	Ingredients (mg)	Formulation Batches								
		F1	F2	F3	F4	F5	F6	F7	F8	F9
1	**Doxofylline**	400	400	400	400	400	400	400	400	400
2	**HPMC K100M**	40	80	120	40	80	120	40	80	120
3	**Xanthan Gum**	40	40	40	80	80	80	120	120	120
4	**Avicel 101**	160	120	80	120	80	40	80	40	0
5	**PVP K90D**	5	5	5	5	5	5	5	5	5
6	**Magnesium Stearate**	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
7	**Talc**	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
8	**IPA**	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s

Optimization Data Analysis and Optimization-Model Validation

Statistical validation of the Polynomial equation generated by design expert 9.0.4 was established on the basis of ANOVA provision in the software. A total of 10 runs with one center points were generated. The models were evaluated in terms of statistically significant coefficients and R^2 values. Various feasibility and grid searches were concluded to find the composition of optimized formulations. Various 3-D response surface graphs were provided by the design expert software. By intensive grid search performed over the whole experimental region, two optimum check point formulations were selected to validate the chosen experimental domain and polynomial equations. The check point formulation were prepared and evaluated for various response properties. The resultant experimental values of the response were quantitatively compared with the predicted values to calculate the percentage prediction error. Also, linear regression plots between actual and

predicted values of the response were produced using MS-excel.

Contour plot and surface plot of design

The optimization of formulation was carried out by plotting contour plots (3-D) and surface plot (2-D) for all observed dependent variable. Here, contour plot and surface plots were drawn using the design expert 9.0.4 software. These types of plots are useful in study of the effect of 2 factors on the response at one time. Various contour plots and response surface plots are depicted in figures respectively.

5.3.5 Stability Study of Optimized Formulation

Optimized batch was placed for stability study at **40±0.5˚C/75±5 %** RH for 1 month. Sample was collected after that and evaluated for physical parameters and *in vitro* dissolution study.

6. RESULTS AND DISCUSSION

6.1 Analysis of drug candidate

6.1.1 Melting Point

Table 6.1: Melting Point of Doxofylline.

Test	Specification	Observation
Melting Point	144-145.5^{0}C	144^{0}C

Thus, it has been identified that Observed Melting Point of Doxofylline is within the Specific Range so it conform that Doxofylline drug is pure.

6.1.2 Drug identification

1. UV spectroscopy

Determination of maximum wave length in 0.1 N HCl buffer pH 1.2[23]

From the UV spectroscopic analysis the maximum wavelength is found at 274.20 nm which is near to the standard reported value 274nm. Hence, 274.20 nm is taken as a maximum wavelength.

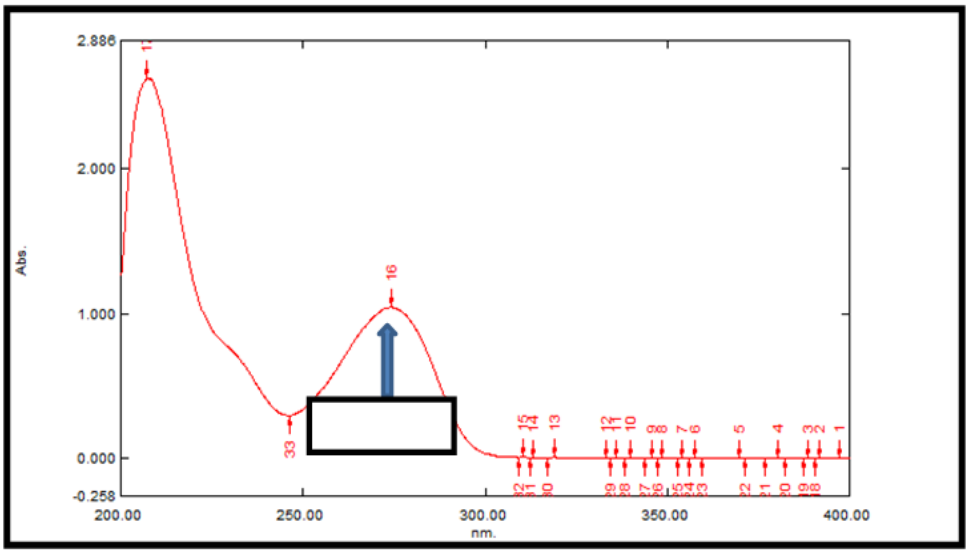


Figure 6.1: Spectra of Doxofylline 5 µg/ml solution in 0.1 N HCl buffer pH 1.2.

Calibration curve of Doxofylline in 0.1 N HCl buffer pH 1.2

The calibration curve taken in 0.1 N HCl buffer pH 1.2 showed a linear relation with a regression coefficient (r^2) of 0.999. The absorbance was well within the range of Beer and Lambert law.

Table 6.2: Absorbance of Doxofylline in 0.1 N HCl pH 1.2 at 274.20 nm.

Concentration (µg/ml)	Absorbance			Avg. Absorbance (n=3, Mean±SD)
	I	II	III	
0	0	0	0	0
5	0.225	0.232	0.215	0.224±0.008
10	0.408	0.444	0.437	0.429±0.019
15	0.689	0.623	0.602	0.638±0.050
20	0.858	0.826	0.820	0.834±0.020
25	1.022	1.044	1.065	1.043±0.021

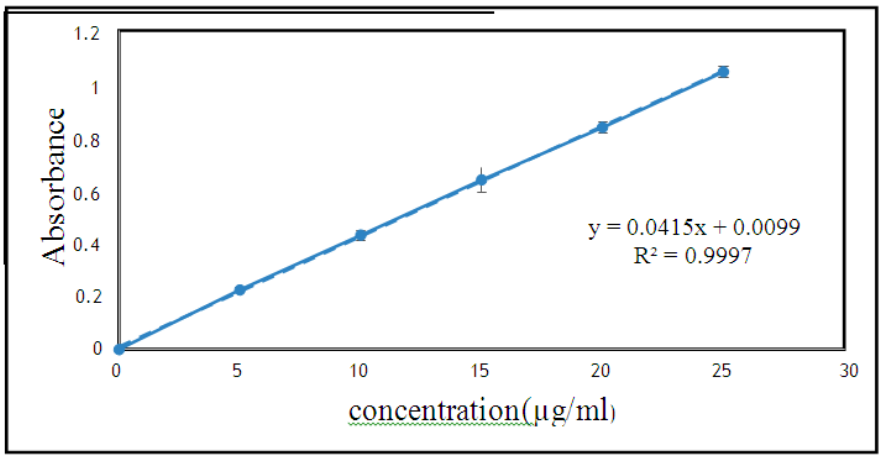


Figure 6.2: Calibration curve of Doxofylline in 0.1 N HCl pH 1.2.

Determination of maximum wave length in Phosphate buffer pH 6.8[32]

From the UV spectroscopic analysis the maximum wavelength is found at 273.60 nm which is near to the standard reported value 274nm. Hence, 273.60 nm is taken as a maximum wavelength

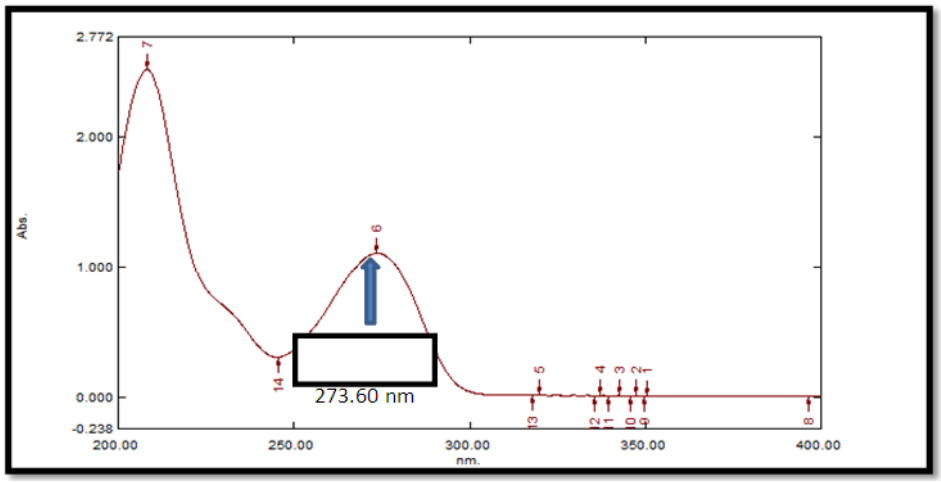


Figure 6.3: Spectra of Doxofylline 5 µg/ml solution in Phosphate buffer pH 6.8.

Calibration curve of Doxofylline in Phosphate buffer pH 6.8

The calibration curve taken in Phosphate buffer pH 6.8 showed a linear relation with a regression coefficient (r^2) of 0.998. The absorbance was well within the range of Beers and Lamberts law.

Table 6.3: Absorbance of Doxofylline in Phosphate buffer pH 6.8 at 273.60 nm.

Concentration (µg/ml)	Absorbance			Avg. Absorbance (n=3, Mean±SD)
	I	II	III	
0	**0**	**0**	**0**	**0**
5	0.213	0.192	0.162	0.189 ± 0.004
10	0.392	0.378	0.330	0.366 ± 0.018
15	0.593	0.557	0.50	0.549 ± 0.029
20	0.797	0.726	0.639	0.72 ± 0.016
25	1.112	0.805	0.938	0.885 ± 0.012

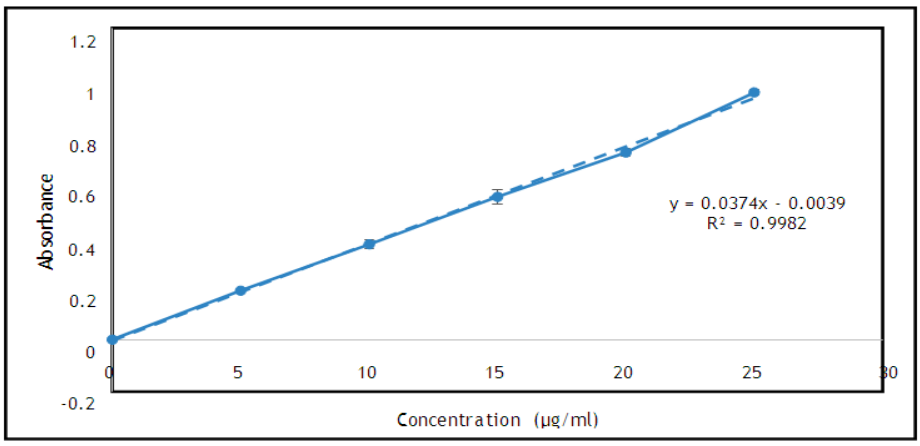
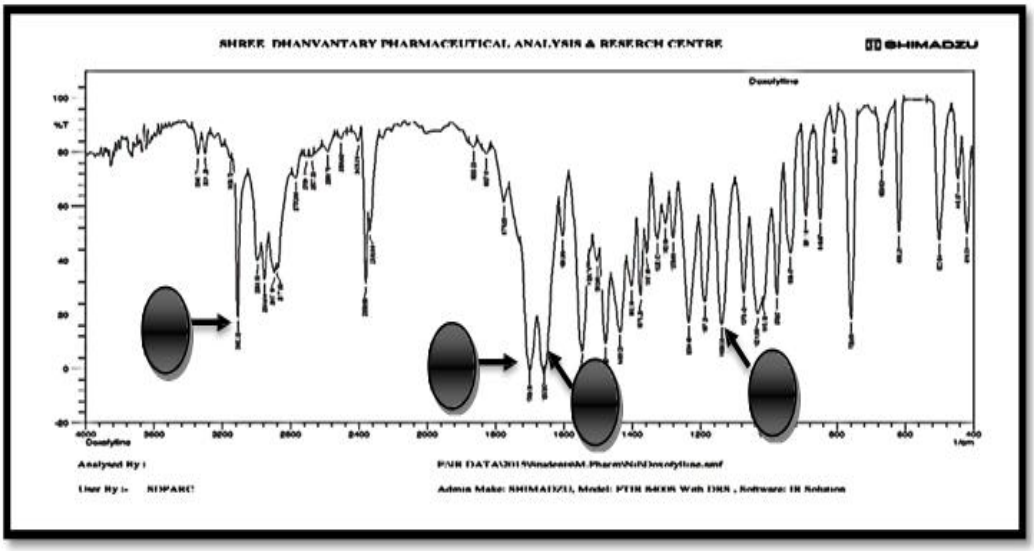


Figure 6.4: Calibration curve of Doxofylline in Phosphate buffer pH 6.8.

2. FTIR characterization

From the data shown in table 6.4 and figure 6.5, it was observed that the FTIR peaks of sample Doxofylline drug is nearly equal to the peaks reported for the standard Doxofylline drug. Therefore it can be concluded that the given sample is pure Doxofylline drug.



6.3 Evaluation of Matrix Tablet of Trial Batches

1. Pre compressional parameters of Trial Batches

Flow property of granules for all formulated batches is shown in table no. 6.6. The bulk density varies between 0.41 to 0.51 gm/ml, the tapped density varied between 0.48 to 0.56 gm/ml, the Carr's index varies between 7.90 to 15.68 % and Hausner's ratio 1.08 to 1.18 %. Further, angle of repose 21.33 to 28.81 was found. So that prepared granules shows a good flow property.

Table 6.6: Pre-compressional parameter for trial batches.

Formulation	Parameters				
	Angle of repose (^{0}) (n=3, Mean±SD)	Bulk density (g/ml) (n=3, Mean±SD)	Tapped density (g/ml) (n=3, Mean±SD)	Carr's index (%) (n=3, Mean±SD)	Hausner ratio (%) (n=3, Mean±SD)
T1	23.79±0.82	0.45±0.02	0.50±0.01	11.18±2.95	1.12±0.03
T2	28.81±1.64	0.51±0.01	0.56±0.02	7.90±0.63	1.08±0.01
T3	24.90±0.78	0.48±0.01	0.55±0.01	12.52±2.83	1.14±0.03
T4	21.33±1.28	0.41±0.02	0.48±0.01	14.46±3.07	1.16±0.06
T5	26.60±0.73	0.46±0.00	0.52±0.00	11.53±2.05	1.13±0.02
T6	25.28±1.09	0.43±0.16	0.51±0.05	15.68±1.39	1.18±0.09

2. Post compressional parameters of Trial Batches

From table no. 6.7 it was seen that all tablets passes the weight variation test as per IP. Further the parameters like hardness and thickness meet the criteria. The low value of % friability indicated the mechanical stability of the formulation. Drug content in the different formulations of trial batches were found to be 96.57 to 100.4

Table 6.7: Post-compressional parameter for trial batches.

Formulation	Parameters					
	Thickness (mm) (n=3, Mean±SD)	Diameter (mm) (n=3, Mean±SD)	Hardness (kg/cm^3) (n=3, Mean±SD)	Weight Variation (NMT 5%) (n=3, Mean±SD)	Friability (%) (n=3, Mean±SD)	%Drug content (n=3, Mean±SD)
T1	4.28±0.001	12.56±0.09	6.56±0.15	Pass	0.24%±1.56	97.96
T2	4.75±0.004	12.94±0.11	6.33±0.15	Pass	0.37%±0.43	99.70
T3	3.96±0.006	12.73±0.49	6.1±0.1	Pass	0.19%±2.48	100.4
T4	4.91±0.003	12.85±0.56	7.03±0.25	Pass	0.33%±0.91	98.36
T5	4.88±0.008	12.42±0.20	6.7±0.17	Pass	0.41%±0.20	97.14
T6	4.59±0.005	12.63±0.94	6.89±0.36	pass	0.35%±1.08	96.57

3. *In vitro* Drug Release study of Trial batches of Doxofylline matrix tablets

In vitro drug release of matrix tablets was performed using two different dissolution medium i.e. in pH 1.2 acid buffer for initial 2h followed by pH 6.8 phosphate buffer for next 24h to mimicking passage of dosage form from stomach to ileum. The result of drug release in different media is shown in Table. No 6.8. Results indicated that formulation T3 releases 83.59 % of drug in 12h. Formulation T1 and T2 releases 85.39% and 93.18% of drug in 15 h. Formulation T6 releases 89.01% of drug in 18 h. Formulation T5 releases 87.44% of drug in 21h. So that they did not match with the prefixed goal of the sustained the drug release for 24h. But in case of formulation T4 drug release was found to be 94.25% in 24h. This meet the prefixed criteria for sustained the drug release for 24h time period. So that it can be concluded that among the six formulations T1, T2, T3, T4, T5 and T6; formulation T4 was most suitable for sustained the drug release for 24h.So optimization of T4 batch was done by using 3^k factorial design.

Table 6.8: % Drug Release for Trial batches.

Time (hr)	Cumulative % Drug release (n=3, Mean ± SD)					
	T1	T2	T3	T4	T5	T6
0	0	0	0	0	0	0
1	9.56 ± 0.23	10.35 ± 0.73	14.57 ± 1.43	6.64 ± 0.14	8.04 ± 0.79	9.24 ± 1.73
2	13.45 ± 0.68	16.04 ± 0.92	19.89 ± 0.96	11.25 ± 0.57	11.95 ± 1.25	12.38 ± 1.49
3	16.88 ± 0.16	19.25 ± 1.33	24.87 ± 1.09	15.55 ± 2.49	16.11 ± 0.14	16.53 ± 0.84
4	21.14 ± 1.76	24.01 ± 0.84	30.42 ± 0.77	17.93 ± 0.27	19.22 ± 3.28	20.17 ± 0.88
5	23.52 ± 0.45	28.52 ± 0.66	35.94 ± 0.43	19.82 ± 0.95	21.17 ± 0.66	22.84 ± 1.68
6	28.54 ± 2.84	31.60 ± 0.57	42.73 ± 2.79	22.89 ± 0.76	25.45 ± 0.73	27.31 ± 3.04
7	32.81 ± 0.28	36.99 ± 2.41	49.55 ± 0.15	24.10 ± 1.55	28.46 ± 1.58	30.76 ± 1.66
8	40.84 ± 3.19	44.78 ± 0.79	55.66 ± 2.00	24.99 ± 2.09	33.04 ± 2.91	37.01 ±0.92
9	45.74 ± 0.41	52.07 ± 1.61	63.05 ± 1.86	27.44 ± 0.86	35.82 ± 0.44	40.39 ± 1.57
10	52.53 ± 1.46	58.68 ± 0.13	68.95 ± 0.94	29.71 ± 0.22	38.08 ± 0.89	45.25 ± 0.82
11	60.46 ± 2.17	65.70 ± 3.72	76.38 ± 1.73	31.42 ± 1.41	40.55 ± 0.16	49.93 ± 1.60
12	65.97 ± 1.03	73.42 ± 0.49	**83.59 ± 0.31**	34.20 ± 0.69	42.66 ± 0.93	54.12 ± 0.25
15	**85.39 ± 2.18**	**93.18 ± 1.43**	---	46.79 ± 0.76	56.09 ± 0.49	72.94 ± 1.18
18	---	---	---	60.11 ± 1.55	71.6 ± 2.07	**89.01 ± 1.73**
21	---	---	---	79.53 ± 2.04	**87.44 ± 0.89**	---
24	---	---	---	**94.25 ± 1.68**	---	---

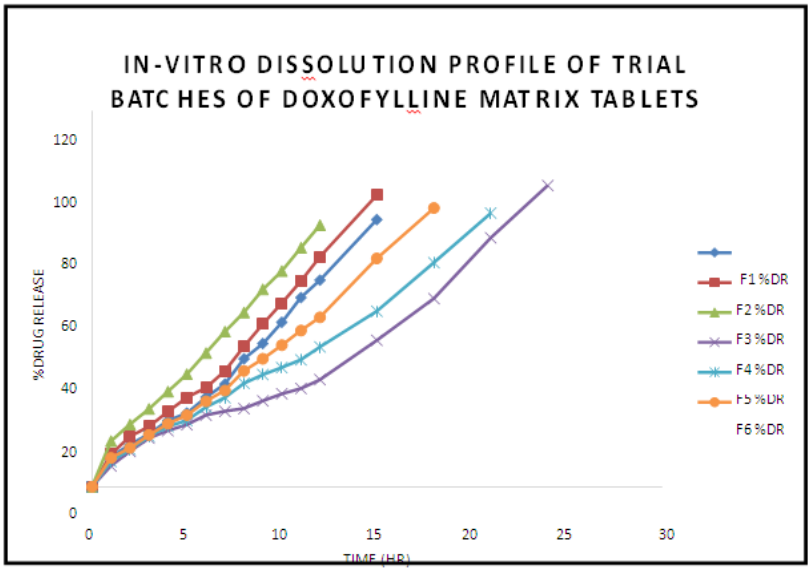


Figure 6.12: Comparision of % drug release of Trial batches.

6.4 Evaluation of matrix tablets of factorial batches

1. Pre compressional parameters of Factorial Batches

Flow property of granules for all formulated batches is shown in table no. 6.9. The bulk density varies between 0.41 to 0.51 gm/ml, the tapped density varied between 0.48 to 0.56 gm/ml, the Carr's index varies between 7.90 to 15.68 % and Hausner's ratio 1.08 to 1.18 %. Further, angle of repose 21.33 to 28.81 was found. So that prepared granules shows a good flow property.

Table 6.9: Pre-compressional parameter for factorial batches.

Formulation	Parameters				
	Angle of Repose (^{0}) (n=3, Mean±SD)	Bulk density (g/ml) (n=3, Mean±SD)	Tapped density (g/ml) (n=3, Mean±SD)	Carr's index (%) (n=3, Mean±SD)	Hausner ratio (%) (n=3, Mean±SD)
F1	22.16±1.09	0.48±0.05	0.53±0.06	9.43±0.58	1.10±0.06
F2	23.73±0.57	0.52±0.06	0.56±0.02	7.14±1.24	1.07±0.09
F3	25.09±0.61	0.43±0.03	0.47±0.08	8.51±1.86	1.09±0.03
F4	23.28±1.28	0.46±0.16	0.52±0.06	11.53±0.31	1.13±0.02
F5	26.49±0.99	0.50±0.10	0.56±0.14	10.71±0.90	1.12±0.07
F6	28.31±1.37	0.54±0.02	0.60±0.05	10±2.56	1.11±0.22
F7	24.95±0.64	0.42±0.19	0.46±0.09	8.69±0.64	1.09±0.10
F8	28.67±1.12	0.51±0.05	0.57±0.01	10.52±1.20	1.11±0.03
F9	27.46±0.83	0.45±0.12	0.52±0.10	13.46±2.07	1.15±0.08

2. Post compressional parameters of Factorial Batches

From table no. 6.10 it was seen that all tablets passes the weight variation test as per IP. Further the parameters like hardness and thickness meet the criteria. The low value of % friability indicated the mechanical stability of the formulation. Drug content in the different formulations of Factorial batches were found to be 96.91 to 105.02.

Table 6.10: Post-compressional parameter for factorial batches.

Formulation	Parameters					
	Thickness (mm) (n=3, Mean±SD)	Diameter (mm) (n=3, Mean±SD)	Hardness (kg/cm^3) (n=3, Mean±SD)	Weight Variation (NMT5%) (n=3, Mean±SD)	Friability (%) (n=3, Mean±SD)	% Drug Content (n=3, Mean±SD)
F1	4.28±0.005	12.33±0.17	5.86±0.43	Pass	0.43±1.26	99.21
F2	4.67±0.001	12.59±0.69	6.42±0.15	Pass	0.66±2.81	102.58
F3	3.99±0.009	12.24±0.53	6.79±0.28	Pass	0.81±0.77	97.84
F4	4.81±0.003	12.65±0.24	5.62±0.12	Pass	0.29±1.90	105.02
F5	4.32±0.004	12.83±0.88	7.10±0.59	Pass	0.37±0.64	104.19
F6	4.75±0.001	12.46±0.97	7.25±0.64	Pass	0.54±2.12	99.10
F7	4.43±0.008	12.52±0.34	6.31±0.95	Pass	0.70±0.58	102.67
F8	4.96±0.005	12.71±0.30	6.97±0.11	Pass	0.25±0.76	96.91
F9	4.50±0.009	12.78±0.72	7.73±0.39	Pass	0.79±1.05	100.36

6.5 Statistical analysis of 3^2 Factorial Design

6.5.1 Fitting of data to the model

A two-factor, three-level full factorial statistical experimental design requires 9 experiments. All the responses observed for 9 formulations prepared were simultaneously fit to quadratic model using Design Expert 9.0.2.0. It was observed that the best fit model was quadratic model and the comparative values of R2, SD, and %CV are given in table along with the regression equation generated for each response as shown in table. A positive value represents an effect that favours the optimization, while a negative value indicates an inverse relationship between the factor and the response. It is evident that both independent variables, viz., HPMC K100M (X1) and Xanthan Gum (X2) have positive effects on the responses, viz., Y1 (T50 %) and Y2 (T80 %).

The criteria for selection of feasible region of were as shown in table 6.12

Table 6.12: Desirable values of dependent variables for optimization.

Response	Desirable values
T50%	12-18 hrs.
T80%	18-21 hrs.

Table 6.13: Experimental values of dependent variables for optimization.

Formulation code	Formulation Component		T50% (Y1)	T80% (Y2)
	HPMC K100M	Xanthan Gum		
F1	-1	-1	11.28	15.25
F2	0	-1	11.63	16.97
F3	+1	-1	14.71	22.05
F4	-1	0	11.09	16.75
F5	0	0	12.06	18.45
F6	+1	0	17	23.42
F7	-1	+1	15.28	20.51
F8	0	+1	15.61	22.02
F9	+1	+1	17.29	23.93

Table 6.14: Summary of results of multiple regression analysis for Y1 and Y2.

Dependent variable	T50%(Y1)		T80%(Y2)	
	P value	Coefficient	P value	Coefficient
Intercept	0.0234	12.43	0.0019	18.70
X1	0.0097	1.89	0.0005	2.76
X2	0.0124	1.76	0.0015	2.08
X1X2	0.5160	-0.35	0.0488	-0.92
X11	0.1029	1.37	0.0460	1.23
X22	0.2192	0.95	0.2104	0.64

Table 6.15: Summary of results of regression analysis for responses Y1-Y2 for fitting to quadratic model.

Quadratic model	R^2	Adjusted R^2	Predicted R^2	Adequate precision	%CV
Y1	0.9239	0.8287	0.1957	9.451	7.21
Y2	0.9789	0.9525	0.7767	18.994	3.32

6.5.2 Data analysis of Y1 (T50%)

The observed value for T50% for all 9 batches varied from 11.09 to 17 hr. The result clearly indicates that Y1 is strongly affected by the independent variables selected for the study. The response (Y1) obtained at various levels of two independent variables were subjected to multiple regression to give a quadratic polynomial equation no. 1

Equation 1

$Y_1 = 12.43 + 1.89\ X_1 + 1.76\ X_2 - 0.35\ X_1X_2 + 1.37\ X_{11} + 0.95\ X_{22}$

The above equation clearly shows that coefficient b_1 (+1.89) and coefficient b_2 (+1.76) bear a Positive sign. Therefore, increasing the values of X_1 and X_2 expected to increases the values of T50% of the formulation. Variable X_1 and X_2 were also found to be significant ($P<0.05$).

6.5.3 Data analysis of Y2 (T80%)

The observed value for T80% for all 9 batches varied from 15.25 to 23.93 hr. The result clearly indicates that Y_2 is strongly affected by the independent variables selected for the study. The response (Y_2) obtained at various levels of two independent variables were subjected to multiple regression to give a quadratic polynomial equation no. 2

Equation 2

$Y_2 = 18.70 + 2.76\ X_1 + 2.08\ X_2 - 0.92\ X_1X_2 + 1.23\ X_{11} + 0.64\ X_{22}$

The above equation clearly shows that coefficient b_1 (+2.76) and coefficient b_2 (+2.08) bear a Positive sign. Therefore, increasing the values of X_1 and X_2 expected to increases the values of T80% of the formulation. Variable X_1 and X_2 were also found to be significant ($P<0.05$).

Table 6.16: Summary of Quadratic polynomial equation for responses Y1 and Y2 for fitting to quadratic model.

Quadraticmodel	Quadratic polynomial equation
Y_1	$Y_1 = 12.43 + 1.89\ X_1 + 1.76\ X_2 - 0.35\ X_1X_2 + 1.37\ X_{11} + 0.95\ X_{22}$
Y_2	$Y_2 = 18.70 + 2.76\ X_1 + 2.08\ X_2 - 0.92\ X_1X_2 + 1.23\ X_{11} + 0.64\ X_{22}$

6.5.7 Evaluation of Optimized Batch

Table 6.19: Evaluation parameter of optimized batch.

Evaluation Parameters	Experimental Values (n=3, Mean±SD)
Angle of repose (^{0})	24.95±0.64
Bulk density (g/ml)	0.42±0.19
Tapped density (g/ml)	0.46±0.09
Carr's index (%)	8.69±0.64
Hausner ratio (%)	1.09±0.10
Thickness (mm)	4.43±0.008
Diameter (mm)	12.52±0.34
Hardness (kg/cm^3)	6.31±0.95
Friability (%)	0.70±0.58

Table 6.20: In vitro drug release of optimized batch.

Time (hr)	%CDR (n=3, Mean±SD)
0	0
1	6.97±0.73
2	11.30±0.98
3	14.00±1.52
4	16.99±1.30
5	20.47±0.56
6	22.29±2.07

7	24.38±0.19
8	27.86±2.43
9	30.05±2.28
10	33.16±1.11
11	35.01±0.09
12	37.52±0.21
15	49.18±1.73
18	66.82±0.29
21	81.36±0.67
24	**95.2±0.1.25**

T50% For Optimized Formulation = 15.28 hr
T80% For Optimized Formulation = 20.82 hr

Table 6.21: Result of optimized batch for response variables.

Response Variable	Predicted values	Experimental values
Y_1 (HPMC K100M)	14.98	15.28
Y_2 (Xanthan Gum)	20.51	20.82

6.6 Stability Study

The stability study of optimized formulation was carried out at 40ºC±0.5% and 75% RH using stability chamber for one month. The different parameters that were studied are shape, colour, hardness, thickness and dissolution rate. The Optimized formulation were found to be stable in terms of physical appearance, hardness and *in vitro drug* release.

Table 6.22: Stability study of Optimized batch.

Parameters	Initial	After 1 month
Shape	Convex	Convex
Colour	White	White
Thickness (mm)	4.43	4.43
Hardness (Kg/cm^2)	6.31	6.31
% Drug release	95.2	93.57

7. CONCLUSION

From the results and discussion following conclusion were drawn:

The present investigation deals with the formulation and evaluation of sustained release matrix tablets of Doxofylline for asthma using polymers such as HPMC K100M, Xanthan Gum and Guar Gum. As per trial batches concluded that combination of HPMC K100M and Xanthan Gum were suitable as release rate controlling polymers for sustaining of drug release for 24 hrs. So thus Doxofylline could be successfully delivered to provide 24 hrs relief of asthmatic effect by design of a sustained release matrix formulation.

The FTIR and DSC study showed no sign of incompatibility, thus concluding the selected polymers are likely to be suitable for preparation of sustained release matrix tablet.

The formulation was optimized using a two factor, three level full factorial Design. The amount of independent variables HPMC K100M (X_1) and Xanthan Gum (X_2) showed a significant effect on the dependent variables T50% (Y_1) and T80% (Y_2). The quantitative effect of these factors at different levels was predicated by using polynomial equations. Linearity observed between the actual and predicted values of the response variables suggested the prognostic ability of the Response surface methodology design. Response surface methodology was the used to predict the levels of the factors X_1 and X_2 required to obtain an optimum formulation with good T50% and T80%. A optimized formulation was prepared according to these levels.

From evaluation parameters of factorial batches it should be concluded that if the concentration of HPMC K100M and Xanthan Gum increase than T50% and T80% will be increase. After all evaluation of optimize batch was selected for the 1 month stability study and the result revealed that there is no significant change in drug release profile and physical parameters which indicates that the selected formulation is stable.

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