ISSN 0975-6299 Vol 2/Issue 1/ Jan-Mar 2011

International Journal of Pharma and Bio Sciences

RESEARCH ARTICLE

PHARMACEUTICS

FORMULATION AND EVALUATION OF ORODISSOLVING TABLET OF FLUOXETINE USING SUPERDISINTEGRANTS

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ABSTRACT

Mouth dissolving tablet offers a solution for pediatrics, geriatrics; psychiatric or mentally ill people and those have difficulty in swallowing tablets/capsules resulting in improved patient compliance. The aim is to formulate fifteen formulations of fast dissolving tablet of fluoxetine using different superdisintegrants (Sodium Starch Glycolate, Croscarmellose, Crospovidone Pregelatinized starch) by wet granulation method and the tablets were evaluated for various physiochemical properties and found to be within the permissible limit. *In vitro* dissolution studies show the release is in the following order of superdisintegrants: **Crospovidone > Pregelatinized starch > Croscarmellose > Sodium Starch Glycolate**. From the study it has been found and concluded, crospovidone at a concentration of 5% w/w **(**F-XII) shows maximum in-vitro dissolution profile, this is also confirmed by *In vivo* pharmacokinetic studies, and hence it emerged as the overall best formulation hence suitable for preparing fast dissolving tablet of fluoxetine.

KEY WORDS

Fluoxetine, Antidepressant drug, superdisintegrants, oro-dissolving tablet, wet granulation.

INTRODUCTION

The need for delivering drugs to patients efficiently and with few side effects has prompted pharmaceutical companies to engage in the development of new drug delivery system. A solid dosage form that dissolves or disintegrates rapidly in oral cavity, resulting in solution or suspension without the need of water is known as fast dissolving dosage form or mouth dissolving tablets [1]. When this type of tablet is placed into the mouth, the saliva will serve to rapidly dissolve the tablet. They are also known as oro-dissolving, rapid –dissolve oro-dispersible, melt in mouth, rapimelt, quick dissolving, fast melts, and porous tablets.

For treatment of depression various conventional oral dosage forms like tablets, capsules, oral suspension, syrups etc are available in market but the major drawbacks with these are many patients find it difficult to swallow (dysphagia) tablets and hard gelatin capsules. Dysphagia [2] is a common problem encountered in all age groups in concern to solid dosage forms, which results in high incidence of non-compliance and ineffective therapy. The difficulty experienced in particular by pediatrics and geriatrics patients. Other groups that may experience problems include the mentally ill, developmentally disable and patients who are uncooperative and hence do not take their medicines as prescribed leading to patient non-compliance. Recent advances in novel drug delivery systems (NDDS) aim to enhance safety and efficacy of drug molecule by formulating a convenient dosage form for administration and to achieve better patient compliance i.e., one, which will rapidly disintegrate in the mouth without need of water (fast dissolving tablet). Advantages of this drug delivery system include administration without water, accuracy of dosage, easy portability, alternative to liquid dosage forms, ideal for pediatric and geriatric patients and rapid onset of action.

Fluoxetine [3] have become first line drug in the pharmacotherapy of patients with depression. This is because the drug possesses tolerability and safety advantages over the tricyclic agents. The concept of formulating orodispersible tablets containing fluoxetine offers a suitable and practical approach in serving desired objective of rapid disintegration and dissolution characteristics with increased bioavailability.

MATERIALS AND METHOD

Crospovidone (Paxmy Speciality Chemicals, Chennai) croscarmellose (Paxmy Speciality Chemicals, Chennai) fluoxetine (Paxmy Speciality Chemicals, Chennai) pregelatinized starch (Colorcon Ltd., Goa), sodium starch glycolate (SD Fine Chemicals Ltd., Mumbai).

Wet granulation method using superdisintegrants

Fifteen formulations were prepared by wet granulation method using different superdisintegrants in various ratios (designated as F-1, F-2, F-3, F-4, F-5, F-6, F-7, F-8, F-9, F-10, F-11, F-12, F-13, F14 and F-15) and formulation F-1 prepared without superdisintegrant is used as control (Table 1, 2).

Table 1 & 2

Composition of different batch's of orodispersible tablets of Fluoxetine Hydrochloride from F-1 to F-15

Ingredients	Quantity per tablet (mg)							
	F-1	**F-2**	**F-3**	**F-4**	**F-5**	**F-6**	**F-7**	**F-8**
Sodium starch glycolate	-	4	6	8	-	-	-	-
Croscarmellose	-	-	-	-	4	6	8	10
Cellulose microcrystalline	-	68	66	64	68	66	64	62
Lactose	172				100			
Fluoxetine					10			
Mannitol					3			
Saccharin sodium					1			
Starch paste					10			
Magnesium stearate					1			
Talc					1			
Colloidal silicon dioxide					1			
Total					**200**			

Ingredients	Quantity per tablet (mg)						
	F-9	**F-10**	**F-11**	**F-12**	**F-13**	**F-14**	**F-15**
Crospovidone	4	6	8	10	-	-	-
Pregelatinized starch	-	-	-	-	10	15	20
Cellulose microcrystalline	68	66	64	62	62	57	52
Lactose				100			
Fluoxetine				10			
Mannitol				3			
Saccharin sodium				1			
Starch paste				10			
Magnesium stearate				1			
Talc				1			
Colloidal silicon dioxide				1			
Total				**200**			

Fluoxetine raw material and all excipients were passed through sieve no.60 before granulation and lubrication. The required quantity of Fluoxetine and other excipients (except lubricants and glidants) were weighed and mixed uniformly. Then the mixture was made to a damp mass using starch paste. Then the prepared mass was passed through sieve no. 16. The prepared granules were dried in an oven at a temperature of 50^{o}C for one hour. The granules obtained were lubricated by adding and mixing with talc, magnesium stearate and

colloidal silicon dioxide. The lubricated granules were evaluated and punched into tablets with an average weight of 200 mg, using Cadmach tabletting machine.

RAW MATERIAL EVALUATION OF FLUOXETINE HYDROCHLORIDE

Identification: By Infrared Absorption spectroscopy (Fig 1).

EVALUATION OF GRANULES

Bulk density [4]:

Apparent bulk density is the ratio of weight of the powder to the bulk volume it occupies, expressed in gm/ml.

Tapped Density [4]:

After determining the poured bulk density the granules were tapped mechanically for 100 times till a constant volume called tapped bulk density was obtained .The minimum volume occupied in the cylinder and the weight of granules was measured.

Carr's index:

Using poured bulk density and tapped bulk density the percentage compressibility of granules was determined, which is given as Carr's compressibility index [4].

$$\text{Carr's compressibility index (\%)} = \frac{\text{Tapped bulk density} - \text{poured bulk density}}{\text{Tapped bulk density}}$$

Hausner ratio:

Hausner ratio [4] is an indirect index of ease of powder flow. It is the ratio of tapped density to bulk density. Lower hausner ratio (<1.25) indicates better flow properties than higher ones (>1.25).

Angle of repose [4]:

It is the maximum angle that can be obtained between the free surface of a powder heap and horizontal plane. The angle of repose is given as.

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

Where θ = angle of repose

h = height of the heap

r = radius of the base of the heap

The results of all the parameters were shown in Table 3.

Table 3

Evaluation of granules for Fluoxetine Hydrochloride orodissolving tablet

S.No	Formulations	Bulk density (gm/cm³)	Tapped density (gm/cm³)	Carr's index (%)	Angle of repose (º)	Hausner ratio
1	F-I	0.392	0.399	18.03	28º 37'	1.018
2	F-II	0.361	0.408	11.59	28º 07'	1.131
3	F-III	0.387	0.434	10.86	29º 37'	1.121
4	F-IV	0.414	0.462	10.31	27º 21'	1.115
5	F-V	0.372	0.421	11.52	27º 21'	1.130
6	F-VI	0.359	0.421	14.62	28º 22'	1.170
7	F-VII	0.375	0.414	9.42	27º 28'	1.104
8	F-VIII	0.366	0.411	10.90	27º 18'	1.122
9	F-IX	0.376	0.442	15.08	28º 22'	1.177
10	F-X	0.332	0.391	14.99	29º 37'	1.176

11	F-XI	0.333	0.385	13.33	29º 05’	1.154
12	F-XII	0.328	0.376	12.91	29º 21’	1.148
13	F-XIII	0.378	0.427	11.53	29º 22’	1.130
14	F-XIV	0.406	0.464	12.49	28º 26’	1.143
15	F-XV	0.369	0.436	15.39	27º 21’	1.182

EVALUATION OF TABLETS

Thickness:

The thickness[5] of the tablets was measured by using digital vernier callipers.

Weight Variation test [5]:

Twenty tablets were selected at random and weighed individually on shimdzu BL-220. The individual weights were compared with the average weight for determination of weight variation.

Drug content [5]:

20 tablets of each formulation were weighed and powdered. The quantity of powder equivalent to 10 mg of fluoxetine was transferred into a 100 ml standard flask and volume was made up with 0.1N hydrochloric acid. Further 1ml of the above solution was diluted to 10 ml with 0.1N hydrochloric acid and absorbance of the resulting solution was observed at 225nm.

Hardness [5]:

Hardness of the tablet was determined using the Monsanto hardness tester.

Friability test [5]:

20 previously weighed tablets were placed in the apparatus, which was given 100 revolutions and the tablets were reweighed.

$$\text{Percentage friability} = \frac{\text{Initial weight-Final weight}}{\text{Initial weight}} \times 100$$

Wetting time [5]:

For determination of wetting time, a piece of tissue paper folded twice was placed in a small petri dish (having internal diameter of 5 cm) containing 6 ml of water. A tablet was placed on the paper and the time required for complete wetting was measured. The wetted tablet was then weighed.

Disintegration test [5]:

Fast dissolving tablets should disintegrate within 3 mts. 6 tablets of each formulation were taken and placed in 6 tubes of disintegration apparatus. The time taken for complete disintegration was noted.

The results of physical parameters of mouth dissolving tablet was shown in Table 4.

Table 4
Evaluation of Fluoxetine Hydrochloride orodissolving tablets

Formulations	Weight Variation test	Thickness (mm) Mean ± S.D	Hardness (Kg/cm^2)	Friability (%w/w) Mean ±S.D	Percent drug content Mean ±S.D	Wetting time (secs) Mean ±S.D	Disintegration test (secs) Mean±SD
F-I	Passes	5.22	4 - 4.5	0.49±0.041	9.57±0.00	92± 0.74	90±1.73

F-II	Passes	5.20	3.5- 4	0.65±0.00	9.94±0.07	41± 2.13	40±2.886
F-III	Passes	5.18	4	0.82±0.021	9.88±0.24	37± 0.15	36±1.0
F-IV	Passes	5.20	3.5 - 4	0.30±0.01	9.97±0.19	35± 1.89	33±1.429
F-V	Passes	5.19	4	0.54±0.053	9.98±0.12	72±1.65	70±2.161
F-VI	Passes	5.21	3.5 - 4	0.79±0.048	9.97±0.00	62±2.54	61±3.564
F-VII	Passes	5.19	3.5 - 4	0.96±0.047	10.03±0.07	61±0.89	60±2.659
F-VIII	Passes	5.14	3.5	0.59±0.00	9.63±0.21	83± 1.04	81±2.56
F-IX	Passes	5.19	3.5- 4	0.40±0.021	10.09±0.00	43± 0.88	40±0.10
F-X	Passes	5.17	3.5- 4	0.61±0.01	9.91±0.00	17± 1.56	15±3.441
F-XI	Passes	5.22	3.5	0.92±0.042	9.57±0.11	14± 1.89	12±2.12
F-XII	Passes	5.22	3- 3.5	0.87±0.038	9.78±0.20	11± 1.56	10±1.00
F-XIII	Passes	5.21	4	0.91±0.01	10.05±0.07	27± 1.04	25±1.561
F-XIV	Passes	5.20	3.5 - 4	0.58±0.021	9.88±0.24	32± 2.78	30±3.213
F-XV	Passes	5.17	3.5	0.58±0.047	9.83±0.00	32± 0.89	30±2.56

 # All the values are expressed as mean ± SD

In- vitro dissolution studies [6]:

The dissolution test has been carried out for all the formulations. The in vitro drug release is performed using USP dissolution apparatus- II, 24 type paddle apparatus using 900 ml of 0.1 N HCL at paddle rotation of 50 rpm at 37±0.5 0. 5 ml of the samples were withdrawn at predetermined time intervals of 5,10,15,20,30,45,60 mins for a period of 60 mins and replaced with the fresh medium of 0.1 N HCL. The samples were filtered through 0.45 mm membrane filter, suitably diluted and analyzed at 225 nm using double beam UV/Visible spectrophotometer (Shimadzu Corporation, UV-1601, Japan). The content of drug was calculated using equation generated from standard calibration curve. The observation for different batches was shown in (Table 5). The percentage release of Fluoxetine with respect to time for each batch, were graphically shown in (Fig. 2, 3, 4, 5) and comparison bar graph was shown in (Fig 6).

Figure: 2

In-vitro dissolution profile of fluoxetine hydrochloride tablet from F-1 to F-4

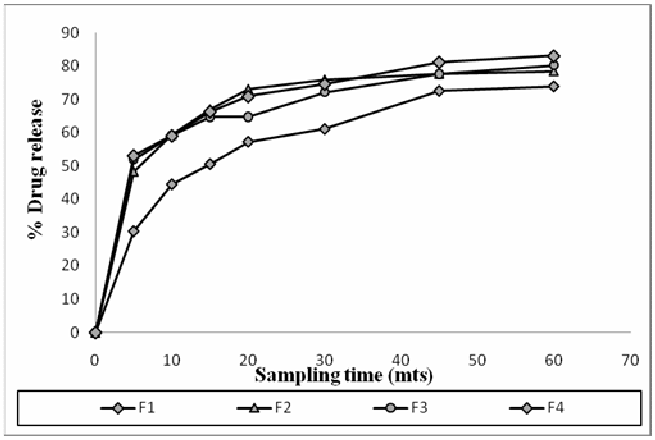


Figure : 3

In-vitro dissolution profile of fluoxetine hydrochloride tablet from F-5 to F-8

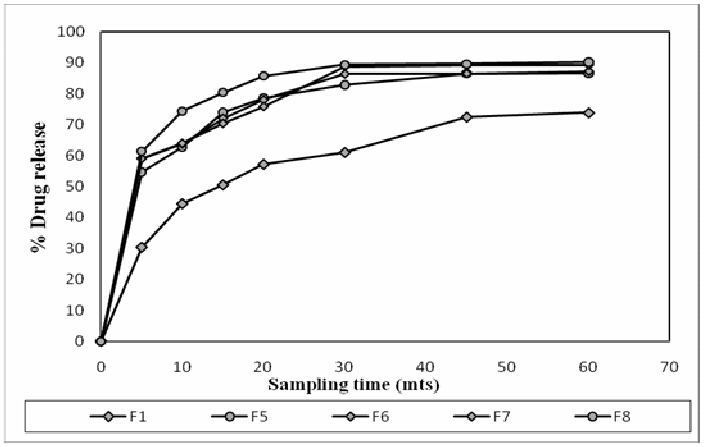


Figure : 4

In-vitro dissolution profile of fluoxetine hydrochloride tablet from F-9 to F-12

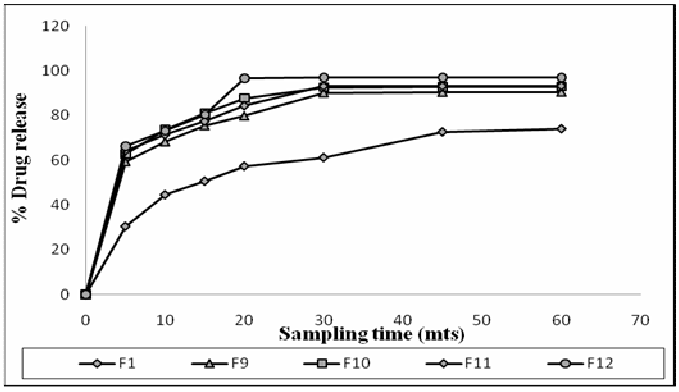


Figure : 5

In-vitro dissolution profile of fluoxetine hydrochloride tablet from F-13 to F-15

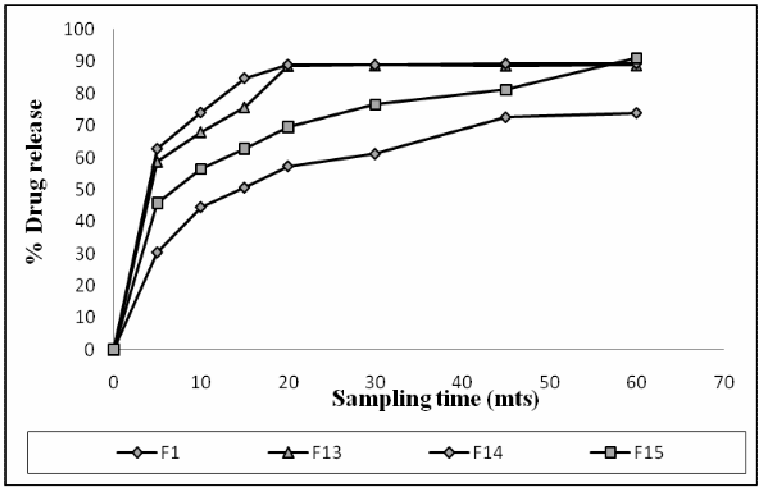


Figure: 6

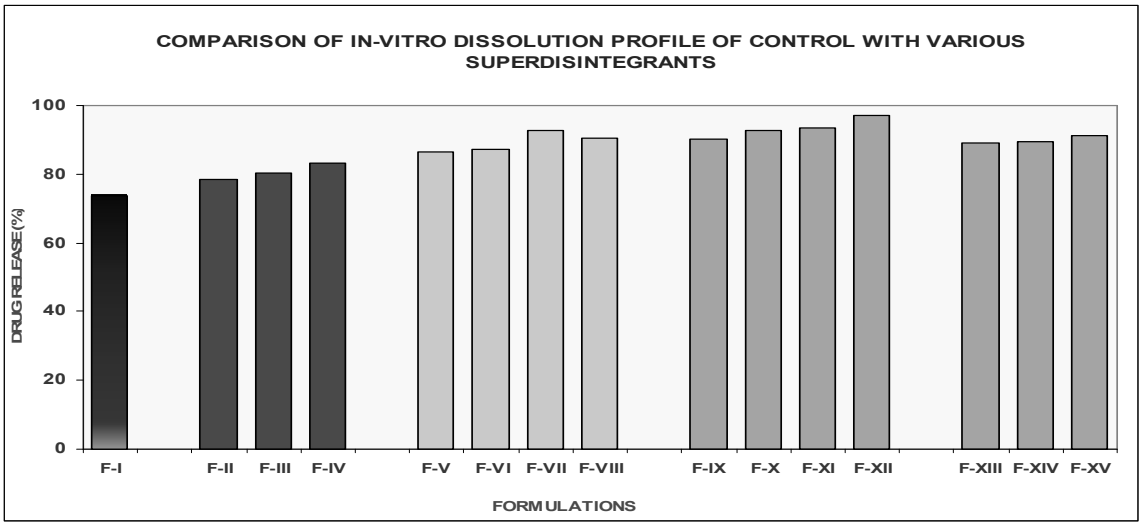


Table 5

Comparison of percentage drug release of Fluoxetine Hydrochloride orodissolving tablet

S.No	Formulations	Superdisintegrants	Ratio (% w/w)	*In vitro* release in 60 minutes (%)
1	F-I	Control	-	73.85
2	F-II	Sodium Starch Glycolate	2%	78.38
3	F-III		3%	80.25
4	F-IV		4%	83.07

5	F-V	Croscarmellose	2%	86.63
6	F-VI		3%	87.18
7	F-VII		4%	89.17
8	F-VIII		5%	90.39
9	F-IX	Crospovidone	2%	90.16
10	F-X		3%	92.88
11	F-XI		4%	93.26
12	F-XII		5%	96.97
13	F-XIII	Pregelatinized starch	5%	88.90
14	F-XIV		7.5%	89.39
15	F-XV		10%	91.12

Stability studies [7]:

All the formulations were stored in stability chamber at 45°C ± 2°C temperature and 75 % ± 5% relative humidity. Samples of tablets were analyzed at 1st day, 15th day and 45th day for hardness, disintegration time, and *in vitro* dissolution test. The results were given in Table 9.

Table 9
Accelerated Stability Studies

S. No	F-1	F-2	F-3	F-4	F-5	F-6	F-7	F-8	F-9	F-10	F-11	F-12	F-13	F-14	F-15
Hardnes	4-4.5	3.5-4	4	3.5-4	4	3.5-4	3.5-4	3.5	3.5-4	3.5-4	3.5	3.5	4	3.5-4	3.5
D.T (secs)	90± 1.73	40± 2.89	36± 5.0	33± 6.43	70± 2.16	70± 2.16	60± 4.65	81± 2.56	81± 2.56	15± 3.44	12± 2.12	10± 1.00	25± 4.56	30± 3.21	30± 2.56
Invito Dissolution	73.85	78.38	80.25	83.07	86.63	87.18	89.17	90.39	90.16	92.88	93.26	96.97	88.90	89.39	91.12

EVALUATION OF BEST FORMULATION

The formulation exhibiting faster disintegration, better *in vitro* dissolution profile and other optimum properties was considered as best among the other formulations and were subjected to the following tests,

Determination of interaction between drug and excipients [8]:

The drug and drug-excipient mixture of formulation F-XII were subjected to Infra-red (IR) studies to check drug-excipient interaction, shown in (Fig 1& 7).

Figure: 1
IR Spectroscopy of Fluoxetine Hydrochloride

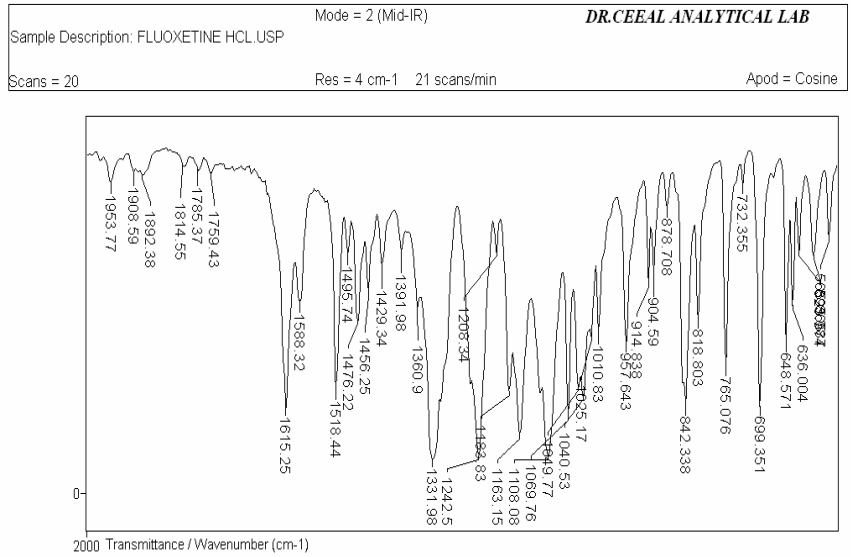
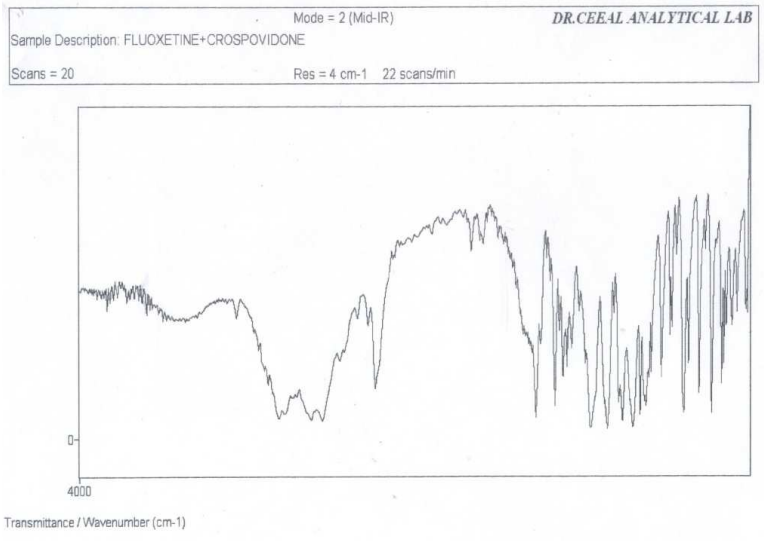


Figure: 7
IR study to determine the Interaction between drug and excipient



Differential scanning calorimetric (DSC) study [9]:

The pure fluoxetine drug and formulation F-XII were subjected to differential scanning calorimetric study performed on a NETZSCH DSC 204 instrument to asses drug- excipient compatibility, shown in (Fig 8 & 9).

Figure: 8
Differential Scanning Calorimetric Study for the Drug

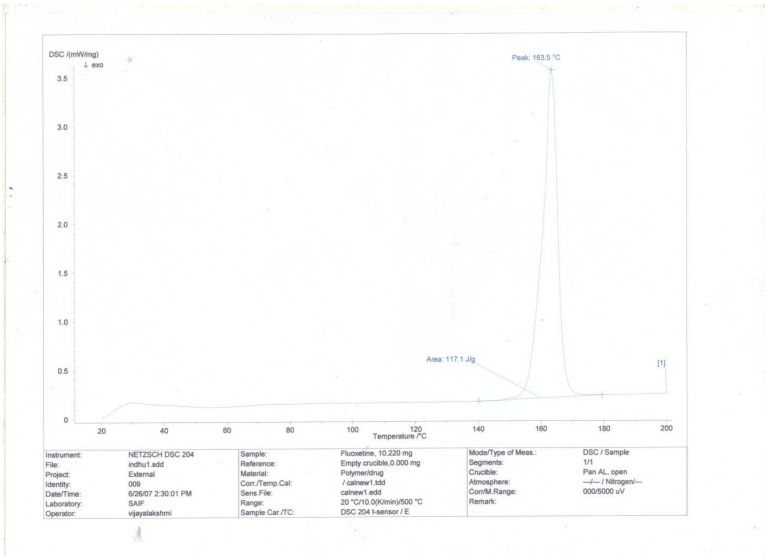
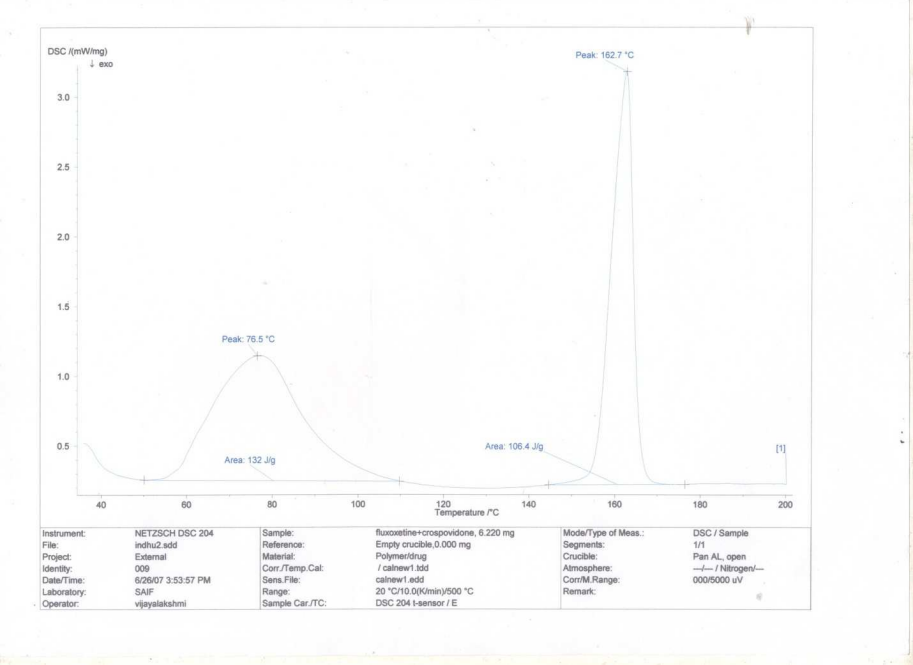


Figure: 9
Differential Scanning Calorimetric Study for drug and excipient



Release kinetics [10]:

The *in vitro* release data of F-XII was fitted in the kinetic equations to find out the mechanism of fluoxetine release from the fast dissolving tablet. The kinetic models used were zero order and first order equation. Correlation coefficient was determined for both the

equations. The results were summarized in Table 6 and shown in (Fig 10 & 11).

Table 6
Release kinetics analysis of Fluoxetine Hydrochloride orodissolving tablet

S.No	Time (mts)	Zero order Cumulative drug release (mg) (Q_t)	First order Log % of drug release {Log (Q_0-Q_t)}
1.	5	6.479	0.547
2.	10	7.145	0.456
3.	15	7.851	0.332
4.	20	9.445	-0.255
5.	30	9.474	-0.279
6.	45	9.483	-0.287
7.	60	9.483	-0.287

Figure: 10
Pharmacokinetic Profile of the drug

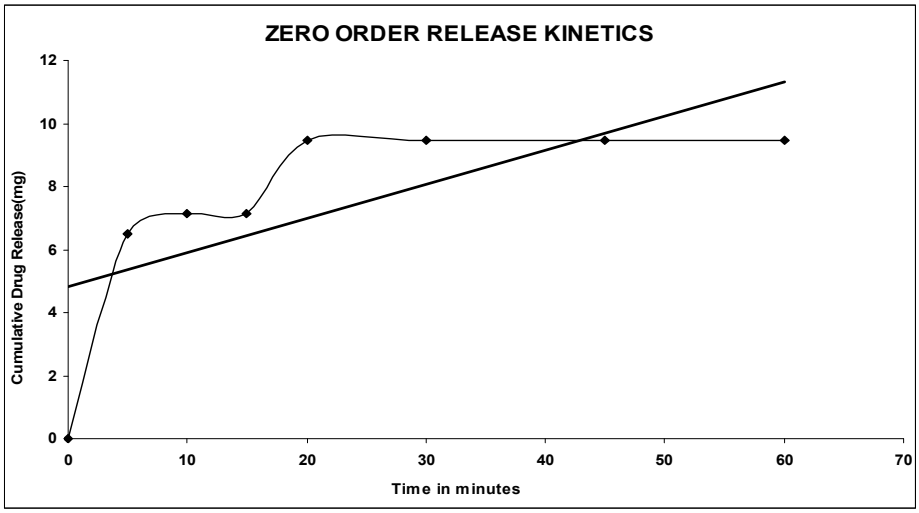
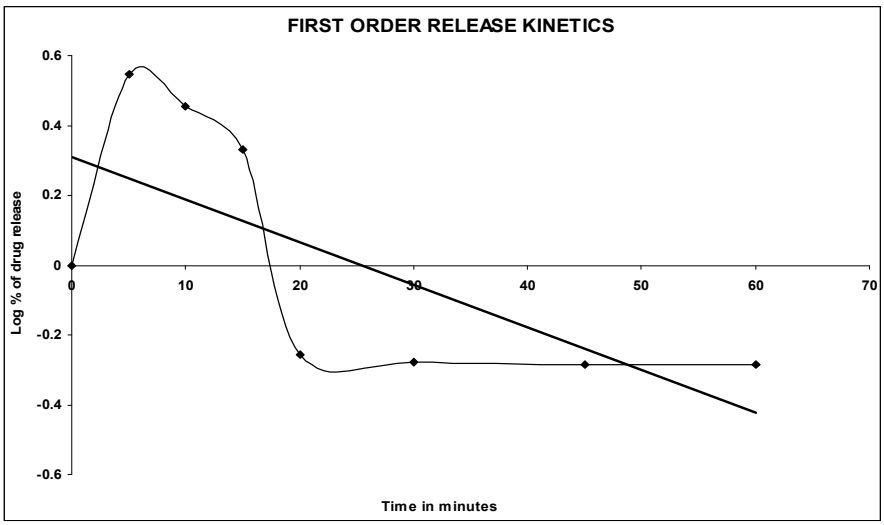


Figure: 11
Pharmacokinetic Profile of the drug



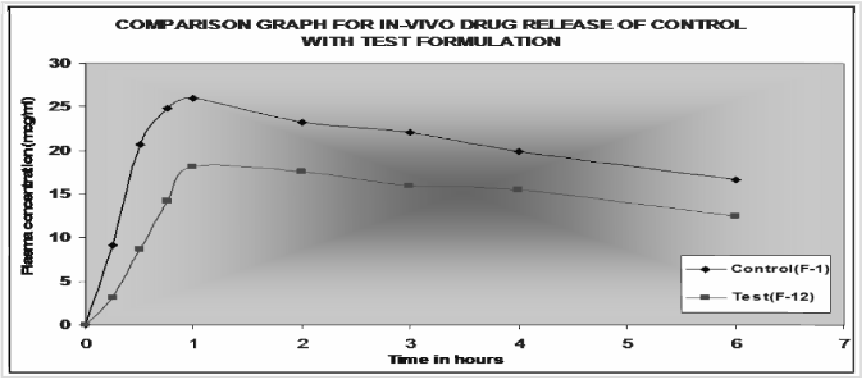
In vivo release study [11]***:***

Formulation F- XII (test) and F-1 (control) were subjected to *in vivo* release studies using rabbit as animal model. Six male rabbits weighing 1.5 kg and 12 months old were selected for the study. They were divided into two groups of 3 in each and the study was conducted as single dose randomized parallel design. The animals were housed individually under (23 ± 2 ^{o}C, 55 ± 5 % RH, 12 hours light/dark cycle) environmental conditions. The rabbits were fasted overnight and allowed free access to tap water only.

The test formulation F-XII and control formulation F-1 were administered to the rabbits by gastric intubation method [12] after calculating the animal dose[70]. 1 ml of blood samples were withdrawn from the marginal ear vein of rabbit at 0.25, 0.50, 0.75, 1, 2, 3, 4 and 6 hrs. The plasma samples were separated by centrifugation and the drug was extracted. Then the samples were assayed by high performance liquid chromatography.

The results were summarized in Table 7 & 8 and shown in (Fig 12).

Figure: 12



RESULTS AND DISCUSSION

The granules were well within the specific limit indicating good flowability. With this the granules were found to be free flowing material and showed suitability to be compressed as tablets of expected weight. The results were shown in (Table 2)

Thickness ranged from 5.18 – 5.22. Uniformity of weight was observed to be within the I.P. limits. Hardness was observed to be within the limit in the range of 3.5 – 4.0 except for control formulation the hardness was found to be 4.5 kg/cm^2. Friability was observed between percent 0.30 – 0.96 % w/w hence within the limit of > 1%. The results of drug content for all formulations were found to be between 95 % – 101.0 % hence within the IP limit of 85.0 % - 115.0 %.

Disintegration time was found to be between 10 -90 seconds. The recommended limit for fast dissolving tablets is that it should disintegrate within 3 minutes. Therefore, all formulations are within this limit and pass the test. The disintegration time (D.T) is higher for control (90 secs) and for F-XII it is only 10 seconds at (5%) and hence this was considered to be good compared to other formulations. The results were shown in (Figure 2).

In vitro dissolution test reveals the release increases from 73% to a maximum of almost 97%. The release is in the following order of superdisintegrants: Crospovidone > Pregelatinized starch > Croscarmellose > Sodium Starch Glycolate. The maximum *in vitro* dissolution was found to be with formulation F-XII. The control formulation has the least *in vitro* dissolution (73.85 %) and the formulation F-XII was found to contain maximum in vitro dissolution of 96.97%. It clearly shows due to the superdisintegrant – (crospovidone at 5%) and it seems to be the best. The reason is its highly porous structure and water wicking mechanism into porous network of tablet and hence increases in concentration of crospovidone accounts for rapid drug release.

The IR spectrum of fluoxetine shows us that there is no interaction between the drug and the excipient (Figure 8). The DSC curves observed in the case of fluoxetine shows a single sharp exothermic effect corresponding to the melting of drug was observed. **T_{peak} = 163.5º C** and ∆Ht = 117.1 J/g. The DSC record of the formulation F-XII corresponds to a single exothermic peak **T_{peak} = 162.7º C** and ∆Ht = 106.4 J/g and a broad exothermic peak T_{peak} = 76.5º C and ∆Ht = 132 J/g due to the excipients. And the DSC thermogram shows no change in the exotherm of the pure drug of fluoxetine. From this, it was inferred that there is no interaction between the drug and excipients.

The release kinetic analysis was studied for formulation F-XII for both first order and zero order kinetics. The correlation coefficient was determined and found to be -0.4214 for first order kinetics and 0.8630 for zero order kinetics. From the above data it was inferred that the dissolution profile of formulation F-XII follows zero order kinetics.

Following are the results obtained from the *in vivo* studies of both F-XII and control formulation F-I. For formulation F-XII, the C_{max} was found to be 26.01µg/ml and the t_{max} is 60 mins. The AUC $_{(0-\alpha)}$ was found to be 312 µg-hr/ml. For control formulation F-I the C_{max} was found to be 17.8µg/ml and the t_{max} is 60 mins. The AUC $_{(0-\alpha)}$ was found to be 199 µg-hr/ml. The control shows a difference of percentage with that of formulation F-XII. The In-vivo graph shows the increase in plasma drug concentration of test (F-XII) formulation when compared to the control (F-I) formulation.

Short term accelerated stability studies were conducted for all the formulations and results observed reveals that there was no significant difference in the evaluated parameters namely thickness, hardness, disintegration time, percentage drug content and percentage drug release. This inference shows that the formulations should be stable.

CONCLUSION

The results have shown that Crospovidone 5% as a superdisintegrant (F-XII) shows fastest disintegration (10 secs) and maximum drug release (96%) within 20 minutes, when compared with other formulations. This was further ascertained by the *in vivo* studies in rabbit models where formulation F-XII has shown a marked increase in drug release profile when compared to that of control and other formulations. To conclude, crospovidone at a concentration of 5% w/w is suitable for preparing fast dissolving tablet of fluoxetine.

ACKNOWLEDGEMENT

I sincerely thank Mrs. K. Surya Prabha, Asst Professor, Hindhu College of Pharmacy, Guntur, Andhra Pradesh and Mrs. Grace Rathnam, Director, C.L. Baid Metha College of Pharmacy, Chennai for providing the facilities to carry out this work successfully.

REFERENCES

1. Panagrahi, D., Baghel, S., Mishra, B., Mouth dissolving tablets – An overview of preparation techniques, evaluation and patented technologies. *J Pharm Res.* 2005, *4*, 33-38.
2. Gilbert, S., Banker., Rhodes, C.T., *Modern pharmaceutics*, Marcel Dekker Inc, New York 1996, pp. 372-379.
3. Goodman., Gilman., *The Pharmaceutical Basis of Therapeutics*, McGraw Hill, New York 2001, pp. 451-469.
4. United States Pharmacopoeia, Official monographs Twinbrook Parkway 2000, pp. 738 – 740, 2051, 2270.
5. Lachman, L., Liberman, H. A., Kanig J. L. The Theory and Practice of Industrial Pharmacy, Varghese Publishing House , Bombay 1987.
6. Thorsteinn Loftsson., Dagny Hreinsdottir., Determination of Aqueous Solubility by Heating and Equilibration: A Technical Note. *AAPS Pharm Sci Tech* 2006, *7(1)*.
7. Richard Byed, A., Janet Markham, K., Developmental Toxicology Studies of Fluoxetine Hydrochloride Administered Orally to Rats and Rabbits. *Toxicol Appl Pharmacol.* 1993.
8. Silverstein, R.M., Webster, F.X., *Spectrometric Identification of Organic Compounds*, 1998.
9. Yee Lily., Wong Steven, H.Y., Skrinska Victor, A., Chiral high-performance liquid chromatographic analysis of fluoxetine and norfluoxetine in rabbi plasma, urine, and vitreous humor using an acetylated β-cyclodextrin column. *J Anal Toxicol.* 2000, *24*, 651-655
10. Peterson, R.S., Risley, D.S., Anderson, P.N., Hostettler, K.F., Stability of fluoxetine hydrochloride in fluoxetine solution diluted with common pharmaceutical diluents. *Amer J Hosp Pharm* 2005, *51*, 1342-1345.
11. Madan, P. L., *Biopharmaceutics and Pharmacokinetics*, New Delhi 2000, pp. 27-29.
12. Ghosh, M. N., .*Fundamentals of Experimental Pharmacology*, Scientific Book Agency, Calcutta 1984, pp. 192-193