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Research Article

Design and Evaluation of Delayed and Controlled Release Formulation of Leukotriene Inhibitor Zileuton

SHETE AMOL[1], PATIL VISHAL[2], YADAV ADHIKRAO[3], SAKHARE SFURTI[3], SHIRKE SUPRIYA[4]

[1] Research Group Department of Pharmaceutics and Quality Assurance, Shree Santkrupa College of Pharmacy, Ghogaon, Karad-415111, India.

[2] Department of Formulation and development Sun Pharma advanced research centre, vadodara. Gujarat. India

[3] Gourishankar Institute of Pharmaceutical Education and Research, Limb, Satara- 415002, India.

[4] Department of pharmaceutics, Satara College of pharmacy, Satara. India.

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ABSTRACT

The objectives of present investigation were to design and evaluate stable, delayed Controlled release formulation of zileuton, which may provide peak plasma concentration early morning and compare with marketed formulation available in USA under the brand name of "Zyflo® CR" .The matrix coated and bialyer tablets, were prepared by wet granulation technology and some critical processing parameters were studied like in granulation time and extent of granulation. Loss on drying of the granules, coating pan speed, inlet temperature, bed temperature, Distance between spray gun and product bed. The prepared tablets were evaluated for official evaluation parameters and effect of speed of agitation and Ph of dissolution media were studied. ZIB/03 showed the same dissolution profile that of the innovator at pH 6.8 and 7.2 tris buffer but, compare to innovator at pH 1.2 and 4.5 acetate buffer it showed dissolution dissimilarity. One month accelerated stability study of the batch no. ZIB/03 showed a slightly faster release than the initial. Developing delayed and controlled release formulation of zileuton might provide peak plasma concentration in the early morning. So it could be said that a superior formulation than currently marketed formulation (Zyflo CR) was achieved.

INTRODUCTION

Chronopharmaceutics is a branch of pharmaceutics devoted to the design and evaluation of drug delivery systems that release a bioactive agent at a rhythm that ideally matches the biological requirement of a given disease therapy. Ideally, chronopharmaceutical drug delivery systems (ChrDDS) should embody time-controlled release mechanism. Ideal Chr DDS should: be non-toxic within approved limits of use, have a real-time and specific triggering biomarker for a given disease state, have a feedback control system (e.g. self-regulated and adaptative capability to circadian rhythm and individual patient to differentiate between awake–sleep status), biocompatible and biodegradable, especially for parenteral administration, easy to manufacture at economic cost, and easy to administer in to patients in order to enhance compliance to dosage regimen.

Author for Correspondence:
Email: amol.shete@rediffmail.com

Some of the early chronotherapies simply involved either the unequal morning and evening dosing of conventional 12-hrs, sustained-release capsule and tablet preparations or the once-daily morning or evening dosing of ultra-slow-release drug-delivery systems. Such simple chronotherapeutic approaches were, and are still, used today in many countries to improve the treatment of allergic rhinitis, bronchial asthma, peptic ulcer, rheumatoid arthritis, osteoarthritis, and hypertension [1-6].Leukotriene has significant role in asthma. It is one of the mediator of asthma. Zileuton (N-(1-benzo[b]thien-2-ylethyl)-N- hydroxyurea) is an orally active inhibitor of 5- lipoxygenase, the enzyme that catalyzes the formation of leukotrienes from arachidonic acid. It is claimed to be a potent anti-inflammatory and anti-asthmatic agent. Thus inhibits leukotriens (LTB4, LTC4, and LTD4) formation so the compound inhibits leukotriens – dependent brochospasm. Zileuton is indicated for the prophylaxis and chronic treatment of asthma in adults and

children of 12 years of age and older. Zileuton is not indicated for use in the reversal of bronchospasm in acute asthma attacks. Therapy with zileuton can be continued during acute exacerbations of asthma. Zileuton is indicated in patients with chronic asthma to improve asthma symptoms, improve pulmonary function (forced expiratory volume in 1 second [FEV 1], morning and evening peak expiratory flow rates [7-9].

Current formulation is available in market under the trade name of "Zyflo® (Zileuton) CR". Short elimination half life (3hrs) of drug, it is difficult to maintain peak plasma concentration at early morning after oral administration. So it may be difficult to treat the asthma at early morning. The objectives of present investigation were to design, development and evaluate delayed Controlled release formulation of Zileuton, which may provide peak plasma concentration early morning and compare with marketed formulation available in USA under the brand name of "Zyflo® CR", obtain delayed release formulation which is pharmaceutically equivalent product with Reference ("Zyflo® CR tablet") by i*n vitro* methods, develop simple, robust dosage form, investigate and optimize various factors affecting the in vitro performance of the designed formulation, carry out the Drug-excipient Compatibility studies and stability studies of developed product as per guidelines.

MATERIALS AND METHODS

Materials

Zileuton, Hyperomellose USP NF (HPMCK 100, HPMCK 100 M, LVCR), Polyplasdone XL10, KOLLIDONE K 30, purchase from Changzhou Huaren Chem. Co. Ltd., Colorcorn and BASF respectively. All other chemicals used were of analytical reagent grade and used as received without any further purification.

Methods

Drug - Excipients Compatibility Study (Physical Observation)

Zileuton was mixed with different proportions with all excipients to be used in our formulation in different rations and kept at 40°C/75% Relative humidity (RH) conditions for one month. The physical properties (Colour change) were monitored regularly. The suspected change in colour in any mixture is discarded from study.

Strategy I: Development of Zileuton matrix coated tablet by wet granulation:

Zileuton was passed through sieve no 30# mixed with mannitol and granules were prepared by using PVP K 30 as a binder and water as a binder solvent. Granules were dried at at 65ºC till LOD comes to NMT2%, dry granules were sifted through sieve no. 18, HPMC K100M, MCC PH 102 were added extragranularly as a hydrophilic polymer and diluent respectively.

Magnesium Stearate was added as a lubricant in the usual manner, and tablets were compressed by 16 station compression machine (Cadmach Ahmadabad).Compressed tablets were coated by Eudragit RSPO and RLPO coating machine dissolved in isopropyl alcohol and acetone as a coating solution in different ratio (Table 1) by using Ganson setting all essential parameter of coating machine.

Strategy II: Development of Zileuton Bilayer tablet by wet granulation

Preparation of first layer

Zileuton was passed through sieve No. 30# mixed with mannitol and granules were prepared by using pregelatinized starch as a binder and water as a binder solvent. Granules were dried at at 65ºC till LOD comes to NMT2%, dry granules were sifted through sieve No. 18, and crosspovidone and MCC PH 102 were added extragranularly.

Preparation of second layer and coating

Zileuton was passed through sieve no 30# mixed with mannitol and granules were prepared by using PVP K 30 as a binder and water as a binder solvent. Granules were dried at at 65ºC till LOD comes to NMT2%, dry granules were sifted through sieve no. 18, HPMC K100M, MCC PH 102 were added extragranularly as a hydrophilic polymer and diluent respectively. Magnesium Stearate was added as a lubricant in the usual manner, and tablets were compressed by 16 station compression machine (Cadmach Ahmadabad). Compressed tablets were coated by Eudragit RSPO and RLPO (80:20) dissolved in isopropyl alcohol: acetone: DBS as a coating solution in different ratio (table 2) to gain a constant weight gain of 8% by using Ganson coating machine setting all essential parameter of coating machine (Table 3).

Evaluation of granules [10]

Prepared granules were evaluated for granular properties like angle of repose, bulkiness, porosity, void volume and percentage compressibility. Compressibility Index and Hausner Ratio are measures of the propensity of a powder to be compressed.

Table 1: Batches of Zileuton matrix coated tablet by wet granulation

Sr.No	Ingredients	Mg/Tablets					
	Core tablet	**ZIM/01**	**ZIM/02**	**ZIM/03**			
1	Zeliuton	600	600	600			
2	Mannitol (Mannitol 160)	-	100	200			
3	Povidone (PVPK30)	55	55	55			
4	Hypromellose (K100 M)	200	200	100			
5	Microcrystalline cellulose(Avicel pH102)	80	80	80			
6	Mg. stearate	10	10	10			
	Total weight	**945**	**1045**	**1045**			
	Coating			**RLPO**	**RLPO:RSPO (50:50)**	**RLPO:RSPO (20:80)**	
		Coating not carried out	**Coating not carried out**	**6% coating**	**8% coating**	**6% coating**	**8% coating**
1	Eudragit RLPO	-	-	51.66	34.58	10.33	13.83
2	Eudragit RSPO	-	-	-	34.58	41.33	55.33
3	DBS	-	-	10.33	13.83	10.33	13.83
4	IPA	-	-	q.s.	q.s.	q.s.	q.s.
5	Acetone	-	-	q.s.	q.s.	q.s.	q.s
	Total weight	**-**	**-**	**1107**	**1128**	**1107**	**1128**

As such, they are measures of the relative importance of interparticulate interactions. In a free-flowing powder, such interactions are generally less significant, and the bulk and tapped densities will be closer in value. For poorer flowing materials, there are frequently greater interparticle interactions, and a greater difference between the bulk and tapped densities will be observed.

Table 2: Batches of zileuton matrix coated tablet by eudragit RSPO an RLPO (20:80) at an 8% constant weight gain

Sr. No.	STRATEGY I		
	Ingredient	**Mg/ tablet**	
	Core tablet	**ZIM/ 04**	**ZIM/ 05**
1	Zileuton	600	600
2	Mannitol (160)	200	150
3	Povidone (PVP K 30)	55	55
4	Hypermellose (K100 M)	150	150
5	Microcrystalline cellulose (Avicel Ph 102)	80	80
6	Magnesium Stereate	10	10
Total weight		1095	1045

Table 3: Essential parameter of coating machine

Sr.No	Parmaeter	Range
1	Pump RPM	5-7
2	Atomization	1.8 – 2.1
3	Pan width	0.9
4	Inlet lower capacity	35 -39 %
5	Outlet	35 – 38 %
6	Pan RPM	22 – 26
7	Bed temperature	30
8	Inlet temperature	40

Evaluation of tablets

Prepared tablets were evaluated for official and unofficial test in pharmacopoeia.

In vitro dissolution study

The *in vitro* drug release of prepared tablets were measured in triplicate by using dissolution apparatus (Lab India, Model Disso 2000 Tablet dissolution test apparatus, Mumbai, India) using apparatus USP Type II. Dissolution studies were carried out by using 900mL water with 0.05M SLS because Zileuton has more solubility in this medium compared with other medium such as 0.1N HCl, 4.5 Acetate buffer and 6.8 Phosphate

buffer, 7.2 Phosphate buffer with 0.05M SLS at 50, 75 rpm and 37.0° 0.5°C. Samples were withdrawn after suitable time intervals and replaced each time with 5 mL dissolution medium. The solutions were immediately filtered through 0.45 mm membrane filter, diluted and the concentration of zileuton determined spectrophotometrically at 260 nm. Effect of stirring rate and ph of dissolution medium on in vitro drug release was studied. In vitro dissolution profiles of prepared tablets were compared with marketed formulation and similarity-dissimilarity factor were calculated.

Stability Studies [11]

In order to assess the stability of drug product, accelerated stability studies were conducted for the batches of Zileuton Tablets. The batches were kept for stability in 60cc wide mouth HDPE container containing 1g silica gel canisters and Pharma grade polyester cotton closed with CRC closure with HS123 printed liner at the condition of 40°C ±2 °C/75% RH ±5% for the period of 1, 3, months, tablets were evaluated for appearance, drug content, water content and in vitro dissolution study.

RESULTS AND DISCUSSION

Pre-formulation Study

From the detailed Pre-formulation study it is concluded that, Moisture uptake study was carried out at 75% RH for 48 hrs and water content was determined by using KF titration. From the study it was observed that drug does not absorb significant moisture at 75% RH, so it was concluded that drug is not moisture sensitive. pH dependent solubility of Zileuton was carried out, from that study it was observed that Zileuton is insoluble at pH 1.2 (0.1N HCL), Water, pH 7.2 and pH 6.8 ,pH 4.5. Solution stability study of Zileuton in different media was carried out. It was observed that Zileuton is stable in 0.1N HCl, 4.5 acetate buffer, 6.8 phosphate buffer, 7.2 buffer & Water with 0.05M SLS for 24 hrs.

Drug-Excipients Compatibility study

From the physical observation it was found that Zileuton does not have significant colour change with specified excipients.

Physical Parameters evaluation of Granules, tablet and coating

The physical methods involved characterizing granules for their flow property, compressibility and weight variation. Since the Carr's index (%CI) indirectly measures the flowability of lubricated granules, the %CI values of all batches were determined and it was found that all batches showed passable CI value. All the batches passed the test for hausner ratio (Table 4). Hardness was optimized between 150 N to 180N range (Table 5). Hardness below 150N showed breaking of edges during coating, whereas Hardness above 180N showed collaring effect and layer separation in case of bi-layer compression. All the batches showed more than 95% w/w of the drug content as shown in Table 6. Tablets were coated with Eudragit polymer with different weight gain and different coating parameters were evaluated. Among this parameter gun distance, spray RPM, Pan RPM, inlet temperature and bed temperature plays crucial role during coating. It was found that bed temperature above 40°C causes sticking of tablets and non uniformity during film formation. Also if gun distance is kept above the optimized distance, spray drying of the polymer and non uniformity during film formation was observed. Curing of coated tablet played significant effect on dissolution study. If dissolution was performed without curing for sufficient time (30 min.) tablets showed 90% release within 3 hrs.The same coated tablet with same coating showed slow dissolution profile ,after curing (2 hrs).

In vitro Dissolution study

Zileuton matrix coated tablets by wet granulation

Batch ZIM/01 dissolution of uncoated tablet was performed in a selected dissolution condition. It was observed that only 52.5% was released after 12 hrs.By comparison with innovator dissolution profile, the release was retarded to greater extent. This may be due to high concentration of hypromellose K 100M or absence of water-soluble diluent such as mannitol due to which Zileuton (low water soluble drug) did not diffuse out quickly from the swelled matrix [12]. Batch ZIM/02 was prepared by using HPMC 20 % based matrix with the addition of 9 to 10 % mannitol. The In-Vitro release study showed that there was still retardation in dissolution profile. This might be due to high concentration of hypromellose K 100M.

Batch ZIM/03 decreased concentration of HPMC K100M to 10 % and increased concentration of mannitol to 20%. In vitro release profile showed that there was increased drug released upto 98 % in 12 hrs.

Table 4: Micromeritic properties of prepared granules

Batch	Bulk density (BD)	Tapped density	Compressibility index (%)	Hausners ratio
ZIM/01	0.54	0.69	21.73	1.27
ZIM/02	0.56	0.71	21.1	1.26
ZIM/03	0.51	0.67	23.8	1.31
ZIM/04	0.49	0.65	24.6	1.32
ZIM/05	0.52	0.69	24.6	1.32
ZIB/01	0.48	0.62	22.5	1.29
ZIB/02	0.51	0.67	23.8	1.31
ZIB/03	0.49	0.65	24.6	1.32

Table 5: Evaluation parameters for prepared tablets

Batch	Hardness(N)	Friability(%)	Thickness(mm)	Weight variation (mg)
ZIM/01	150+/- 10N	0.57	7.24+/- 0.1	945+/-5.1
ZIM/02	150+/- 10N	0.42	7.25+/- 0.1	1045+/-5.1
ZIM/03	150+/- 10N	0.67	7.24+/- 0.1	1095+/-5.1
ZIM/04	150+/- 10N	0.65	7.25+/- 0.1	1045+/-5.1
ZIM/05	150+/- 10N	0.52	7.23+/- 0.1	1045+/-5.1
ZIB/01	140+/- 10N	0.45	6.39+/- 0.1	901+/-5.1
ZIB/02	140+/- 10N	0.34	6.40+/- 0.1	912+/-5.1
ZIB/03	140+/- 10N	0.52	6.37+/- 0.1	881+/-5.1

Table 6: % w/w Drug content of formulation

Sr. No	Batch	% W/W
1	ZIM/01	96.22
2	ZIM/02	95.01
3	ZIM/03	99.03
4	ZIM/04	98.04
5	ZIM/05	97.75
6	ZIB/01	97.84
7	ZIB/02	97.51
8	ZIB/03	98.21

To obtain a delay of 3 hrs, ZIM/03 was coated in different lot with different ratio and different weight gain of Eudragit RLPO and RSPO as shown in Figure 1. First lot (ZIM/03A) was coated with Eudragit RLPO only up to 6% wt. gain, and the in vitro release profile did not show the delay for 3 hrs. Second lot (ZIM/03B) was coated with Eudragit RLPO: RSPO (50:50) upto 6% wt.gain,and the in vitro release profile showed delay of upto 1 hr. Third lot (ZIM/03C) was coated with eudragit RSPO: RLPO (80:20) upto 6% wt.gain in vitro release profile showed the delay of 2 hrs. Fourth lot (ZIM/03C) was coated with eudragit RSPO: RLPO (80:20) upto 8% wt.gain in vitro release profile showed the delay of 3 hrs. But inspite of the delay the dissolution profile was faster than that of innovator. This may be attributed to the lower concentration of HPMC K100M in the matrix.

Batch ZIM/04 increased concentration of HPMC K100M to 15% and the dissolution of uncoated tablet was performed in a selected dissolution condition. It was observed that only 70% released after 12 hrs. By comparing with dissolution profile of the innovator it was observed that the release was retarded, to a longer extent. Batch ZIM/05 same concentration of HPMC K100M (15%) and mannitol 15%, Dissolution of uncoated tablets was performed in a selected dissolution condition. It was observed that only 70% released after 12 hrs. As innovator exhibited 60% drug release after 4 hrs, however any batch from strategy I did not give desired drug release profile to that of innovator. So next strategy was designed with bilayer formulation, out of which one layer is IR and another layer being CR. This strategy was planned because after delay there should be burst release, which was achieved with IR portion.

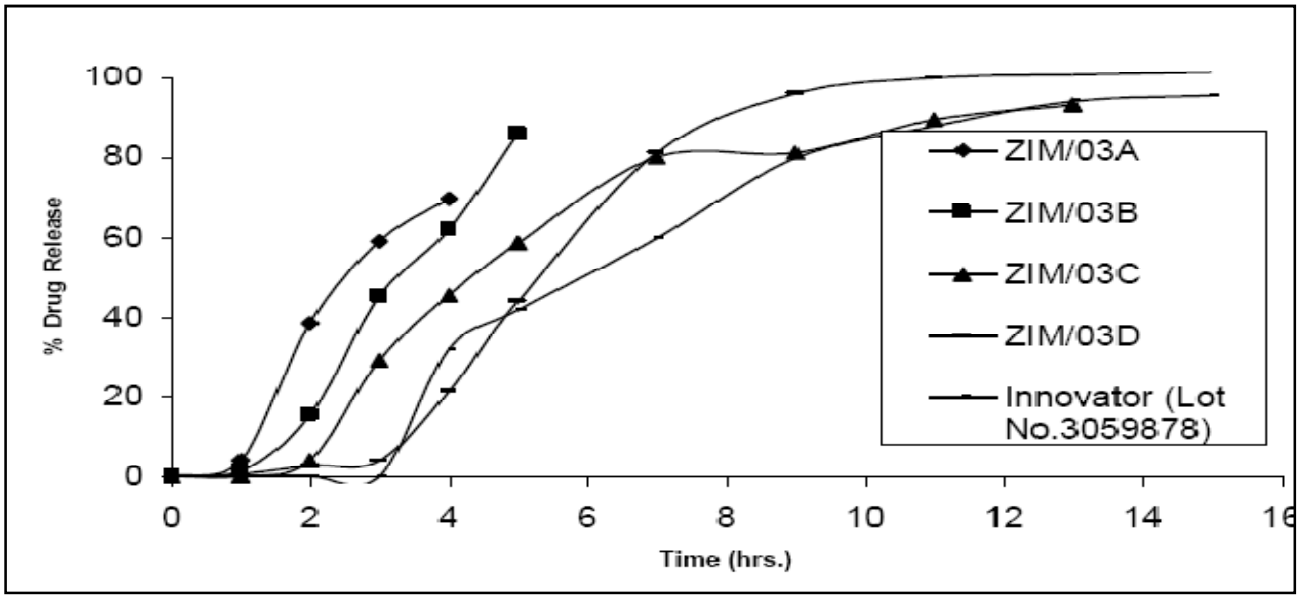

Figure 1: Effect of ratio and different weight gain of Eudragit RLPO and RSPO on in vitro drug release.

Zileuton Bilayer tablet by wet granulation

In vitro drug release profile of all batches of bilayer tablets is shown in Figure 2.Batch ZIB/01 the dissolution of uncoated tablet was performed in a selected dissolution condition. In vitro dissolution profile showed, slow dissolution profile compared to that of innovator. This batch was coated with eudragit RSPO: RLPO (80:20) with 8% weight gain. Results showed that 3 hrs delays were obtained (as shown in Fig 3) but dissolution profile is slower than that of innovator. This might be due high viscosity of HPMCK100M[13]; and drug might be entrapped in a matrix.

Batch ZIB/02 dose of IR portion was increased and also there was use of only HPMC K100 (12%) LVCR. Dissolution of uncoated tablet was performed in a selected dissolution condition. In vitro dissolution showed that f2 value of ZIB/02 as compared to the innovator is 70. Batch was coated with eudragit RSPO: RLPO (80:20) with 8% weight gain.

Batch ZIB/02A there was burst release (after 1 hr lag) with eudragit RSPO: RLPO coating (80:20) of 8 % weight gain. This might be due to the lesser curing of tablet after coating. Same coated tablet after curing for 2 hrs. showed slower dissolution profile. In vitro dissolution showed that f2 value between innovator and ZIB/02B is 43. This might be due to the slightly high concentration of HPMC K100LVCR. Batch ZIB/03 there was decreased concentration of HPMC K100 LVCR (8%). Dissolution of uncoated tablet was performed in a selected dissolution condition. In vitro dissolution showed that f2 value between innovator and ZIB/03 is 59. It showed slightly faster dissolution Batch was coated with eudragit RSPO: RLPO (80:20) with 8% weight gain.

Batch ZIB/03A coated with eudragit RSPO: RLPO (80:20) with 8% weight gain. In vitro dissolution showed that there were three hrs delays obtained with f2 value 65.

Effect of Dissolution variables on *In-Vitro* Drug release:

Effect of speed of agitation on drug release

The effect of speed of agitation on drug release was studied with different rpm like 50 and 75. It was observed that, in the case of innovator stirred at 50 rpm, that a 84.5% drug was released, whereas at 75 rpm 100 % drug was released. Thus it was concluded that faster release profile was seen with increasing of paddle rpm. Final batch ZIB/03 was studied at 50 and 75-rpm .At both the rpm lag periods of three hours was obtained but release profile at 50 rpm was slow.

Effect of pH on drug release:

The multimedia study was done for final batch ZIB/03 in different pH media like pH 1.2, 6.8, 7.2 Tris buffer and 4.5 acetate buffer. Study showed that at all pH, delay of 3 hrs was obtained. Dissolution profile in all the media was not significantly different, which showed that their pH independent release profile. ZIB/03 showed the same dissolution profile that of the innovator at pH 6.8 and 7.2 tris buffer but, compare to innovator at pH 1.2 and 4.5 acetate buffer it showed dissolution dissimilarity.

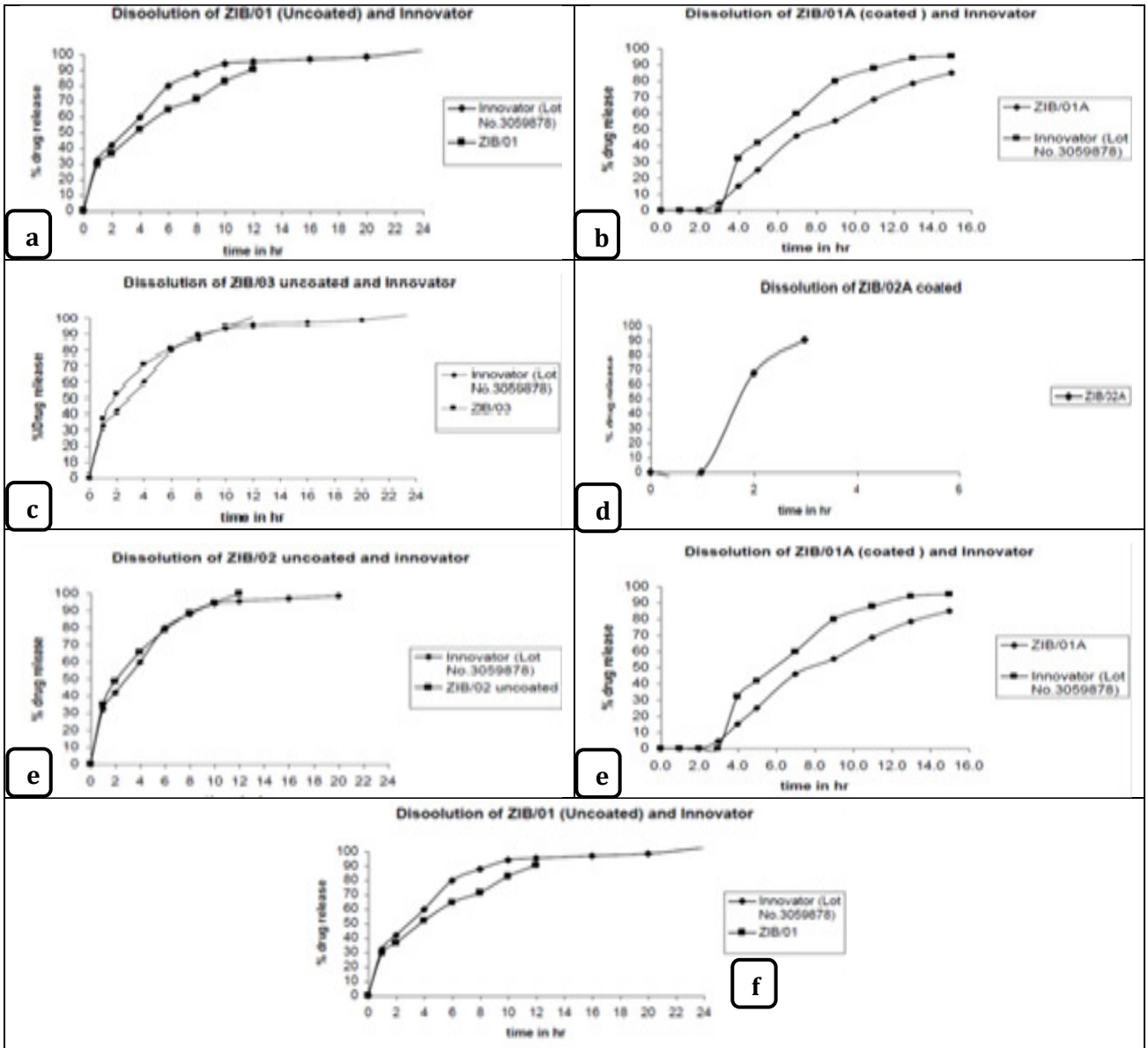

Figure 2: *In vitro* drug release profile of all batches of bilayer tablets of Zileuton

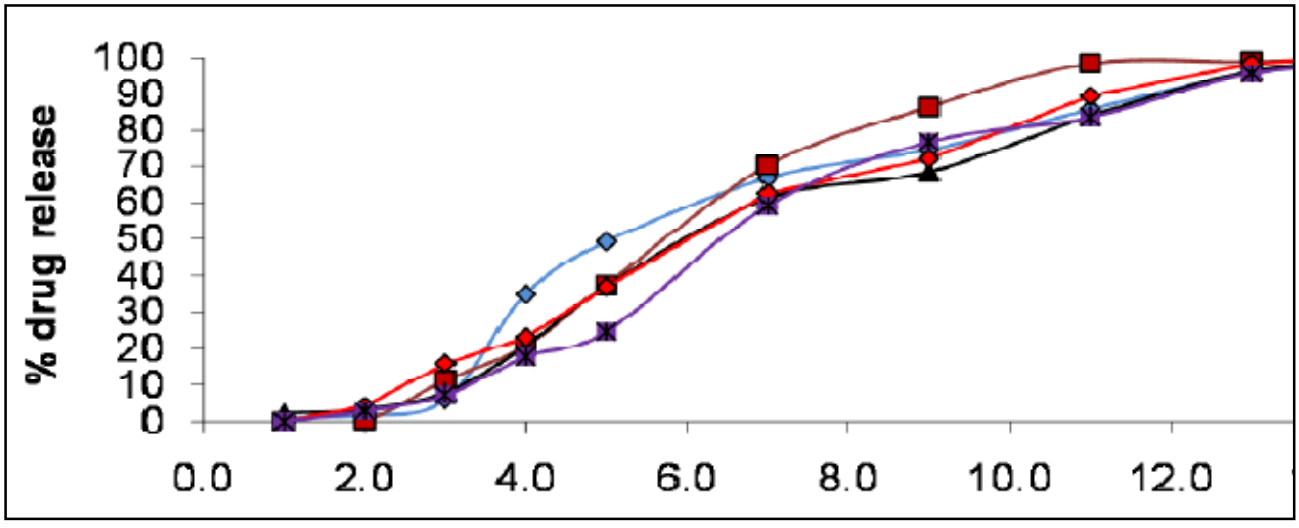

Figure 3: *In vitro* multimedia dissolution study

CONCLUSION

From above results it was concluded that a promising formulation has been developed for Zileuton Delayed and Controlled release Tablets, 600 mg. The physical parameters, analytical data and dissolution profile of Zileuton Delayed and Controlled release Tablets, 600mg was found to be satisfactory range. *In vitro* dissolution study with this composition was found to be reasonably good. Also dissolution profile in all the media is not significantly different which showed that their pH independent release profile. ZIB/03 showed the same dissolution profile that of the innovator at pH 6.8 and 7.2 tris buffer but, compare to innovator at pH 1.2 and 4.5 acetate buffer it showed dissolution dissimilarity.

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