FORMULATION AND EVALUATION OF RITONAVIR TABLETS BY USING HOT MELT EXTRUSION METHOD

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Abstract:

In the pre-formulation studies, solubility, drug-excipient compatibility and flow properties of API were studied. All formulations were prepared by wet granulation method by using microcrystalline cellulose PH102, Croscarmellose sodium, povidonek30, sodium lauryl sulfate, colloidal silicon dioxidePH200, and magnesium stearate. Dry granulation batch was taken and results were compared with wet granulated batch of same composition. Film coating was done by using Opadry white YS-1-7059 to protect ritonavir from moisture. The tablets prepared were found to be within the official limits with respect to weight variation, thickness, hardness, friability, disintegration and dissolution. The stability study was performed for F6 formulation as per ICH guidelines. Stability study was carried out for 3 months at 40°C/75%RH. The tablets were tested for release and results were found within the limits. Among the all formulations the release profile of trial F6 was found to be similar to the marketed product release profile.

Introduction

ORAL DRUG DELIVERY

Oral drug delivery is the most widely utilized route of administration among all the routes that have been explored for systemic delivery of drugs via pharmaceutical products of different dosage forms. Oral route is considered as most natural, uncomplicated, convenient and safe due to ease of administration, patient acceptability and cost-effective manufacturing process.

TABLETS

Tablets are defined as solid pharmaceutical dosage forms containing drug substances with or without suitable diluents and prepared either by compression or molding methods. Tablets remain popular as a dosage form because of the advantages, afforded both to the manufacturer (simplicity & economy of preparation, stability and convenience in packing, shipping and dispensing). Tablets are in different shapes like round, oval, oblong, cylindrical or triangular. They may differ greatly in size and weight depending on the amount of drug substance present and intended method of administration.1

Properties of tablets

- It should have sufficient strength and resistance to shock and abrasion to with stand handling, manufacturing, packing, shipping and use.
- Tablets must be uniform in weight and in drug content of individual tablet.
- Tablets must be elegant in appearance and must have characteristic shape, colour and other markings necessary to identify the product.
- Tablets must retain all these functional attributes, which include drug stability and efficacy.

Advantages of tablets

- High level of patient acceptability
- They lend themselves to certain special release profile products,

such as enteric or delayed release products.

- These are best suited to large scale production than other unit oral forms.

Disadvantages of tablets

- Drugs with poor wetting, slow dissolution properties, optimum absorption high in GIT or combination of these features may be difficult or impossible to formulate.
- Slow onset of action compared to parentals, liquid orals and capsules.
- It is difficult to convert a high dose poorly compressible API in to a tablet of suitable size for human use.

INTRODUCTION TO RITONAVIR

Ritonavir (RTV) is an antiretroviral drug and used in advance Human Immunodeficiency Virus (HIV). It is used as viral Protease Inhibitor (PI) as well as a booster for other PI drugs. It was first developed by AbbVie ltd in 1996, and is currently marketed as brand Norvir in white tablet form. The British HIV association (BHIVA) guideline recommends the drug as a second line treatment for HIV, in combination with other antiretroviral drugs called Nucleoside and Nucleotide Reverse Transcriptase (NRTI). Development of viral resistance to the drug has been reported in clinical practice. Experiments have been carried out to improve the drug's efficacy.

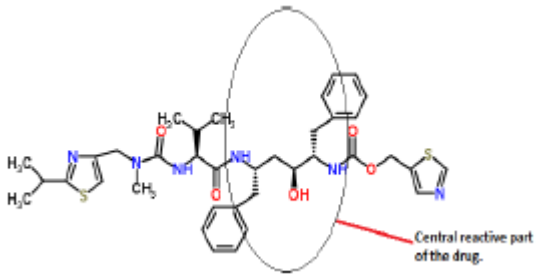
HIV life cycle:

The Human Immunodeficiency Virus (HIV) is a retrovirus, which contains a ribonucleic acid (RNA) as genetic material. It incorporates the RNA into the human DNA during its life cycle. The life cycle completes in three stages. It begins with the virus attaching and entering into the immune cell called CD4 cell. Second stage starts after entering in the CD4 cell. The virus gets uncoated and releases the genetic material into cytosol. The HIV reverse transcriptase (RT) produces viral DNA, which are transported to nucleus of the infected cell. The viral DNA is integrated to host genome by Integrase enzymes. In the third phase, the mRNA synthesises the complex of viral functional introns. They are transported out of the nucleus. Viral polypeptides called Gag and Pol are synthesized in cytosol from the introns. The viral mRNA also synthesises another polypeptide called Env in endoplasmic reticulum (ER). The Gag and Pol assemble in the cytosol and constitute viral core and reverse transcriptase of mature virus respectively. The core contains the antigenic parts such as protease (PR), integrase, RT and RNA. The Env makes up envelop of the virus. The Gag and Pol are cleaved by proteolysis process before conversion into mature functional viruses from the infected cell.

Ritonavir as a protease inhibitor:

RTV inhibits the proteolysis process. The inhibitory function of the drug is based on peptidomimicking and C2 symmetric characters. It mimics the peptide bond of the viral polypeptide and competes as a substrate forming the tetrahedral intermediate with viral protease. The key structure of the drug that mimics dipeptide bond is isosteric to the viral peptide. The isosteric structures have the same arrangement of electrons in the similar space. The similarity in the electron arrangement enables the nucleophilic

attack on the hydroxyl carbon of the drug by water in protease. Hence, the drug molecule competes with viral polypeptide in binding the viral protease.



Showing the highlighted part of the RTV that has peptidomimetic charcter

LITERATUREREVIEW

Ashish Chandwani1 and Jonathan Shuter2 et, al., Ritonavir is the first and only formulated HIV-1 protease inhibitor (PI). Large clinical trials have demonstrated ritonavir's clinical efficacy in both antiretroviral-naïve and -experienced patients. The immunologic, virologic benefits of treatment with this agent have proven in HIV-infected adults, adolescents, and children. Smaller studies use of ritonavir monotherapy as a therapeutic option in certain patients. The drug is characterized by a high genetic barrier to resistance, appears to more forgiving of non-adherence than earlier, unbooted PIs. The most frequent side effects observed are diarrhea, nausea, and vomiting. This gastrointestinal adverse effectis generally mild to moderate. Metabolic derangements,hyperlipidemia and glucose intolerance, have also observed inritonavir recipients. Antiretroviralagents continue to expand,ritonavir remains a proven and effective drug for the treatment of HIV infection.

AnjaiahSrirangam, PhD, Monica Milani, PhD, Ranjana Mitra, PhD, Zhijun Guo, PhD, Mariangellys Rodriguez, BS, Hitesh Kathuria, PhD, Seiji Fukuda, MD PhD, Anthony Rizzardi, BS, Stephen Schmechel, MD PhD, David G. Skalnik, PhD, Louis M. Pelus, PhD, and David A. Potter, MD PhD et, al,. Ritonavir is therapeutic agent in lung cancer,its targets in lung adenocarcinoma are unknown, as are candidate biomarkers for its activity. RNAwas used to identify genes whose expression affects ritonavir sensitivity. Synergy between ritonavir, gemcitabine and cisplatin was tested by isobolo gram analysis.

C. L. Cooper R. P. G. van Heeswijk K. Gallicano D. W. Cameron The pharmacokinetics of protease inhibitors center around the microsomal enzyme cytochrome P-450 3A4. As a potent inhibitor of this enzyme, ritonavir can increase the bioavailability and half-life of co-administered protease inhibitors. Evidence suggests that increased exposure to protease inhibitors is clinically relevant. Antiretroviral treatment with low-dose ritonavirboosted lopinavir, indinavir, and saquinavir as durable virological activity and shows impressive immune reconstitution. Although tolerable in most cases, gastrointestinal side effects, hepatotoxicity, and blood lipid abnormalities remain relevant issues. Additional study will explain the advantages and disadvantages of twice-daily, low-dose ritonavirboosted regimens and determine whether once-daily regimens based on this principle will have a lasting role in clinical practice.

AIM AND OBJECTIVE

The main aim and objectiveof the present study is to improve the solubility and dissolution of the ritonavir tablets by hot melt extrusion technique

During this technology, the low soluble crystalline polymorphic form will convert to amorphous form where polymer will use to decrease the recrystallization of the amorphous ritonavir.

This should conclude by XRD methodology and different concentrations to be check to know about polymorphic form of ritonavir API.

To achieve the goal various prototype formulation trials will be taken and evaluated with respect to the various quality control such as dissolution. The formula will be finalized by comparing the invitro dissolution profile with that of the marketed tablets.

DRUG AND EXCIPIENT PROFILE

Chemical name	:	1,3-thiazol-5-ylmethyl ~{N}-[(2~{S},3~{S},5~{S})-3-hydroxy-5-[[(2~{S})-3-methyl-2-[[methyl-[(2-propan-2-yl-1,3-thiazol-4-yl)methyl]carbamoyl]amino]butanoyl]amino]-1,6-diphenylhexan-2-yl]carbamate
Synonyms	:	ABT 538, ABT-538, ABT538, Norvir, Ritonavi
Molecular formula	:	C37 H48N6O5S2
CAS registry number	:	5155213-67-5
Molecular weight	:	720.946 g/mol
Melting point	:	126-132℃
Description	:	White to light tan powder
Solubility	:	Practically insoluble in water, Freely soluble in methanol,ethanol and isopropanolol.
Category	:	Antiretroviral activity
BCS classification	:	Ritonavir is a BCS class IV compound (low solubility & low permeability)
pKa	:	13.68
Partition coefficient (log p)	:	3.9
Half life	:	6.74 h
Storage	:	Ritonavir stored in a cool (2-25℃) dry place, protected from light and in a well closed container.

Pharmacodynamic parameters: Studies of pharmacodynamic effects with respect to the proposed indication showed that ritonavir has been demonstrated to be approximately 500fold more specific for HIV. Minimal effects were observed on the cardiovascular system of conscious rats and anaesthetized dogs. In the isolated guinea pig ileum, no antagonist or agonist effect was found. However, the low exposure to ritonavir in these tests in comparison to human patients allows a very low extent of extrapolation.

3.3.1.1. MICROCRYSTALLINE CELLULOSE

Chemical name: Cellulose.

Functional Category: Tablet and capsule diluent, Adsorbent; Suspending agent; Tablet Disintegrant.

Description: Microcrystalline cellulose is a purified, partially depolymerized cellulose that occurs as a white, odourless, tasteless, crystalline powder composed of porous particles. It is available in different particle sizes and moisture grades that have different properties and applications.

Solubility: Slightly soluble in sodium hydroxide solution, practically insoluble in water, dilute acids, and most organic solvents.

Melting Point: Chars at 260-2700c.

Storage: Microcrystalline cellulose is a stable though hygroscopic material and stored in a well-closed container in a cool, dry place.

REF: Raymond C. Rowe, Paul J. Shesky and Marian E. Quinn; Handbook of Excipients, 6th Edition, London, UK: Pharmaceutical Press; 2009: 96-99.

MATERIAL USED IN THE DESIGN OF FORMULATION

LIST OF MATERIAL USED IN THE DESIGN OF FORMULATION

S.No	INGREDIENTS AND BRAND NAME	SUPPLIER/ MANUFACTURER	Category
1.	Rotinavir	Aurobindo Pharma Ltd	Active Pharmaceutical Ingredient
2.	Copovidone USNF (plasdone S- 630)	BASF	Binder
3.	Colloidal silicone dioxide USNF (Aerosil 200)	Evonik, India	Glidant/Lubricant
4	Dibasic calcium phosphate	Caliphamra-A	Diluent
5	Sodium stearyl fumarate	PRUV	Lubricant
6	Hypromellose USP	Methocel E5 LV	Polymer
7	Polyethylene glycol 400 USNF	Polyglycol	Platisizer
8	Polysorbate 80 USP	Tween 80	Solubilizer
9	Titanium dioxide USP	-	Opacifier
10	Talc USP	Luzenac pharma	Anti caking agent
11	Polyethylene glycol 6000 USNF	Macrogol 6000	Platisizer

EQUIPMENT'S USED IN THE STUDY

S.No	Process	Equipment	Make/Model
1.	Weighing	Electronic weighing balance	Essae-Teraoka Ltd/PG6000
		Electronic weighing balance	Shimadzu/Aux220
2.	Sifting	Sieve No-20 & 40	United Engineering Ltd
3.	Granulator	Rapid mixer granulator	Make-Sainath boilers
4.	Hot melt extrusion	Hot melt extrutor	
5.	Drier	Rapid fluid bed drier	Retsch Model-TG200
6.	Moisture Analyzer	Moisture Analyzer	Sartorius/MA150C
7.	Milling	Multimill	Anchor Mark/MML
8.	Blending	Pillar type bin blender	Tapasya Engineering Pvt Ltd/Lab model
9.	Compression	8 Station Tablet compression machine	Make: Kambert Machinery Model: KMP-8
	Punches	Tooling type-D tooling	Pacific Tools Pvt Limited
10.	Coating Machine	Conventional Coating Pan	Anchor Mark
11.	Packing	Blister Packing Machine	Make: Mechtek Model: EzeeBlist
12.	Disintegration	Disintegration test	Veego&VTD_DV – ED-2L
13.	Sealing	Induction sealer	Electronic device sigma J.R(CSP300)
14.	Hardness (Kp)	Tablet Hard ness tester	D.R schlevniger&8m
15.	Dissolution test apparatus type II	UV-Pharmaspec – 1700	DBK Instruments Ltd., Mumbai.

EXPERIMENTAL METHODOLOGY

PREFORMULATION STUDIES:

Preformulation studies were performed to investigate the physical and chemical properties of a drug substance alone and also when combined with other substances such as excipients. It was the first step in the rational development of dosage forms.

Scope: The use of preformulation parameters maximizes the chances in formulating an acceptable, safe, efficacious and stable product and at same time provides the basis for optimization of the drug product quality.

a. Purpose:

1. To finalize specifications of active pharmaceutical ingredient (API)

2. To study the compatibility between active and inactive ingredient

3. Characterization of reference product

b. Scope:

Preformulation parameter maximizes the chances in formulating an acceptable, safe, efficacious and stable product.

c. Physical characteristics:

Loss on drying:

This characterization is recommended in EP, BP and USP. Although the loss in weight, in the samples so tested, is principally due to water, a small amount of other volatile materials will also contribute to the weight loss. The moisture balance combines both the drying process and weight recording. A moisture analyzer balance is suitable where large numbers of samples are handled and where a continuous record of loss in weight with time is required.

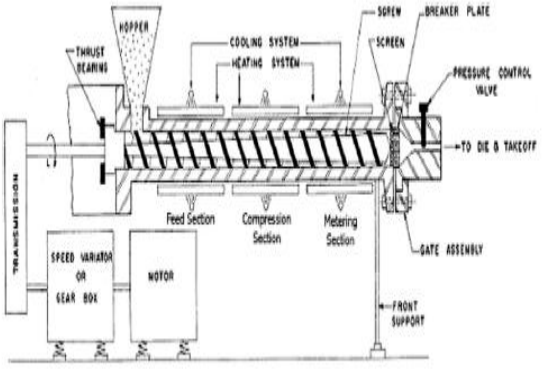
Procedure:

About 1gm of Venlafaxine HCl was placed in the plate of the digital moisture balance instrument. The temperature was set to 105oC and the instrument was run till a constant weight was obtained. Finally, the percentage loss on drying was read out automatically on the panel.

Single-Screw and Twin-Screw Extruder

A single-screw extruder consists of one rotating screw positioned inside a stationary barrel at the most fundamental level. In the more advanced twin-screw systems, extrusion of materials is performed by either a corotating or counter rotating screw configuration. Irrespective of type and complexity of the function and process, the extruder must be capable of rotating the screw at a selected predetermined speed while compensating for the torque and shear generated from both the material being extruded and the screws beingused. However, regardless of

the size and type of the screwinside the stationary barrel a typical extrusion set up consists of a motor which acts as a drive unit, an extrusion barrel, arotating screw, and an extrusion die. A central electronic control unit is connected to the extrusion unit in order to control the process parameters such as screw speed, temperature, and therefore pressure. This electronic control unit acts as a monitoring device as well. The typical length diameter ratios (L/D) of screws positioned inside the stationary barrel are another important characteristic to consider whether the extrusion equipment is a single-screw or twins crew extruder. The L/D of the screw either in a single-screwextruder or a twin-screw extruder typically ranges from 20 to 40: 1 (mm). In case of the application of pilot plant extruders the diameters of the screws significantly ranges from 18 to30 mm. In pharmaceutical scale up, the production machines are much larger with diameters typically exceeding 50– 60mm. In addition, the dimensions of a screw changeover the length of the barrel. In the most advanced processing equipment for extrusion, the screws could be separated byclamps or be extended in proportion to the length of the barrel itself. A basic single-screw extruder consists of three discrete zones: feed zone, compression, and a metering zone.



Schematic diagram of a single-screw extruder

FORMULATION OF RITONAVIR IMMEDIATE RELEASE TABLETS

Wet granulation

Sifting

1. Sift the ritonavir USP (micronized) through ASTM ≠ 20 mesh. 2. Sift copovidone through ASTM ≠ 40 mesh. 3. Heat the sorbitan monolaurate to 60-70° C or till it forms a clear transparent solution into a stainless steel container. 4. Ingredients of step 1 were loaded into 3 ltr Rapid Mixer Granulator (RMG) and spray sorbitan mono laurate of step 3 slowly on this material with impellar at fast speed and chopper at slow speed then continue the mixing for 2 minutes after the complete spraying of sorbitan monolaurate with impellar at fast speed and chopper at slow speed. 5. Unload the material of step 4 with impeller at slow speed and check the weight of the material. 6. Calculate and weigh the actual quantity of material office step 5 required for the required for the batch. 7. Load about 25 % w/w of sifted copovidone of step 2, material of step 6 and about 25% w/w of sifted copovidone of step 2 into Rapid mixer granulator and mix for 5 minutes with impeller at slow speed. 8. Add the remaining quantity of

sifted copovidone of step 2 to the material of step 7 and mix for 5 minutes with impeller at slow speed. 9. Unload the material of step 8 with impeller at slow speed. 10. Load the material of step 9 into a feeding Hopper of hot melt extruder and start the melt extrusion process. 11. Record the actual parameters observed 12. Collect and mill the extrudes of step 10 in Multi Mill through 0.5 mm screen at fast speed with knives forward direction and collect the past extrudes. 13. Weigh the milled extrudes of steps for 12 based on the yield of mild extrudes of step 12 calculate the quantities of the dibasic calcium phosphate, anhydrous dibasic calcium phosphate dehydrate, powdered cellulose, corn starch 400l mannitol, silicified microcrystalline cellulose (Prosolve SMCC® 90), silicified microcrystalline cellulose(Prosolv SMCC® 50), microcrystalline cellulose and sift together through 40 ASTM mesh 14. Calculate the required quantity of sodium stearyl fumarate based on the end of milled extrudes of step 12 where the quantity of sodium stearyl fumarate and sift through ASTM ≠ 60 mesh 15. Load the mild extrudes of step 12 and shifted material of a step 13 into a low shear blender and blend for 25 minutes 16. Add the sifted sodium lauryl stearyl fumarate of a step 14 to the material of step 15 and blend for 5 minutes and unload. 17. Compress the blend of step 16. 18. Prepare coating suspension by dispersing Aquarius prime 118010 white in specified amount of purified water to achieve 15% w/w solid content using suitable stirrer. Stir the suspension for about 45 minutes 19. Load the core tablets of step 17 in the coating pan and pre warm the tablets at an inlet air temperature of 50 ± 10°C with an intermittent inching of the coating pan. 20. Start the spray of coating suspension of step 18 after the product temperature reaches 35 ± 5°C, continue the coating till a coat weight build up of 2.08% w/w ± 0.50°C w/w per tablet was obtained.

Results and Discussion

The present study was undertaken to formulate ritonavir ER coated tablets.
The study involves preformulation studies of drug and excipients, formulation and processing development along with evaluation of tablets made with the optimized formulation. Finally film coated tablets were evaluated by *invitro* methods.

Drug Excipients physical compatibility studies

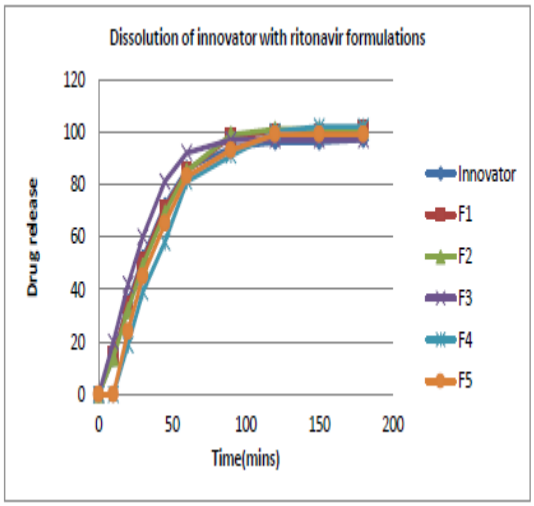
S.No	Ritonavir +excipients	Ratio	Initial Observation	Observation after 1month		
				40^0C/75%RH (open)	40^0C/75%RH (closed)	2 – 8^0C closed
1.	Ritonavir	N/A	White color	No change in color	No change in color	No change in color
2.	Ritonavir + MCC	1 : 1	White color	No change in color	No change in color	No change in color
3.	Ritonavir + Lactose monohydrate	1 : 1	White color	No change in color	No change in color	No change in color
4.	Ritonavir + HPMC	1 : 1	White color	No change in color	No change in color	No change in color
5.	Ritonavir + Ethyl Cellulose	1 : 1	White color	No change in color	No change in color	No change in color

Precompression Studies

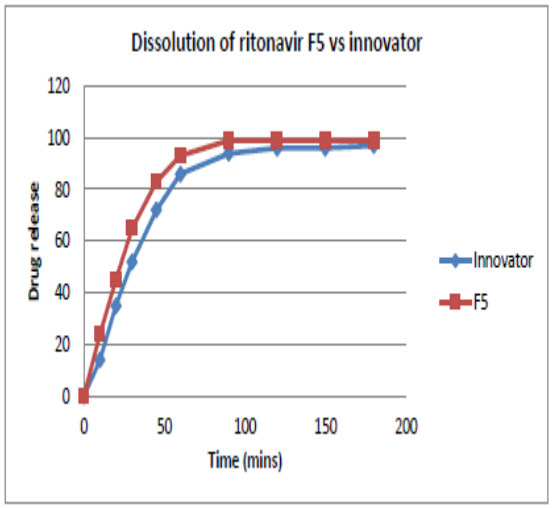
Bulk density, tapped density, compressibility index and hausner's ratio test was performed. The prepared blends of various formulations showed bulk density in the range of 0.30 to 0.51, which indicates the flow of blends are fair to good; tapped density in the range of 0.42 to 0.71 g/ml respectively, which indicates that there is good flow. The % compressibility index range was found to be 24 to 33%, which indicates the flow is poor and passable of the blends and Hausner's ratio in the range of 1.32 to 1.50, Hausner's ratio indicates the flow is poor.

Precompression data

S.No	Formula	Bulk density(g/ml)	Tapped density (g/ml)	Compressibility index (%)	Hausner's ratio
1	F1	0.595	0.735	19.05	1.235
2	F2	0.581	0.757	23.27	1.303
3	F3	0.581	0.735	20.93	1.265
4	F4	0.581	0.781	25.58	1.344
5	F5	0.555	0.735	24.44	1.323

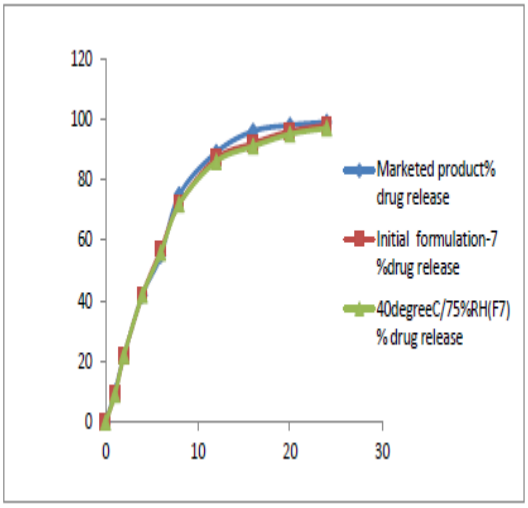


Comparison of Dissolution profile of ritonavir formulations Vs Innovator



Stability Data of formulation F7

Dissolution of trial F-7 tablets was comparable with marketed product. So tablets of this batch were kept for stability studies. The results of stability studies are shown in Table after 3 months the physical parameters of the tablets were same. Water content and related substance are within limits. The tablets were tested for Physical appearance, assay, relative substances, dissolution, moisture content at initial, 1st month, 2nd month and 3rd month in accelerated conditions (40+20C & 75+5%RH).



Comparison of Drug release of Reference, Formulation 7 and Stability loaded Formulation 7

CONCLUSION

Attempts were made in the present investigation to develop a pharmaceutically stable formulation of ritonavir immediate release tablets. Ritonavir were indicated for the treatment of antibacterial activity. In this study, ritonavir immediate release tablets were formulated by wet granulation method and moisture protective film coating was given. These results clearly reflect that the prepared formulation releasing the drug immediately within the specifications.

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