Pharmacokinetic evaluation of ritonavir crystallo co-agglomerates in rat module

Santosh V. Gandhi[1,*], Rasika R. Korhale[2], Swati U. Kolhe[3]

[1]Professor, [2]Research Student, Dept. of Quality Assurance, [3]Associate Professor, Dept. of Pharmacology, AISSMS College of Pharmacy, Maharashtra

*Corresponding Author:
Email: santoshvgandhi@rediffmail.com

Abstract

The present work was done to evaluate pharmacokinetic parameters of ritonavir standard, ritonavir crystallo co agglomerates and marketed formulation of ritonavir. The aim of formulation of Ritonavir crystallo co agglomerates was immediate drug release and solubility enhancement. The analysis was carried out by HPLC. The parameters evaluated were C_{max}, Tmax, area under curve (AUC $_{0-t}$, AUC $_{0-\infty}$), $t_{1/2}$, k elimination (k_e).

KEY WORDS: Ritonavir, Pharmacokinetic study, HPLC, Crystallo co agglomerates

Introduction

Retrovirus is the etiologic causative agent of acquired immunodeficiency syndrome (AIDS). Protease is an enzyme which is essential for the viral growth. These enzyme actions can be inhibited by the protease inhibitors, mainly in this class Indinavir, Ritonavir, Amprenavir, Nelfinavir, Atazanzvir, Saquinavir drugs are used in the treatment of HAART (Highly Active Anti-Retro viral Therapy)[1]. Ritonavir is characterised by low aqueous solubility, a lack of bioavailability when given in the solid state, instability once in solution under ambient conditions and a metallic taste. It belongs to BCS class II, i.e. low solubility high permeability. Ritonavir is used to inhibit a particular liver enzyme that normally metabolizes protease inhibitors, cytochrome P450-3A4 (CYP3A4)[2,3]. Generally Ritonavir is degraded by CYP3A4[4]. To avoid this the crystallo co agglomerates were prepared to enhance the extent of absorption. Ritonavir has the structural formula as shown in (Fig. 1)[5].
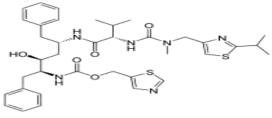

Fig. 1: Structure of Ritonavir

Materials and Method
Drugs and chemicals
Pure Ritonavir is used as working standard, was received as gift from Lupin Laboratories Ltd., India. All chemicals and reagents i.e. Acetonitrile (HPLC), glacial acetic acid (AR) and ammonium acetate (AR) employed were purchased from LobaChemie, Mumbai.

Instrument
Separation of Ritonavir was performed on HPLC system (Make-JASCO) equipped with HiQSil C18 column (250×4.6 mm;5μm particle size), Rheodyne injector (50 μL) and Jasco UV 2075 plus detector. The data acquisition was performed by Borwin chromatography software(version 1.5). Digital Balance Shimadzu make (Model AY 120) was used for weighing chemicals. Separation was carried out at a flow rate of 1 mL/min using acetonitrile: 10mM ammonium acetate buffer(85:15 v/v) as mobile phase and detection at 239 nm.

Preparation of optimized crystallo co agglomerates
In a crystallization vessel, Ritonavir was dissolved in required amount of acetone (good solvent) to make saturated solution. This was added to aqueous solution of PVP K-30 (bad solvent) with stirring using a mechanical stirrer (Remi motors, Mumbai) for 15 min, following which dichloromethane (DCM) was added slowly which acted as bridging liquid. The temperature of the crystallization system was maintained below 5°C. The stirring was continued to obtain agglomerates, which were then filtered and dried overnight at room temperature.

Preparation of tablets of optimized crystallo co agglomerates
All the materials are shown in the formula (Table 1) were mixed by geometric mixing technique. Mixing was continued for about 30 minutes until a homogenous powder blend was obtained. Lactose was used as

diluent, PVP K-30 was used as dry binder, SLS was used as dispersing agent, talc was used as lubricant and starch as disintegrant. Tablets were prepared by direct compression method using standard 10.5 mm concave punches on rotary tablet compression machine (Rimek Mini Press II MT). All the product and process variables like mixing time and hardness, were kept constant and within permissible limits.

Table 1: Formula for preparation of tablets

Sr. No	Ingredient	Amount (mg)
1	Ritonavircrytalllo co agglomerates	100
2	Starch	18
3	SLS	12
4	PVP K-30	48
5	Talc	12
6	Lactose	QS

#Total weight of the tablets was kept 200 mg

Experimental animals

Experiments were performed with Wister rats weighing between 250 ± 20 gms. The animals were housed in colony cages under conditions of standard lighting, temperature (22 ± 1^0c) and humidity for at least one week before the beginning of experiment, to adjust to the new environment and to overcome stress possibly incurred during transit. During this period, we provided food and water. The experiments were planned after the approval of Institutional Animal Ethical Committee (IEAC), AISSMS College of Pharmacy (257/PO/ReBi/S/2000/CPCSEA).

Chromatographic conditions

The mobile phase consisted of acetonitrile: 10 mM ammonium acetate buffer in the proportion of 85:15 v/v. The mobile phase was filtered through $0.45\mu m$ membrane filter. The flow rate was 1 ml/min and the effluent was monitored at 239nm. The total run time of the method was set at 10 min.

Preparation of calibration curve of Ritonavir

Preparation of stock solutions: A stock solution representing $100\mu g/ml$ of ritonavir was prepared in acetonitrile, and the solution was stored at -20^0C. The working standard solutions were prepared prior to use from stock solution by sequential dilution with acetonitrile to yield final concentrations of 1, 2, 3, 4, 5, 10 and 15 $\mu g/ml$ of Ritonavir. Calibration curve of Ritonavir standard is shown in (Fig. 2).
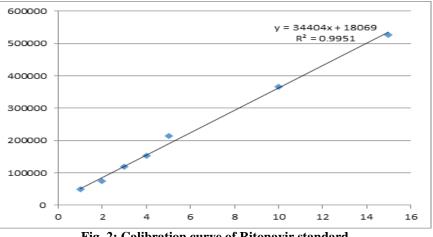

$$y = 34404x + 18069$$
$$R^2 = 0.9951$$

Fig. 2: Calibration curve of Ritonavir standard

Extraction procedure

Volumes of 0.25ml blank plasma, 0.25 ml of working standards of Ritonavir (4 to 60 µg/ml) were added separately and gently vortex for 5 min. Then add 0.5ml of acetonitrile (to get concentration range 1 - 15 µg/ml). The mixture was centrifuged for 10min at 3000rpm. Then the supernatant was transferred into tube and 50µl was injected into the HPLC. The calibration curve of Ritonavir in spiked plasma is shown in (Fig. 3).
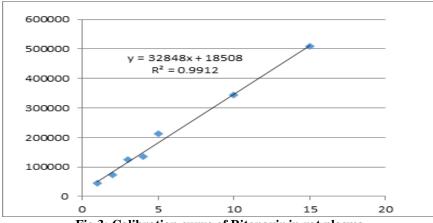
Fig 3: Calibration curve of Ritonavir in rat plasma

Pharmacokinetic studies in rats[6-8]

Male Wister rats were randomly distributed into four groups of six animals in each group; they were housed in well ventilated plastic cages and maintained on uniform diet and temperature with 12h light and dark cycle. Before the experiment all animals were fasted for 24hours.

Group I - Normal Saline (2 ml/kg)
Group II - Ritonavir (2 mg/kg)
Group III - Ritonavir crystallo co- agglomerates (10 mg/kg)
Group IV -Ritonavir marketed formulation (10 mg/kg)

Blood samples were withdrawn at 0, 0.5, 1, 2, 4, 6, 24hour time intervals from tail vein using heparinized capillaries. Plasma was separated by centrifugation and stored in vials at −20°C until further estimated.

Sample administration

The pure ritonavir standard, ritonavir crystallo co-agglomerate tablets and marketed formulation were administered to rats orally as suspension. The sodium CMC suspension was prepared and given according to dose.

Treatment of bioavailability data

The various pharmacokinetic parameters like elimination half-life (t1/2), overall elimination rate constant (Ke), area under concentration time curve (AUC), C_{max}, T_{max} for the drug under consideration were obtained in each subject from plasma concentration verses time profile and statistical work done by two-way ANOVA.

Results and Discussion

The ritonavir pure drug, ritonavir crystal co-agglomerates and marketed formulation were evaluated for pharmacokinetic parameters like area under curve (AUC $_{0-t}$, AUC $_{0-\infty}$), C_{max}, $t_{1/2}$, k elimination (k_e), T_{max}. The results are shown on (Table 2).

Table 2: Results

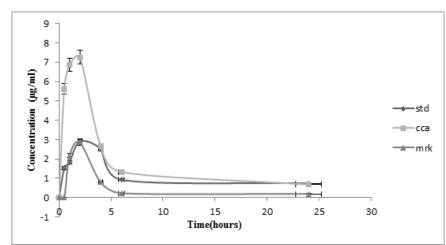| Parameter	Ritonavir	Ritonavir crystallo co-agglomerates	Marketed formulation
C_{max}(µg/ml)	2.8968	7.2625	2.8491
Tmax (hr)	2	2	2
AUC_{0-t}(µg/ml/h)	27.5447	43.5540	12.0304
AUC $_{0-\infty}$(µg/ml/h)	18.334	39.948	8.438
$K_{(e)}$(hr^{-1})	0.0798	0.188	0.0547
$t_{1/2}$(hr)	8.684	3.686	12.66

Fig. 3: Drug release pattern of ritonavir standard (std), ritonavir crystallo co agglomerates (cca) and marketed formulation (mrk)

The pharmacokinetic data obtained was subjected to Two way ANOVA. The module was significant according to two way ANOVA. There is no significant change in T_{max}. But other parameters are significantly increased.

This concludes that ritonavir crystallo co agglomerates showed good absorption and bioavailability than the Ritonavir standard and marketed formulation.

Acknowledgement

Reference

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