Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 6, Issue 2, 2014

Research Article

UTILIZATION OF CO-CRYSTALLIZATION FOR SOLUBILITY ENHANCEMENT OF A POORLY SOLUBLE ANTIRETROVIRAL DRUG – RITONAVIR

KUSHAL SHAH, SHEETAL BORHADE, VAISHALI LONDHE*

Shobhaben Pratapbhai Patel School of Pharmacy & Technology Management, SVKM's NMIMS, Mumbai, India.
Email: Vaishali.londhe@nmims.edu

Received: 20 Jan 2014, Revised and Accepted: 03 Mar 2014

ABSTRACT

Objective: The main objective of this work is to explore co-crystallization approach for increasing solubility of an antiretroviral drug, Ritonavir (RTN).

Methods: In this study, different co-formers with different functional groups like carboxylic acid, acid amide, amino acids were tried in ratio of 1:5 (RTN : Co-former) using solvent grinding method and methanol as a cosolvent. Co-formers used were succinic acid (SUC), adipic acid (ADP), nicotinamide (NIC) and D-alanine (ALA).

Results: The co-crystals formed were characterized by melting point determination, Fourier transform infra red (FTIR), differential scanning calorimetry (DSC), X ray diffraction (XRD) and solubility studies. Dissolution studies were performed with drug alone and co-crystals of drug with SUC, ALA and ADP as they showed 3 - 4 folds increase in solubility.

Conclusion: Melting point, DSC, FTIR spectra of co-crystals were different than pure drug and co-formers indicating their interaction. XRD patterns of co-crystals were not completely amorphous but less intense compared to drug alone. Faster dissolution was seen for co-crystals of SUC and ADP as compared with ALA.

Keywords: Ritonavir; Co-crystallization; Adipic acid, Succinic acid, D – alanine, Nicotinamide, Solubility enhancement.

INTRODUCTION

In Pharmaceutical industry, screening studies of NCEs as well as formulation development frequently encounters problem of solubilisation of poorly soluble drugs. Poorly soluble drugs have reduced systemic circulation and hence less bioavailability. Different techniques have been applied and studied to improve solubility and bioavailability of such drugs. The techniques include use of surfactants, solid dispersion, drug micronisation, polymorphism etc. [1]. Ritonavir (RTN) is an antiretroviral drug; the protease inhibitor class used to treat HIV infection and AIDS. It belongs to BCS class II, i.e. low solubility high permeability [2, 3]. RTN is used to inhibit a particular liver enzyme that normally metabolizes protease inhibitors, cytochrome P450-3A4 (CYP3A4) [4]. Various techniques were used to enhance solubility and dissolution of RTN as solid dispersions, complexation as described further. Ritonavir-polyvinyl pyrrolidone vinyl acetate solid dispersion prepared by spray drying was found to improve RTN solubility markedly [5]. Hot melt extrusion of RTN using cremophor, soluplus showed significant increase in solubility [6]. The solubility of RTN in combination with beta cyclodextrin (βCD), Solutol HS15 and polyvinyl pyrrolidone (PVP) K30 was more as compared to βCD alone [7]. The process of antisolvent precipitation using HPMC and sodium dodecyl sulfate yielded small, uniform and stable ritonavir nanoparticles with evidently enhanced dissolution rate [8]. Microparticles for the controlled release of RTN using cellulose polymer were prepared by the solvent evaporation method. The release kinetics data and characterization studies indicated that drug release from microcapsules was diffusion –controlled [9].

Co-crystals incorporate pharmaceutically acceptable guest molecules, called as co-formers, into a crystal lattice along with the API. Co-crystals have regained attention as an attractive alternate solid form for drug development [10, 11]. Extensive research work has been carried out in this field to improve the aqueous solubility of poorly water-soluble drugs like Aceclofenac, Atorvastatin, Carbamazepine [12 - 14]. This approach is also tried for antiretroviral drugs like Nevirapine and Acyclovir. Nevirapine co-cystals using malic acid as co-former resulted in fivefold increase in Nevirapine solubility [15]. Acyclovir was co-crystallized and amorphisized using tartaric acid, citric acid and PEG400 separately to improve its dissolution [16]. Co-crystals have the potential to alter and optimize physical properties such as crystalline form, solubility, and stability of an active pharmaceutical ingredient (API) without detrimentally affecting its activity [17]. Nicotinamide (NIC), Adipic acid (ADP), Succinic acid (SUC) and DL- Alanine (DAL) are GRAS status compound which can be used as co crystallization agents [18].

Therefore, in this study co-crystals of RTN were prepared using different co-formers and characterized.

MATERIALS AND METHODS

Materials

RTN pure drug was purchased from Hetero Lab, Hyderabad, All co-formers having 99% purity were purchased from local suppliers. Other chemicals used in the study were of analytical grade.

Preparation of co-crystals

Co-crystals were prepared using liquid assisted co-grinding. API and co-former in ratio of 1:5 were ground manually in a mortar and pestle at room temperature for 1 hour with slow addition of small volume of methanol drop wise. Resulted mass was dried further and evaporated at room temperature.

Co-crystals with nicotinamide (RTNNIC), succinic acid (RTNSUC), adipic acid (RTNADP), and D-alanine (RTNDAL) were obtained as dry mass. They were characterized further.

Characterization of co-crystals

Melting point determination

Melting point of pure API, co-formers and co-crystals were obtained by capillary method using liquid paraffin.

Differential Scanning Calorimetry (DSC)

Thermal analysis of API, co-former and co-crystal were recorded individually on DSC (Q200), Waters. The samples were scanned at 10°C/min over a temperature range of 50º- 300°C with nitrogen purging.

Fourier Transform Infrared (FT-IR) Studies

FT-IR of pure API, co-formers and selected co-crystals spectra were recorded individually by a Spectrum RXI, Perkin Elmer FTIR

spectrophotometer by mixing them with potassium bromide. Scans were recorded in the range of 400-4000 cm^{-1} at spectral resolution of 4 cm^{-1}.

Powder X-Ray Diffraction

The X-ray diffractogram were generated using a Bruker diffractometer D8 Advance (Software: Diffrac. Suite). Multiscans over 10-60 minutes were employed over the 2θ- range 0-40°.

Drug Solubility study

The solubility studies of RTN and co-crystal were performed by shake flask method. The RTN was added in excess to the distilled water in vials. The vials were sealed and kept in the rotary shaker (Orbitek- Scigenics Biotech, India) at 25°C ± 0.5 and 100 rpm. After equilibrium the solutions were filtered using 0.45 µ membrane filter (Pall Corporation, Pall India Pvt. Ltd., Mumbai, India), diluted suitably and were quantified using HPLC(Perkin Elmer series 200 with PDA detector).

The samples were analysed using Kromasil C8, 25 cm X 4.6mm X 5 µ column. Mobile phase used was phosphate buffer pH 6.8: ACN (45:55) v/v. Injection volume and column flow rate were 10 µl and 1.5 ml/min respectively, at ambient temperature.

Drug Dissolution study

As co-crystals of adipic acid, D-alanine, succinic acid showed good solubility, their dissolution was performed. The dissolution study for RTN, co-crystal was carried out using USP type II (Paddle type) Dissolution apparatus with 500 mL 0.1N hydrochloric acid as dissolution medium at 37°C. The samples were withdrawn at definite time intervals for one hour and were quantified using UV spectrophotometer (Perkin Elmer lambda 25) at 240 nm.

RESULTS AND DISCUSSION

RTN was co-crystallized using NIC, SUC, DAL and ADP as co-formers and methanol as co-solvent by solvent assisted grinding method. They were characterised and evaluated by different techniques.

Melting Point

It was found that the melting point of the co-crystals showed a significant deviation with respect to the melting point of pure drug and the individual co-formers indicating their interaction. This test was used as a preliminary test for confirmation of co-crystal formation for further characterization.

Characterization by DSC

From the DSC thermograms (fig.1), it was observed that the thermograms of the co-crystals were different in pattern and intensity as compared to RTN and co-formers which indicates their interaction. This shift in the melting point is due to the change in crystal lattice of the RTN in presence of co-former, forming a relatively different crystal lattice in co-crystals.

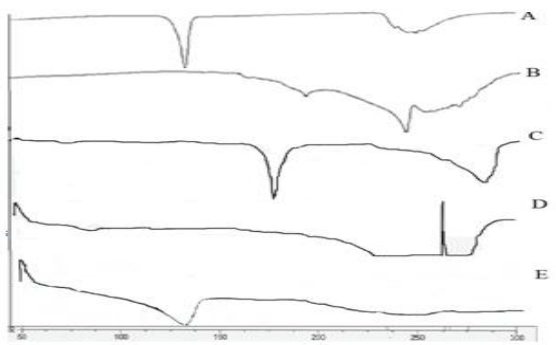

Fig. 1: DSC pattern of (A) RTN, (B) RTNNIC (C) RTNSUC, (D) RTNDAL, (E) RTNADP

Fourier Transform Infrared (FT-IR) Studies

As shown in Fig.2, the FT-IR spectrum of RTN showed presence of C=O amide peak at 1622.14 cm^{-1} C=O ester peak at 1716.04 cm^{-1} -NH- stretch at 3357.91 cm^{-1}, Amide –NH-bend at 1527.03 cm^{-1}. There is a shift in the functional groups present in the co-crystals compared to that of drug, showing presence of new bond formation.

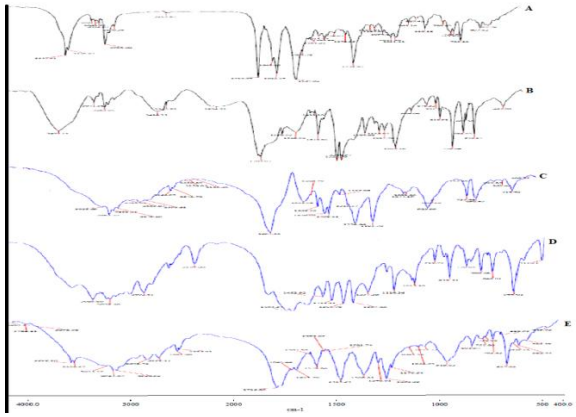

Fig. 2: FTIR spectra of (A) RTN, (B) RTNNIC, (C) RTNADP, (D) RTNDAL (E) RTNSUC

Powder X-Ray Diffraction

The crystallogram pattern of co-crystals shows a variation in peak intensity as compared to that of the pure RTN and co-formers as shown in Fig.3. Hence it was concluded that, some physical and or chemical bonding incurred between pure RTN and co-formers resulting in the formation of respective co-crystals. This change in the crystallogram pattern can be due to change in the crystal lattice structure owing to the interaction between the drug and the co –former.

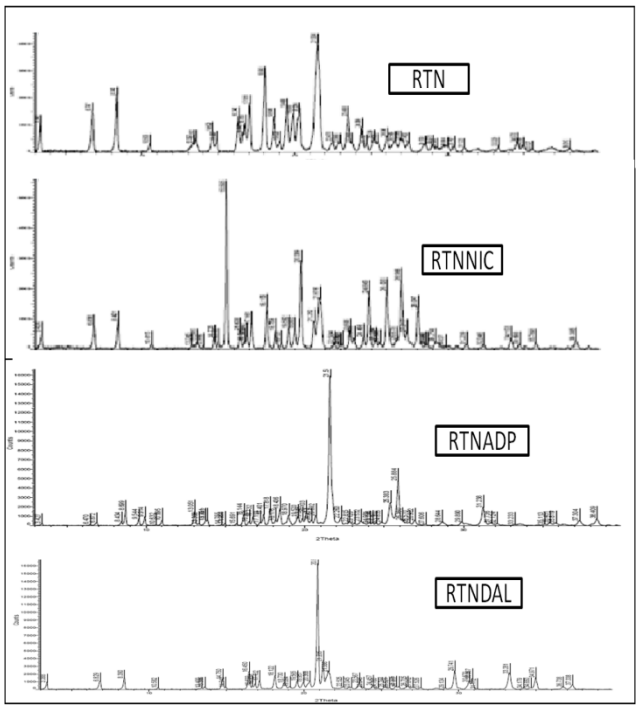

Fig. 3: Overlay of XRD pattern (A) RTN, (B) RTNNIC, (C) RTNADP, (D) RTNDAL

Drug Solubility study

Saturation solubility data (Fig.4) showed around 6 folds increase for cocrystals with ADP and SUC whereas around 3 and 4 folds increase was obtained with cocrystals of NIC and DAL respectively.

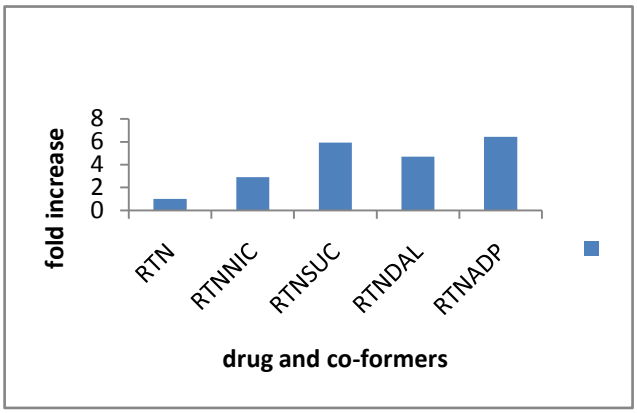

Fig. 4: Saturation solubility studies

Drug Dissolution study

From the dissolution data (Fig.5) it was observed that cocrystals RTNSUC and RTNADP showed two times drug release at initial time points as compared to RTN alone but at the end of 1 hr, only 15% increase in drug release was found. RTNDAL showed marginal increase in drug dissolution.

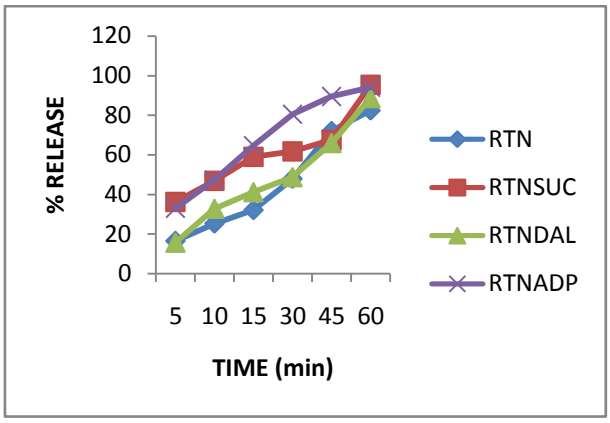

Fig. 5: Dissolution Profile for co-crystals & RTN

CONCLUSION

The co-crystals were prepared successfully using different coformers by solvent drop grinding method. These co-crystals were characterized by melting point, FTIR, DSC and XRD. These studies indicated formation of new crystal phases due to physical and or chemical interactions between API and co-former. For RTN, dicarboxylic acids co-crystals showed better solubility and dissolution as compared to amino acid than acid amide.

List of abbreviations

Ritonavir=RTN, succinic acid=SUC, adipic acid=ADP, nicotinamide=NIC and D-alanine=ALA.

Competing interests

The authors declare that they have no competing interests.

Authors' contribution

All authors contributed equally. All authors read and approved the final manuscript.

ACKNOWLEDGEMENTS

The authors thank Microlabs Ltd; Mumbai, India and Alkem Laboratories for carrying out DSC and XRD studies respectively. The authors are also thankful to Ms. Mrunmayi Gore, SPPSPTM for manuscript preparation.

REFERENCES

1. Strickley RG: Solubilizing Excipients in Oral and Injectable Formulations, Pharm Res, Feb 2004; 21, 2; 201 -230.
2. www.hiv-druginteractions.org, July 2011, (as referred on 26th Feb 2013)
3. Lemke TL, Williams DA *et al*: Foye's Principles of Medicinal chemistry, sixth edition, Lippincott Williams & Wilkins, 1220-1222.
4. http://www.hivandhepatitis.com/hiv_and_aids/norvir_1.htm. 30.04.13, (as referred on 26th Feb 2013)
5. Poddar SS, Nigade SU, Singh DK: Designing of Ritonavir Solid Dispersion through Spray Drying, Scholars Research Library, Der Pharmacia Lettre, 2011: 3 (5) 213-223.
6. Jevprasesphant R et al: Improving the solubility and stability of ritonavir using hot melt extrusion technique. AIDS 2010 - XVIII International AIDS Conference: Abstract no. THPE0155.
7. Chowdary KP, AnnammaDevi G.S, Anusha B: A factorial study on enhancement of solubility and Dissolution rate of ritonavir by Hydroxy propyl β cyclodextrin and solutol HS15, Int J Chem, 2012v02i4 (01-07).
8. Kshirsagar SJ: Preparation and Characterization of Amorphous Nanoparticles for Solubility Enhancement of Ritonavir, Int Journal of Pharmaceutical Invention, July 2012, 2(6), 27-35.
9. Jenita JJL: Formulation and characterization of ritonavir loaded Ethyl cellulose microspheres for oral delivery, World J Pharm Res, 1(2), 207-215.
10. Shah N, Zaworotko MJ: The role of co crystal in pharmaceutical science, Drug Discovery Today, 13,440-446.
11. Vishweshwar P, McMahon JA, Bis JA: Pharmaceutical co-crystals, J Pharm Sci 2006; 95,499-516.
12. Sevukaranjan M: Synthesis and characterisation of co-crystals: aceclofenac- nicotinamide, J Pharm Sci & Res, 3(6), 2011, 1288-1293.
13. Ahayanfar A. Ghavimi H: coamorphous atorvastatin calcium to improve its physicochemical and pharmacokinetic properties, J Pharm Pharm Sci, 16(4)577, 2013.
14. Tomaszewsk, Karki S, Shu J, *et al*: Pharmaceutical characterization and evaluation of cocrystals: Importance of in vitro dissolution conditions and type of conformer, Int J Pharm, 453 (2013) 380– 388.
15. Mino RC: Co-crystals of antiretroviral drug Nevirapine: crystal structures, thermal analysis and dissolution behavior, Cryst Eng Comm, 2012, 14, 2541.
16. Masuda T, Yoshihashi Y, *et al*: Co-crystallization and amorphization induced by drug-excipient interaction improves the physical properties of acyclovir, Int J Pharm. 2012 Jan 17; 422(1-2), 160-9.
17. McNamara DP Childs SL: Pharmaceutical co-crystals, Pharm Res, 2006, 23, 1888-1897.
18. Basavoju S, Bostrom D, Velaga SP: Indomethacin-saccharin co-crystal: Design, synthesis and preliminary pharmaceutical characterization, Pharm Res, 2008, 25, 530-541.