European Journal of Pharmaceutics and Biopharmaceutics 89 (2015) 329–338

Contents lists available at ScienceDirect

European Journal of Pharmaceutics and Biopharmaceutics

journal homepage: www.elsevier.com/locate/ejpb

Research paper

Fabrication, solid state characterization and bioavailability assessment of stable binary amorphous phases of Ritonavir with Quercetin

Swapnil J. Dengale [a,*], Syed Sajjad Hussen [a], B.S.M. Krishna [a], Prashant B. Musmade [a], G. Gautham Shenoy [b], Krishnamurthy Bhat [a]

[a] Department of Pharmaceutical Quality Assurance, Manipal College of Pharmaceutical Sciences, Manipal University, Manipal, India
[b] Department of Pharmaceutical Chemistry, Manipal College of Pharmaceutical Sciences, Manipal University, Manipal, India

ARTICLE INFO

Article history:
Received 25 August 2014
Accepted in revised form 16 December 2014
Available online 24 December 2014

Keywords:
Binary amorphous forms
Ritonavir
Quercetin
Solubility
Solid-state characterization
Bioavailability

ABSTRACT

In the current study, Quercetin (QRT) was characterized for thermodynamic and kinetic parameters and found as an excellent glass former. QRT was paired with Ritonavir (RTV) (BCS class-IV antiretroviral) to form stable amorphous form and pharmacologically relevant combination. Binary amorphous forms of RTV and QRT in molar ratios 1:1, 1:2 and 2:1 were prepared by solvent evaporation technique and characterized by XRPD, DSC and FTIR. The prepared binary phases were found to become amorphous after solvent evaporation which was confirmed by disappearance of crystalline peaks from X-ray diffractograms and detecting single T_g in DSC studies. The physical stability studies at 40 °C for 90 days found RTV:QRT 1:2 and RTV:QRT 2:1 phases stable, while trace crystallinity was detected for 1:1 M ratio. The temperature stability of RTV:QRT 1:2 and RTV:QRT 2:1 amorphous forms can be attributed to phase solubility of both components where the drug in excess acts as a crystallization inhibitor. Except for RTV:QRT 1:2 ratio, there was no evidence of intermolecular interactions between two components. Almost 5 fold increase in the saturation solubility was achieved for RTV, compared to crystalline counterpart. While for QRT, the solubility advantage was not achieved. *In vivo* oral bioavailability study was conducted for 1:2 binary amorphous form by using pure RTV as a control. C_{max} was improved by 1.26 fold and T_{max} was decreased by 2 h after comparing with control indicating improved absorption. However no significant enhancement of oral bioavailability (1.12 fold after comparing with control) was found for RTV.

1. Introduction

Human immunodeficiency virus (HIV) related acquired immune deficiency syndrome (AIDS) has claimed over 25 million lives since its discovery in 1981 [1]. Based on the knowledge of the HIV replication cycle, several drug targets have been identified over the years and a number of effective treatment options are currently available, protease inhibitors being one such class of drugs. In spite of intensive research and availability of pharmacologically effective molecules, a cure for HIV infection remains elusive. According to the 12th edition of World Health Organization Essential Core Drug List (year 2004), the main reason for failure of effective treatment for HIV is low bioavailability of the antiretrovirals. This leads to short residence times, resulting in low concentrations of antiretroviral drugs at certain inaccessible viral reservoir sites such as the central nervous system and lungs [2]. Low bioavailability is due to poor solubility of antiretrovirals, their ability to act as substrate of CYP3A4 metabolising enzyme and P-gp efflux transport system. This fact contributes to increased clearance of anti-retrovirals from body and forms the impediment to effective bioavailability.

Since the inception of the HAART therapy in the management of AIDS in the late 90s, the mortality and morbidity related to HIV is significantly reduced. Ritonavir (RTV) belongs to the class protease inhibitor (PI), given in triple drug HAART therapy [3,4]. Further, RTV was the first PI for which efficacy was proven in a study with clinical endpoints. It has poor aqueous solubility (1.26 μg/ml) and low bioavailability (5%) [5,6]. By virtue of RTV's peptidomimetic structure, it is substrate/inhibitor of efflux system mediated by transporters of the ATP-binding cassette (ABC) P-glycoprotein (P-gp). RTV is also a known potent inhibitor of CYP3A4 enzyme family [6–9].

Quercetin (QRT) is a bio-flavonoid usually found in onions, apples and various fruit juices. Reports from various studies showed the protective effects of QRT against oxidative damage,

* Corresponding author. Department of Pharmaceutical Quality Assurance, Manipal College of Pharmaceutical Sciences, Manipal University, Manipal 576104, Karnataka, India. Tel.: +91 8202922482, mobile: +91 9916311176; fax: +91 8202571998.
E-mail address: swapnil.jd@manipal.edu (S.J. Dengale).

http://dx.doi.org/10.1016/j.ejpb.2014.12.025
0939-6411/

cardiovascular disease and it is found to possess anti-tumor activity. Interestingly, QRT was found to inhibit HIV-1 reverse transcriptase with IC_{50} value 60 μM. [10,11]. Due to its health benefits, QRT is available as a dietary supplement which is sold over the counter in markets [12]. QRT is shown to inhibit ABC transporters including P-gp, MRP1, MRP4 and BRCP in vitro, though it's *in vivo* inhibiting potential is controversial [13–16]. QRT exhibits poor aqueous solubility and it is practically insoluble in water. Given with the above mentioned health benefits, a number of studies have been reported to enhance solubility and thereby bioavailability of QRT [17–19].

In the field of amorphous drug stabilization, scientists are compelled to search for new avenues, owing to the certain disadvantages of solid dispersion technology which includes tackiness of systems, reduced glass transition temperature due to hygroscopicity, and phase separation. In order to avoid many disadvantages of solid dispersion technology in amorphous phase stabilization, the interest has been shifted to novel and more efficacious binary amorphous systems. In these systems, a combination of two small molecules (drugs or excipients) is used instead of drug–polymer mixtures. These systems have been found to provide better stability and enhanced dissolution rates for the studied drugs [20]. The primary reason for the enhanced stability and dissolution has been found to be linked with the solid state interactions between the two small molecules present in the system. There are several reports after 2002 on the area of binary amorphous systems. Yamamura et al. combined two drug molecules and found that cimetidine formed an amorphous binary mixture with the nonsteroidal anti-inflammatory drugs (NSAIDs) naproxen [21], indomethacin [22] and diflunisal [23] upon precipitation from ethanol solution. Alleso et al. reported binary mixtures of naproxen and cimetidine which were found to become amorphous upon ball milling for 60 min at 4 °C [24]. Lobmann et al. reported improvement in the stability and solubility of Glipizide while combined with Simvastatin [25]. Chieng et al. showed that crystalline indomethacin can be made amorphous and stabilized by co-milling with crystalline ranitidine hydrochloride [26].

In the current study, the feasibility of QRT as a glass former in conjugation with RTV was investigated. QRT was screened for glass forming ability, Kauzmann's temperature (T_{ks}) and fragility (both kinetic and dynamic). The study conducted in our laboratory has found RTV as a potential molecule having good glass forming ability when processed either with polymers or with small molecules. The combination of QRT with RTV in particular opted for the following reasons. First, the cocktail of RTV (protease inhibitor) and QRT (reverse transcriptase inhibitor) was anticipated to show additive therapeutic response, such as HAART therapy. Second, RTV is an anti-retroviral drug with very low oral bioavailability due to its poor aqueous solubility. Solubility could be improved by presenting the drug as a high energy amorphous solid employing binary amorphous technology to prepare amorphous forms of RTV and QRT, which in turn would make more drug available in the gut fluids, neutralizing the solubility barrier mentioned in the BCS classification. Third, RTV is prone to being effluxed by the gut membranes due to transporters (P-gp) and to metabolic enzymes (CYP3A4). Transporter-enzyme interplay in the intestine as well as in the liver is important primarily for low solubility compounds. Fortunately, the transporter-enzyme system is subject to saturation if sufficient amount of drug(s) were to be present in the gut and blood. The saturation of efflux transporters could be achieved using QRT, which is known P-gp substrate as well as inhibitor. This in turn would make more drug available in blood, neutralizing the effects of P-gp and thereby increasing the permeability of RTV i.e. second barrier mentioned in BCS classification.

The proposed approach was designed in anticipation of development of platform technology which would help minimize the effects of low solubility and low permeability on poorly water soluble drugs such as RTV.

2. Materials and methods

2.1. Materials

Ritonavir (RTV) was obtained from Lupin pharmaceuticals, as gift sample. Quercetin (QRT) was purchased from sigma Aldrich, Germany. Chemical structures of RTV and QRT are shown in Fig. 1. Both drugs were used as received.

2.2. Methods

2.2.1. Thermodynamic characterization of QRT

2.2.1.1. Determination of experimental fragility. The ratio of melting temperature to glass transition temperature (T_m/T_g) was calculated to determine experimental fragility [27].

2.2.1.2. Determination of Kauzmann's temperature. Kauzmanns temperature (T_{ks}) was determined by entropy based approach using following equation.

$$\frac{1}{T_{ks}} = \frac{1}{T_m}\left(1 + \frac{\Delta H_m}{K}\right) \tag{1}$$

where T_m is melting temperature, ΔH_m is enthalpy related to melting event and K is constant. Constant K was calculated by plotting product of configurational heat capacity and temperature ($C_{pconf} * T$) as a function of difference between corresponding temperature and glass transition temperature of compound ($T - T_g$). K was taken as y-intercept of the graph [28] (Supplementary data).

2.2.1.3. Determination of kinetic fragility (m). Kinetic fragility was estimated by determining the dependence of T_g of amorphous compound on the heating rate (q). Amorphous compound was heated at heating rates (5, 10, 15 and 20 K/min) in DSC and corresponding change in the T_g was monitored. Kinetic fragility index (m) was calculated by following equation,

$$m = \frac{E_a}{2.303 \cdot R \cdot T_g} \tag{2}$$

where E_a is activation energy, and R is gas constant. The value of the term E_a/R was taken from the slope of the plot of $\ln q$ versus $1/T_g$ [27] (Supplementary data).

2.2.1.4. Determination of strength parameter (D).

$$m = D(T_0/T_g)\Big/\left[(\ln 10)\{1 - (T_0/T_g)\}^2\right] \tag{3}$$

In Eq. (3), for practical purpose the value of T_0 is taken as the value of Kauzmann's temperature (T_{ks}) and m is the value of kinetic fragility index [27].

2.2.2. Preparation of amorphous forms

2.2.2.1. Preparation of binary amorphous forms of RTV–QRT. A total of 1000 mg RTV and QRT in molar ratios of 1:1 (704.8 mg of RTV and 295.2 mg of QRT), 1:2 (544.2 mg of RTV and 456.9 mg of QRT), 2:1 (826.9 mg of RTV and 173.2 mg of QRT) were taken in round bottom flask and then dissolved with 50 ml methanol (ICH Class-2, Solvents to be limited) to form a clear solution. The solvent was evaporated using rotatory evaporator under reduced pressure at 40 °C temperature. The residual solvent in the prepared precipitates is then completely removed by placing the precipitates inside a vacuum desiccator containing $CaCO_3$ crystals at least for 2 days. Further precipitates were stored in desiccator until their use in the experiment.