Chemometric Methods for the Quantification of Crystalline Tacrolimus in Solid Dispersion by Powder X-Ray Diffractometry

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Received 3 December 2013; revised 5 February 2014; accepted 7 February 2014

Published online 1 March 2014 in Wiley Online Library (wileyonlinelibrary.com). DOI 10.1002/jps.23912

ABSTRACT: The objective of this study was to develop powder X-ray diffraction (XRPD) chemometric model for quantifying crystalline tacrolimus from solid dispersion (SD). Three SDs (amorphous tacrolimus component) with varying drug to excipient ratios (24.4%, 6.7%, and 4.3% drug) were prepared. Placebo SDs were mixed with crystalline tacrolimus to make their composition equivalent to three SD (crystalline tacrolimus component). These two components were mixed to cover 0%–100% of crystalline drug. Uniformity of the sample mixtures was confirmed by near-infrared chemical imaging. XRPD showed three distinct peaks of crystalline drug at 8.5°, 10.3°, and 11.2° (2θ), which were nonoverlapping with the excipients. Principal component regressions (PCR) and partial least square (PLS) regression used in model development showed high R^2 (>0.99) for all the mixtures. Overall, the model showed low root mean square of standard error, standard error, and bias, which was smaller in PLS than PCR-based model. Furthermore, the model performance was evaluated on the formulations with known percentage of crystalline drug. Model-calculated crystalline drug percentage values were close to actual value. Therefore, these studies strongly suggest the application of chemometric-XRPD models as a quality control tool to quantitatively predict the crystalline drug in the formulation. © 2014 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci 103:2819–2828, 2014

Keywords: tacrolimus; solid dispersion; powder X-ray diffraction; chemometric; principal component analysis; partial least square; multivariate analysis

INTRODUCTION

Tacrolimus, a potent immunosuppressive agent, is the clinician's drug of choice to prevent rejection of the transplanted solid organ in patients if cyclosporine A or steroid-based regimen fails.[1,2] Despite its life saving potential in such patients, the drug has biopharmaceutical and solubility issues.[3–5] Under Biopharmaceutical Classification System (BCS), it is placed in BCS class II drug exhibiting high permeability and low solubility. Even though a plethora of formulation and drug delivery modalities have been reported to improve bioavailability, which include self microemulsifying drug delivery system, prodrug, oily solution, complexation with cyclodextrin, micro or nano-based drug delivery system, and solid dispersion (SD)[5,6], only a few formulations have made it to the market. Commercially, it is available as injection (5 mg/mL), capsules (Prograf®, 0.5, 1, and 5 mg, a SD formulation,[4,7,8] and ointment[8] (Protopic® 0.03, and 1%).

The main focus of formulating the poorly aqueous soluble drug is to present the drug in its molecular form to the absorbing surface, that is, gut mucosa. Any intrinsic or extrinsic factor triggering crystallization of the drug in the formulation can impact an overall absorption and thereby bioavailability of the drug, which is of particular concern to a drug like tacrolimus possessing a narrow therapeutic index (5–15 μg/mL).[9] Sub-potent dose of tacrolimus can increase the potential of a graft rejection, whereas overdose can cause dose-dependent neuro/nephrotoxicity. Therefore, a dosage form or delivery strategy is needed that can reliably deliver a calibrated concentration of the drug for the graft survival and minimization of the drug-induced toxicity. Because tacrolimus is recommended to be administered orally after initial intravenous infusion,[10,11] SD of tacrolimus offers one of the several other options to improve bioavailability. SD is a formulation strategy to enhance drug solubility by molecularly dispersing the poorly aqueous soluble drug in hydrophilic polymer and converting crystalline to amorphous drug, enhancing wettability, and affecting carrier-mediated solubilization of the poorly aqueous soluble drug.[12] Various dispersion techniques to prepare SD have been reported including solvent evaporation method, melting method, solvent wetting method, and surface attachment methods.[9,13] Although SD approach improves the physicochemical properties of the drug, it is not a thermodynamically stable system leading to reversion of the molecules to its thermodynamically more stable form by crystallization over a period of time.[14] Depending upon the manner the finished product is handled during the excursion of product from the industry to the pharmacy or end user, the onset of crystallization process or chances of drug falling out of specification may be sooner than later and thereby patients will not get the timely therapeutic benefit of the drug. There were instances of drug products recalled by United States Food and Drug Administration (US FDA) because of the crystallization of the amorphous drug in the product.[15] Therefore, a universal evaluation technique that can be used to detect and quantitatively differentiate the crystallinity in SD or its product intermediate is required to monitor the state of drug (percentage crystalline/amorphous) in drug products. This determination could allow for the development of crystallinity-discrimination dissolution methods.

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Journal of Pharmaceutical Sciences, Vol. 103, 2819–2828 (2014)

Powder X-ray diffraction (XRPD) is one of the several techniques commonly used in evaluating solid-state property of the drug. Because polycrystallites in the powder always contain crystallite at an angle that satisfy Braggs law to produce diffraction, XRPD can produce constituent-specific diffraction at an angle (2θ) whose position and intensities depend upon crystal lattice and unit cells contents, respectively [United States Pharmacopeia (USP) 35, Chapter 941]. In case of amorphous drug, absence of crystal lattice produces halo XRPD. Furthermore, proportionality of the peak intensity to the crystalline fraction present in the powder has been reported, which means fraction of crystalline constituent in the powder can be obtained by establishing correlation between drug and peak intensity.[16] Because XRPD is a multivariate process, responses of the powder diffraction can be fit into a model by establishing multivariate calibration curve using samples of known crystallinity. Chemometrics, which is a method of relating data on chemical system to the state of the system using statistics,[17] employs multivariate analysis techniques for model development. In this method, structures of the data are evaluated using principal component analysis (PCA) and assessments are performed whether data pretreatment that can improve the overall structure of the data. Multivariate model of the property of interest (percentage crystalline or amorphous) is then developed using principal component regression (PCR) or partial least square (PLS) regression. These regression models are then used to predict the property of interest in the unknown samples. Previously, chemometric models for estimating nimodipine polymorphs in the mixtures were built and their performances were evaluated using spectroscopic techniques, that is, FTIR, near infrared (NIR), and Raman.[18] Therefore, the focus of this work was to build and validate chemometric-XRPD models for tacrolimus SD and evaluate their performance in predicting percentage crystalline (or amorphous) tacrolimus in tacrolimus product intermediates.

MATERIALS AND METHODS

Materials

Tacrolimus (Ria International LLC, East Hanover, New Jersey), hydroxypropyl methyl cellulose (HPMC, 6cps viscosity substitution type 2910 USP) (Shin Etsu Chemical Company, Tokyo, Japan), croscarmellose sodium (FMC Biopolymer, Philadelphia, Pennsylvania), lactose monohydrate (Sigma–Aldrich, St. Louis, Missouri), and ethyl alcohol (Decon Lab, Inc., King of Prussia, Pennsylvania) were purchased and used as received. All other chemicals and solvents used were of analytical grade.

Methods

Preparation of SD

The commercial tacrolimus product (Prograf®) and its generic forms are the SD formulations, and contain HPMC (hypromellose), lactose monohydrate (LM), croscarmellose sodium (CC), and magnesium stearate as its inactive ingredients (US FDA Prograf® capsule USP label). The capsule consists of two components: SD granules and extragranular ingredients.[19,20]

Tacrolimus SD formulations were prepared by solvent evaporation methods described by Yamashita et al.[21] with some modification. Briefly, HPMC was added to the ethanolic drug solution. The mixtures were kept stirring for an hour to hydrate the polymer, then croscarmellose sodium and lactose monohydrate were added under stirring. Ethyl alcohol was allowed to evaporate under stirring. Residual alcohol was removed by drying under reduced pressure at 25°C for 24 h. The dried mass was then pulverized using glass mortar and pestle and passed through USP sieve no 80/120. The formulations proportions that passed through USP sieve no 80 but retained on 120 were used in analysis. Because SD can be prepared in different drug to excipients ratios, drug to excipients ratios (Table1) 1:3.10 (SD-1), 1:14 (SD-2), and 1:22 (SD-3), which represent 24.4%, 6.7%, and 4.3% of tacrolimus with respect to excipients, were chosen in the present work to prepare three tacrolimus SD granules and called amorphous tacrolimus component (SD-1, SD-2, and SD-3). Their amorphous state was confirmed by differential scanning calorimetry and XRPD. Furthermore, three placebo formulations were prepared in a similar manner having identical qualitative and quantitative excipients composition as amorphous SD components (SD-1, SD-2, and SD-3). Crystalline tacrolimus equivalent to SD-1, SD-2, and SD-3 was added to placebo and labeled as crystalline tacrolimus component (PM-1, PM-2, and PM-3).

Table 1. Composition of Tacrolimus Solid Dispersion Formulations

	Drug:Excipients	
Formulation	Tacrolimus	Excipients (HPMC, CC, LM)
SD1	1	3
SD2	1	14
SD3	1	22

Preparation of Tacrolimus Samples

SD-1 and PM-1, SD-2 and PM-2, and SD-3 and PM-3 were mixed in various ratios to make samples that have 0%–100% of added crystalline tacrolimus of the formulations. These sets of mixtures were labeled as FD-1, FD-2, and FD-3. These mixtures were then swirled 100 times in horizontal and vertical direction to allow mixing of the powder components.

NIR *Chemical Imaging (NIR-CI)*

The components distribution was tested by NIR chemical imaging (NIR-CI) technique. NIR-CI of FD-1, FD-2, and FD-3 samples sets was acquired by a Sapphire imaging system using SapphireGo software (Malvern, Worcestershire, UK). The instrument is equipped with indium-gallium-arsenide focal-plane array detector and liquid-crystal tunable filter to allow diffuse light from the sample and produces 320 × 256 pixel images. Before capturing images, the background reference and dark response were collected using Spectralon 99 (Labsphere, North Sutton, New Hampshire) and a clean stainless steel mirror, respectively. In the NIR range starting from 1400 to 2450 nm, the data were obtained with an increment of 10 nm, and eight scans were coadded to produce an average spectrum. The obtained data were analyzed by ISys chemical imaging software (Malvern). Data were treated before analysis, which include transformation of reflectance to absorbance, masked, truncated, and normalized by mean centering and scaling to unit variance by spectrum. The libraries for each set of sample (FD-1, FD-2, or FD-3) consisting of two components namely amorphous tacrolimus component (added 100% amorphous drug, SD-1, SD-2, or SD-3) and crystalline tacrolimus component