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Crystalline phase transition of ezetimibe in final product, after packing, promoted by the humidity of excipients: Monitoring and quantification by Raman spectroscopy

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ABSTRACT

Ezetimibe (EZT), in its anhydrous form, is a drug used for cholesterol and lipids reduction in blood plasma. The presence of EZT monohydrate in commercial tablets can change the solubility rate of the API, decreasing its activity. The objective of this work was to verify if the humidity present in the excipients could promote the phase transition from EZT anhydrous to hydrate. Initially the stability of the pure anhydrous form was monitored by Raman, at room temperature (23 °C) and relative humidity (75%). The MCR-ALS method showed that almost all EZT changed to hydrated form in 30 min. Then tablets of ezetimibe in the presence of its excipients were prepared and vacuum packed using a polyethylene film. Such tablet was monitored by Raman spectroscopy for 24 h in order to quantify the mixture of the crystalline forms. A multivariate calibration model using Raman spectroscopy and Partial Least Square (PLS) regression was built, with validation and cross validation errors around 0.6% (wt/wt), for both crystalline forms, and R^2 higher than 0.96. The PLS model was used to quantify the crystalline mixture of ezetimibe in the monitored tablet, after 24 h more than 70% of ezetimibe changed to the hydrated form.

1. Introduction

The change in the crystalline form of active pharmaceutical ingredients (API) is a phenomenon of huge importance due to the possibility of such changes to affect some properties of the API such as melting point, apparent solubility, density and others [1]. However, for pharmaceutical companies, the most problematic change among different crystalline forms is the dissolution rate. The final solubility of an API is not altered by polymorphism or hydration phenomena (considering the solubility after equilibrium), but the dissolution rate can be affected. This property is determinant for the success of a therapy, because the API has a residence time in the organism before its elimination. Real cases where changes in the crystalline form affect the final product are not rare, the most emblematic case being the Norvir® from the Abbott Company [2].

Also the hydrates usually present lower dissolution rates than the correspondent anhydrous [3] and this is one of the biggest problems in the pharmaceutical industry [4,5]. Thus an accurate monitoring of different transitions that might appear during API handling is necessary. The presence of hydrates in final products depends on the tablet manufacturing and storage conditions [6] and, obviously, the tendency of the molecule to form hydrates [7].

Ezetimibe is a class of lipid-lowering compounds that is used to control cholesterol levels in the organism. It is classified as a Class II drug according to the Biopharmaceutical Classification System. It means that this API has low solubility in water but is highly absorbed by the organism [8,9]. Some studies regarding the increase of bioavailability of the final ezetimibe product can be found in the literature [10,11]. As with most of the APIs which present a hydrate form, the dissolution rate of the hydrate form of ezetimibe, in water, is also lower than its anhydrous counterpart [12]. This API was approved for treatment of hypercholesterolemia in 2002 and since then has been commercialized under the name of Zetia® by Merck. Ezetimibe is generally administered in combination with other drugs, depending on the treatment necessity and patient limitations. Despite the time ezetimibe has been commercialized, studies in the literature about different crystalline forms of ezetimibe are limited [11,13].

There are many analytical techniques, suggested by the regulatory agencies, to analyze the crystalline form of APIs. The main techniques used for this purpose are: powder X-ray diffraction (PXRD), mid infrared (IR) and Raman spectroscopies and calorimetric techniques such as differential scanning calorimetry (DSC) and

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thermogravimetric analyses (TGA), which are useful to evaluate the formation of hydrates and solvates, and others [14]. The combination of such techniques can be used to characterize and quantify mixtures of polymorphs. Usually, the spectroscopy techniques (such as Raman and infrared) need be associated to multivariate analysis in order to monitor possible changes in the crystalline form of the API and to quantify the mixture of crystalline forms [6,15,16].

Multivariate Curve Resolution—Alternated Least Squares is a chemometric tool that enables the monitoring of crystalline transitions in APIs when associated to spectroscopic techniques [17]. By definition MCR-ALS is a bilinear resolution method that decomposes the original data matrix (D, which contains the spectra of samples) into two other matrices: the matrix C, which contains the concentration profiles of *n* components recovered by the model, and the matrix S, which contains the spectral profiles of the substances related to the matrix C [18]. This technique is quite relevant owing to its capacity to be used in association with several spectroscopic methods to monitor in line process in the industry [19]. MCR-ALS allied to Raman spectroscopy was used to perform in-line monitoring of the synthesis of ibuprofen–nicotinamide cocrystal [20]. Another chemometric tool widely used is the Partial Least Squares regression, which makes possible to build multivariate regression models that can be used to determine the concentration of each crystalline form (or amorphous content) in the final product [6] and API [15]. PLS is one of the most used chemometric tools and its application includes spectroscopic data such as infrared and Raman spectroscopies [21]. Raman is an ideal technique to deal with drug products, mainly final products, because most of the excipients generate low Raman scattering while APIs present good intensity [19]. In addition, each polymorph or solvatomorph form presents unique spectra, an essential characteristic for quantification and identification purposes [22]. Therefore, PLS and Raman spectroscopy is a powerful combination to quantify the crystalline states of the API in the solid dosages [6].

In this work we performed a study about the hydration of the ezetimibe under two conditions: the pure API at room condition (23 °C and 75% R.H.) and the hydration promoted by the humidity of excipients in the formulation. These studies were conducted by the monitoring of the hydration process by Raman spectroscopy, followed by the quantification of the crystalline forms (hydrated and anhydrous) by use of Partial Least Squares and Multivariate Curve Resolution.

2. Materials and methods

2.1. Reagents

All reagents used in this work: ezetimibe, sodium croscarmellose, lactose monohydrated, magnesium stearate, microcrystalline cellulose, povidone and sodium laurilsulfate; were pharmaceutical grade, kindly donated by a local pharmaceutical company and were used as received.

2.2. Synthesis of EZT-H

The hydrated ezetimibe (EZT-H) was synthesized using ezetimibe anhydrous (EZT-An) as precursor material. The synthesis consists in leaving the precursor material in an atmosphere with saturated humidity for one week. Such atmosphere was prepared using a desiccator bowl and two Petri dishes. An amount of nearly 200 mg of EZT-An was allocated inside a Petri dish. In another Petri dish water was added. Both dishes were let side by side inside the desiccator bowl (without silica). Pure EZT-H was obtained to build the multivariate calibration curve, in order to quantify the mixture of EZT-H and EZT-An in the final product.

2.3. Characterization of EZT-H, EZT-An and excipients

The EZT-H and EZT-An were characterized by powder X-ray diffraction (PXRD), thermogravimetric analyses (TGA) and Raman spectroscopy. The PXRD analyses were made using an X-ray diffractometerRigakuDmax 2500 PC (Tokyo, Japan) with a copper source (wavelength of 0.154 nm), 30 kV voltage and 40 mA emission current. The samples were analyzed from 5° to 45° (2°θ) at a scan rate of 0.2° min^{-1}.

The thermogravimetric experiments were made in a Thermogravimetric Analyzer 209 F3 Tarsus (NETZSCH-Gerätebau GmbH, Selb, Germany) under nitrogen atmosphere with a flow rate of 40 mL min^{-1} at heating rates of 10 °C min^{-1} in the range of 45–120 °C. In the analysis, platinum crucibles were used and a sample mass around 5.0 mg.

The Raman analyses were performed using an *i*-Raman BWS 415-785H (B & W Tek, Inc., Newark, DE, USA), with red laser of 785 nm, spectral resolution of 3.5 cm^{-1}, coupled to a microscope BAC151B(B & W Tek, Inc.) with 20× objective lens. The parameters used at Raman analyses were integration time of 30 s and a laser power of 31.6 mW (10%).

The excipients were tested as to their humidity content using a moisture analyzer MOC63u from Shimadzu (Tokyo, Japan). All analyses were performed using around 1 g of excipient, heated until 120 °C during 60 min.

2.4. Monitoring of hydration of pure EZT-An by Raman and MCR-ALS

The hydration of anhydrous ezetimibe was evaluated in two ways: in the pure API at room temperature and relative humidity and; in a tablet vacuum packed in the presence of its excipients.

To monitor the hydration of the pure EZT-An, a small amount of the API was placed in a Petri dish at 75% of relative humidity and 23 °C (ambient conditions). The spectra were taken every minute during 50 min. The acquired spectra were organized in a matrix with 50 rows (one spectrum/min) and 991 columns (Raman shifts), which corresponds to the Raman shift range of 206–1774 cm^{-1}. Baseline correction was performed and the spectra were normalized by the area. MCR-ALS was used for the deconvolution of the overlapped signals from the crystalline forms. For that, the ALS routine presented in the MCR—ALS toolbox was used [23,24]. To identify the number of components presented in the crystalline transition, the SVD routine from Matlab® was used. The MCR-ALS was performed using two components and non-negativity in the concentration profile as the only constraint.

2.5. Monitoring of hydration of EZT-An promoted by the humidity of excipients, by Raman and PLS

In order to evaluate if the humidity in the excipients could promote a crystalline transition of the API (EZT-An to EZT-H), a "query" tablet containing the excipients and just the anhydrous form of ezetimibe (EZT-An) was prepared. To prepare the tablet, a package insert of Zetia® was consulted to know the excipients used in this formulation. As the proportions of the excipients are not described in the package, the concentration of the excipients was estimated using the Handbook of Excipients [25], to get acceptable proportions in the formulation. The objective was not to produce tablets with the same excipient composition of Zetia®, but verify if these excipients could promote a phase transition by water transfer from the excipients to the crystalline lattice of the ezetimibe. The used formulation is given in Table 1, adding 10.00 mg of EZT-An for the "query" tablet, compressed at 100 kg/cm^2 and vacuum packed. The Raman spectra were collected in different times, through the package, at 0, 2, 6, 12 and 24 h after the preparation. In addition,