IDENTIFICATION AND STABILITY OF CALCIPOTRIOL PSEUDOPOLYMORPHS IN PHARMACEUTICAL CREAMS

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ABSTRACT

Calcipotriol is a vitamin D analogue and is used as active pharmaceutical ingredient in pharmaceutical formulations against psoriasis. It exists in two pseudopolymorphs, anhydrate and monohydrate, the latter of which, can be found in the commercially available cream Dovonex®. The percentage of the API in the cream is as low as 0.005 wt %.

Methodologies were developed for monitoring calcipotriol’s hydrate state in creams exhibiting pharmaceutical strength considerably lower than the detection ability of all commonly used techniques. The methods were based on X-Ray Powder Diffraction and micro-Raman spectroscopy. Transformation of calcipotriol anhydrate crystals to monohydrate was found to take place in the presence of cream’s excipients.

INTRODUCTION

Polymorphs of an Active Pharmaceutical Ingredient (API) can have different chemical and physical properties and, thus, a direct impact on the process-ability of the drug substance and the quality/performance of the drug product, such as stability, dissolution, and bioavailability. One polymorph may convert to another during manufacturing and storage, particularly. Therefore, identification of the crystal phase of an API is of outmost importance.

Polymorph identification in pharmaceutical formulations is often very difficult due to the small strength of API in the formulation. The analytical techniques used for polymorph identification are X-Ray Powder Diffraction (XRPD), Raman and IR spectroscopy. The detection limits of these techniques vary between 0.5 and 2 wt % and sometimes are two orders of magnitude higher than the percentage of API in formulation. Such an example is the presence of calcipotriol in cream.

Calcipotriol is a vitamin D analogue and is used as API in pharmaceutical formulations against psoriasis. It exists in two pseudopolymorphs, anhydrate and monohydrate [1], the latter of which, can be found in the commercially available cream Dovonex®. The percentage of the API in the cream is as low as 0.005 wt %.

Since the monohydrate API is patent protected [2], tracing of anhydrate crystals in the cream as well as evaluation of their stability is needed.

The scope of the present work was the development of a methodology for the identification of the hydrate state of calcipotriol crystals in the pharmaceutical cream. Two approaches were followed: Most of the excipients in the cream are in non-
crystalline form having no XRD pattern. On the other hand, the respective patterns of the pure pseudopolymorphs showed no overlapping reflections. Thus, identification of both of hydrate state in a cream formulation by XRPD would be an easy case as long as their percentage exceeds the detection limit of the method. Mixtures of the placebo with large amounts of anhydrate calcipotriol were prepared, XRD spectra of which, were regularly acquired and the stability of the polymorph in time was monitored. A major drawback of the technique was the large detection limit as well as the fact that XRD is a bulk technique.

Study of the crystals in a microscopic level was also attempted. Mixtures of anhydrate polymorph with placebo, in quantities simulating the formulation strength, were prepared and their Raman signal was recorded focusing the laser beam on the crystals through a microscope. In this way minimal intervention from cream excipients was expected.

EXPERIMENTAL

Chemicals and reagents
Calcipotriol anhydrate, Calcipotriol monohydrate, cream placebo and finished product (Cipocal) with 0.005 wt% anhydrate API were provided by Pharmex SA. Dovonex®, having monohydrate calcipotriol, was purchased by a local pharmacy store. The calcipotriol anhydrate was kept at 4 °C and found to be stable for a period of approximately 3 months, according to the periodically recorded XRD patterns.

Instrumentation
The micro-Raman spectra were recorded using a T-64000 Jobin-Yvon/Horiba with a monochromator slit 300 µm and grating with 600 grooves/mm. Detector type: 2D CCD (operating at 140 K); Source: 785 nm CrystaLaser (CW diode laser); Laser Power was set at 3 mW; Acquisition time: 2x400 sec; Resolution: Approximately 8 cm⁻¹; Other Parameters: A proper interference filter rejected plasma lines. The excitation beam was directed toward the microscope and with the use of a beam splitter and a microscope objective (50X/0.55 Olympus) it was focused on the sample. Raman scattered radiation was collected in a backscattering geometry by the same microscope objective and by passing through the beam splitter and a notch filter it was focused on the slit of a single monochromator.

The x-ray powder diffraction analysis (XRPD) was performed on a Bruker AXS D8 Advance using the Cu Kα (λ=1.54°A) radiation and Ni as Kβ filter. Detector type was LynxEye. Voltage was set at 40kV and current at 40mA. A 0.02 2-theta step-size was used. Samples were spread on PMMA sample holders having a 25 mm diameter and 1.5 mm depth circular cavity.

Crystal morphology studies were done with a Leica DM 2500M optic microscope equipped with a Leica DFC 420C 5 Megapixel camera.

RESULTS AND DISCUSSION

Differences between monohydrate and anhydrate Calcipotriol crystals
The diffraction patterns and the Raman spectra of both hydrate states were compared to each other and can be seen in Figs 1a, 1b and 2a, 2f, respectively. The anhydrate XRD differs significantly from the XRD pattern of the calcipotriol monohydrate
while the anhydrate Raman spectrum exhibits also several differences from the respective calcipotriol monohydrate spectrum.

Fig. 1. XRD patterns of calcipotriol monohydrate, calcipotriol anhydrate, calcipotriol anhydrate after mixing with Cipocal placebo cream.

Fig. 2. Raman spectra of calcipotriol monohydrate (a), calcipotriol anhydrate (f). Transformation of anhydrate to monohydrate after mixing with placebo takes place (e-b).
Identification of calcipotriol form in cream

The differences observed between the anhydride and monohydrate forms can be used for the identification of the calcipotriol form in a formulation. Since the formulations in question was cream and most of the excipients were non-crystalline material it was decided to rely on XRD for this study since a simplified pattern with no overlapping reflections is expected as opposed to the spectral information obtained from the other two widely used techniques for polymorph identification in formulations i.e. IR and Raman spectroscopy.

In Fig. 3 the XRD patterns of Dovonex cream and Cipocal cream are presented. The patterns are characterized by the lack of significant reflections mainly due to the absence of significant percentage of crystalline compounds in the above mentioned formulations. No calcipotriol diffraction peaks were observed and their absence should be attributed to the very small percentage of the API in the formulations (50 µg/g or 0.005 %). Usually the detections limits of the techniques such as XRD, IR and Raman, that are used to characterize the different polymorphs and pseudo-polymorphs are around to 0.5 wt%, two orders of magnitude higher that the current percentage of calcipotriol in the formulations.

![XRD Patterns]

Fig. 3. XRD patterns of Dovonex cream and Cipocal cream.

Thus, no conclusion can be drawn on the API form in Cipocal cream and Cipocal ointment i.e. if the anhydrate form added remained unchanged, based on the XRD patterns of the cream formulations.

Monitoring Calcipotriol anhydrate in Cipocal placebo using XRD

In order to circumvent the problem it was decided to mix placebo cream with larger amounts of the anhydrate calcipotriol and to regularly check its stability through time.

A dispersion of 20 mg anhydrate calcipotriol in 0.5 g of cream excipients was prepared. The mixture was kept at room temperature and was protected from the light but not from the atmosphere. The XRD patterns of the mixture are in fig 1.
The diffraction patterns of the API-cream placebo mixture indicate that anhydrate transforms gradually to monohydrate. Characteristic monohydrate reflections are marked in Fig. 1 with red arrows and the letter M.

**Monitoring Calcipotriol anhydrate using micro-Raman spectroscopy**
The second method used was micro-Raman spectroscopy. Micro-Raman can focus on individual crystals and thus signal interference from placebo ingredients can be avoided. On the other hand using this technique no quantitative information can be obtained since Raman signal from few individual crystals has no statistical value and thus the conclusion(s) drawn can be considered as strong indications rather than conclusive evidence.

Calcipotriol anhydrate was added in proper quantities in Cipocal placebo cream. Raman spectra were recorded with time, having as $t=0$ the mixing time of API and placebo, from different API crystals.

From Fig 2 it can be concluded that a transformation of the anhydrate crystals to monohydrate also takes place. The characteristic anhydrate vibration is marked with blue arrows and the letter A in Fig. 2 while the characteristic monohydrate peak with the letter M and a red line. The anhydrate peak gradually disappears and the monohydrate emerges.

**Monitoring anhydrate transformation using optic microscope**
The evolution of elongated monohydrate crystals from their anhydrate analogs in the presence of cream placebo was also monitored employing optic microscopy. Aggregate anhydrate particles with irregular morphology added in the placebo (Fig. 4a) were pictured to co-exist with elongated monohydrate crystals during their gradual transformation (Fig. 5) before the latter dominate the sample (Fig. 4b).

![Figure 4. Microphotographs of: a) Calcipotriol anhydrate crystals after the initial mixing with placebo, b) Calcipotriol monohydrate crystals after their transformation from their anhydrate analogue. Objective: 40x](image)
CONCLUSIONS
All methodologies have demonstrated that calcipotriol anhydrate is transformed to monohydrate in the presence of Cipocal cream excipients. This finding is not unexpected since for most of the anhydrate compounds that are known to exhibit also hydrate state(s) this is a naturally occurring process.

REFERENCES