IDENTIFICATION AND QUANTITATIVE ANALYSIS OF POSACONAZOLE API IN NOXAFIL® ORAL SUSPENSION

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ABSTRACT

X-ray Powder Diffraction (XRPD) and micro-Raman Spectroscopy were applied in the present work in order to develop methods capable of identifying and quantifying the Active Pharmaceutical Ingredient Posaconazole in Noxafil® oral suspension.

For both analytical techniques calibration lines were constructed by calculating the relative ratios of characteristic posaconazole and placebo vibration peaks. The non overlapping peaks at 18 2-theta for the API and 25.6 2-theta for the placebo were used for the XRD calibration line. Respectively for the construction of micro-Raman calibration line, the vibration peak at 1613 cm^{-1} was used as characteristic of the API and since all placebo peaks overlapped with the API ones the vibration peak at 644 cm^{-1} was used for the ratio I_{644} /I_{1613}. Limits of Detection and Quantification were calculated for the two methods. The DL of XRD method was found approximately 10mg/ml while the respected DL of micro –Raman about 3 mg/ml.

Identification of Posaconazole's polymorphic form in the commercial oral suspension was also attempted using X-ray Powder Diffraction (XRPD).

INTRODUCTION

Noxafil® is an azole antifungal agent available as concentrated solution to be diluted before intravenous administration (18 mg/ mL), delayed-release tablet (100 mg), or suspension for oral administration. Noxafil® oral suspension is a white, cherry-flavored immediate-release suspension containing 40 mg of posaconazole per mL as active substance. The active pharmaceutical ingredient (API) posaconazole is a triazole antifungal agent containing 4 chiral centres. [1],[2].

Polymorphism is a phenomenon relating to the occurrence of different crystal forms for one molecule. There may be several different crystalline forms for the same molecule with distinct crystal structures and distinct and varying physical properties like melting point, XRPD pattern, IR-spectrum and solubility profile. Six polymorphic forms (I, II, III, IV, X, Y) along with the amorphous are known but Form I is the more stable [1], [2]. In this study XRPD was used for the identification of the different posaconazole polymorphs in the oral suspension.

For the quantification of posaconazole several methods have been reported [3] such as HPLC, capillary electrophoresis, liquid chromatography–tandem mass spectrometry and bioassay. In this work it will be attempted to apply two non-destructive and easy to apply methods, Raman Spectroscopy and X-ray Powder Diffraction (XRPD), for the quantitative determination of posaconazole in noxafil® oral suspension.

EXPERIMENTAL

Chemicals and reagents

Noxafil® oral suspension (40mg/ml), posaconazole Form I, as well as the placebo mixture were provided by a Greek Pharmaceutical company. etc

Preparing the mixtures

Six dispersions were prepared in order to construct the calibration lines for both methods. The first five were made by adding placebo to commercially available syrup to achieve lower concentrations than the initial (2.5 mg/ml, 5mg/ml, 10mg/ml, 20mg/ml and 40mg/ml). The fifth was prepared by enriching the syrup with solid API in order to reach a concentration of 60mg/ml. In order to calculate the detection and quantification limit an additional dispersion was made of 2.5 mg/ml concentration.

Instrumentation

***Raman Spectroscopy*:**

A Renishaw, InVia, Raman microscope equipped with a laser with a 785 nm excitation line was used.

***Recording the spectra*:**
Analysis was done employing a Raman microscope (InVia Raman Microscope, Renishaw, UK). Spectra were acquired focusing a laser line at 785nm through an objective lens (20x) onto the sample. One drop of each dispersion was placed on a slide with gold substrate and reflected surface (mirror).The investigated area was 200-2500cm^{-1} with 5 accumulations each, under 80% laser power and 10 s exposure time.

XRPD
PXRD patterns were obtained at room temperature using an X-ray diffractometer (Bruker AXS D8 Advance with Bragg-Bretano geometry), LynxEye detector and Cu Ka spectral line (l=1,540562 A) with the generator voltage and current set at 40 kV and 40 mA, respectively. The sample holders had a central circular cavity of diameter 2 cm. In every sample holder was placed 1 ml of each dispersion. Samples were scanned from 3 to 35° 2-theta at a step size of 0.02(θ) and scan speed 1s/step.

In order to investigate further the identification of posaconazole in the oral suspension (40mg/ml) along with pre-commercial suspensions prepared by the Greek Pharmaceutical Company were left in fume hood for the water to be evaporated. In addition both suspensions were centrifuged (Biofuge Stratos, Heraeus, 15000 rpm for 10 minutes) for the same reason.

RESULTS AND DISCUSSION
Identification of API
X-ray diffractogramms of Noxafil® oral suspension (40mg/ml) were compared against Form I and placebo patterns (fig.1). Although several diffraction peaks are present the pattern was too noisy for a positive identification to be made probably due to low posaconazole's wt % in the dispersion. In order to overcome this problem it was attempted to increase posaconazole's wt % in order to facilitate the detection of Form I. For this the suspension was left in 25^{o}C for 24hr for the water to be evaporated and the XRPD pattern of the concentrated dispersion was re-recorded (Fig. 1). The diffraction peaks of the evaporated pattern can easily be attributed to Form I. An alternative path that was explored was to centrifuge Noxafil® oral suspension. Indeed, the data obtained from both the experiments were compared to Form I pattern (fig. 1) and the diffractograms were practically identical to each other.

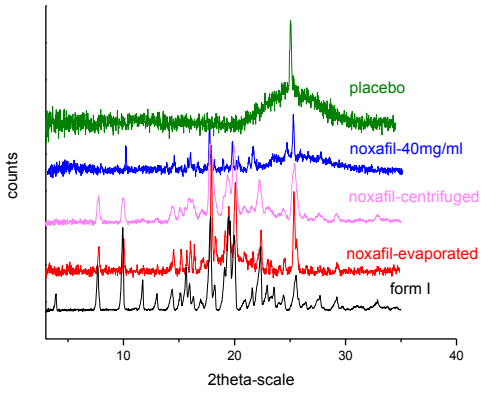

Figure 1.XRD diffraction patterns of Noxafil 40mg/ml Oral Suspension (blue line), Form I (black line), evaporated Noxafil 40mg/ml Oral Suspension (red line), centrifuged noxafil (pink line) and placebo pattern(green line).

Quantitative analysis of API

Raman
Raman spectra of form I, placebo and Noxafil® oral suspension (Fig.2) were obtained. The API characteristic vibration peak lies at 1613 cm^{-1} and so can be used for the quantitative analysis of posaconazole. All placebo vibrations overlap with API peaks. So for the placebo metrics the strong vibration at 644 cm^{-1} was chosen which is the sum of the API and placebo presence.

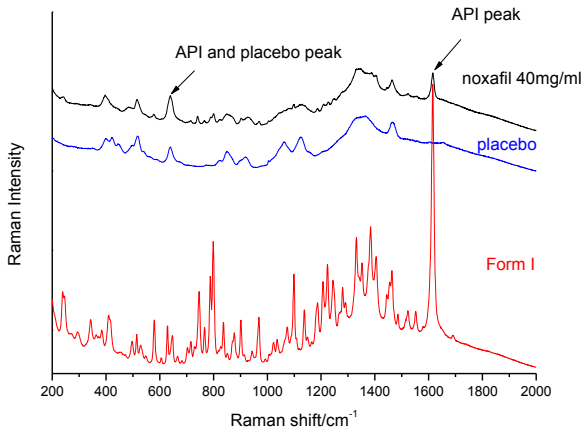

Fig. 2. Raman spectra of Form I (red line), noxafil 40mg/ml(black line) and placebo.

Calibration line

The equation of intensity of a Raman line depends on a variety of factors. The measured Raman intensity, I(v), can be represented as:

$$\boldsymbol{I_{(v)} = I_0 K_{(v)} C} \quad (1)$$

Where I_o is the intensity of the excitation laser line, v is the Raman shift, and K(v) is a factor which includes the frequency dependent terms: the overall spectrophotometer response, the self-absorption of the medium and the molecular scattering properties. C is the concentration of the Raman active species.

For the quantitative analysis the ratios of vibration peaks of API (1613 cm^{-1}) to the area of a simultaneous participation at 644 cm^{-1} were calculated. From equation (1), the ratio of the Raman areas of API's vibrations to the placebo combined with API peak against the concentration of API, is expected as:

$$\frac{A^{644}_{posaconazole,placebo}}{A^{1613}_{posaconazole}} = \frac{I_o}{Io}\frac{k^{posaconazole}_{644}}{k^{posaconazole}_{1613}}C_{posaconazole} + \frac{I_o}{I_o}\frac{k^{placebo}_{644}}{k^{posaconazole}_{1613}}C_{placebo} = \frac{k^{posaconazole}_{644}}{k^{posaconazole}_{1613}} + \frac{k^{placebo}_{644}}{k^{posaconazole}_{1613}}C_{posaconazole} \quad (2)$$

And assuming that placebo concentration in the final suspension is constant the plot is expected to yield a straight line:

$$\frac{A^{644}_{posaconazole,placebo}}{A^{1613}_{posaconazole}} = \boldsymbol{A} + \boldsymbol{B}\frac{1}{C_{API}} \quad (3)$$

Where $A=\frac{k^{posaconazole}_{644}}{k^{posaconazole}_{1613}}$ and $B=\frac{k^{posaconazole}_{644}C_{placebo}}{k^{posaconazole}_{1613}}$

After integration of these two peaks for each artificial dispersion with the appropriate software (OriginPro© 8) the calibration line (fig.3) was constructed and the final equation was as followed:

$$\frac{A^{644}_{posaconazole,placebo}}{A^{1613}_{posaconazole}} = \{3.69(\pm 0.14)\}\frac{1}{C_{API}} + \{0.91(\pm 0.14)\}, \mathbf{R^2 = 0.99453}$$

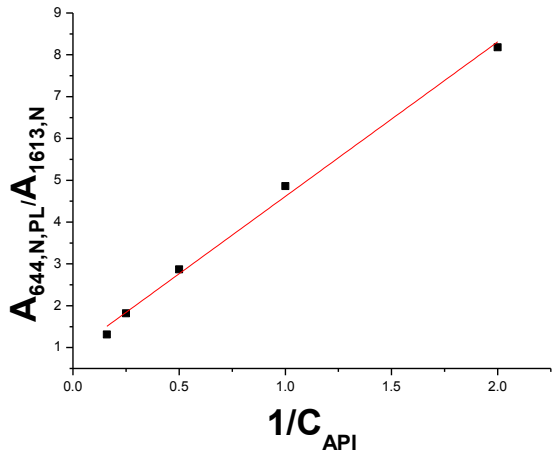

Fig.3. Calibration line of Raman Spectroscopy

Limit Of Detection (L.O.D.) of posaconazole in this method was calculated by visual evaluation. The detection limit was determined by the analysis of samples with known concentrations of analyte and by establishing the minimum level at which the analyte can be reliably detected.[4] The Raman spectra of 2.5mg/ml, 5mg/ml (fig.4) determined the detection limit approximately 5mg/ml.

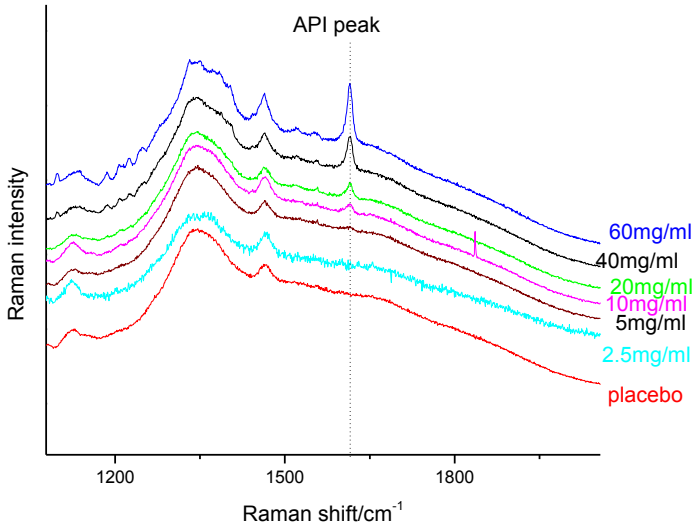

Fig.4. Raman spectra of artificial dispersions and placebo in the region of API peak.

The Limit of Quantification (L.O.Q.) of Posaconazole is: *L.O.Q=3xL.O.D.=9mg/ml*

XRPD

Despite the noisy diffractogram of the as –received oral suspension it was attempted to construct a calibration line using the no overlapping, characteristic API peak at 18 2-theta for the API and the respective placebo peak at 25.6 2-theta (fig.5). Accordingly, the equation of the calibration line was determined as:

$$\frac{A^{18}_{posaconazole}}{A^{25}_{placebo}} = \frac{I_o}{Io}\frac{k^{posaconazole}_{18} C_{posaconazole}}{k^{placebo}_{25} C_{placebo}} = \boldsymbol{k_{total} C_{posaconazole}} (3)$$

$$\frac{A^{18}_{posaconazole}}{A^{25}_{placebo}} = \{\mathbf{0.027}(\pm \mathbf{0.001})\} \boldsymbol{C_{API}} (4),\ \mathbf{R^2 = 0.9922}$$

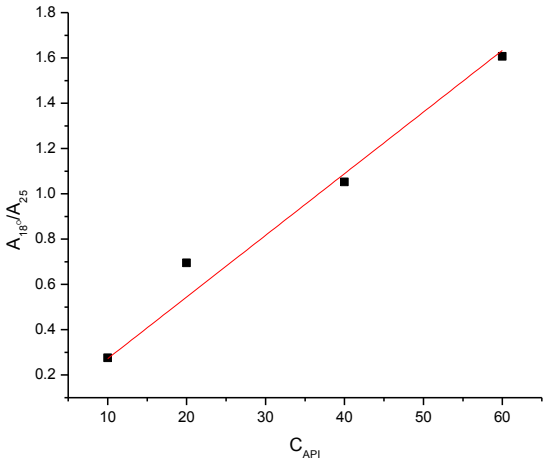

Fig 5. XRPD calibration line for quantification of posaconazole API.

As before Limit Of Detection (L.O.D.) of posaconazole in this method was calculated by visual evaluation. The XRD diffractograms of 2.5 mg/ml, 5mg/ml and 10 mg/ml (fig.6) were used. The detection limit found to be approximately 10mg/ml.

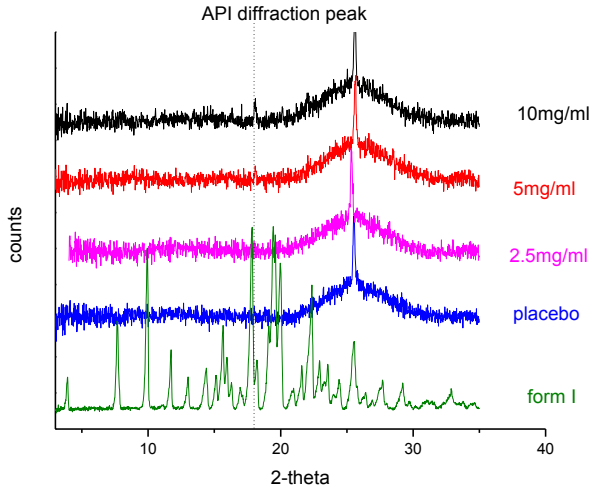

Fig.6. XRD diffraction patterns of Noxafil 10mg/ml Oral Suspension (black line), 5mg/ml(red line), 2.5mg/ml(pink line), Form I (green line), and placebo pattern (blue line).

The Limit of Quantification (L.O.Q.) of Warfarin Sodium is: *L.O.Q=3xL.O.D.=30mg/ml*

CONCLUSIONS

XRPD was successfully used for the identification of the polymorph phase of posaconazole in the oral suspension. XRPD and Raman spectroscopy were also applied for the quantitative determination of the API in the suspension. Detection limits were also determined as 3mg/ml for Raman Spectroscopy and 10 mg/ml for XRPD.

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