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Monotropically More Stable Novel Polymorph of Nitazoxanide with Improved Aqueous Solubility

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Nitazoxanide [2-[(5-nitro-1,3-thiazol-2-yl)carbamoyl]phenyl]ethanoate] is a drug used for antiprotozoal treatment. This drug belongs to biopharmaceutics classification system (II) and presently available in two high oral dosage forms-tablet (500 mg) and oral suspension (100 mg per 5 mL when reconstituted). A monotropically more stable novel polymorphic form (Form II) of nitazoxanide with improved aqueous solubility has been identified and fully characterized by a variety of analytical techniques such as powder X-ray diffraction, differential scanning calorimeter, thermogravimetric analysis, Karl Fischer titration, infrared spectroscopy, solution state nuclear magnetic resonance, polarized microscopy and high performance liquid chromatography techniques. Aqueous solubility of Form II is increased by 2.76 folds. Form II is obtained from less toxic and environment friendly Class III organic solvent ethanol.

Keywords: Polymorphs, Nitazoxanide, Antiparastic agent, Aqueous solubility, Powder X-ray diffraction.

INTRODUCTION

Nitazoxanide, chemically is 2-acetyloxy-N-(5-nitro-2-thiazolyl)benzamide used as antiprotozoal drug. The molecular formula is $C_{12}H_9N_3O_5S$ (Fig. 1). Nitazoxanide uses in medicine and is a prodrug. Nitazoxanide was successful in the treatment of metronidazole-resistant giardiasis. This novel agent has a broad spectrum of activity against many other gastrointestinal pathogens, including bacteria, roundworms, flatworms and flukes[1]. It is the first and only USFDA approved drug for treatment of *Cryptosporidium parvum* infection and also the first new drug approved for treatment of *Giardia lamblia* infection in ≥ 40 years[2,3]. Nitazoxanide belongs to biopharmaceutics classification system (II) and it has low aqueous solubility[1]. Nitazoxanide is presently available in two high oral dosage forms-tablet (500 mg) and oral suspension (100 mg per 5 mL when reconstituted)[2]. Nitazoxanide is first reported in USA patent US 3,950,351[4].

Polymorphism is the ability of a substance to crystallize in different crystal modifications, each of them having the same chemical structure but different arrangements or conformations of the molecules in crystal lattice[5]. Polymorphism has the potential to affect many aspects of drug development in the pharmaceutical industry. Multiple crystal forms with different solid-state properties can exhibit differences in bioavailability

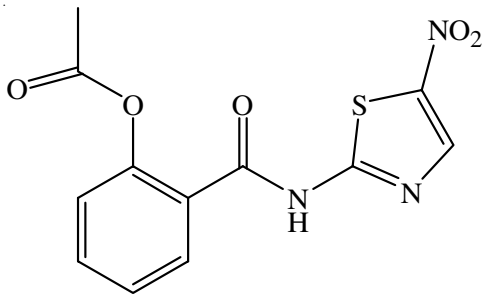

Fig. 1. Structure of nitazoxanide

of the active drug substance, shelf life of the drug and behave differently during processing. Different polymorphs exhibit different physicochemical properties such as solubility, dissolution rate, bioavailability, chemical and physical stabilities[6-8].

Solubility and dosage of drug are the important parameters and are always interlinked with each other in the treatment of a disease. In general, when solubility of drug is less, high dosage of drug is required. Increasing the solubility of a drug to certain extent has the following indirect advantages *viz.*, (a) dosage of the drug is reduced for the treatment of a disease (b) the manufacturing cost and (c) the toxic side effect caused by the drug. The various other techniques that are used to increase the solubility of the drug are (i) salt preparation (ii) particle size reduction (iii) co-crystals preparation, (iv) polymorphs, *etc.*[9,10].

Extensive literature survey revealed that until now no literature precedence is available pertaining to the polymorphs of nitazoxanide. As part of our ongoing research program, the present paper reports the monotropically more stable, reproducible polymorph (Form II) of nitazoxanide with improved aqueous solubility. Polymorphic modifications of nitazoxanide was characterized by means of typical structure-sensitive analytical techniques such as powder X-ray diffraction, DSC, TGA, FT-IR, NMR, polarized microscopy, Karl Fischer titration and HPLC analysis.

EXPERIMENTAL

Nitazoxanide (designated as Form I) is obtained from SUVEN Life Sciences Limited, Hyderabad, India and the purity of this drug is > 99.8 %. The solvents used in the study are of analytical grade. All the solvents used were purchased from Sigma-Aldrich.

Preparation of Form II of nitazoxanide: Form I of nitazoxanide (3.0 g) was taken in a round bottom flask containing 60 mL of ethanol. The mixture was slowly heated under constant stirring (400 rpm). The solids dissolved and a clear solution formed at reflux temperature. The mass was cooled to 0-5 °C and maintained at the same temperature under stirring (200 rpm) for 3 h. The crystalline solids obtained were then filtered and the mass was dried under reduced pressure to obtain crystalline product, Form II (2.8 g). Yield: 90 %.

Polarized microscopy (PM): Microphotographs were obtained by using polarized microscopy (Nikon LV100). Images were generated under transmitted light with partially crossed polarizers.

Determination of particle size: Determination of particle size was carried out using optical microscopy with a calibrated eye piece micrometer and stage micrometer by taking a small quantity of formulation on slide. About 100 microcrystal size was measured individually, average was taken and their size range and mean diameter frequency was calculated.

Average particle size is calculated by the formula:

$$\text{Average particle size} = \varepsilon nd/n$$

Differential scanning calorimeter: Differential scanning calorimeter (DSC) thermograms were obtained by a differential scanning calorimeter (Model Q100, TA instruments). The measurements were made using aluminium sample pan, using about 2-10 mg samples under nitrogen atmosphere, at a scanning speed of 2 °C/min.

Solution state nuclear magnetic resonance spectra: NMR spectra were obtainted in a Bruker NMR with a Bruker AC 200 console (Bruker, Germany). The spectra were processed with Win NMR software (Bruker, Germany). The samples were prepared dissolving 5 mg of each form in 0.5 mL of DMSO-d_6 with 0.03 % of tetramethylsilane (TMS) used as reference for $\delta_H = 0$.

Thermogravimetric analysis (TGA): Thermogravimetry (TG) curves were obtained with a thermogravimeter (Model Q500, TA instruments). The measurements were made using a 50 mg platinum pan (sample weight about 10 mg) under nitrogen atmosphere at a scanning speed of 2 °C/min. Mass loss (%) was calculated based on the mass of the original sample.

Karl Fischer titration (KFT): Water content (% w/w) of the samples (200 mg) was determined by Karl Fischer titrimetry (716 DMS Titrino, Metrohm Limited, Switzerland). The instrument was calibrated by using deionized water, before sample analysis.

Powder X-ray diffractometry (PXRD): The powder X-ray diffraction pattern was measured with an X-ray diffractometer (Model RINT Ultima, Rigaku Denki). The conditions of measurement were as follows: target Cu, monochrometer graphite, voltage 45 kV and current 40 mA, with a scanning speed of 1 °C/min. Approximately 200 mg of sample were loaded into the sample holder.

Fourier transform infrared spectroscopy (FT-IR): FT-IR spectra were recorded on a Bomem MB-120 infrared spectrometer. Spectra over a range of 5000 to 500 cm^{-1} with a resolution of 1 cm^{-1} (32 scans) were recorded using KBr pellets. For diffuse reflection analysis, samples weighing approximately 2 mg were mixed with 200 mg KBr by meas of an agate motor and pestle and placed in sample cups for fast sampling.

Aqueous solubility measurement by high performance liquid chromatography: Aqueous solubility is measured by Agilent HPLC system (1100 series) with YMC ODS-AM, 250 × 4.6 mm, 5 µm column having a UV visible detector. The mobile phase was a mixture of buffer (5 mM ammonium acetate in HPLC grade water, adjust the pH to 3.0 with formic acid) and acetonitrile (Gradient) and the flow rate was 1.0 mL/min. The detection wavelength is set at 232 nm. Sample volume of 10 µL was injected with an automatic injector.

Procedure: The sample was prepared by weighing accurately about 2 mg of sample and transferring it into a 2 mL eppendorf tube. Added 1 mL HPLC grade water and vortexed for 2-5 min. The eppendorf tube was placed on a rugged rotator and kept under rotation at 30 % speed by adjusting the knob. It was rotated for 48 h and then removed the eppendorf tube from the rotator. It was centrifuged for 2 min at 3000 rpm and transfer supernatant solution into sample vial. The standard was prepared by weighing accurately about 2 mg of the sample. It was transferred to 10 mL volumetric flask, added 1 mL methanol and vortexed for dissolution. Finally it was made up to the mark with water. Separately injected water as blank. Sample and standard were injected three times and recorded the areas of standard and sample.

Aqueous solubility calculation:

$$\text{Sample concentration (µg/mL)} = \frac{\text{Area of sample}}{\text{Area of standard}} \times \text{Standard concentration (µg/mL)}$$

Accelerated stability study: Form I and Form II was packed in polyethylene laminated aluminium foils of thickness 0.04 mm and stored for stability under ICH specified accelerated stability conditions of storage for zones III and IV at 40 °C/75 % RH. The samples were characterized after 90 days by using powder X-ray diffraction, DSC, TGA, FT-IR, NMR, polarized microscopy, Karl Fischer titration and HPLC.

RESULTS AND DISCUSSION

Nitazoxanide is soluble in DMSO and THF, slightly soluble in acetone and ethanol and practically insoluble in other Class II and Class III organic solvents. However, it is slightly

soluble in acetone and ethanol. Therefore, only these two solvents were used for screening polymorphic forms of nitazoxanide.

Crystallization of Form I from acetone always gave the same type of crystals (Form I), while different type of crystals were obtainted in the crystallization from ethanol (Class III solvent), which is less toxic and enivornment friendly. Hence, this new form of crystals was named as Form II. Form II is analyzed by HPLC showing that no by products or decomposition compounds had been formed during the preparation of the crystals.

In principle, the first and the foremost technique that is adopted after preparation of the new form of crystal (in this case Form II) is to analyze the morphology of Form II. Images of crystals representing the morphologies of Form I and Form II are presented in Figs. 2 and 3. They showed difference in morphology between the two forms. Both the forms are found crystalline in nature. Crystals of Form I are in block shape with average particle size of 110 mm to 120 mm (Fig. 2) and crystals of Form II are in thin diamond shape with average particle size of 30 mm to 40 mm (Fig. 3). The difference in birefringence of Form I and Form II is the preliminary evidence for the existence of polymorphism.

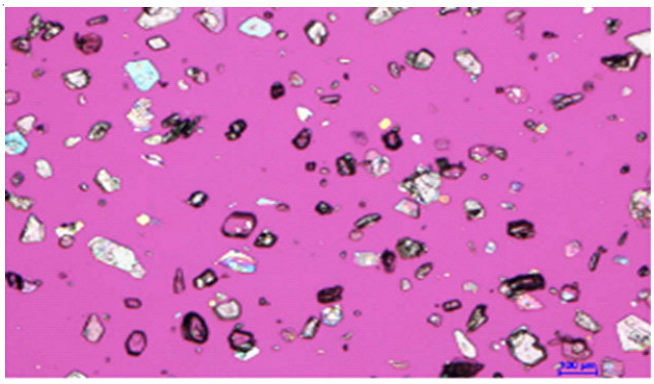

Fig. 2. Microphotograph of Form I

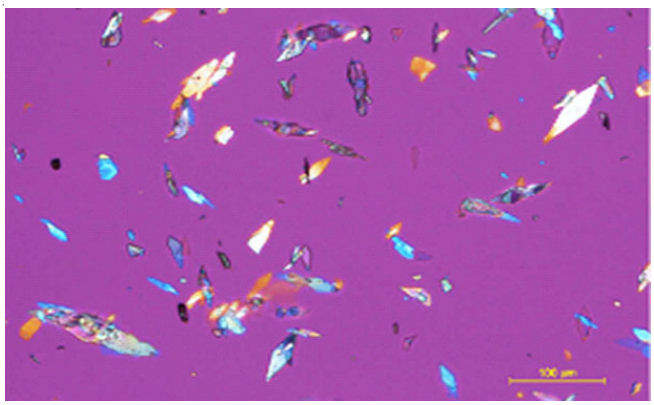

Fig. 3. Microphotograph of Form II

The evidence for the two polymorphs, Form I and Form II was further supported by powder X-ray diffraction patterns (Figs. 4 and 5). These show distinct differences in positins and relative intensities. Form I shows characteristic peaks at 12.96, 17.71 and 20.88 (2θ ± 0.2° 2θ), while Form II shows characteristic peaks at 15.88, 21. 22 and 26.62 (2θ ± 0.2° 2θ).

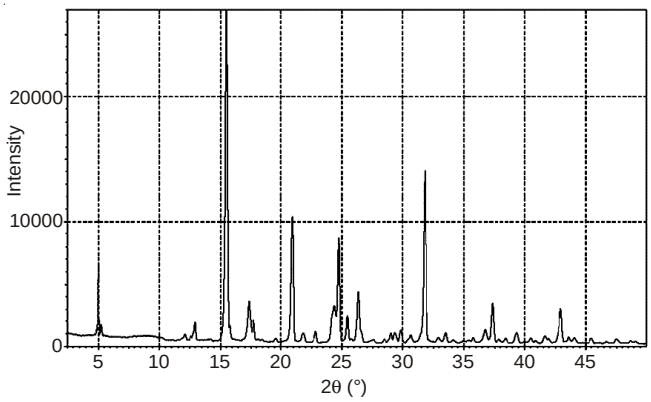

Fig. 4. Powder X-ray diffraction pattern of Form I

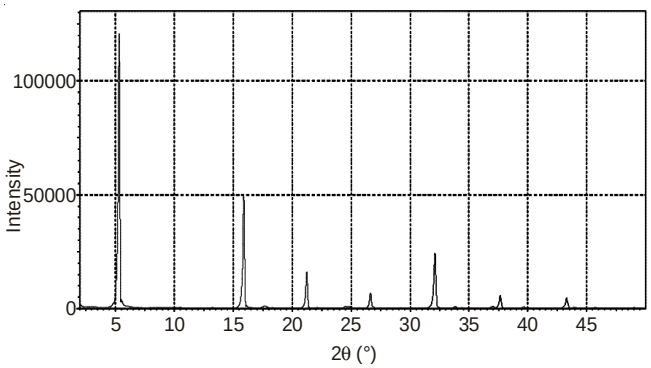

Fig. 5. Powder X-ray diffraction pattern of Form II

The observed variation patterns in the powder X-ray diffraction indicates that they have different crystal structure and therefore two polymorphic forms of nitazoxanide. The sharp diffraction peaks of Form I and Form II indicates both forms are in crystalline nature.

A careful observation of ^{1}H NMR of Form I and Form II indicated that both the crystals did not correspond to solvates or hydrates and the possibility of presence of impurities was also discarded.

The crystalline nature and relative stability of Form I and Form II was determined by DSC thermogram (Figs. 6 and 7). Form I showed single sharp exothermic peak at 201.14 °C with a heat of fusion of 43.1 kJ/mol and Form II showed single sharp exothermic peak at 204.71 °C with a heat of fusion of 50.5 kJ/mol. These results suggest both Form I and Form II are crystalline nature. The DSC data provided insight into the relative stability of Form I and Form II. The presence of single exothermic peak of DSC thermogram in Figs. 4 and 5 indicate absence of solvate or hydrate in Form I and Form II. Based on the heat of fusion rule[11], the higher melting point form with higher enthalpy of fusion is monotropically more stable form. Thus, Form II is thermodynamically more stable than Form I at all temperatures up to melting points of either forms. Since, thermodynamically most stable form is always preferred choice for pharmaceutical development. Therefore, Form II is suitable for doing further research on nitazoxanide.

The TG curve of Form I and Form II (Fig. 8) showed no weight loss in melting. These results suggests both Form I and Form II are neither solvated nor hydrated, which was also confirmed by liquid ^{1}H NMR data.

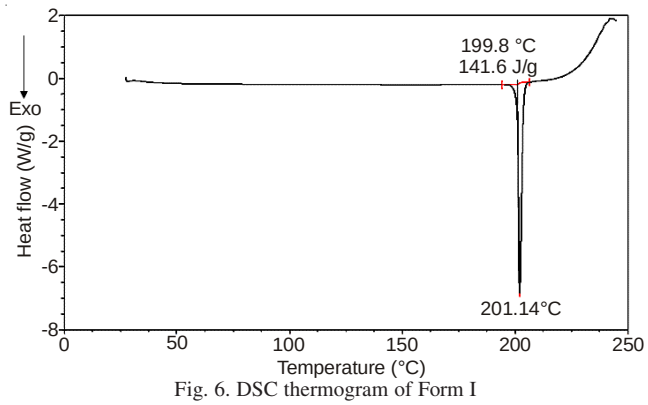

Fig. 6. DSC thermogram of Form I

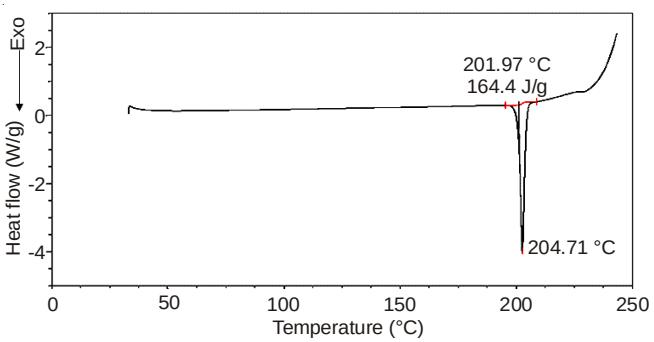

Fig. 7. DSC thermogram of Form II

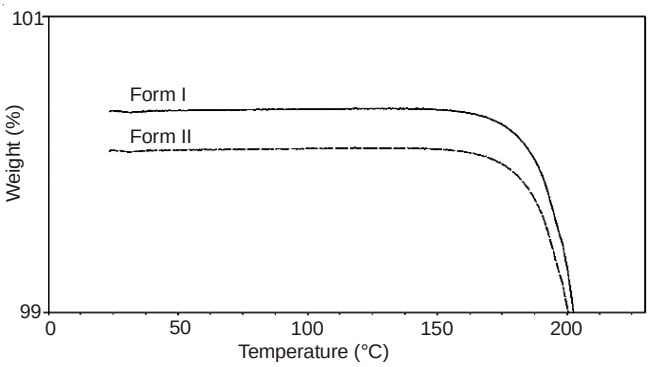

Fig. 8. TG curve of Form I and Form II

Further evidence for the absence of solvates and hydrates was supported Karl Fischer titration, which clearly demonstarted that no water content is found in Form I and Form II, further more these data is in conccurence with the results observed in TGA and DSC.

Most of the absorption peaks in the finger print region of Form I were comparable to the corresponding peaks in the Form II spectrum. A part from some similarities between the two spectra, significant difference in wavelengths between Form I and Form II can be seen in the whole spectral region. For instance, following peaks of Form I: 3049 cm^{-1}, 1829.56 cm^{-1}, 1470 cm^{-1} and following peaks of Form II: 3044.77 cm^{-1}, 1825.70 cm^{-1}, 1472.71 cm^{-1} are having difference. This suggests difference in molecular packing of Form I and Form II.

Aqueous solubility of Form I and Form II are measured by using HPLC. Aqueous solubility of Form I is 2.11 µg/mL and Form II is 5.83 µg/mL. These results clearly indicating increase in aqueous solubility of Form II when compared to the aqueous solubility of Form I by 2.76 folds. The average particle size of Form II is reduced nearly to three folds and aqueous solubility results are co-relating with the particle size results

Form I and Form II were subjected for accelerated stability study and were found to be stable under ICH specified accelerated stability conditions of storage for zones III and IV at 40 °C/75 % RH. No change is observed in aqueous solubility, morphology, desolvation, melting behaviours and powder X-ray diffraction pattern.

Conclusion

A montropically more stable novel polymorphic form (Form II) of nitazoxanide with improved aqueous solubility has been identified. From the DSC results, it concluded that the most physically stable form of nitazoxanide is Form II. The X-ray diffractograms as well as DSC themograms of Form I and II are very different and enable a clear, fast identification of the polymorphs. HPLC analysis showed the increase in aqueous solubility of Form II by 2.76 folds. Therefore, Form II of nitazoxanide can be used for carrying out further research aiming to decrease the dosage of nitazoxanide drug in anti-protozoal treatment.

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