Structural Determination of the Stable and Meta-Stable Forms of Atomoxetine HCl Using Single Crystal and Powder X-Ray Diffraction Methods

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ABSTRACT: Strattera™ is the first FDA-approved nonstimulant medication for the treatment of Attention Deficit Hyperactivity Disorder (ADHD) in children, adolescents, and adults. Two polymorphic forms and an amorphous form of the active pharmaceutical ingredient, atomoxetine HCl, were discovered during drug development. The thermodynamically stable polymorphic form was selected for the commercial product. The stable form readily grows as crystals suitable for single crystal diffraction. The meta-stable crystal form is isolated by rapid crystallization, providing crystals that are too small for routine single crystal methods; consequently its structure was determined by X-ray powder diffraction. © 2006 Wiley-Liss, Inc. and the American Pharmacists Association J Pharm Sci 95:1677–1683, 2006

Keywords: Strattera™; atomoxetine HCl; polymorphism; single crystal; structure; X-ray powder diffraction.

INTRODUCTION

Polymorphism is an important property of pharmaceutical solids, since it can potentially impact the performance of the drug product. As a consequence, it is important to identify its existence and apply analytical technologies that ensure consistency and control of the active pharmaceutical ingredient that is used in the drug product.[1] Determination of crystallographic structure is one of the most powerful methods for characterizing and ensuring the purity of crystalline standards; by comparing calculated powder patterns to experimental patterns,[2] understanding the organizational arrangement that lead to its facial development and morphology, and composition that influence its physical properties. In fact, once the crystallographic structure has been established, morphology modification can readily be made by analysis of forces that cause directional growth and through the addition of "tailor made" inhibitors that disrupt growth and alter crystal morphology in a reasonably predictable way.[3] Fortunately, recent developments in extracting information from powder patterns,[4] computational algorithms for pattern idexation,[5] structure solution,[6] and refinement has led to our increased ability to solve crystal structures from powder diffraction.

Usually it is simple to obtain single crystals of the stable polymorphic form, since growth conditions can proceed under conditions that lead to single crystals of sufficient size and quality, that is, under thermodynamic control. Typically more challenge is involved in growing meta-stable forms where growth and isolation occurs under kinetically controlled conditions. These conditions often produce smaller crystals of poor quality relative to the stable form. This is the exactly the situation faced with atomoxetine HCl.

Atomoxetine,[*R*(−)-*N*-methyl-gamma-(2-methylphenoxy) benzenepropanamine], is a highly

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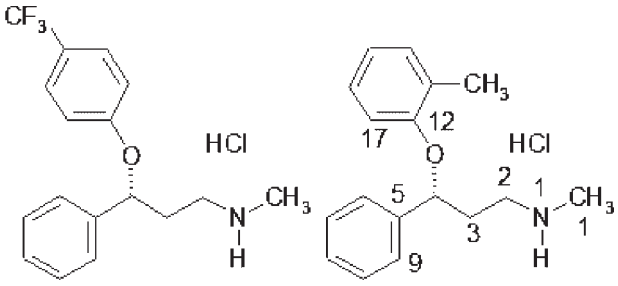

Figure 1. Molecular structure and numbering scheme used for fluoxetine HCl (left) and atomoxetine HCl (right).

selective norepinephrine reuptake inhibitor (Fig. 1). Atomoxetine HCl hydrochloride has been approved for the treatment of Attention Deficit Hyperactivity Disorder (ADHD) in children and adults. The following report describes the two crystalline forms of atomoxetine HCl that have been identified. To date, the crystal structure of this compound has not been reported, although the X-ray powder diffraction patterns for one of the two polymorphs, that is Form I, was reported in the powder diffraction file (PDF 00-038-1605) in 1987.[7] The following report provides the crystallographic structure of both the meta-stable and stable polymorphic forms of atomoxetine HCl. The crystal structure of the stable crystalline form was determined by single crystal X-ray diffraction. Form II crystals of suitable size could not be grown so its structure was determined by X-ray powder diffraction.

During initial attempts to scale up the crystallization process, the meta-stable crystalline form was crystallized on two of the first three campaigns. Later attempts to isolate this form were unsuccessful. As a consequence, the crystal structure was determined by using the X-ray powder diffraction technique using Monte Carlo Simulated Annealing procedures in combination with energy optimization leading to the solution of its structure. This study provides an illustration of the utility of the X-ray powder diffraction method for structure determination of meta-stable crystalline forms.

MATERIALS AND METHODS

Materials

Preparation of solid forms—Form I crystals have been produced by evaporative crystallization from a number of different solvents including ethanol, acetone, and ethyl acetate. Form II crystals are produced by rapid precipitation from solutions. One method for doing so is to add 1 molar equivalent of HCl gas to a concentrated ethyl acetate solution of the free base form of atomoxetine, at room temperature, followed by isolation of the precipitated crystalline form. Amorphous materials were produced by the melt-quench technique; liquid nitrogen or a DSC refrigerated cooler was used as the cooling medium.

Single Crystal Diffraction

A clear single crystal of approximate dimensions 0.25 mm × 0.25 mm × 0.25 mm was mounted on a thin glass fiber and immersed in a stream of nitrogen at −100°C. Data were collected using a CuK_α radiation source ($\lambda = 1.54178$ Å) and a Bruker–Nonius Kappa geometry diffractometer equipped with a SMART 6000 CCD area detector.[8] Cell refinement and data reduction were performed using the *SAINT* program. The unit cell was indexed, having orthorhombic parameters and systematic absences consistent with the space group P 2(1)2(1)2(1). The structure was solved by direct methods.[9] All nonhydrogen atom atomic parameters were independently refined. The hydrogen atoms on the amine nitrogen were located in the successive difference Fourier maps and refined independently. The hydrogen atoms on the carbon atoms were placed at calculated positions and added to the structure factor calculations. The space group choice was confirmed by successful convergence of the full-matrix least-squares refinement on F^2.[10] Structural details are provided in Table 1 and in supplement.

X-Ray Powder Diffraction

Patterns were obtained on a Siemens D5000 X-ray powder diffractometer, equipped with a CuK_α source ($\lambda = 1.54056$ Å) and a Kevex solid-state detector, operated at 50 kV and 40 mA. Each sample was scanned from 4 to 35° 2θ at a step size of 0.03°, a scan rate of 10 s/step. Fixed slits were used with a 0.6-mm aperture slit, 1-mm anti-scatter slit, and 0.2-mm detector slit.

Differential Scanning Calorimetry

A Seiko DSC 210 was used under the following conditions: scan rate 10°C /min, crimped Al pan, 50 mL/min N_2 purge. The instrument was calibrated using indium and antimony according

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Table 1. Crystal Data and Structure Refinement for Atomoxetine HCl Form I and Form II

Crystal Form	I	II
Empirical formula	C_{17} H_{22} Cl N O	C_{17} H_{22} Cl N O
Formula weight	291.81	291.81
Temperature	173(2) K	293(2) K
Wavelength	1.54178 Å	1.54056 Å
Crystal system	Orthorhombic	Monoclinic
Space group	P2(1)2(1)2(1)	P2(1)
Unit cell dimensions	$a = 7.2708(2)$ Å, $b = 13.2106(4)$ Å, $c = 16.6775(6)$ Å	$a = 11.36$ Å, $b = 7.39$ Å, $c = 10.02$ Å, $\beta = 93.77^\circ$
Volume, Z	1601.90(9) $Å^3$, 4	839.9 $Å^3$, 2
Density (calculated)	1.210 g/cm^3	1.154 g/cm^3
Crystal size	0.25 mm × 0.25 mm × 0.25 mm	<10 micron powder
Theta range for data collection	4.27–63.84°	5.00–30.00°
Final R indices [I>2sigma(I)]	$R1 = 0.0450$, $wR2 = 0.0761$	$R_w = 0.1163$, $R_p = 0.0883$
Absolute structure parameter	0.01(2)	
Extinction coefficient	0.013(2)	
Largest diff. peak and hole	0.285, −0.228 e· $Å^{-3}$	

to the manufacturer's procedure. All melting temperatures were the onsets of melting endotherms. The melting data reported (Tab. 1) were the average values of 2–3 measurements. Estimated standard errors were ±0.05°C for temperatures and ±0.1 kJ/mole for enthalpies.

Determination of T_g was accomplished using a TA DSC2920 operated under these conditions: heating rate 10°C/min, crimped aluminum pan, 40 mL/min N_2 purge. Subambient cooling was provided by the refrigerated cooling system (RCS). The temperature and heat flow were calibrated using indium. The procedure for T_g measurement was as follows: (1) heat 5–10 mg of the atomoxetine HCl sample (Form I) to about 2°C above the end of the melting endotherm; (2) quench the melted sample through contact with dry ice; (3) scan the sample from −30°C for T_g. Rapid cooling was found to be necessary to prevent the crystallization of atomoxetine HCl.

Crystal Structure Determination from X-Ray Powder Diffraction Data

Computational methods--All simulations were carried out using Accelrys Materials Studio modeling and simulation product suite.[11] The X-ray powder diffraction data collected for atomoxetine HCl Form II were indexed by X-Cell using the first 20 reflections.[12] Using the successive dichotomy approach, which provides a complete list of all possible cells, along with the zero-point shift of the diffraction pattern, the space group was determined to be P2(1) ($Z = 2$). All subsequent steps of the crystal structure determination procedure were carried out using Reflex Plus and VAMP.[13] The cell parameters, background, zero-point shift, profile parameters, and peak intensities were refined by the Pawley method[14] implemented within Reflex Plus. The following conditions produced a structure for Form II with $R_{wp} = 0.0885$: The Pseudo–Voigt profile function was used for simulating the peak shape. The background was determined by linear interpolation using 20 coefficients. The Finger-Cox-Jephcoat method of asymmetry correction was used due to axial divergence.[15]

After an initial (AM1[16]) geometry optimization of the atomoxetine cation was performed using the semiempirical molecular orbital software package, VAMP, the structure solution was performed by the parallel tempering method[17] employed in Powder Solve (Reflex Plus). A close contact energy penalty function was applied during structure determination to prevent solutions with overlapping atoms being generated. The March–Dollase method[18] was used for modeling preferred orientation during the structure solution search. The asymmetric unit was defined with six torsional degrees of freedom (DOFs) for the six single bonds, which define the backbone of the cation, five (two[19] translational and three rotational) additional DOFs for the cation motion group, and three translational DOFs for the chlorine anion, bringing the total DOFs for the asymmetric unit to 14. The search was repeated five times, each with 18.7 million steps, to ensure a high probability that the best solution to the crystal structure was

found. The best solution to the crystal structure was further refined by the Rietveld method.[20]

Pareto Optimization

Rietveld refinement with energy represents a multiobjective optimization problem where a solution should have a low energy and a good match between simulated and experimental pattern at the same time. Generally, both criteria cannot be met optimally simultaneously and there will not be a single "best" solution. The decision on what solution to select depends on specific refinement task: is it more important in this particular case to have a good fit with the experimental pattern or is it critical that the energy is close to its minimum Pareto optimization finds sets of optimal solutions based on the optimization of a combined figure of merit ($R_{comb} = (1 - \omega)R_{wp} + \omega R_{energy}$).[21] In this context, it means that no other solutions could have a lower energy and a lower value of R_{WP} at the same time, although there might be solutions with either lower R_{WP} or lower energy, but not both. Pareto optimization only provides the set of optimum solutions, the decision which of the optimum solutions to select is taken by the user according to specific criteria. In an energy versus R_{wp} chart, the optimal solutions form a curve, the Pareto front, separating the continuum of all possible solutions to the right from the (energy, R_{WP}) region to the left, where no solutions exist. In nearly all cases, the absolute optimum solution having minimum energy and minimum R_{WP} is part of an inaccessible region.

RESULTS AND DISCUSSION

Stability Relationship between Atomoxetine HCl Polymorphs

The stability relationship between the polymorphs of atomoxetine HCl (I and II) has been determined using differential scanning calorimetry. The higher melting Form I, 167.9°C, was found to have a higher heat of fusion, 38.9 kJ/mole. Form II melting point was 164.3°C with an enthalpy of fusion of 36.8 kJ/mole. Therefore, according to the Heat of Fusion Rule,[22] Form I is more stable than Form II at any temperature (monotropy). The glass transition of the amorphous form of atomoxetine HCl was determined to be 30.1°C.

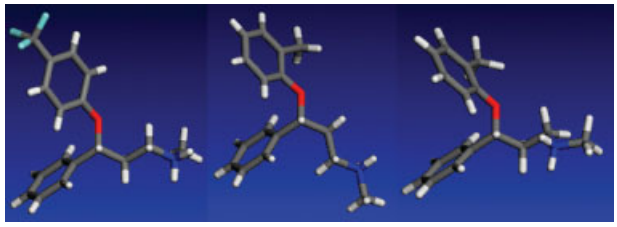

Figure 2. Molecular modeling of conformation found in fluoxetine HCl (left), atomoxetine HCl Form I (middle), and Form II (right).

Single Crystal Structure

Atomoxetine HCl crystallizes in two polymorphic forms, Forms I and II. Whereas the crystal structure of Form I was readily determined using single crystal X-ray diffraction methods, the structure of the meta-stable form could only be determined by a X-ray powder diffraction method (Tab. 1).

Molecular Conformation

One chiral center is present in the molecular structure of atomoxetine. The stereochemistry was confirmed by anomalous dispersion; the absolute structure parameter refined to the value of 0.01(2) indicating the *R*-configuration about atomoxetine's chrial center.[23] One independent molecule was observed in the asymmetric unit the structure of both polymorphic forms of atomoxetine HCl and this was consistent with the observations in ^{13}C CPMAS spectra collected using solid-state NMR. The molecular conformation of the aromatic ring portions of the molecules in the two polymorphic forms of atomoxetine HCl were similar to one another as well as to that of the structurally similar molecule fluoxetine HCl.

Unlike fluoxetine HCl, whose amino side chain exists in *gauche* and *trans* conformations about the

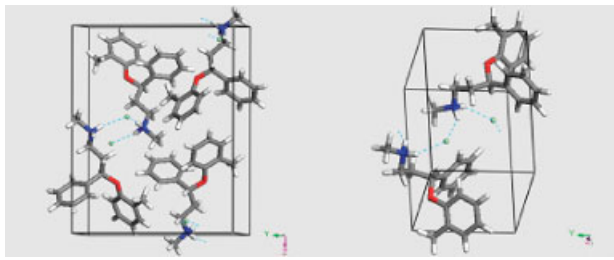

Figure 3. The molecular packing in Form I (left) and Form II (right).

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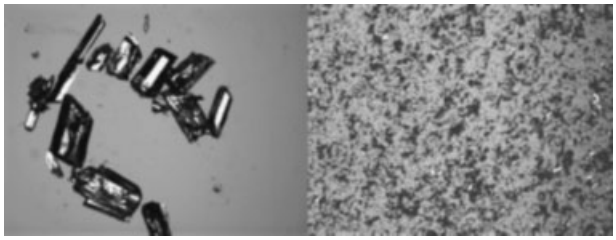

Figure 4. Photomicrographs of crystals of atomoxetine HCl Form I (left) and Form II (right.)

N1 C2 and C2 C3 bonds, respectively, Form I of atomoxetine's side chain exists in an extended *trans* conformation. Atomoxetine HCl Form II side chain exists in the *trans* and *gauche* conformation about the corresponding torsions and could be considered as conformational polymorphism due to the free rotation about the single bonds of the side-chain.[24] Figure 2 provides a comparison of the molecular conformations present in the three related molecular structures.

Crystal Packing

Figure 3 provides the unit cell packing of atomoxetine HCl in Forms I and II. The hydrogen bonding network in both crystal forms are composed of extended chains where the chloride ion serves as a hydrogen bond acceptor for two amino protons on neighboring screw-related atomoxetine molecules. Table 1 presents details of the crystal structure of the two polymorphic forms. The atomic coordinates, torsion angles, bond lengths, and bond angles are provided in supplementary material.

Crystal Morphology

The crystal faces of the "equant"shaped Form I crystals were indexed using a single crystal X-ray diffractometer. Crystals of atomoxetine HCl grown from ethanol have essentially the morphology depicted in Figure 4. Different solvent systems provide morphologies in which the faces may be developed to different extents. Crystals of atomoxetine HCl Form II have a much finer particle size causing difficulty in its structure determination by standard methods. A comparison between the experimentally observed X-ray powder diffraction pattern, Figure 5, for the meta-stable crystalline form versus the powder pattern that is calculated from the best structural solutions shows a high degree of similarity and provides confidence in the correctness of the solution.

X-Ray Powder Diffraction Patterns of Crystal Forms

The X-ray powder diffraction measurements revealed different crystalline forms of atomoxetine HCl. Comparison of the X-ray powder diffraction

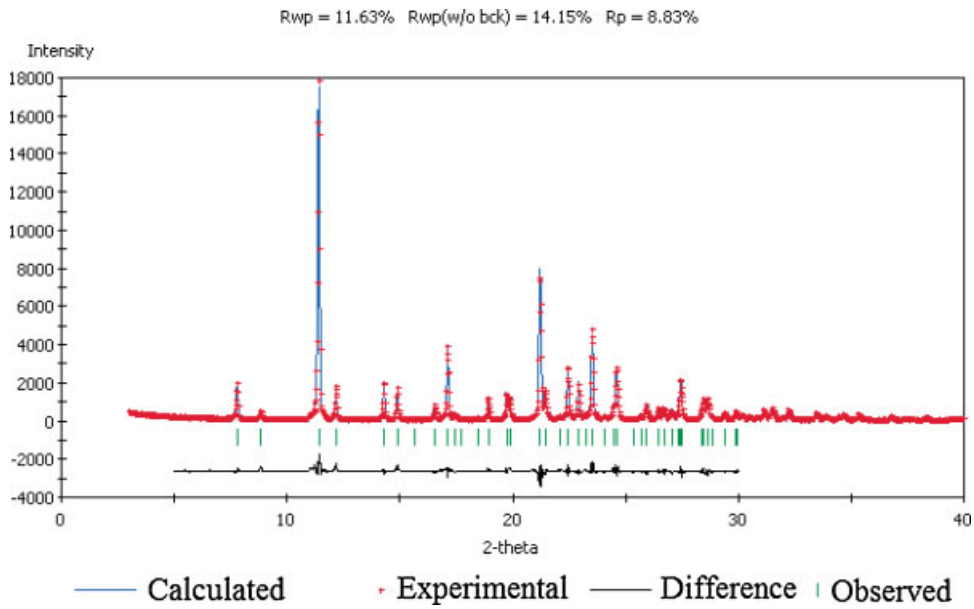

Figure 5. Comparison of the experimental pattern and pattern calculated from the structure solution by the X-ray powder diffraction method.

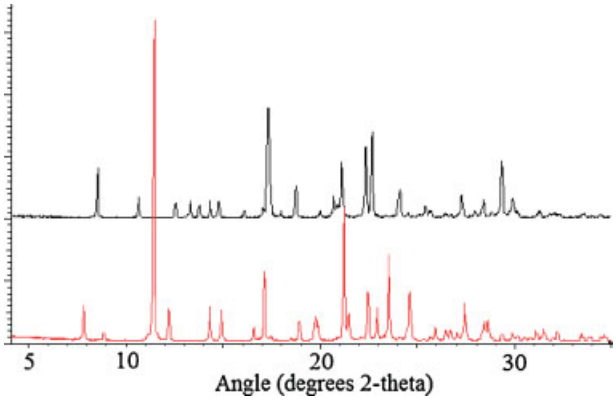

Figure 6. Experimental X-ray powder diffraction pattern of atomoxetine HCl Form I (top) and Form II (bottom).

pattern of atomoxetine HCl, Form I versus Form II, indicates that the molecular packing in the two crystal forms is substantially different, Figure 6. Form II can be readily detected at 7.9°, 11.5°, and 12.2° 2θ. As a result, X-ray powder diffraction serves as a highly specific technique for distinguishing the two crystal forms and may be used to detect low levels of Form II (the meta-stable crystal form) in atomoxetine HCl Form I API.

CONCLUSIONS

Two crystal forms of atomoxetine HCl and an amorphous form exist. Form I is monotropically related and more stable than Form II. The amorphous form of atomoxetine HCl has a glass transition temperature at 30.1°C and readily crystallizes to the stable polymorphic form when exposed to ambient conditions. The single crystal structure of atomoxetine HCl Form I was determined and the absolute configuration of the molecule was determined to be *R*-configuration. The structure of the crystalline Form II, the meta-stable form, was determined using the X-ray powder diffraction method and a combination of Monte Carlo Simulated Annealing and Pareto Energy Optimization. This report demonstrates the application of structure solution using X-ray powder diffraction as a viable alternative to single crystal methods for determination of structures of meta-stable crystalline forms where isolation procedures do not permit growth of crystals of suitable size or quality for single crystal methods.

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