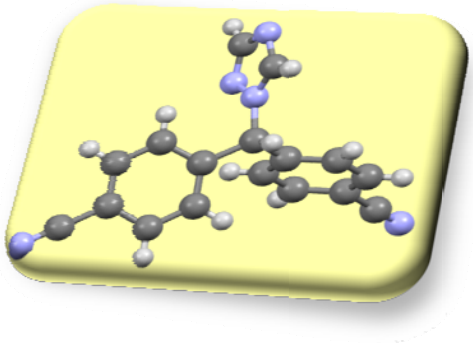
Chapter 4: Serendipity leading To "Polymorphism"

Contents



Abstract:

Crystallization of Letrozole, a well marketed drug for hormone receptor positive breast cancer, is a subject of this chapter. Letrozole molecule in spite of having many pro-hydrogen-bonding sites and planar aromatic ring did not show its ‘expression’ in actual solid state crystal structure. On this background, to enhance various non-covalent interactions in this molecule, mainly hydrogen bonding, π-π interaction, electron-donor-electron-acceptor ability, can one ‘add’ or ‘insert’ a structurally complimentary compound? This hypothesis leads to an experiment where hydroquinone and pyrogallol were added, separately, in systematic proportion during crystal growth. Detailed investigation of thus obtained products using DSC was performed, which showed interesting small but distinct shift in endotherms. Literature single crystal data and powder XRD data reveals observation of new polymorph. That means in our experiments hydroquinone acted as a ‘template’ for crystallization for Letrozole, without actually taking part in ‘lattice’ formation. This new form crystallizes in orthorhombic crystal system with *C-1* space group. One can expect difference in solubility behavior, thermal stability, chemical inertness and hence bioavailability for the new polymorph of already marked drug.

4.1 Introduction

Crystallization is an important separation and purification process [1]. The crystals thus formed have highly regular internal structure; hence structure prohibits foreign molecules from being incorporated into the lattice. In this process a solid of highest purity is obtained [2].

Solids have different degrees of mutual solubility, which determines the arrangement of atoms in the mixed crystal lattice. The mixed lattice can be of two types, substitutional or interstitial. The substances may be soluble over a partial or even a complete range of relative concentrations, producing a crystal whose properties vary continuously over the range. This provides a way to tailor the properties of the resulting crystal for specific applications.

Pharmaceuticals co-crystals comprising active pharmaceutical ingredients (API) and a co-crystal former have emerged as an innovative strategy to improve the performance of medicines by modifying their physical properties without changing any covalent bonds in either of the species [3].

Polymorphs by definition are the crystals of same molecule having different physical properties as a result of the order of changes in the molecule in the crystal lattice. The different physical properties of polymorph affect the pharmaceutical parameters such as stability of drug, structural phase transitions, compressibility and density. Thus, ‘polymorphs’ can have different dissolution rates and hence bio availability of the drug, most critical phenomena in action of drug [4].

It has been already reported that crystal growth is affected by solvent, temperature, seed crystals, templates and interfaces [5]. Inducing polymorphism using templates both in heterogeneous or homogenous form is relatively common [6]. Sometimes the weak interactions play a structure directing role and perturb the strong interactions [7]. In literature, the template induced polymorphism has already been presented by many research groups [8].

To the best of our knowledge four polymorphs of Letrozole are reported till date [4,9-10]. In this study to express this concept, Letrozole and hydroquinone were considered primarily. Hydroquinone which qualifies GRAS (Generally recognized as safe) is used as a coformer in this system [11]. Experiments were also carried out with pyrogallol.

4.2 Why *Letrozole* (Let)?

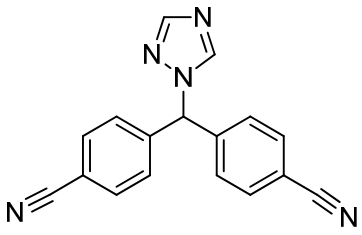


Figure 4.1: Structure of *Letrozole*

Hormone receptor positive type of breast cancer is very common. The diagnosis is done when excess amount of estrogen or progesterone receptors are found in the tumor cells. A well-known example of drug to treat this type of cancer is tamoxifen, which acts as a selective estrogen receptor down regulator since its mode of action is to bind to the estrogen receptor. Second way is to block the action of enzyme called aromatase, a member of the cytochrome p450 family, consisting of an iron centre surrounded by a porphyrin [12]. Aromatase converts androgens to estrogens which is responsible for growth of breast cancer tumors. Letrozole (*Figure 4.1*) is an example of drugs that can co-ordinate with aromatase and hence stop the production of estrogen [13]. Letrozole chemically 4,4'-((1*H*-1,2,4-triazol-1-yl)methylene)dibenzonitrile was approved by the FDA. It is marketed under the brand name FEMARA by Novartis Pharmaceuticals as a film coated tablet containing 2.5mg of Letrozole as a free base. Letrozole is a white to yellowish crystalline powder, practically odourless; m.p: 184-185°C [14]. Attractive features for considering Let are: (i) practically insoluble in water, (ii) electron deficient system because of cyano group, giving a space to incorporate electron donor and acceptor concept for designing of polymorphs (iii) planar molecule (iv) heterocyclic structure (v) slightly basic.

4.3 Complimentary structures of Letrozole

In our initial experiments we planned two 'phenol' based structures.

4.3.1. Why Hydroquinone (Hyq) ?

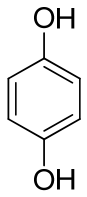


Figure 4.2: Structure of hydroquinone

Hydroquinone (*Figure 4.2*), chemically benzene-1,4-diol or quinol, is an organic compound with phenol in its core structure having the chemical formula $C_6H_4(OH)_2$. It has two hydroxyl groups bonded to a benzene ring in a *para* position. It is a white granular solid; m.p: 172-173°C [15]. Hydroquinone's O-H group traditionally is slightly acidic. The resulting conjugate base can undergo O-alkylation to give mono- and diethers. Hydroquinone has variety of uses principally associated with its action as a reducing agent that is soluble in water. Hydroquinone is a major component in photographic developers for film and paper where, it reduces silver halides to elemental silver [16]. Hydroquinone is a drug which has been used for many years to lessen the appearance of sun-related skin discolorations and other undesired hyper pigmentation disorders. It is approved by FDA that oral ingestion of hydroquinone in stipulated amount is also possible. Attractive features of Hyq : (i) soluble in water (ii) through hydrogen bonding it may interact to form cocrystals (iii) hydroxyl group in it makes it a π electron rich system, giving a space to incorporate electron donor and acceptor concept for designing of polymorphs and (iv) slightly acidic.

4.3.2. Why Pyrogallol ?

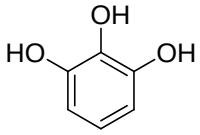


Figure 4.3: Structure of pyrogallol

Pyrogallol (*Figure 4.3*) chemically known as benzene 1,2,3-triol with molecular formula $C_6H_3(OH)_3$. It is an active ingredient of Emblica Officinalis, the Indian gooseberry and is known for its anti-inflammatory and anti-pyretic properties [17]. In the search of new phenols to see the interactions with our system, we also tried pyrogallol as a coformer. Pyrogallol acts as a powerful reducing agent and as a drying material. It is used to indicate the amount of oxygen in air and dry nitrogen gas lines. As hydroquinone, it was also used in photograpy as developing agent [18]. In pharmaceuticals pyrogallol is being tested as a drug candidate for nonsmall cell lung cancer (NSCLC) [19] and cystic fibrosis (CF) [17]. Pyrogallol can form hydrogen bonds to several coformers [20]. The reasons for using pyrogallol are similar to that of hydroquinone.

4.4 Experimental

Experiments were planned with Letrozole (API, Active Pharmaceutical Agent), a gift sample from Sun Pharmaceuticals (SPARC, Vadodara). Numerous batches of Letrozole and hydroquinone (template) were applied in the study, all of which were of Good manufacturing processes (GMP) quality.

The crystals were grown in glass vials. A solution of hydroquinone in methanol was added slowly into a solution of Letrozole in methanol at room temperature. The resultant solution was allowed to crystallize at ambient temperature (slow evaporation method). After crystallization the crop was harvested as first crop from mother liquor and then the second crop was also isolated from the solution. First crop for case 1 was isolated after four days, while for rest of the cases it was isolated after five days. The resultant crystals were air dried and used for respective analysis. Crystals were harvested on an average

after four to five days. Crystals in all cases were isolated from the bottom of the glass vial.

Mole ratios tried for these set of experiments are listed in the table below (*Table 4.1*).

Table 4.1: Experiments planned for crystallization using different mole ratios of Letrozole and hydroquinone

Entry	*Letrozole* (mole equivalent)	Hydroquinone (mole equivalent)
Case 1	1	0
Case 2	1	0.25
Case 3	1	0.5
Case 4	1	1
Case 5	1	2
Case 6	1	3
Case 7	1	4

Pyrogallol was also used in a similar way, as the coformer in our study, but we didn't have good quality 'understandable' data and therefore it's not mentioned further in this chapter.

4.5 Strategy for analysis:

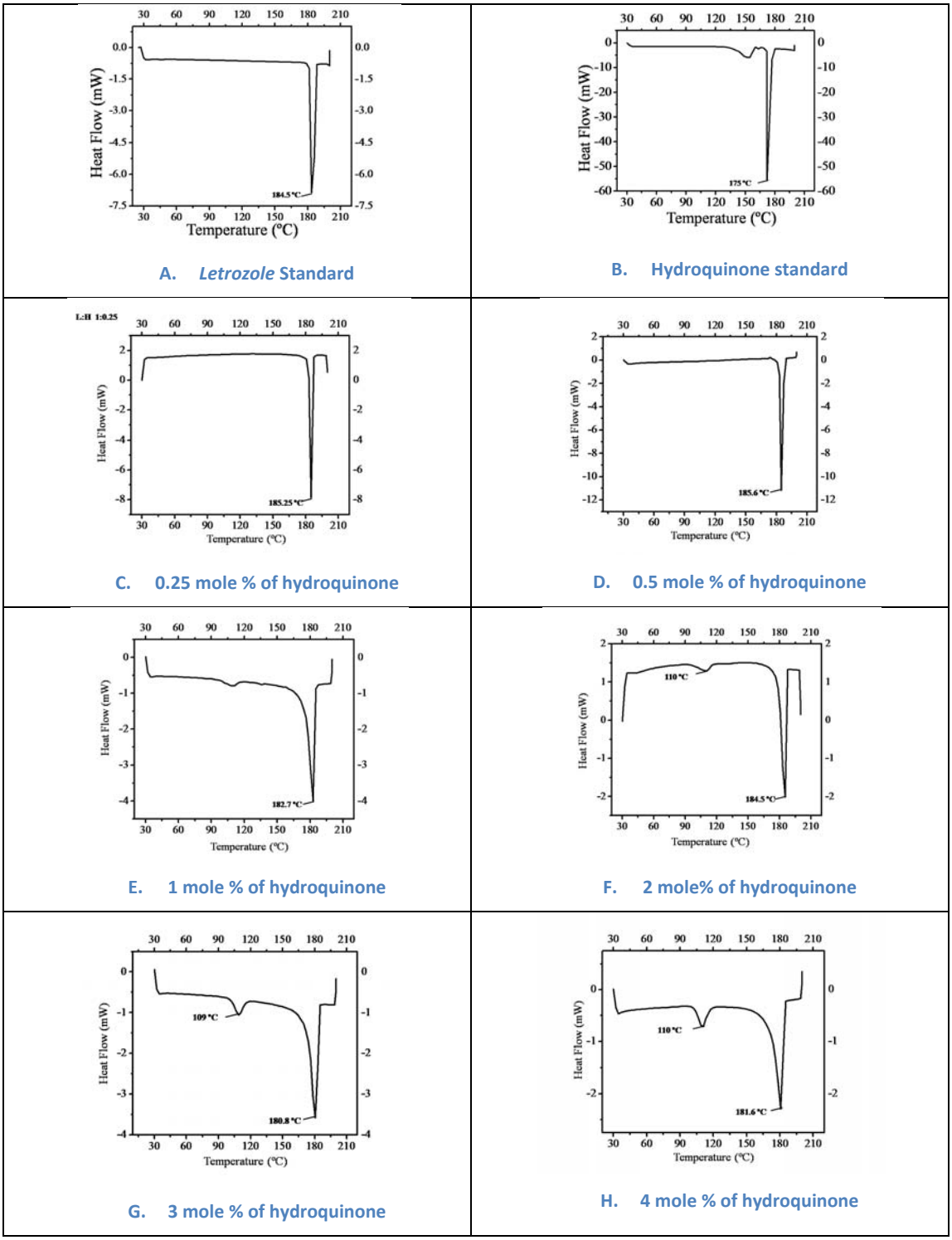
After obtaining the crystals following studies were planned for detailed investigation.

1. DSC
2. Single crystal XRD
3. Powder XRD

4.6 DSC Studies

DSC was performed using instrument Mettler Toledo DSC822.

Figure 4.4 presents the DSC thermograms of various experiments performed. For Letrozole and hydroquinone, endotherms were observed at 184.5° and 175.0°C respectively (*Figure 4.4 A and B*). For case 2 to case 7 the endotherms were observed respectively at 185.2°C, 185.6°C, 182.7°C, 184.5°C/110.0°C, 180.8°C/109.0°C and 181.1°C/110.0°C. Small hump was observed for cases 3, 5 and 6 (*Figure 4.4 C to H*).



A. *Letrozole* Standard

B. Hydroquinone standard

C. 0.25 mole % of hydroquinone

D. 0.5 mole % of hydroquinone

E. 1 mole % of hydroquinone

F. 2 mole% of hydroquinone

G. 3 mole % of hydroquinone

H. 4 mole % of hydroquinone

Figure 4.4: DSC thermograms of respective mole % of hydroquinone

Figure 4.5 presents plot with temperature versus concentration of hydroquinone in percentage (Case 1 to 7). As we can observe that in *Figure 4.5*, X axis represents

changing percentage of hydroquinone. Y1 and Y2 axis represents the temperature in Celsius scale for endothermic transitions in DSC. Peak, onset, end temperatures were observed and concluded.

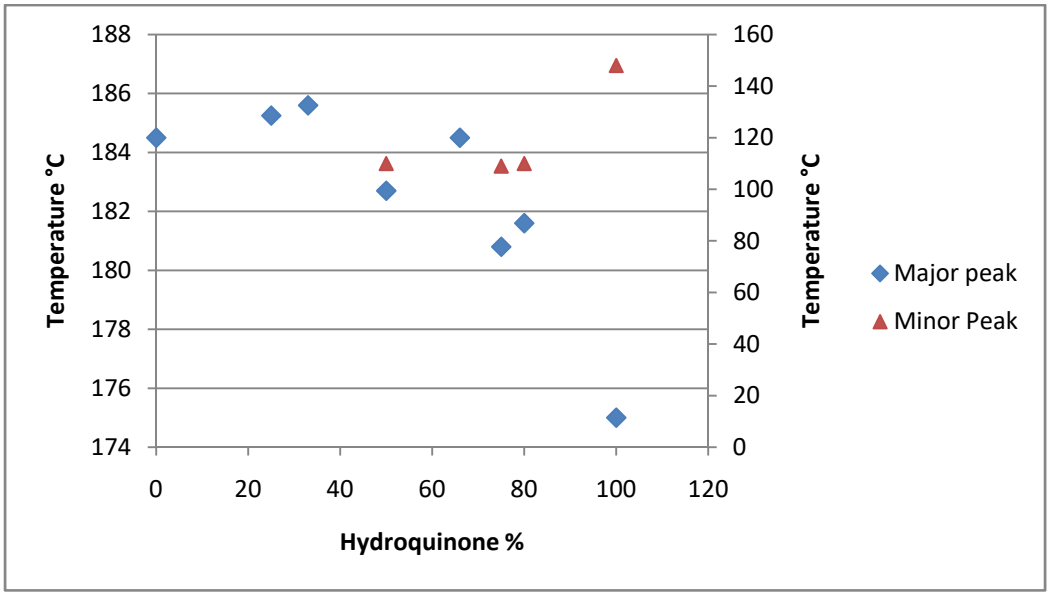


Figure 4.5: Graph with temperature versus hydroquinone concentration in percentage

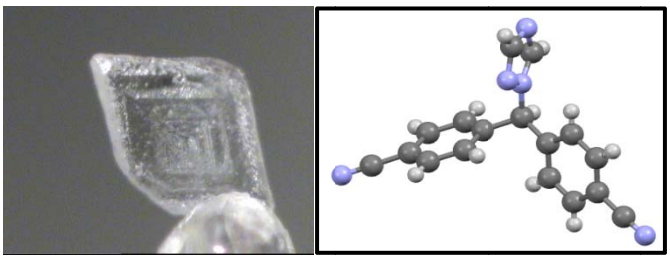
From Figure 4.5, we can conclude that for cases 2 and 3, where ratio of hydroquinone is less than one equivalent, there is slight increase in thermal stability or melting point with respect to pure Letrozole and Hydroquinone. On the other hand, in all remaining cases, where ratio of hydroquinone is equal to or more than one equivalent, temperature is lower than or equal to melting points of pure compounds. Ideally impurity should lower the melting point. Then why in present study, cases 2 and 3 show a higher melting point? Is it due to co-crystal formation or due to new polymorph generation? This curiosity forced us to further investigate the system.

4.7 Single Crystal XRD

The crystallographic data for already reported and new form of Letrozole is given in *Table 4.2*. Crystals of suitable size of the already reported form and new form were obtained by slow evaporation of the solvent. The data was collected on Oxford X-CALIBUR-S diffractometer with MoKα radiation (λ = 0.71073) at 293K. The data interpretations were processed with CrysAlis Pro, Agilent Technologies, and version 1.171.35.19. An absorption correction based on multi-scan method was applied. All structures were solved by direct methods and refined by the full matrix least-square based on F^2 technique using SHELXL-97 program package. All calculations were carried out using WinGX system Ver- 1.64.

Two types of single crystals were 'isolated' during present investigation. Good sugar like crystals was harvested in all the cases (Case 1 to 7).

1. Crystal structure of Letrozole obtained in almost all the cases (already reported form)



a b

Figure 4.6: (a) Habit of crystal found in majority of the cases *Letrozole* (Form I) (b) Ball and stick model of *Letrozole* form 1.

Here the habit of crystal is shown in *Figure 4.6 (a)*. Sample of Letrozole without hydroquinone was also crystallized. As a result it gave us form I, given below are the details of crystal we obtained.

The description for crystal reported in literature is as follows: The atomic structure based on single crystal X-ray data of Letrozole was reported by Xu et al. (2002) [21] and Wang et al. (2004) [22], the average values of cell parameters for monoclinic crystal system are

a=7.030(0) Å, *b*=16.170(3) Å, *c*=13.360(3) Å, *α*=*γ*=90°, *β*=104.80°, unit cell volume V= 1468.3 (5) Å, Z=4, and space group $P2_1/c$ [21].

The description of crystal harvested in present study, recorded at 293 K for form 1 is as follows: Letrozole crystallizes in monoclinic system. The average values of observed cell parameters are *a*=7.030(2) Å, *b*=16.233(3) Å, *c*=13.335(3) Å, *α*=*γ*=90°, *β*=104.89° (3), unit cell volume V= 1470.8(6) Å, Z=4, and space group $P2_1/n$ (*Table 4.2*). Asymmetric unit has four molecules in a unit cell.

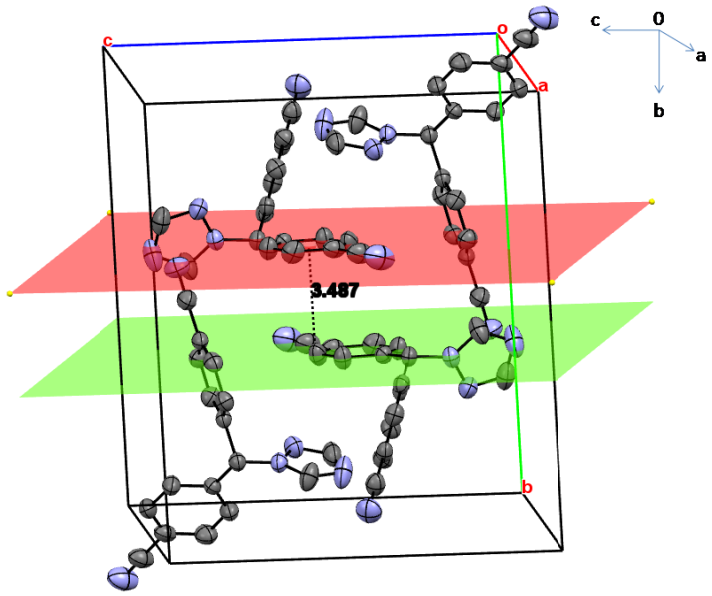


Figure 4.7: Molecular view of *Letrozole* in its form I with π-π stacking

Most striking interactions in the X-ray data is shown in figure 4.7, *π*-*π* stacking, a distance of 3.487 Å along *b*-axis *hkl* value of (12,15,30). This stacking is not continuous, but observed in generating dimers.

2. Crystal obtained from experiment of L:H (1:0.5).

Hydroquinone here acts as template. Hydroquinone forces the Letrozole molecules to appear in a different polymorph; i.e to arrange in different order. The mechanism of role of hydroquinone is not clear.

The description for crystal of new metastable form is as follows: Letrozole crystallizes in orthorhombic system. The average values of cell parameters are *a*=7.0317(9) Å, *b*=25.754(3) Å, *c*=16.180(2) Å, $\alpha=\beta=\gamma=90°$, unit cell volume V= 2930.1(6) Å, Z=4, and space group *C-1*. *Figure 4.8* shows the habit of crystal. *Figure 4.9* shows ORTEP view of Letrozole with thermal ellipsoids drawn at 50% probability. Here asymmetric unit has eight molecules.

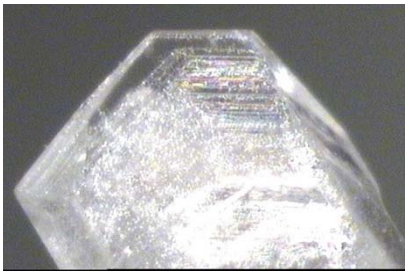


Figure 4.8: Habit of crystal shown by the new form of Letrozole

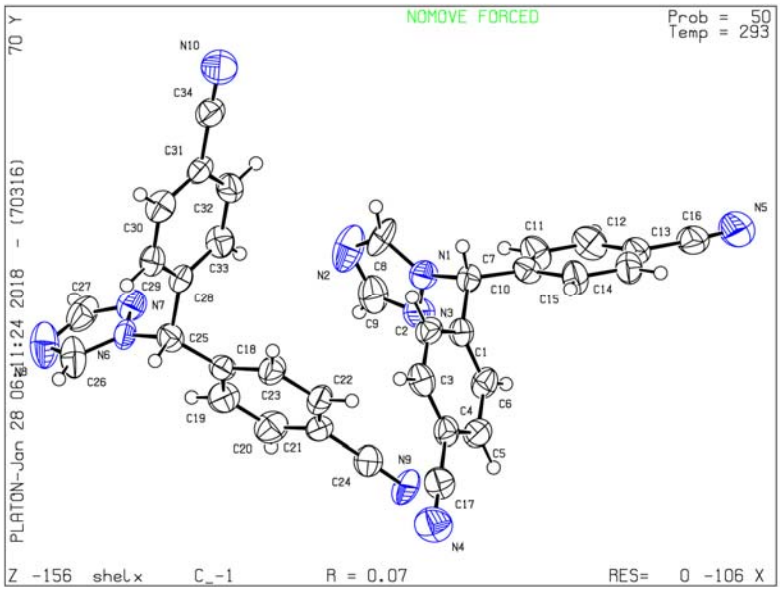
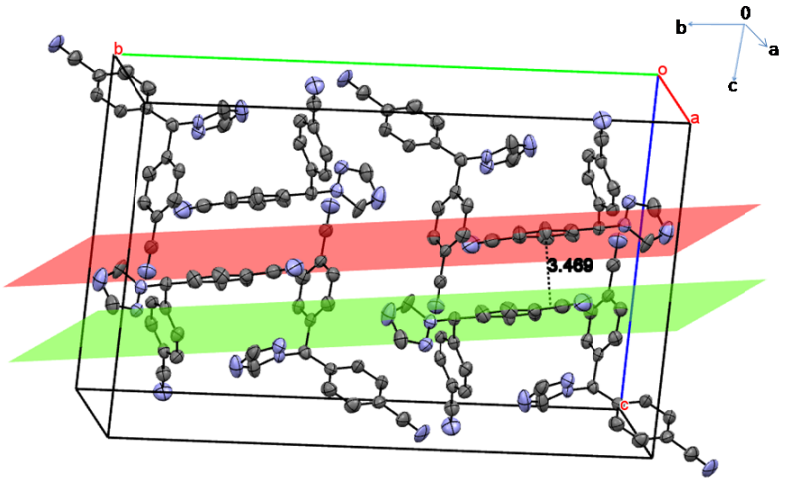


Figure 4.9: Molecular view of new form of Letrozole having thermal ellipsoids drawn at the 50% probability.

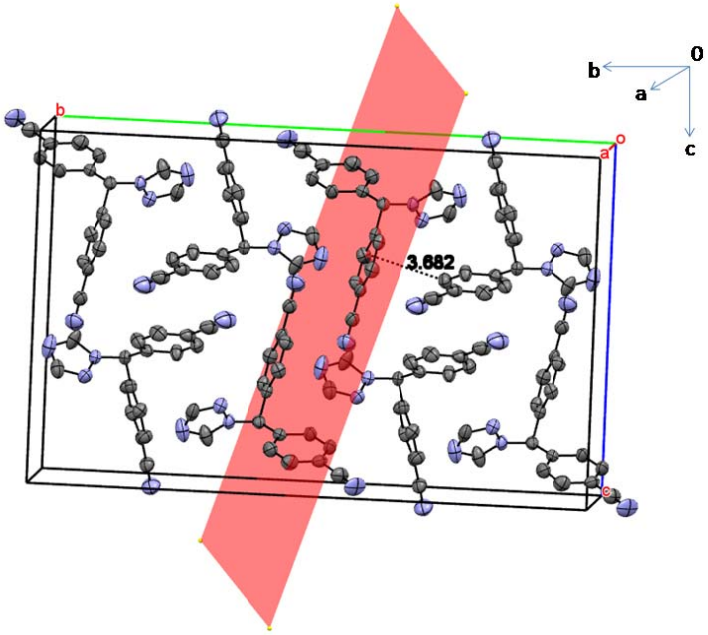
As seen in the *Figure 4.10(a) and (b)*, the π-π stacking is observed with a distance of 3.469Å along *c*-axis with *hkl* value of (8,32,10). π-π Stacking observed is not continuous and exists for dimeric structure. The C-π interaction was also observed with a distance of 3.682 Å along b axis with *hkl* value of (12,15,12). C-π stacking also forms dimeric

structure with C-H-π (C-H-centroid) angle 167°. Both these interactions are hosted by the same ring but with two different neighbors (See *Figure 4.10 (a) and (b)*). For C-π interaction both the symmetric ring with cyano benzene group participates in the similar kind of π-π and C-π interactions.

While comparing these interactions in new polymorph with the repoted structure of Letrozole, we observed that: (i) Unit cell parameters for new polymorph *a*, *b*, *c* is 7.031, 25.754, 16.180 respectively and for reported polymorph *a*, *b*, *c* are respectively 7.030, 16.233 and 13.335, here one can note that *c*-axis of new form is similar to *b*-axis of old form; (ii) for new polymorph π-π stacking is along *c*-axis, while for reported polymorph it is along *b*-axis; (iii) for new polymorph extra C-π stacking is observed along *b*-axis; (iv) the *hkl* value for π-π stacking for reported form is (12,15,30) and for new polymorph it is (8,32,10); while C-π stacking for new polymorph exists with *hkl* (12,15,12).



a



b

Figure 4.10: Molecular view of Letrozole in its new polymorph showing *π*-*π* stacking and orthogonal C-*π* interactions

Table 4.2 compares and summarizes the crystallographic data we obtained for already reported and new polymorph of Letrozole.

Table 4.2: Crystallographic data and structure refinements for reported form 1 and new form of Letrozole

	Reported form of Letrozole	**New polymorph of Letrozole**
CCDC No	-	1820481
Empirical formula	$C_{17}H_{11}N_5$	$C_{33}H_{22}N_{11}$
Formula weight	287.29	572.62
Temperature/K	293(2)	293(2)
Crystal system	Monoclinic	Orthorhombic
Space group	$P2_1/n$	C-1
a/Å	7.030(2)	7.0317(9)
b/Å	16.233(3)	25.754(3)
c/Å	13.335(3)	16.180(2)
α/°	90.00	90.00
β/°	104.89(3)	90.00
γ/°	90.00	90.00
Volume/Å^3	1470.8(6)	2930.1(6)
Z	4	4
ρ_{calc}g/cm^3	1.297	1.298
μ/mm^{-1}	0.086	0.083
F(000)	592.0	1188.0
Radiation	MoKα (λ = 0.71073)	MoKα (λ = 0.71073)
2Θ range for data collection/°	6.52 to 58.28	6.32 to 57.54
Index ranges	$-9 \le h \le 4$, $-21 \le k \le 20$, $-14 \le l \le 16$	$-9 \le h \le 4$, $-32 \le k \le 26$, $-18 \le l \le 21$
Reflections collected	4401	4217
Independent reflections	2595 [R_{int} = 0.0248, R_{sigma} = 0.0569]	3583 [R_{int} = 0.0688, R_{sigma} = 0.1122]
Data/restraints/parameters	2595/0/199	3583/0/397
Goodness-of-fit on F^2	1.045	0.875

From single crystal X-ray structure we can conclude that hydroquinone in our experiments acts as a template and forces Letrozole molecules to crystallize in the new crystal lattice. The exact role of hydroquinone here is not clear. The data from single crystal XRD forced to record powder XRD for the bulk behavior.

4.8 Powder XRD

In 1912, physicist Max Von Laue discovered that crystalline substances diffract X-rays by acting as three dimensional diffracting systems similar to spacing of the planes in crystal lattice [23]. X-ray diffractions were generated by constructive interference of monochromatic X-rays and a crystalline sample. While scanning a sample through a range of 2θ angles, all possible diffraction directions of lattice should get diffracted due to the random orientation of powdered material. Typically, *d*-spacing are compared with standard reference patterns to get more insights into the crystal. A key component of all the diffraction is the angle between incident and diffracted rays [24]. Diffraction pattern is a product of the unique crystal structure of molecule. The crystal structure describes arrangement of every atom and hence the molecule. When atoms are arranged differently a different diffraction pattern is produced leading to polymorphism. Miller indices (*hkl*) are used to identify different planes of atoms. Observed diffraction patterns are related to planes of atoms helps in analyzing the atomic structure and microstructure of a sample.

Powder X-ray diffraction (XRD) patterns were recorded with a Bruker axs D8 advance diffractometer in Electrical Research and Development Association (ERDA) by using the following instrument parameters; X-ray source: Cu K-alpha radiation (λ = 0.15406 nm) (25°C), current: 40mA, voltage: 40kV; start angle (2θ): 5° and stop angle (2θ) : 60°; step size: 0.050°, step time: 2sec and scan speed: 2˚/min.

Table 4.3 summarizes the PXRD data. First column list 2θ (with percentage relative intensity inside the bracket) values for pure API (Let) obtained from the source. Next column presents the 2θ values (simulated data) for the new form of Letrozole (Case 3) crystallized from the methanol. Next four columns lists 2θ values for pure hydroquinone and different forms of Letrozole reported in the literature for comparison.

The PXRD patterns of Hyq at room temperature [25] are presented in *Table 4.3, Column 3*. The PXRD exhibited sharp characteristics, revealing its high crystalline property, which is used as finger prints. The sharp intensity at 2θ° value 20.25° (100%) and at 9.42°, 16.07°, 16.27°, 16.84°, 21.36°, 30.60° are observed. These diffraction 2θ values of hydroquinone don't appear in the crystals harvested by us, which eliminates the possibility of hydroquinone sitting in the crystal system. This was also confirmed in single crystal study, where no hydroquinone insertion in the crystal lattice was observed.

The XRD patterns for Letrozole reported in the literature and Letrozole crude (what we have procured as gift sample) and crystallized by us without adding template (Hyq) are in good agreement with each other. The diffraction pattern corresponding to the highest intensity (100%) was observed at θ value of 14.23° for crude Let. θ value of 17.19° with 100% intensity was observed for blank experiment. Also the other important diffraction 2θ values were observed for crude at 13.18°, 16.33°, 17.22°, 17.68°, 19.80°, 21.15°, 21.53°, 22.18°, 23.39°, 25.77°, 26.33°, 27.63°, 28.29°, 29.68° and 31.20°. The other important diffraction patterns for blank Let were observed at 14.22°, 16.32°, 17.69°, 19.78°, 21.12°, 21.52°, 22.13°, 23.36°, 25.73°, 27.59°, 28.25°, 29.67°, 30.45° and 31.18°. The evaluation of the XRD of above two samples clearly shows that the important diffraction 2θ values, which are characteristics of Letrozole, remain practically unchanged. Minor changes were observed in the relative intensity, which are observed due to artifacts.

The simulated XRD pattern for new form of Letrozole was obtained from single crystal data. The diffraction observed at 2θ value of 25.62° was of 100% relative intensity. Also other important diffraction 2θ values for new polymorph were at 10.92°, 13.04°, 14.14°, 16.28°, 17.04°, 17.18°, 17.59°, 19.64°, 21.02°, 21.37°, 21.95°, 23.20°, 24.05°, 25.99°, 26.25°, 27.45°, 27.66°, 28.24°, 28.41°, 29.5° and 29.84°.

Table 4.3: Tabulated Raw data, *2ϑ* values obtained from PXRD, compared with the values reported in literature

***Letrozole* (Crude)**	**Case 3 (Simulated data)***	**Lit Hyd[1]**	**Lit Let form 1[2]**	**Lit Let form 2[2]**	**Lit Let US Patent[3]**	**Lit Let Form L[4]**
2ϑ (% relative intensity)						
8.865 (3.2)						
		9.42				
11.039 (7.1)	10.97 (12.38)					10.887 (3.5)
13.182 (82.2)	13.04 (50.04)		13	13.2	13.2	13.069 (100)
13.825 (7)						
14.234 (100)	14.14 (53)		14.2	14.2	14.2	14.1 (46.3)
14.862 (6.6)	14.83 (6.94)					14.695 (2.2)
		16.07				
		16.27		16.2	16.2	16.17 (6.6)
16.332 (17.1)	16.281 (14)					
		16.84				
	17.04 (60.35)					17.062 (23.7)
17.222 (82.4)	17.18 (53.58)		17.2		17.2	
17.687 (12.4)	17.59 (14.28)			17.4		
19.807 (47.3)	19.64 (51.4)		19.7	19.7	19.7	19.656 (11.4)
		20.25 (100)				
21.158 (15.7)	21.02 (19.52)					20.989 (5.1)

***Letrozole* (Crude)**	**Case 3 (Simulated data)***	**Lit Hyd[1]**	**Lit Let form 1[2]**	**Lit Let form 2[2]**	**Lit Let US Patent[3]**	**Lit Let Form L[4]**
2ϑ (% relative intensity)						
	21.37 (69.33)	21.36		21.4	21.4	21.393 (27.3)
21.531 (70.4)						
	21.95 (14.38)					21.955 (7.5)
22.182 (12.3)						
	23.20 (15.82)					23.229 (5.2)
23.395 (12.8)						
24.108 (9.6)	24.05 (11.91)					23.938 (2.7)
25.772 (48.7)	25.62 (100)		25.7	25.7	25.7	25.601 (26.7)
	25.99 (10.27)					
	26.25 (9.65)					26.164 (8.6)
26.336 (14.3)						
27.134 (9.7)	27.06 (11.09)					26.960 (2.3)
	27.45 (14.59)					27.450 (4.5)
27.630 (19.8)	27.66 (13.59)					
28.294 (11.8)	28.24 (16.71)					28.161 (6.9)

***Letrozole* (Crude)**	**Case 3 (Simulated data)***	**Lit Hyd[1]**	**Lit Let form 1[2]**	**Lit Let form 2[2]**	**Lit Let US Patent[3]**	**Lit Let Form L[4]**
2ϑ (% relative intensity)						
	28.415 (10.82)					
	29.5 (33.35)					
29.683 (26.7)	29.84 (10.41)					29.48 (4.9)
						30.240 (1.9)
		30.6				
						31.081 (2.2)
31.209 (8.5)						
						31.891 (2.1)
32.075 (8.4)						
34.723 (7.5)						34.572 (4.6)
35.782 (8.1)						
40.486 (5.0)						40.002 (2.6)
						42.783 (1.9)

* Simulated data

1. Literature reference [25]
2. Literature reference [9]
3. Literature reference [10]
4. Literature reference [4]

Indexing of the PXRD patterns was carried out and is tabulated in *Table 4.4*. In spite of small changes in intensities in the PXRD, *hkl* values for case 2, 4, 5, 6 and 7 suggested that all the PXRD have the same crystal system. The common *hkl* value for these cases were 020, 101, 111, 102, 112, 122, 032, 123, 141 and133. Variation in *hkl* value and hence new crystal system supports our observation of new crystal polymorph. The common *hkl* values for new polymorph were 002, 110, 111, 130, 112, 131, 132, 150, 114, 153, 134 and 172. Analysis of these planes was carried out to find out exact molecular fragments which are: (i) For 111 (at 14.23° 2θ value) plane in both polymorphs (100% intensity for old and 50% intensity for new) passes through same molecular fragments, which suggests that this plane remains intact in the new polymorph; (ii) the new polymorph has 100% intensity at 2θ 25.62° and 114 plane passes through it.

In literature it is reported that template plays a critical role in generating polymorphism [16].

Table 4.4: Indexing of the PXRD peaks*

Letrozole (Purified)	***hkl***	**Case 3 (0.5 eq)**	***hkl***	**Case 4 (1 eq)**	***hkl***	**Case 5 (2 eq)**	***hkl***	**Case 7 (4 eq)**	***hkl***
2ϑ(% relative intensity)									
8.865 (3.2)	011							8.875 (5.2)	011
11.039 (7.1)	020	10.927 (12.38)	002	11.055 (6.6)	020			11.054 (9.6)	020
13.182 (82.2)	101	13.04 (50.04)	110	13.177 (36.7)	101	13.141 (10.9)	101	13.187 (54.3)	101
13.825 (7)				13.820 (5.6)				13.848 (9.1)	
14.234 (100)	111	14.145 (53.007)	111	14.216 (26.3)	111	14.235 (6.4)	111	14.228 (42.1)	111
14.862 (6.6)	012			14.862 (5.4)	012			14.871 (9.1)	012
16.332 (17.1)	102	16.281 (14)	130	16.313 (7.3)	102	16.295 (1.7)	102	16.316 (22.7)	102
		17.046 (60.35)	112						
17.222 (82.4)	112	17.181 (53.58)	131	17.192 (42.1)	112	17.245 (23.9)	112	17.230 (100)	112
17.687 (12.4)	031	17.59 (14.28)	042	17.675 (11.8)	031			17.703 (18.7)	031
19.807 (47.3)	122	19.649 (51.4)	132	19.799 (25.5)	122	19.814 (16.1)	122	19.805 (61.6)	122
								20.188 (9)	
21.158		21.026	113	21.529	032			21.178	

Letrozole (Purified)	***hkl***	**Case 3 (0.5 eq)**	***hkl***	**Case 4 (1 eq)**	***hkl***	**Case 5 (2 eq)**	***hkl***	**Case 7 (4 eq)**	***hkl***
2ϑ(% relative intensity)									
(15.7)		(19.52)		(100)				(38.8)	
21.531 (70.4)	032	21.371 (69.33)	150			21.569 (100)	32	21.531 (81.1)	032
22.182 (12.3)	113	21.958 (14.38)	004	22.083 (43)	113			22.211 (22.3)	113
23.395 (12.8)	023	23.204 (15.82)	133	23.423 (12.3)	023			23.492 (27.6)	023
24.108 (9.6)	123	24.057 (11.91)	152	24.075 (13.4)	123	24.031 (2.1)	123	24.089 (23.9)	123
25.772 (48.7)	141	25.62 (100)	114	25.749 (50.6)	141	25.723 (3.7)		25.762 (57.7)	141
		25.991 (10.27)	044						
26.336 (14.3)	202	26.254 (9.65)	220	26.325 (23)	202			26.124 (16.6)	
27.134 (9.7)	133	27.066 (11.09)	153					27.099 (11.2)	133
		27.455 (14.59)	134						
27.630 (19.8)	004	27.667 (13.59)	202	27.685 (26.4)	221	27.723 (1.3)		27.763 (21.6)	113
28.294 (11.8)	051	28.242 (16.71)	081			28.258 (1.5)			
		28.415 (10.82)	025					28.352 (30.4)	051
29.683 (26.7)	024	29.5 (33.35)	172			29.527 (2.6)		29.667 (28.7)	024
		29.845	082						

Letrozole (Purified)	***hkl***	**Case 3 (0.5 eq)**	***hkl***	**Case 4 (1 eq)**	***hkl***	**Case 5 (2 eq)**	***hkl***	**Case 7 (4 eq)**	***hkl***
2ϑ(% relative intensity)									
		(10.41)							
				30.479 (9)	150				
31.209 (8.5)	232							31.157 (13.9)	230
32.075 (8.4)	134			32.071 (11)	134			32.091 (23.2)	134
								33.643 (22.4)	104
34.723 (7.5)	005								
				35.371 (12.6)		35.313 (1.9)			
35.782 (8.1)	160			35.769 (14.2)	161			35.791 (20.6)	
								38.613 (6)	302
								39.210 (5.4)	154
								40.073 (6.8)	244
40.486 (5.0)	144			40.365 (12.4)	313			40.484 (6.8)	
								43.021 (6.1)	

* Powder X Software was used for indexing.

Calculations of Miller indices:

The calculation was done using Powder X software:

X-ray wavelength= 1.54nm (Cu Source)

2θ=any value from the graph

Bragg's equation,

$$\lambda = 2d\sin\theta$$

Where,

λ = Wavelength of X-ray

d = interplane spacing

θ = angle of X-ray incident on the sample

$$d = \lambda/2\sin\theta$$

$$d_{hkl} = a/\sqrt{h2 + k2 + l2}$$

Where,

a = Unit cell dimension

h,k,l = Miller indices

For crystal system,

$$a = 2r\sqrt{2}$$

Where,

r = atomic radius

After obtaining the $h^2 + k^2 + l^2$ value, for a particular crystal packing, principal diffraction planes are considered and miller indices are obtained.

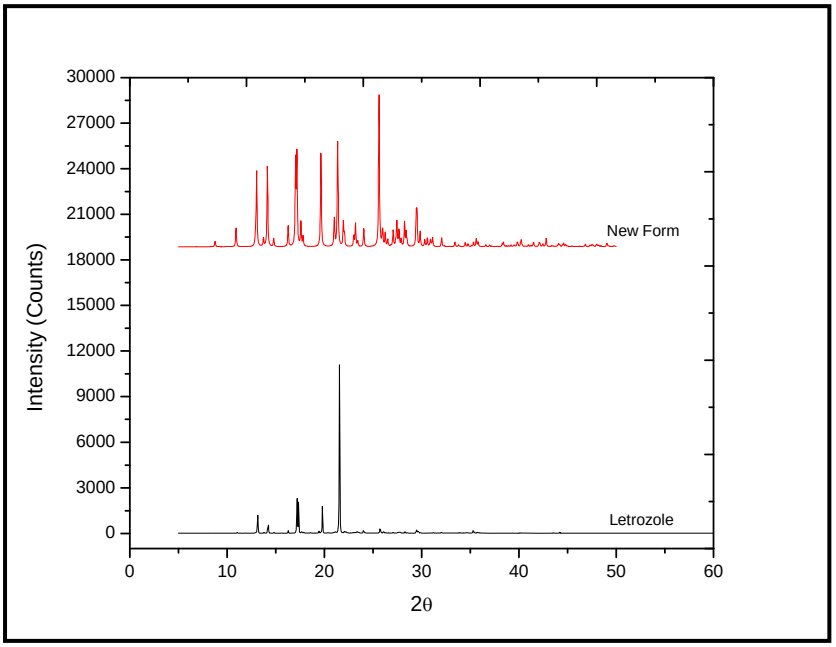


Figure 4.11: Powder XRD pattern of New polymorph (simulated) and already reported form of Letrozole

As seen in Figure 4.11 the PXRD of new and old polymorph of Letrozole are completely different. 2θ values were observed at 14.23° and 25.64° for polymorph I and II.

4.9 Conclusion

- Experiments for crystallization of Letrozole (API) with different proportions of hydroquinone were planned.

- DSC studies reveals that slight increase in thermal stability or melting point, with respect to pure Letrozole and Hydroquinone is observed for case 2 and case 3, where in those cases ratio of hydroquinone was less than one equivalent. On the other hand, in all remaining cases, where hydroquinone is equal to or more than one equivalent, endotherm is lower than or equal to melting points of pure compounds. Ideally impurity should lower melting point. This curiosity forced us to further investigate the system.

- Crystallization of Letrozole from methanol with various ratios of hydroquinone, yielded sugar like crystals belonging to monoclinic $P2_1/n$ space group, as reported in literature. We used hydroquinone as template here. Additionally, the crystallization from one of the stoichiometric ratio (Let:Hyq, 1:0.5) once gave a polymorph of Letrozole (new form), which crystallized in orthorhombic system *C-1* space group. However, the new polymorph crystals could not be reproduced again by simply varying the mole ratios of the hydroquinone. The curious occurrence of the disappearing metastable polymorph left us in ambiguous state.

- Salient features of new and reported polymorphs of Letrozole are: (i) unit cell parameters for new polymorph *a*, *b*, *c* is 7.031, 25.754, 16.180 respectively and for reported polymorph *a*, *b*, *c* are respectively 7.030, 16.233 and 13.335, here one can note that *c*-axis of new form is similar to *b*-axis of old form; (ii) for new polymorph π-π stacking is along *c*-axis, while for reported polymorph it is along *b*-axis; (iii) for new polymorph extra C-π stacking is observed along *b*-axis; (iv) the *hkl* value for π-π stacking for reported form is (12,15,30) and for new polymorph it is (8,32,10); while C-π stacking for new polymorph exists with *hkl* (12,15,12).

- To reconfirm new polymorph the simulated powder X-ray pattern was checked and compared with different powder XRD patterns of four different forms of Letrozole reported in literature.

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