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COLLECTIVE INSIGHTS ON THE POLYMORPHS, BIOAVAILABILITY, AND BINDING PROPERTIES OF ATOVAQUONE (ANTIMALARIAL DRUG): AN OVERVIEW

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Abstract

This review initiative had interrelated key aspects like crystal structure, bioavailability, and stereospecific binding capabilities of Atovaquone. Surprisingly, very little literature was available regarding the exploration of different polymorphs of Atovaquone. Interestingly, extensive literature was found towards the bioavailability features and factors specifically related with Atovaquone. Several researchers had attempted to correlate crystal morphology and orientation with the binding properties of Atovaquone and its structurally related compounds. The polymorphic stability of the molecule will play a crucial role in drug formulation and contributes towards the bioavailability of the drug through variations in solubility. Hence for Atovaquone, two factors must be considered: its polymorphic nature and the presence of stereospecific isomers to explain its bioavailability and binding properties. The trans*-isomer of Atovaquone, having a specific polymorphic form had provided higher bioavailability, more efficient binding, and an expectedly higher inhibitory activity.*

Keywords: *Atovaquone; Bioavailability; Crystal packing; Cytochrome bc1; Isomers; Polymorphs; Solubility*

Introduction

Atovaquone (ATQ) is a widely used antimicrobial drug for treating infections such as *Pneumocystis carinii* pneumonia, toxoplasmosis, and malaria. It is a versatile compound with established antiprotozoal properties and promising anticancer potential due to its ability to inhibit mitochondrial function and alter cancer cell metabolism. Its broad-spectrum activity and specific mechanisms of action make it a strong candidate for repurposing in cancer therapy alongside its traditional use against parasitic infections (Ashton et al., 2016; Baggish & Hill, 2002; Cheng et al., 2020; Gupta & Srivastava, 2019; Looareesuwan et al., 1999; Ridley, 2002; Spencer & Goa, 1995; Srivastava & Vaidya, 1999; Srivastava et al., 1997; Stevens et al., 2019; Xiang et al., 2016). Regarding its mechanism of inhibition, ATQ binds to the cytochrome bc_1 complex,

thereby inhibiting its function. This binding is facilitated by specific interactions with conserved residues within the complex. The IUPAC name for ATQ is 2-[4-(4-chlorophenyl) cyclohexyl]-3-hydroxy-1,4-naphthoquinone. It features a hydroxynaphthoquinone core with a chlorophenyl-cyclohexyl side chain (see **Figure 1**), which plays a significant role in its binding and inhibitory action on the cytochrome bc_1 complex complex (Birth et al., 2014). Numerous studies have highlighted that the 1,2-dioxo moiety of ATQ is critical for establishing binding with cytochrome *b* (Barton et al., 2010; Kessl et al., 2007; Verdaguer et al., 2021).

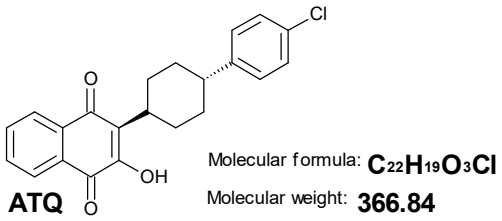

Figure 1. Structure of ATQ

Note on binding ability and bioavailability of ATQ

Among the two isomeric forms of ATQ, *trans*-ATQ is the active form used therapeutically. It effectively binds to cytochrome bc_1, a key component of the electron transport chain in parasites. This binding involves a hydrogen bond between His-181 of cytochrome bc_1 and the carbonyl (C=O) group of *trans*-ATQ, with a bond distance of 2.85 Å. This interaction is crucial for its potency in inhibiting the electron transport chain. In contrast, *cis*-ATQ is not used as a drug due to its significantly weaker binding affinity; the hydrogen bond distance to cytochrome bc_1 is 5.3 Å, indicating less effective interaction and reduced inhibitory potency (Basumallick & Row, 2015; Fontaine et al., 1998). Both *cis*- and *trans*-ATQ, along with their derivatives, had exhibited characteristic intermolecular interactions in their crystal structures. These include O–H···O hydrogen bond dimer motifs as well as weaker interactions such as C–H···O and C–H···Cl hydrogen bonds. These interactions are important for understanding binding preferences and for designing more therapeutically effective derivatives (see **Figure 2**). The presence of halogen atoms in the binding pocket of cytochrome bc_1 significantly influences the binding affinity and stability of the drug-protein complex. This insight is essential for drug design, highlighting the importance of subtle energetic contributions controlled by weak intermolecular interactions (Fry & Pudney, 1992; Nayak et al., 2013).

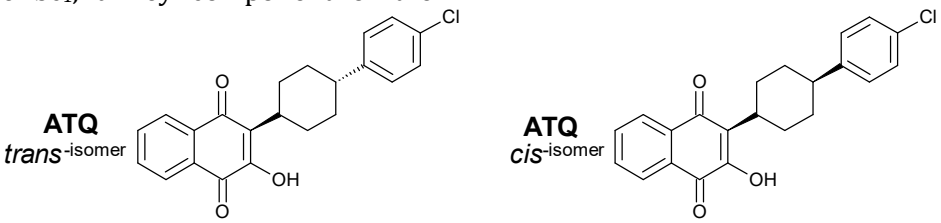

Figure 2. Isomers of ATQ

ATQ exists in crystalline and amorphous forms, each exhibiting distinct properties that influence solubility and bioavailability. Among the crystalline forms, two predominant polymorphs, referred to as crystal phases I and III, are well characterized. These polymorphs differ primarily in the orientation of their dimers within the crystal packing, which are stabilized by strong hydrogen-bond interactions. A crystal-to-crystal phase transition occurs upon heating, specifically from phase I to phase III at approximately 210°C. The crystalline forms of ATQ have been extensively investigated using techniques such as differential scanning calorimetry (DSC), X-ray powder diffraction (PXRD), and single-crystal X-ray diffraction. These analytical methods provide critical insights into the thermal stability and structural properties of ATQ, which are essential for the design of stable formulations with enhanced bioavailability (Malpezzi et al., 2010; Teoh et al., 2022). Both the crystalline and amorphous forms of ATQ offer unique advantages and present

distinct challenges. While crystalline forms confer the necessary structural stability, the amorphous form enhances solubility and bioavailability. However, despite the solubility benefits of the amorphous form, its practical use is complicated by its rapid tendency to crystallize, which poses challenges in quantification and formulation stability. Therefore, a comprehensive understanding of these physicochemical properties is critical for optimizing ATQ formulations for therapeutic use (Takabe et al., 2018; Teoh et al., 2020). In clinical contexts, the bioavailability of ATQ remains low and variable, primarily due to its poor aqueous solubility (less than 0.1 µg/mL). Its absorption is heavily dependent on formulation strategies and concomitant food intake. For instance, the bioavailability of ATQ suspension has been reported to be approximately 47% when administered with food and only 23% in the fasted state (Dressman & Reppas, 2000; Haile & Flaherty, 1993; Kate et al., 2016; Kathpalia et al., 2021; Olan et al., 1994; Sek et al., 2006]. Consequently, numerous approaches have been pursued to enhance the bioavailability of ATQ, given its high cost and therapeutic importance.

Hint on Polymorphism

Polymorphism refers to the ability of a compound to exist in two or more crystalline phases, each differing in the arrangement or conformation of molecules within the crystal lattice. Different polymorphs of the same compound may exhibit variations in physicochemical properties, such as melting point, solubility, and X-ray diffraction patterns. Although these differences disappear upon dissolution, they can significantly affect the properties of the solid form, including handling characteristics, dissolution rate, and chemical stability. These solid-state properties, in turn, directly influence drug processing, shelf life, and commercial viability. Therefore, it is crucial to thoroughly characterize all solid forms of a drug, including its possible polymorphic variants. Polymorphic forms can be distinguished using well-established analytical techniques such as powder X-ray diffraction (PXRD), differential scanning calorimetry (DSC), and infrared spectroscopy (IR) (Brog et al., 2013; Caira, 1998; Gavezzotti, 2007; Kersten et al., 2018; Purohit & Venugopalan, 2009; Raza, 2014; Santos et al., 2014).

Previous studies on polymorphs, binding ability, and bioavailability of ATQ

Numerous past studies have provided deeper insights into the binding ability, bioavailability, and polymorphic forms of ATQ. These findings have been disseminated over time through various scientific platforms, including filed patents and peer-reviewed journal articles. A review article by the authors comprehensively covered all the initiatives pertaining to synthesize ATQ on a commercial scale (Saralaya & Kanakamajalu, 2023). Similarly, the authors had explored prodrug strategies with modest success to enhance ATQ solubility (Sanjay et al., 2024).

The key disclosures relevant to the objectives of the present review were systematically tabulated below in chronological order:

Latter and Gutteridge (1991) described a methodology to isolate the *trans*-isomer of ATQ. The crude ATQ residue, exhibiting a broad melting range of 200–209°C, was recrystallized from acetonitrile to yield the pure *trans*-isomer with a sharp melting point of 216–219°C.

Hudson and Randall (1991) reported an analogous method to obtain stable and pure *trans*-ATQ with an identical melting range of 216–219°C.

Sordet et al. (1998) demonstrated the efficacy of ATQ-loaded nano-capsules designed to increase local drug concentration in infected tissues. The enhanced bioavailability observed was attributed to the solubilization of ATQ within the oily core of the nano-capsules, representing an innovative formulation approach.

Cauchetier et al. (1999) investigated the preparation and physicochemical properties of ATQ-loaded liposomes. They observed that ATQ release occurred in alkaline media via desorption upon fourfold dilution of

liposomes, whereas no release was detected in acidic or neutral environments, indicating efficient encapsulation.

Dearn (2000, 2003) reported the preparation of micro-fluidized ATQ particles with improved bioavailability based on the principle that reducing particle size enhances drug absorption. Using a laboratory-scale Model 120B Microfluidizer, they produced particles wherein at least 90% had a volume diameter between 0.1 and 3.0 μm.

Tarur et al. (2006) described the synthesis and characterization of new ATQ polymorphs. Previously reported ATQ was designated as Form I, exhibiting an XRD pattern with characteristic peaks at 7.2, 11.04, 11.77, 19.34, 21.14, 24.61, 25.28, and 28.4±0.2 degrees. The DSC thermogram for Form I showed a small endothermic event at 197°C and a sharp melting endotherm at 222°C. Building on these findings, the study reported the synthesis and characterization of Forms II and III. Form II exhibited XRD peaks at 7.02, 9.68, 10.68, 11.7, 14.25, 14.83, 18.6, 19.29, 23.32, and 24.54±0.2 degrees, with DSC showing a small endotherm at 169°C and a sharp melting endotherm at 222°C. Form III displayed XRD peaks at 6.99, 9.65, 12.67, 20.07, 20.65, 20.99, 21.88, 22.1, and 25.56±0.2 degrees and a DSC thermogram with a sharp endotherm at 222°C. According to the report, Form I was prepared using solvent combinations such as methylene dichloride/methanol and methylene dichloride/n-heptane. Form II was obtained by recrystallizing Form I from 1,4-dioxane. Form III was prepared from Form I using solvent mixtures, including acetone/water, chloroform/methanol, and diisopropyl ether.

Kumar et al. (2009) reported the synthesis and characterization of a novel polymorph of ATQ, designated as "Form IPCA-ATO." This polymorph was obtained from Form I using various solvents or solvent combinations, including methylene dichloride/lyophilization, chloroform/methanol, methylene dichloride/dimethylformamide, and methylene dichloride/chilling. The novel polymorph exhibited XRD peaks at 6.66, 10.05, 13.11, 18.27, and 23.1±0.2 degrees. Its DSC thermogram displayed two endothermic events: a first endotherm between 117–130°C and a second sharp endotherm at 220–222°C. Furthermore, FT-IR spectra showed distinctive absorption peaks at 3369, 2935, 1633, 1383, 1338, 1312, 1231, and 1053 cm^{-1}.

Malpezzi et al. (2010) gave a detailed structural and thermal characterization of ATQ Forms I and II, employing thermogravimetry (TG), DSC, single-crystal X-ray diffraction (SCXRD), X-ray powder diffraction (XRPD), and temperature-dependent XRPD (TXRPD). The study also included crystals of an intermediate compound, 2-[4-(4-chlorophenyl)cyclohexyl]-3-chloro-1,4-naphthoquinone (ATQ-Cl) (see **Figure 3**). Form I was prepared by dissolving crude ATQ in methylene dichloride, followed by the addition of n-heptane as an anti-solvent. Form III was obtained by dissolving crude ATQ in acetonitrile, concentrating to induce turbidity and subsequent crystallization. ATQ-Cl crystals were generated by recrystallizing the crude material from 1,4-dioxane and tetrahydrofuran. The researchers emphasized the importance of strong hydrogen bonds in Forms I and III, which linked adjacent molecules and resulted in varied crystal packing. In contrast, ATQ-Cl showed no evidence of forming a highly stabilizing hydrogen-bond network.

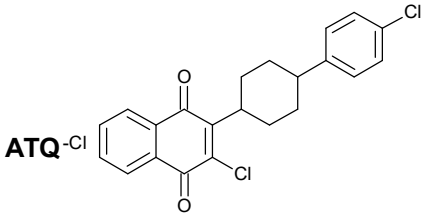

Figure 3. Structure of ATQ-Cl

Narayana et al. (2010) reported the synthesis and characterization of ATQ polymorphs Forms I, II, and III, as confirmed by XRD patterns and DSC thermograms. Form I was obtained from crude ATQ using solvent combinations such as methylene dichloride/methanol and methylene dichloride/n-heptane. Form II was prepared by recrystallizing Form I from 1,4-dioxane, while Form III was synthesized from Form I

using solvents, including acetone/water, chloroform/methanol, and diisopropyl ether alone.

Ceolin and Rietveld (2010) investigated the phase relationship between polymorphic Forms I and III of ATQ. Their work established that the heat-induced transition from Form I to Form III is an enantiotropic transformation, as evidenced by pressure-temperature phase diagrams.

Bandi et al. (2010) described the synthesis and characterization of two novel and stable crystalline forms of ATQ, designated as Forms A and B, with specific surface areas ranging from 0.7 m^2/g to 4.0 m^2/g. Form A was produced by recrystallizing ATQ from acetonitrile with slow cooling, resulting in particles with a specific surface area of 0.76 m^2/g. Form B was prepared by dissolving ATQ in tetrahydrofuran or chloroform, followed by powder isolation via spray drying, yielding particles with specific surface areas between 2.84 and 3.12 m^2/g. The XRD pattern of Form A showed characteristic peaks at 7.3, 10.0, 14.4, 15.1, 18.8, 20.4, 22.2, 23.6, and 24.6±0.2 degrees, and the DSC thermogram displayed a sharp endotherm at 221°C. In contrast, Form B exhibited XRD peaks at 9.7, 18.6, 19.3, 19.9, 20.1, 20.5, 22.2, 22.8, 23.3, 24.4, 24.6, 26.4, 26.9, and 28.8±0.2 degrees, with a DSC thermogram showing a small endotherm around 150–160°C followed by a sharp endotherm at 222–224°C.

Nayak et al. (2013) reported the crystal structure and binding pattern studies of ATQ and several notable derivatives with the cytochrome bc_1 complex. This work provides valuable molecular-level insights to guide drug design initiatives. The study included five naphthoquinone derivatives, the ATQ *cis*-isomer, and ATQ polymorphs Forms I and II (see **Figure 4**). In all cases, hydrogen bonding interactions were identified as critical determinants of binding affinity. Crystals of Form I, isomers of ATQ-Cl, and ATQ-nitro were obtained from a solvent mixture of methylene dichloride and hexane. Additionally, Form II crystals were prepared from aqueous acetone, while ATQ *cis*-isomer, *tert*-butyl ATQ, and Parvaquone crystals were isolated from acetone.

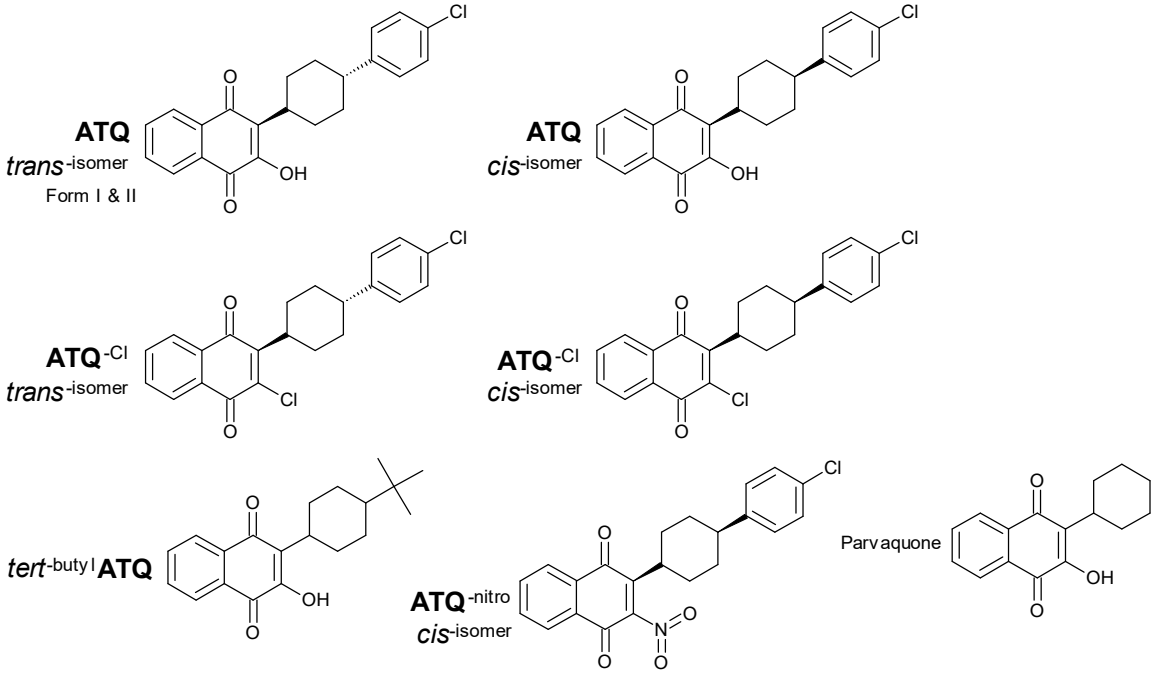

Figure 4. List of compounds subjected to studies by Nayak et al. (2013)

Calvo et al. (2011) demonstrated that incorporating cyclodextrin/poly(anhydride) nanoparticles as carriers for ATQ significantly enhanced its oral bioavailability, achieving approximately 72% bioavailability for this highly lipophilic molecule.

Pangarkar et al. (2013) described the synthesis and characterization of modified ATQ crystals obtained by recrystallization in

the presence of polyethylene glycol 4000 (PEG 4000) in acetonitrile, analyzed via scanning electron microscopy (SEM), FT-IR, DSC, and PXRD. The PEG-4000-mediated crystals had exhibited improved solubility and dissolution rates compared to standard ATQ. DSC analysis revealed a notable shift in the sharp endothermic peak to 215.8°C from the typical 221.0°C, suggesting a reduced melting point linked to enhanced solubility. PXRD results showed variations in peak intensities but no evidence of new polymorph formation, indicating changes in crystal size and habits induced by the additive. This study suggested that specific additives could modulate the crystal habit of ATQ molecules.

Roy et al. (2013) outlined a novel multistep synthetic route for ATQ and emphasized the structural elucidation of intermediates, ATQ, and its isomers using various analytical techniques. Recrystallized *trans*-ATQ from acetonitrile showed a sharp DSC peak at 220.4°C, and the PXRD pattern closely matched Form I, with characteristic peaks at 7.3, 9.7, 10.79, 11.11, 11.83, 15.43, 16.16, 16.89, 17.39, 22.93, 24.62, 24.68, 25.35, 26.18, 26.84, 28.52, 28.7, 29.52, 30.68, 34.23, and 36.84±0.2 degrees.

Birth et al. (2014) provided an in-depth structural analysis elucidating the molecular basis of ATQ's antimalarial action by inhibiting the cytochrome bc_1 complex. Their findings revealed that conserved residues of cytochrome *b* formed multiple non-polar interactions with the naphthoquinone moiety of ATQ, whereas less conserved residues interacted with the cyclohexyl-chlorophenyl side chain. This understanding has been instrumental in guiding drug modification efforts to combat resistance in malaria parasites. In a related context, Palsdottir et al. (2003) reported site-specific binding and complex formation of yeast cytochrome bc_1 with hydroxyquinone anion.

Borhade et al. (2014) described the formulation and characterization of an ATQ suspension with improved pharmacokinetic and therapeutic efficacy via oral administration. The formulation was prepared using a combination of micro-precipitation, high-pressure homogenization, and freeze-drying techniques. This size reduction strategy significantly enhanced the aqueous solubility and *in vitro* dissolution rate of ATQ.

Basumallick and Row (2015) investigated the energy-minimized binding patterns of *trans*-ATQ and *cis*-ATQ with the cytochrome bc_1 complex using molecular docking techniques. Their study highlighted that the hydrogen bond formed between the –NH group of His-181 in the Rieske protein and the carbonyl (C=O) group of *trans*-ATQ played a critical role in its potency, while the binding pattern of *cis*-ATQ was comparatively less effective.

Mohtar et al. (2015) reported the formulation of solid lipid nanoparticles (SLNs) of ATQ via high shear homogenization. Various lipid matrices such as tripalmitin, trilaurin, and Compritol 888 ATO were employed alongside surfactants, including Phospholipon 90H, Tween 80, and Poloxamer 188. *In vitro* release studies demonstrated immediate release of ATQ from the formulated suspension, contributing to enhanced bioavailability.

Kumar et al. (2016) described the development of compositions containing ATQ or its combination with proguanil designed to improve aqueous solubility. These compositions integrated the drug with hydrophilic polymers, surfactants, and inert excipients, resulting in significantly enhanced solubility and improved bioavailability.

Sodero et al. (2017) conducted molecular modeling studies on the binding of various antimalarial drugs containing hydroxynaphthoquinone chromophores (including ATQ) to cytochrome bc_1. Their findings indicated that the carbonyl group (acting as a hydrogen bond acceptor) was involved in a key hydrogen bonding interaction with His-152 of the Rieske iron-sulfur protein (ISP) subunit. Conversely, the hydroxyl group was found to play a minimal role in binding.

Chavan et al. (2017) examined polymorphic transformations of ATQ (specifically Form III to Form I) induced by

incompatible excipients such as magnesium stearate, polyethylene glycol (PEG) 8000, Poloxamer 188, and hydroxypropyl methylcellulose (HPMC) E15. This study underscored the necessity of excipient compatibility assessments prior to milling and grinding operations to maintain the desired polymorphic form. Analytical techniques employed included DSC, FT-IR, PXRD, and hot-stage microscopy.

Kathpalia et al. (2019) introduced a novel pH-driven precipitation method for preparing ATQ nano-suspensions. This approach combined pH adjustment (using sodium hydroxide) and anti-solvent precipitation (using tetrahydrofuran) as a bottom-up technique, followed by microfluidization (a top-down process) to reduce particle size into the nanoscale range. The nano-suspensions were stabilized with phospholipids and maintained stability for approximately six months. The water solubility of ATQ had increased dramatically from 0.74 to 11.98 µg/mL, contributing significantly to an enhanced bioavailability.

Kathpalia et al. (2021) further investigated the physicochemical properties of micronized ATQ suspensions. They demonstrated that the crystalline nature of ATQ was retained even after milling processes. Additionally, particle size reduction via controlled milling led to a 2.3-fold increase in solubility, highlighting the positive impact of size reduction on drug dissolution.

Teoh et al. (2022) investigated the solid-state properties of ATQ through comprehensive thermal and structural analyses, with implications for the design of stable formulations aimed at enhanced bioavailability. Their study elucidated the thermal degradation profile and sublimation tendency of ATQ upon heating. Surface morphology and physicochemical characteristics were examined using various analytical techniques, reinforcing the foundation for optimizing ATQ formulations.

Daniel et al. (2024) reported the synthesis and evaluation of the antimalarial potency of three novel bioinorganic complexes formed by coordination bonding between ATQ and metal centers Ag, Au, and Cu. The complexes synthesized were $[Ag(ATQ)(PPh_3)_2]$, $[Au(ATQ)(PPh_3)]\cdot 2H_2O$, and $[Cu(ATQ)(PPh_3)_2]$, each incorporating triphenylphosphine (PPh_3) as an auxiliary ligand. Phenotype-based activity assays demonstrated these complexes as potent antimalarials with good stability in solid and liquid states. Furthermore, the study revealed that these bioinorganic complexes inhibited the heme detoxification pathway, a critical mechanism in malaria parasite survival. SCXRD was employed to determine the crystal morphology, while routine analytical techniques were used for detailed structural elucidation.

Conclusion

This review aimed to provide clear insights into different polymorphs of ATQ. Additionally, important factors like bioavailability and binding capabilities of ATQ were also discussed elaborately. The poor water solubility of ATQ had inspired many global researchers to investigate various approaches to enhance the aqueous solubility of ATQ. Additionally, this review venture can serve as a useful platform for future researchers to explore further regarding the possible polymorphs, bioavailability, and binding properties of ATQ.

Acknowledment

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