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About PQM

The Promoting the Quality of Medicines (PQM) program is a cooperative agreement between the U.S. Agency for International Development (USAID) and the U.S. Pharmacopeial Convention (USP). The PQM program provides technical assistance to strengthen medicines regulatory authorities and quality assurance systems and supports manufacturing of quality-assured priority essential medicines for malaria, HIV/AIDS, tuberculosis, neglected tropical diseases, and maternal and child health.

Recommended Citation

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<th>Description</th>
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<tr>
<td>ADE</td>
<td>acceptable daily exposure</td>
</tr>
<tr>
<td>ANDA</td>
<td>Abbreviated New Drug Application</td>
</tr>
<tr>
<td>API</td>
<td>active pharmaceutical ingredient</td>
</tr>
<tr>
<td>COSY</td>
<td>Correlation Spectroscopy</td>
</tr>
<tr>
<td>CQA</td>
<td>critical quality attribute</td>
</tr>
<tr>
<td>CV</td>
<td>cleaning validation</td>
</tr>
<tr>
<td>d-PZQ</td>
<td>dextro-PZQ</td>
</tr>
<tr>
<td>DEPT</td>
<td>Distortionless Enhancement by Polarization Transfer</td>
</tr>
<tr>
<td>DMF</td>
<td>drug master files</td>
</tr>
<tr>
<td>EMA</td>
<td>European Medicines Agency</td>
</tr>
<tr>
<td>FDA</td>
<td>U.S. Food and Drug Administration</td>
</tr>
<tr>
<td>FTIR</td>
<td>Fourier transform infrared</td>
</tr>
<tr>
<td>GMP</td>
<td>good manufacturing practices</td>
</tr>
<tr>
<td>HBEL</td>
<td>health-based exposure limits</td>
</tr>
<tr>
<td>HETCOR</td>
<td>heteronuclear correlation</td>
</tr>
<tr>
<td>HPLC</td>
<td>high-performance liquid chromatography</td>
</tr>
<tr>
<td>IID</td>
<td>Inactive Ingredients Database</td>
</tr>
<tr>
<td>IPQC</td>
<td>in-process quality control</td>
</tr>
<tr>
<td>IUPAC</td>
<td>International Union of Pure and Applied Chemistry</td>
</tr>
<tr>
<td>I-PZQ</td>
<td>levo-PZQ</td>
</tr>
<tr>
<td>MASS</td>
<td>Mass Spectroscopy</td>
</tr>
<tr>
<td>NDA</td>
<td>New Drug Application</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
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<td>--------------</td>
<td>----------------------------------</td>
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<tr>
<td>NMR</td>
<td>nuclear magnetic resonance</td>
</tr>
<tr>
<td>NOEL</td>
<td>no-observed-effect-level</td>
</tr>
<tr>
<td>OEL</td>
<td>occupational exposure limit</td>
</tr>
<tr>
<td>PIR</td>
<td>Product Information Report</td>
</tr>
<tr>
<td>PZQ</td>
<td>Praziquantel</td>
</tr>
<tr>
<td>RH</td>
<td>relative humidity</td>
</tr>
<tr>
<td>RLD</td>
<td>Reference Listed Drug</td>
</tr>
<tr>
<td>RMG</td>
<td>rapid mixer granulator</td>
</tr>
<tr>
<td>RP</td>
<td>reverse phase</td>
</tr>
<tr>
<td>SBA</td>
<td>Summary Basis of Approval</td>
</tr>
<tr>
<td>SDF</td>
<td>solid dosage form</td>
</tr>
<tr>
<td>SEM</td>
<td>Scanning Electron Microscopy</td>
</tr>
<tr>
<td>UV</td>
<td>ultraviolet</td>
</tr>
<tr>
<td>WHO</td>
<td>World Health Organization</td>
</tr>
<tr>
<td>XRD</td>
<td>X-ray diffraction</td>
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</table>
Executive Summary

Praziquantel (PZQ) was discovered in 1972 and was developed first for the veterinary market and then for human treatment of schistosomiasis. By 1985, approximately 1 million persons had been treated with PZQ. Today, PZQ remains the drug of choice for all forms of schistosomiasis occurring in humans, because of its high efficacy, low toxicity, and ease of single, oral administration compared to the two other major medicines currently available for treatment of schistosomiasis: metrifonate and oxamniquine [1].

PZQ was the first anthelminthic drug to fulfill the World Health Organization’s (WHO) requirements for population-based chemotherapy of a broad range of parasitic infections. The chemical entity was discovered in 1972, and was developed first for the veterinary market and then for human treatment of schistosomiasis. Initial studies were carried out by E. Merck in healthy volunteers in 1978. No clinically relevant drug-related changes were detected by the extensive psychological, clinical, neurological, hematological, and clinico-chemical examinations of the volunteers. Subsequently, comprehensive toxicological studies, which employed a wide variety of test systems, were also carried out by WHO in collaboration with the International Agency for Research on Cancer. The tests found no mutagenic, carcinogenic, embryotoxic, or teratogenic activity of PZQ. By 1985, approximately 1 million persons were treated with PZQ. From these perspectives, the development of PZQ can be considered a case of successful drug development for tropical diseases, involving various forms of public-private cooperation.

The Product Information Report (PIR) provides a summary of available literature for the active pharmaceutical ingredient (API), analytical methods, toxicology, and formulation process of the solid dosage form (SDF) for the product.

The basic information provided includes chemical structure/formula, International Union of Pure and Applied Chemistry (IUPAC) name, physicochemical properties, and solubility-related data of PZQ.

PZQ structure has been characterized through various techniques (e.g., ultraviolet (UV) visible, Fourier transform infrared (FTIR), nuclear magnetic resonance (NMR), X-ray diffraction (XRD), Mass Spectroscopy (MASS), Scanning Electron Microscopy (SEM) and has been summarized in the document.
Different synthetic routes to synthesize PZQ are also discussed. β-phenylethylamine is usually the key starting material for the synthesis of PZQ [2].

This PIR also highlights the stability of PZQ in aqueous and dry states and gives the mechanism of the degradation and degradation products formed.

The PZQ API commercial preparation is actually a racemic mixture, in which only the levo-enantiomer possesses anthelmintic activity [3]. PZQ is a white to nearly white crystalline powder of bitter taste. The compound is stable under normal conditions and melts at 136°C–140°C with decomposition. The drug substance is hygroscopic. PZQ is easily soluble in chloroform and dimethyl sulfoxide, soluble in ethanol, and very slightly soluble in water.

The qualitative formula for the leading marketed formulation, Biltricide®, is described with proposed functions of the excipients, along with the U.S. Food and Drug Administration (FDA) Inactive Ingredients Database (IID) limits for the individual excipients.

PZQ tablets can be manufactured by using a wet granulation process. The requirements for manufacturing equipment along with the proposed manufacturing process have been outlined. Possible scale-up requirements are included in accordance with FDA’s Scale-Up and Post-Approval Changes Guidance for Immediate Release Products.

PZQ is rapidly absorbed (80%) following oral administration, with a T_max of approximately 1 to 3 hours. When administered with food, the C_max and AUC of PZQ are higher relative to the fasting state, although the variability is also increased. PZQ should always be taken with food. PZQ induces a rapid contraction of schistosomes by a specific effect on the permeability of the cell membrane. The drug further causes vacuolization and disintegration of the schistosome tegument. However, the mechanism of action is unknown.

Toxicity data of PZQ by administering through various routes in animals are as follows:

- oral LD_{50} (rat): 2840 mg/kg;
- intraperitoneal LD_{50} (rat): 586 mg/kg
- intramuscular LD_{50} (rat): >2 gm/kg
- oral LD_{50} (mouse): 2454 mg/kg
- intraperitoneal LD_{50} (mouse): 376 mg/kg
- subcutaneous LD_{50} (mouse): 7172 mg/kg.
Biltricide®, the Reference Listed Drug (RLD), has a shelf-life of 48 months, indicating the robust nature of the drug product [4].

A dedicated manufacturing area and equipment for fabricating PZQ tablets are not necessary. PZQ has good inherent stability and does not pose any major challenge in the manufacturing of solid dosage form. However, to achieve the desired dissolution profile, particle size distribution of API, polymorphic form selection, and concentration of wetting agent need to be optimized.

As per the USP monograph for praziquantel, the storage instruction for PZQ API is “preserve in well-closed light-resistant containers.” The API is reported to be hygroscopic, but the tablets are reported to be manufactured by wet granulation process [5, 6]. It may be prudent to control relative humidity (RH) during manufacturing steps like dispensing and dry mixing, where API is directly exposed to the environment. The facility of manufacturing should be maintained with optimum temperature and RH conditions to achieve batch-to-batch uniformity.

The acceptable daily exposure (ADE) value of PZQ has been reported as 694 µg/day. Although the draft European Medicines Agency (EMA) Guideline based on health-based exposure limits (HBEL) may be used to justify cleaning limits, traditional cleaning limits used by industry (e.g., 1/1000th of minimum therapeutic dose or 10 ppm of one product in another product) may apply for non-hazardous products such as PZQ.
Key Manufacturing Challenges

The existing PZQ formulation is a racemic mixture of levo-PZQ (l-PZQ) and dextro-PZQ (d-PZQ). Only one of these components (levo-PZQ enantiomer) is pharmacologically active. The other component, d-PZQ, has been shown to be inactive and contributes to the bitter taste that makes treating young children difficult.

WHO has recognized the gravity of the morbidity due to schistosomiasis in the preschool age group. WHO has recommended treating these children on a case-by-case basis because of the absence of a suitable pediatric formulation of PZQ for inclusion in preventive chemotherapy [1]. The challenge in ensuring the correct dosage, safe administration, and conservation of the medicine has led WHO to recommend the flexible solid oral formulation for children younger than 5 years [7]. Masking the taste of the API in a pediatric formulation may also be a challenge.

PZQ belongs to BCS class 2, due to its low solubility. The particle size distribution of API and its polymorphic form would be critical attributes for the quality of the dosage form. Key manufacturing challenges may be dust generation during granulation, wettability during granulation, detection of end point during granulation, strength of granules for down-streaming, and flow of granules during compaction.

Pharmacopeias recommend PZQ API be protected from light storage, which is not well-supported in the literature researched in support of this report or in industry manufacturing practice. As discussed later, the author of this report concluded that it is not required to adopt extreme measures (e.g., use of sodium vapor lamps) during the manufacturing of the formulation. (Such measures are routinely adopted for molecules that are extremely sensitive to photogradation, such as nifedipine, mefloquine, and riboflavin). It can also be inferred that PZQ API should be protected from light during long-term storage.

PZQ API has been reported to be hygroscopic. The storage instructions for PZQ API in USP 41 monograph are “preserve in well-closed light-resistant containers.” Based on review of the Biltricide® (Bayer), praziquantel tablets (Cipla), and praziquantel tablets (MacLeod) product references cited later, it can be inferred that a wet granulation process is being followed in the manufacturing industry for PZQ tablets. The industry manufacturing practice indicates that the stability of the PZQ molecule is not affected during the wet granulation process.
Based on the reported hygroscopicity of PZQ API, it is recommended that, during manufacturing, the PZQ API and PZQ tablets be protected from excessive humidity during the unit processes of raw material weighing, dry mixing, lubrication, and compression.
Active Pharmaceutical Ingredient

PZQ is a white to nearly white crystalline powder having bitter taste. The compound is stable under normal conditions and melts at 136°C-140°C with decomposition. The active substance is hygroscopic. It is easily soluble in chloroform and dimethyl sulfoxide, soluble in ethanol, and very slightly soluble in water [8].

Chemical Structure/Formula

Molecular Formula-C19H24N2O2

![Chemical Structure](image)

Name [9]

IUPAC Name

- 2-(cyclohexylcarbonyl)-1,2,3,6,7,11b-hexahydro-4H-pyrazino(2,1-a) isoquinolin-4-one
- 2-(Cyclohexylcarbonyl)-1,2,3,6,7,11b-hexahydro-4H-pyrazino[2,1-a] isochinolin-4-one
- 2-(Cyclohexylcarbonyl)-1,2,3,6,7,11b-hexahydro-4H-pyrazino[2,1-a] isoquinoléin-4-one
OTHERS\textsuperscript{[10,11]}

Traditional Name

- 4H-Pyrano[2,1-a]isoquinolin-4-one, 2-(cyclohexylcarbon-yl)-1,2,3,6,7,11b-hexahydro-
- (11bS)-2-[cyclohexyl(oxo)methyl]-3,6,7,11b-tetrahydro-1H-pyrazino[2,1-a] isoquinolin-4-one
- 2-(cyclohexanecarbonyl)-1,2,3,6,7,11b-hexahydro-4H-pyrazino[2,1-a] isoquinolin-4-one
- 2-(cyclohexanecarbonyl)-2,3,6,7-tetrahydro-1H-pyrazino[2,1-a] isoquinolin-4(11bH)-one
- 2-(cyclohexanecarbonyl)-3,6,7,11b-tetrahydro-1H-pyrazino[2,1-a] isoquinolin-4-one
- 2-(Cyclohexylcarbonyl)-1,2,3,6,7,11b-hexahydro-4H-pyrazino[2,1-a] isoquinolin-4-one
- 2-(Cyclohexylcarbonyl)-1,2,3,6,7-11b-hexahydro-4H-pyrazino[2,1a] isoquinolin-4-one
- 2-(Cyclohexylcarbonyl)-1,2,3,6,7-11b-hexahydro-4H-pyrazino [2,1-aisoquinolin-4-one
- 2-(Cyclohexylcarbonyl)-1,2,3,6,7-11b-hexahydro-4H-pyrazinoe(2,1a) isoquinolin-4-one
- 2-(cyclohexyl(oxo)methyl)-3,6,7,11b-tetrahydro-1H-pyrazino[2,1-a] isoquinolin-4-one
- 2-Cyclohexanecarbonyl-1,2,3,6,7,11b-hexahydro-pyrazino[2,1-a] isoquinolin-4-one
- 2-cyclohexanecarbonyl-1H,2H,3H,4H,6H,7H,11bH-piperazino[2,1-a] isoquinolin-4-one
- 2-Cyclohexylcarbonyl-1,2,3,6,7,11b-hexahydro-4H-pyrazino[2,1-a] isoquinolin-4-one
**MESH Synonyms**[^2,^10]

Cesol, Azinox, Droncit, Warmnil, Pyquiton, Prazinon, Embay8440, Distocide, Cysticide, Biltricide.

**Physical Properties**

**Particle Size**

PZQ has low solubility in water (around 400 µg/mL). The selection of the API particle size would be a critical issue for the development of a dosage form, which is bioequivalent to the reference product.

**Powder X-Ray Diffraction Study**

PZQ showed multiple characteristic diffraction peaks in powder XRD analysis, confirming its crystalline nature (Figure 1) [2].

![Figure 1. X-ray Diffractogram for Crystalline PZQ](image)

**Melting Point**

136°C-142°C[^9]

**Log P**

2.42[^12,^13]
pKa
19.38\textsuperscript{[12]}

Water Solubility
0.381 mg/mL\textsuperscript{[12]}

Refractive Index
1.615\textsuperscript{[11]}

Bulk Density
0.15 gm/cm\textsuperscript{3} or 150 kg/m\textsuperscript{3}\textsuperscript{[14]}

Surface Tension
55.2 dyne/cm\textsuperscript{[11]}

Solubility
PZQ is soluble in ethanol (97 mg/mL) and chloroform (567 mg/mL), but only sparingly soluble in water (0.381 mg/mL)\textsuperscript{[12]}. With the reported basic pKa value of 19.38, PZQ is expected to have pH independent solubility in the pH range of gastrointestinal tract.

Chemical Properties
Stereochemistry of PZQ\textsuperscript{[2, 10, 12]}

<table>
<thead>
<tr>
<th>Stereochemistry</th>
<th>Racemic mixture</th>
</tr>
</thead>
<tbody>
<tr>
<td>Optical Activity</td>
<td>l-PZQ and d-PZQ</td>
</tr>
<tr>
<td>Defined Sterocenters</td>
<td>1</td>
</tr>
<tr>
<td>Charge</td>
<td>0</td>
</tr>
<tr>
<td>Refractivity</td>
<td>88.79 m\textsuperscript{3}\textpermmol\textsuperscript{-1}</td>
</tr>
<tr>
<td>Polarizability</td>
<td>34.84 Å\textsuperscript{3}</td>
</tr>
</tbody>
</table>

Synthesis of PZQ
Numbers of literature references mentioned in this document describes the process for synthesis of PZQ. U.S. Pat. No. 4001411 describes a process for preparation of PZQ by acylating 4-oxo-1,2,3,6,7,11b-hexahydro-4H-pyrazino.2,1-alisoquinoline with cyclohexanoyl chloride in chloroform and triethylamine.
Most approaches of practical importance to pyrazino (2, 1-a) isoquinolin-4 ones start from the readily available isoquinoline, 1 (Scheme 1). The preparation of intermediate 3 via the Reissert reaction and subsequent high-pressure hydrogenation has been reported [2]. Conversion to the final product, 5, can be accomplished by acylation with chloroacetyl chloride, 4, followed by base catalyzed cyclization.

Scheme 1

Scheme 2, the racemic product, 5, can be resolved into its optically active components R-(-) (5a) and S-(+) (5b). A reaction sequence to recycle the unwanted enantiomer, 5b, and its conversion into its mirror image, 5a, is also shown in Scheme 2. This transformation can be accomplished by the dehydrogenation of 5b to afford 6, followed by its catalytic hydrogenation to 5.
KR2002076486 describes a process for preparation of PZQ by reacting phenyl-ethylamine with chloroacetyl chloride to obtain 2-chloro-N-phenethylacetamide. The compound 2-chloro-N-phenethylacetamide is then reacted with phthalimide to give 2-phthalimido-N-phenethylacetamide, which is then treated with hydrazine monohydrate to give 2-amino-N-phenylethylacetamide. On further treatment with bromoacetal, 2-amino-N-phenylethylacetamide gives 2-2.2-dimethoxy ethyl) amino-N-(2-phenylethyl) acetamide. This compound on further cyclization and acylation using cyclo hexanoyl chloride forms PZQ [15]. Several other synthetic methods for synthesis of PZQ have been reported.
Although the commercially available API is a racemic mixture, attempts have been made to synthesize the pure enantiomeric forms. In 2004, a method was reported to synthesize optically pure (−)-PZQ, which is said to have high efficacy and low toxicity compared with racemic mixture [3]. In 2006, a synthetic method of enantio-selective synthesis of (S)-(−) PZQ was also reported [16].

**Impurity Profiles of PZQ**

Both USP and EP have reported three impurities for PZQ: A, B, and C. Their structures are shown in Figure 2 [9, 17].
Figure 2. Impurities Listed in USP and EP

In addition to the above pharmacopeial impurities, other impurities have also been reported. The following (Figure 3) are their names and structures [18, 19].
Degradation Products

Forced degradation studies on PZQ solution were carried using acid, base, hydrogen peroxide, thermal, and UV-radiation [20]. For acidic degradation, a sample solution (10 μg/mL) was prepared by adding 1.0 mL of stock solution and 2.0 mL of 5 M HCl to a 10.0 mL volumetric flask. The volumetric flask was kept under 60°C reflux conditions for 5 hours and neutralized with 5 M NaOH; then the volume was made up to the mark with distilled water. Similarly, alkaline degradation (5 M NaOH), oxidative degradation (1% H₂O₂), and thermal degradation (heated at 105°C) were performed. UV degradation was performed with 10 mg of PZQ solid sample was placed in a UV cabinet at short wavelength (254 nm), then subjected to the proposed sample procedure, and fluorescence intensity was measured every 1 hour up to 24 hours.

Degradation was observed in 5 M HCl (up to 81% in 60 minutes) and 5 M NaOH (up to 1.0% in 60 minutes) and 1% H₂O₂ (up to 84% in 60 minutes), but there was no evidence of degradation under UV light or thermal conditions. For acidic and oxidative degradation, it was observed that relative fluorescence intensity decreased gradually with increased heating time.
Environment Compatibility

PZQ is reported to be harmful to aquatic life, with long-lasting effects, and hence should not be allowed to enter drains. API may be [21]:

1. Irritate the mucous membranes and upper respiratory tract.
2. Be harmful by inhalation, ingestion, or skin absorption.
3. Cause eye, skin, or respiratory system irritation.

It is suggested to use process enclosures, local exhaust ventilation, or other engineering controls to control airborne levels below recommended exposure limits. Facilities storing or utilizing PZQ should be equipped with eyewash and a safety shower [22].

Safety Data Sheet

PZQ is a fine powder, suitable for use as an API. In case of skin contact, all contaminated clothing should be removed, and the contact area should be rinsed with water. In case of eye contact, the eye should be rinsed out with plenty of water. As per the Safety Data Sheet [21], the toxicity data of PZQ is

- oral LD$_{50}$ (rat): 2840 mg/kg
- intraperitoneal LD$_{50}$ (rat): 586 mg/kg
- intramuscular LD$_{50}$ (rat): >2 gm/kg
- oral LD$_{50}$ (mouse): 2454 mg/kg
- intraperitoneal LD$_{50}$ (mouse): 376 mg/kg
- subcutaneous LD$_{50}$ (mouse): 7172 mg/kg

Structure Characterization

UV Spectrum

PZQ absorbs in the UV range because of specific chromophores in the structure that absorb at a particular wavelength. This fact was successfully employed for quantitative determinations of PZQ using the Varian DMS 90 system and matched 1 cm quartz cells. The UV absorption spectrum of PZQ solution in 95% methanol against the corresponding blank was evaluated. The absorption maxima were found at 257, 263, and 271 nm [2], as shown in Figure 4.
Figure 4. Absorption Spectrum of PZQ in 95% Methanol

FTIR Spectrum

The infrared spectrum of pure PZQ was obtained by the KBr disk method recorded on an FTIR spectrophotometer (Perkin Elmer, Spectrum One). KBr pellets were prepared by gently mixing 1 mg of the sample with 200 mg KBr [23]. The spectrum was obtained by scanning at 400 to 4,000 cm$^{-1}$ at a resolution of 2 cm$^{-1}$ and is shown in Figure 5.
Mass Spectrum

The mass spectrum of PZQ was obtained on a Finnigan T3Q45 spectrometer, interfaced with the SuperIncos data system [2]. The parent ion was allowed to collide with argon at collision energy of 19.8 eV. Figure 6 shows the detailed mass fragmentation pattern, where the base peak appears at m/z=203, and the molecular ion peak at m/z=313.
Nuclear Magnetic Resonance Spectrum

Both the $^1$H and $^{13}$C-NMR spectrum of PZQ were obtained in Chloroform-d (CDCl$_3$), using tetramethylsilane as an internal standard [2]. The $^1$H spectrum of PZQ (Figure 7) can be conveniently divided into the aliphatic and aromatic regions. In the aromatic portion, one multiplet is observed at 7.10–7.40 ppm corresponding to protons 8, 9, 10, and 11. The aliphatic protons were initially assigned on the basis of chemical shift considerations. The multiplet at 1.1–1.90 ppm is assigned to the protons of the cyclohexyl -CH$_2$ group, while the multiplet at 2.35–2.60 ppm is assigned to the protons of the cyclohexyl -CH group. The multiplet at 2.65–3.00 ppm is assigned to protons 1, 6, 7, and 7. Protons 3 and 3’ form an AB quartet at 4.10–4.50 ppm, with a coupling constant of 17 Hz. Protons 1’ and 11 b form a multiplet that resonates at 4.70–4.90 ppm, while proton 6’ yields a doublet at 5.10 ppm (coupling constant of 14 Hz). All $^1$H-NMR assignments were confirmed by means of the two-dimensional Correlation Spectroscopy (COSY) experiment (Figure 8).

Figure 7. $^1$HNMR Spectrum of PZQ
A noise-modulated, broad-band decoupled $^{13}$C-NMR spectrum (Figure 9)$^{[24]}$ showed 17 resonance bands, which is in accordance with what would be anticipated for the 19 carbons of PZQ. Carbon resonance bands at 25.53 ppm, 28.54 ppm, 29.04 ppm, and 40.57 ppm were assigned to the cyclohexyl carbons, while bands at 164.20 ppm and 174.53 ppm were assigned to the two carbonyls of PZQ$^{[2]}$. 

$^{13}$C-NMR spectrum
A Distortionless Enhancement by Polarization Transfer (DEPT) experiment (Figure 10) within the aliphatic region permitted the identification of the methine and methylene carbons \(^2\). Seven \(-\text{CH}_2\) absorptions at 25.53 ppm, 28.54 ppm, 28.81 ppm, 29.04 ppm, 38.90 ppm, 44.96 ppm, and 48.83 ppm were assigned to carbons 1, 3, 6, 7, and those of the cyclohexyl group. In addition, two \(-\text{CH}\) resonance bands at 40.58 ppm and 54.75 ppm were assigned to carbon 11 b and the cyclohexyl \(-\text{CH}\).
These assignments were confirmed through the performance of a heteronuclear correlation (HETCOR) experiment (Figure 11).
Analysis of PZQ

A number of methods for analysis of PZQ are reported in pharmacopeias and literature. A few of these analytical methods are summarized below.

Pharmacopeial Methods

United States Pharmacopoeia (USP)
The liquid chromatograph was equipped with a UV detector set at 210-nm and a 4-mm × 25-cm column with 10-μm packing L1 and mobile phase containing a mixture of acetonitrile and water (60:40). The flow rate was kept about 1.5 mL per minute and the 10 μL of prepared standard and test samples were injected into the system. Responses of major peaks were measured. The tailing factor should not more than 1.5, and the relative standard deviation for replicate injections should not be more than 1.0% [9].

British Pharmacopoeia (BP)
The liquid chromatograph was equipped with a 210-nm detector and a 4-mm × 25-cm column with octadecylsilyl silica gel for chromatography (5 μm) as the stationary phase, with mobile phase containing mixture of acetonitrile and water (45:55). The flow rate was kept about 1.0 mL per minute, and the 20 μL of prepared standard and test samples were injected into the system. Responses of major peaks were measured. The retention time for PZQ was about 10 minutes [25].

European Pharmacopoeia (EP)
The liquid chromatograph was equipped with a 210-nm detector and a 4-mm × 25-cm column with octadecylsilyl silica gel for chromatography (5 μm) as the stationary phase, with mobile phase containing mixture of acetonitrile and water (45:55). The flow rate was kept about 1.0 mL per minute, and the 20 μL of prepared standard and test samples were injected into the system. Responses of major peaks were measured. The retention time for PZQ was about 9 minutes [17].

Indian Pharmacopoeia (IP)
The liquid chromatograph was equipped with a UV detector set at 210-nm and a 4-mm × 25-cm column with 10-μm packing L1 and mobile phase containing mixture of acetonitrile and water (45:55). The flow rate was kept about 1.5 mL per minute, and the 10 μL of prepared standard and test samples were injected into the system. The responses of major peaks were measured. The tailing factor should not more than 1.5, and the relative standard deviation for replicate injections should not be more than 1.0% [26].
Other Reported Methods

Quantitative reverse phase (RP) high-performance liquid chromatography (HPLC) was performed [27] on a Varian Pro Star chromatograph equipped with a solvent delivery module 210, an UV-visible spectrophotometric detector 330 (set at 262 nm) and a Rheodine® injection valve with a 100 μL loop. Chromatographic separation was accomplished using a LiChrospher® 100 RP-18 (Merck) stainless steel column (25 cm x 4 mm id., 5 μm particle size). The flow rate was isocratic at 1 mL/minute. The mobile phase was prepared by mixing acetonitrile with water 60:40 (% v/v). The UV detector was set at 262 nm. The HPLC system was operated at (30 ± 1°C). The mobile phase was filtered through a membrane filter (0.45 μm × 47 mm) and degassed using an ultrasonic bath for 45 minutes. The run time of analysis was around 9 minutes.

The method was validated for parameters including accuracy, linearity, and precision, as per ICH guidelines. The values of relative standard deviation and % recovery were found to be satisfactory, indicating that the proposed method is precise and accurate and can be used for routine analysis of PZQ in bulk and pharmaceutical formulation.

The reported regression analysis data and summary of validation parameter for the RP-HPLC method parameters results are as given below in Table 1.

Table 1. RP-HPLC Method Parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linearity</td>
<td>1.0 to 14.0 μg/mL</td>
</tr>
<tr>
<td>Slope</td>
<td>38.399</td>
</tr>
<tr>
<td>Correlation Coefficient</td>
<td>0.999</td>
</tr>
<tr>
<td>Intercept (c)</td>
<td>7.8284</td>
</tr>
<tr>
<td>Limit of Quantitation (μg/mL)</td>
<td>161.75 ng/mL</td>
</tr>
<tr>
<td>Limit of Detection (ng/mL)</td>
<td>53.37 ng/mL</td>
</tr>
<tr>
<td>Precision (CV)</td>
<td>-</td>
</tr>
<tr>
<td>Intra-day precision</td>
<td>1.86 %</td>
</tr>
<tr>
<td>Inter-day precision</td>
<td>0.77%</td>
</tr>
<tr>
<td>Ruggedness</td>
<td>Rugged</td>
</tr>
</tbody>
</table>
Analysis of Impurities/Related Substances/Degradation Products

United States Pharmacopoeia (USP)

The mobile phase and chromatographic system are similar to that of USP API monograph assay.

- **Standard preparation**—Dissolve accurately weighed quantities of *USP PZQ Related Compound A RS*, *USP PZQ Related Compound B RS*, and *USP PZQ Related Compound C RS* in *Mobile phase* to obtain a single solution having known concentrations of about 0.04 mg of each per mL.

- **Test preparation**—Transfer accurately weighed about 200 mg of PZQ to a 10-mL volumetric flask, dissolve in and dilute with *Mobile phase* to volume, and mix.

- **Procedure**—Separately inject equal volumes (about 10 μL) of the *Standard preparation* and the *Test preparation* into the chromatograph, record the chromatograms, and measure the responses for the peaks. The relative retention times are about 0.8 for PZQ related compound A, 1.0 for PZQ, 1.8 for PZQ related compound B, and 2.1 for PZQ related compound C. Calculate the percentages of 2-benzoyl-1,2,3,6,7,11b-hexahydro-4H-pyrazino[2,1-a]isoquinolin-4-one (PZQ related compound A), 2-(cyclohexylcarbonyl)-2,3,6,7-tetrahydro-4H-pyrazino[2,1-a]isoquinolin-4-one (PZQ related compound B), and 2-(N-formylhexahydrohippuroyl)-1,2,3,4-tetrahydroisoquinolin-1-one (PZQ related compound C) in the portion of PZQ taken by the formula:

\[
1000(C/W) \frac{r_U}{r_S}
\]

in which C is the concentration, in mg per mL, of the respective USP Reference Standard taken to prepare the *Standard preparation*; W is the weight, in mg, of PZQ taken to prepare the *Test preparation*; and rU and rS are the peak responses at corresponding retention times, obtained from the *Test preparation* and the *Standard preparation*, respectively. None of the related substances should be more than 0.2%.
European Pharmacopoeia (EP)

The mobile phase and chromatographic system are similar to that of EP API monograph assay.

- **Test solution (a).** Dissolve 40.0 mg of the substance to be examined in the mobile phase and dilute to 10.0 mL with the mobile phase.

- **Test solution (b).** Dilute 1.0 mL of test solution (a) to 20.0 mL with the mobile phase.

- **Reference solution (a).** Dissolve 40.0 mg of PZQ CRS in the mobile phase and dilute to 10.0 mL with the mobile phase. Dilute 1.0 mL to 20.0 mL with the mobile phase.

- **Reference solution (b).** Dissolve 5 mg of PZQ impurity A CRS in reference solution (a) and dilute to 25.0 mL with reference solution (a). Dilute 2.0 mL of this solution to 20.0 mL with the mobile phase.

- **Reference solution (c).** Dilute 1.0 mL of test solution (a) to 20.0 mL with the mobile phase. Dilute 5.0 mL of this solution to 50.0 mL with the mobile phase.

- **System suitability:** reference solution (b):
  - **Resolution:** minimum 3.0 between the peaks corresponding to impurity A and PZQ.

- **Limits:**
  - **Any impurity:** not more than the area of the principal peak in the chromatogram obtained with reference solution (c) (0.5 per cent), not more than 1 such peak having an area greater than 0.4 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.2 per cent).
  - **Total:** not more than the area of the principal peak in the chromatogram obtained with reference solution (c) (0.5 per cent).
  - **Disregard limit:** 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (c) (0.05 per cent).

The summary of the pharmacopeial test is listed in Table 2.
Table 2. Pharmacopeial Test Limits for PZQ

<table>
<thead>
<tr>
<th>Test</th>
<th>Limit</th>
<th>USP</th>
<th>BP</th>
<th>EP</th>
<th>IP</th>
</tr>
</thead>
<tbody>
<tr>
<td>PZQ – Impurity A</td>
<td>NMT 0.2%</td>
<td>NMT 0.2%</td>
<td></td>
<td>NMT 0.5%</td>
<td>NMT 0.5%</td>
</tr>
<tr>
<td>PZQ – Impurity B</td>
<td>NMT 0.2%</td>
<td>NMT 0.2%</td>
<td></td>
<td>NMT 0.5%</td>
<td></td>
</tr>
<tr>
<td>PZQ – Impurity C</td>
<td>NMT 0.2%</td>
<td>NMT 0.1%</td>
<td></td>
<td>NMT 0.5%</td>
<td></td>
</tr>
<tr>
<td>Impurities all together</td>
<td>NMT 0.5%</td>
<td>NMT 0.5%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Assay – Tablets</td>
<td>90 -110%</td>
<td></td>
<td></td>
<td></td>
<td>90 -110%</td>
</tr>
<tr>
<td>Dissolution – Tablets</td>
<td>NLT 75%</td>
<td></td>
<td></td>
<td></td>
<td>NLT 75%</td>
</tr>
</tbody>
</table>

*NMT = Not more than; NLT = Not less than

Reference Standards Availability

Monographs of PZQ are available in USP, BP, EP and IP. The reference standards of PZQ and related substances are also available commercially [28–31].

Stability of PZQ

Dry Powder Stability

PZQ is stable under normal conditions [8]. The pharmacopeias recommend protecting PZQ API from light storage, which is not well-supported in the literature researched in support of this report and in industry manufacturing practice. The summary of photo-stability literature is provided in Table 3 below.
Table 3. Summary of Literature Information Regarding Photo-Stability of Praziquantel

<table>
<thead>
<tr>
<th>State</th>
<th>Stability Condition</th>
<th>Reference</th>
<th>Storage Recommendation</th>
</tr>
</thead>
<tbody>
<tr>
<td>API (as is)</td>
<td>USP 41</td>
<td>Well-closed, light-resistant</td>
<td>containers</td>
</tr>
<tr>
<td></td>
<td>EP 6.0</td>
<td>Protected from light</td>
<td></td>
</tr>
<tr>
<td></td>
<td>International</td>
<td>Well-closed, light-resistant</td>
<td>containers</td>
</tr>
<tr>
<td></td>
<td>Pharmacopoeia 2018</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>BP 2018</td>
<td>Protected from light</td>
<td></td>
</tr>
<tr>
<td></td>
<td>IP 2018</td>
<td>Protected from light</td>
<td></td>
</tr>
</tbody>
</table>

UV radiation: 254 nm for 24 hours [32] No degradation observed

The recommended storage condition provided in the monographs of all the pharmacopeias referenced above is to store PZQ API in well-closed containers protected from light. However, one photodegradation study on API (for 24 hours) did not lead to any photo degradation. The tablet dosage form Biltricide® is commercially supplied in an HDPE bottle of six tablets, and the labeling recommends the product to be stored below 30°C, with no instructions to protect the product HDPE bottle from light. It is apparent that the primary pack (the HDPE bottle of six tablets) provides adequate protection from light. It can be inferred that PZQ API in long-term storage should be protected from light. Additionally, from the packaging and labeled storage condition of Biltricide® and the published literature cited above, it can be deduced that it is not necessary to adopt extreme measures (e.g., use of sodium vapor lamps) during the manufacturing of the formulation. Such measures are routinely adopted for molecules that are extremely sensitive to photogradation, such as nifedipine, mefloquine, and riboflavin.
# Test Specifications of PZQ

Table 4 provides recommended test specifications for PZQ API, based on USP, EP, and other sources.

## Table 4. Test Specifications for PZQ API

<table>
<thead>
<tr>
<th>Test</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Description</td>
<td>White or almost white, crystalline powder</td>
</tr>
<tr>
<td>Identification by IR spectrum</td>
<td>Should pass</td>
</tr>
<tr>
<td>Loss on drying</td>
<td>Should be less than 0.5%</td>
</tr>
<tr>
<td>Residue on Ignition</td>
<td>Should be less than 0.05%</td>
</tr>
<tr>
<td>Heavy metals</td>
<td>Should be less than 20 ppm</td>
</tr>
<tr>
<td>Sulphated ash</td>
<td>Should be less than 1%</td>
</tr>
<tr>
<td>Residual Solvents</td>
<td>Methanol - Should be less than 3000 ppm</td>
</tr>
<tr>
<td></td>
<td>Dichloromethane - Should be less than 600 ppm</td>
</tr>
<tr>
<td>Assay</td>
<td>98.5-101% (on dry basis)</td>
</tr>
<tr>
<td>Related Substances (all together)</td>
<td>Impurity A - Should be less than 0.2%</td>
</tr>
<tr>
<td>Should be less than 0.5%</td>
<td>Impurity B - Should be less than 0.2%</td>
</tr>
<tr>
<td></td>
<td>Impurity C - Should be less than 0.1%</td>
</tr>
</tbody>
</table>
Solid Dosage Form

General summary

PZQ is indicated for the treatment of infections due to various species of schistosoma, such as *Schistosoma haematobium*, *Schistosoma japonicum*, *Schistosoma mansoni*, and *Schistosoma mekongi*, and infections due to the liver flukes caused by *Clonorchis sinensis/Opisthorchis viverrini* [33].

Active drug master files (DMF) for PZQ are listed in Table 5 [34, 35].

Table 5. List of Active DMFs for PZQ API as of April 2019

<table>
<thead>
<tr>
<th>DMF#</th>
<th>Submit Date</th>
<th>Holder</th>
<th>Subject</th>
<th>CEP from EDQM</th>
</tr>
</thead>
<tbody>
<tr>
<td>14352</td>
<td>19/8/1999</td>
<td>Cipla Ltd.</td>
<td>PZQ</td>
<td>Y</td>
</tr>
<tr>
<td>4287</td>
<td>2/10/1981</td>
<td>Merck KGaA</td>
<td>PZQ 2-(cyclohexylcarbonyl)-1,2,3,6,7,11</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>beta-4hyrazino-(2,1-alpha)</td>
<td></td>
</tr>
<tr>
<td>13999</td>
<td>24/2/1999</td>
<td>PCAS</td>
<td>PZQ</td>
<td>Y</td>
</tr>
<tr>
<td>26076</td>
<td>21/5/2012</td>
<td>Salora A Pharma Sciences Ltd</td>
<td>PZQ</td>
<td>-</td>
</tr>
<tr>
<td>26837</td>
<td>14/1/2013</td>
<td>Shanghai Desano Chemical</td>
<td>PZQ</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pharmaceutical Co Ltd</td>
<td></td>
<td></td>
</tr>
<tr>
<td>29271</td>
<td>15/4.2015</td>
<td>Hisun Pharmaceutical Nantong Co Ltd</td>
<td>PZQ</td>
<td>-</td>
</tr>
</tbody>
</table>

All of them are type II DMFs, which include drug substance and their allied compounds.
Regulatory Status

FDA approved the New Drug Application (NDA) for the RLD Biltricide® oral tablet 600 mg in 1982 as a prescription drug. Biltricide® is supplied as a 600 mg white to orange-tinged, film-coated, oblong tablet with three scores. The tablet is coded with “BAYER” on one side and “LG” on the reverse side. When broken, each of the four segments contain 150 mg of active ingredient so that the dosage can be easily adjusted to the patient’s bodyweight [33].

Segments are broken off by pressing the score (notch) with thumbnails if one-quarter of a tablet dose is desired to be administered. This is best achieved by breaking the segment from the outer end.

FDA approved the Abbreviated New Drug Application (ANDA) for PZQ oral tablet filed by PAR Pharmaceuticals [36] in 2017, which FDA found to be therapeutically equivalent to Biltricide® tablets, 600 mg (RLD) of Bayer HealthCare Pharmaceuticals Inc. It is supplied as a 600 mg white to off white, film-coated, oblong tablets with three scores coded with “PAR” on one side “231” on the reverse side. When broken, each of the four segments contains 150 mg of active ingredient so that the dosage can be easily adjusted to the patient’s bodyweight.
Formulation Barriers to Entry

The currently available dosage forms of PZQ are conventional formulations and do not pose major entry barriers in terms of formulation and manufacturing technology. However, critical properties of API (e.g., particle size distribution, polymorphic form, and availability of economical reference standards) could pose an entry barrier for the formulators. Biltricide® (RLD) is supplied in bottles of six tablets of 600 mg each. It is suggested to be stored below 86°F (30°C) [33]. The generic PZQ tablets (Par Pharmaceutical) are supplied in bottles of six tablets of 600 mg each. It is suggested to be stored at 20°C to 25°C (68°F to 77°F), with excursions permitted between 15°C and 30°C (59°F and 86°F), which is a controlled room temperature condition as defined by the USP [36].

Since PZQ is classified as a BCS class 2 drug, it is not a candidate for bio waiver, but qualitative and quantitative (Q1/Q2) matching of the generic product with an RLD product may allow high assurance of achieving bioequivalence.

Formulation Justification

Reverse Engineering

A qualitative formula for the leading marketed formulation was retrieved from the RLD Biltricide® NDA documents submitted to FDA. Biltricide® for oral administration contained 600 mg of the active ingredient PZQ per tablet. The other inactive ingredients in the tablets include corn starch, magnesium stearate, microcrystalline cellulose, povidone, sodium lauryl sulfate, polyethylene glycol, titanium dioxide, and hypromellose [33, 37].

Discussion on Excipients

A list of excipients with their proposed function in the RLD Biltricide® tablet is provided in Table 6. Biltricide® is a film-coated tablet, and FDA’s Inactive Ingredient Database (IID) can be accessed for individual inactive ingredients. IID provides the dosage forms for which the excipient is approved and the maximum concentration approved for that dosage form. Quantitative limits for excipients were checked for tablets [38].
### Table 6. List of Inactive Ingredients with Their Proposed Function and IID Limits

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Function</th>
<th>Reference (Pages of Reference)</th>
<th>IID Limit for Oral Tablet[^38]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn starch</td>
<td>Binding agent; compression aid; disintegrant; tablet and capsule diluent; tablet and capsule filler</td>
<td>[^39] pp. 200-01</td>
<td>5.28 mg</td>
</tr>
<tr>
<td>Magnesium stearate</td>
<td>Tablet and capsule lubricant</td>
<td>[^39] pp. 404-07</td>
<td>28.31 mg</td>
</tr>
<tr>
<td>Microcrystalline Cellulose</td>
<td>Binder/Diluent/Adsorbent (@20-90% Concentration), Antiadherent/Disintegrant (@5-20% concentration)</td>
<td>[^39] pp. 129-33</td>
<td>736.83 mg</td>
</tr>
<tr>
<td>Povidone K25</td>
<td>Tablet Binder or Coating agent</td>
<td>[^39] pp. 581-85</td>
<td>187.6 mg</td>
</tr>
<tr>
<td>Hypromellose USP</td>
<td>Tablet Binder or Coating agent</td>
<td>[^39] pp. 326-29</td>
<td>1943 mg</td>
</tr>
<tr>
<td>Sodium lauryl sulfate</td>
<td>Wetting agent and/or Tablet lubricant (@1-2%)</td>
<td>[^39] pp. 651-53</td>
<td>51.69 mg</td>
</tr>
<tr>
<td>Polyethylene Glycol 4000</td>
<td>As film coating or polishing material in film coating</td>
<td>[^39] pp. 517-22</td>
<td>167.6 mg</td>
</tr>
<tr>
<td>Titanium dioxide</td>
<td>White Pigment and Opacifier</td>
<td>[^39] pp. 741-44</td>
<td>49.27 mg</td>
</tr>
</tbody>
</table>

**Formulation Challenges**

PZQ is a crystalline powder of bitter taste and is reported to be hygroscopic [4, 36]. According to WHO data published in 2017, an estimated 220 million people are potentially infected with schistosomiasis, 10 percent of whom are probably children under 6 years of age.

The WHO approach to control schistosomiasis is based on regular treatment with a single, oral dose of 40 mg/kg body weight with PZQ. The main target population for treatment is children of school age (6 to 14 years of age). However, the available 600 mg PZQ tablet is difficult to administer to the pediatric population. The tablet needs to be crushed for administration, which may result in inaccuracy of dosing. Moreover,
the current PZQ product has a bitter taste that may be unacceptable to infants and children, leading to dosage compliance issues. Therefore, an opportunity and/or challenge of innovation exists for potential manufacturers to develop a more patient-friendly dosage form than the currently available 600 mg tablet presentation.

PZQ API is reported to be hygroscopic. The hygroscopicity literature is summarized in Table 7.

Table 7. Literature Information Regarding Hygroscopicity of Praziquantel (PZQ)

<table>
<thead>
<tr>
<th>State</th>
<th>Comment</th>
<th>Reference</th>
<th>Packaging</th>
</tr>
</thead>
<tbody>
<tr>
<td>API (as is)</td>
<td>Hygroscopic</td>
<td>Biltricide® Tablets Leaflet</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Hygroscopic</td>
<td>Praziquantel Tablets - Par Pharmaceutical</td>
<td></td>
</tr>
<tr>
<td>Tablets</td>
<td>No special storage conditions</td>
<td>Biltricide® Tablets Leaflet</td>
<td>HDPE Bottle</td>
</tr>
<tr>
<td></td>
<td>Heat and dampness can destroy some medicines</td>
<td>Biltricide® Tablets - Consumer medicine information</td>
<td>HDPE Bottle</td>
</tr>
<tr>
<td></td>
<td>30°C/75%RH - Long term - Stable</td>
<td>Praziquantel tablets - Macleods</td>
<td>HDPE bottles or clear film PVC/PVDC-Aluminum blisters</td>
</tr>
<tr>
<td></td>
<td>40°C/75%RH - 6 months - Stable</td>
<td>Praziquantel tablets - Cipla</td>
<td>HDPE screw cap, having 2 silica gel bags of 1gm and Rayon Sani coil</td>
</tr>
<tr>
<td></td>
<td>30°C/75%RH - Long term - Stable</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>30°C/75%RH - 6 months - Stable</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Tablets can be manufactured using a direct compression, dry granulation, or wet granulation process. A careful assessment of the qualitative composition of the RLD Biltricide® indicates use of a wet granulation process. Polyvinylpyrrolidone (PVP) is present in the formulation as a tablet binder and is usually employed in a wet granulation process. The WHO Summary Basis of Approval (SBA) of the Macleods Product mentions very poor flow properties of API, thus ruling out use of direct compression method. The SBA also mentions the use of organic solvent based wet granulation method. Further, for manufacturing of PZQ tablets manufactured by Cipla Pharma uses wet granulation process [5].
In view of the Biltricide® (Bayer), praziquantel tablet (Cipla), and praziquantel tablet (Macleods) product references cited above, it can be inferred that a wet granulation process is being followed in the manufacturing industry for PZQ tablets. The industry manufacturing practice indicates that the stability of the PZQ molecule is not affected during the wet granulation process.

Based on reported hygroscopicity of the PZQ API, however, it is recommended that, during manufacturing, the API and PZQ tablets be protected from excessive humidity, during the unit processes of raw material weighing, dry mixing, lubrication, and compression.

**Manufacturing Process**

PZQ tablets are preferably prepared using a wet granulation process [5, 6]. Wet granulation is the most common method to obtain granules for compaction. Major unit operations in wet granulation include milling, sieving, blending, granulation, drying, milling, blending compression, and coating.

As indicated above, based on reported hygroscopicity of the PZQ API, API and PZQ tablets should be protected from excessive humidity during the unit processes of raw material weighing, dry mixing, lubrication, and compression.

The first step may be the milling of raw material. However, this is an optional step based on the particle size of the supplied raw material. Airjet mill or Quadro mill can be used for milling. Milled powder is then subjected to sieving to achieve uniform particle size. Bulk powder obtained after sieving is blended with appropriate inactive ingredients. A V blender, double cone blender, bin blender, or octagonal blender can be used for blending. Granulating fluid containing the binder is added to dry powder blend in a rapid mixer granulator (RMG) to obtain wet dough mass and subsequently the granules.

Granules are then dried using equipment such as a fluidized bed dryer. The dried granules are milled, sieved, and mixed with additional inactive ingredients (e.g., lubricants, disintegrants) in a blender. The blend is then compressed in a tablet press to obtain the tablet dosage form. The tablets may then be coated utilizing a film coating pan. The RLD Biltricide® is a film-coated tablet presentation.

Precautions for safe handling include avoiding contact with skin and eyes. Additionally, formation of dust and aerosols should be avoided. Adequate general or local exhaust ventilation should be provided to keep airborne concentrations below the permissible exposure limits. Normal measures should be taken for fire prevention protection. For
nuisance exposures, a type P95 (US) or type P1 (EU EN 143) particle respirator should be used. For higher level protection, type OV/AG/P99 (US) or type ABEK-P2 (EU EN 143) respirator cartridges should be used. Respirators and components tested and approved under appropriate government standards, such as NIOSH (US) or CEN (EU), should be used.

**Analytical Methods**

**Pharmacopeial Methods**

**USP**

**Dissolution** - The medium used for dissolution is 900 mL of 0.1 N hydrochloric acid containing 2.0 mg of sodium lauryl sulfate per mL. USP 2 apparatus is used at 50 rpm for 60 minutes. UV absorbance values of resultant filtered dissolution samples were measured at 263 nm and compared with standard preparation. The amount dissolved should not be less than 75% (Q) of the labeled amount of PZQ in 60 minutes.

**Assay** - The mobile phase and chromatographic system are similar to that of API. Weigh and finely powder not less than 20 tablets. Transfer an accurately weighed portion of the powder, equivalent to about 150 mg of PZQ, to a 100-mL volumetric flask, add 70 mL of mobile phase, sonicate for 5 minutes, dilute with mobile phase to make up the volume, mix, and filter. Transfer 3.0 mL of the filtrate to a 25-mL volumetric flask, dilute with mobile phase to volume, and mix. Calculate the quantity, in mg, of PZQ in the portion of tablets taken by the formula:

\[
2500\left(\frac{C}{3}\right)\left(\frac{r_U}{r_S}\right)
\]

in which C is the concentration, in mg per mL, of USP PZQ RS in the standard preparation, and rU and rS are the peak responses obtained from the assay preparation and the standard preparation, respectively.

**WHO International Pharmacopoeia**

**Assay** - Weigh and powder 20 tablets. To a quantity of the powder equivalent to about 25mg of PZQ add 50 mL of ethanol (~750g/L) test solution (TS), shake, and dilute to volume with the same solvent. Filter and discard the first 5 mL of the filtrate. Measure the UV absorbance of a 1-cm wide quartz glass cuvette at the wavelength of 264nm against a solvent cuvette containing ethanol (~750g/L) TS. Calculate the percentage content of PZQ by comparison of the absorbance at the same wavelength of a solution containing 0.50mg of PZQ RS per mL of ethanol (~750g/L) TS.
Other Methods

In a reported method [27], analysis of API and tablets was performed using a LiChro-spher® 100 RP-18 (Merck) stainless steel column (25 cm x 4 mm id., 5μm particle size). The flow rate was isocratic at 1 mL/minutes. The mobile phase was prepared by mixing acetonitrile with water 60:40 (% v/v). The UV detector was set at 262 nm. The HPLC column chamber was operated at (30°C ± 1°C). The run time of sample analysis was less than 9 minutes. The content of 10 tablets, each containing 150 mg of PZQ, was pulverised using a mortar and pestle. A weighed amount tablet containing 150 mg was transferred to a 100 mL volumetric flask, and the volume was completed with mobile phase. An aliquot of 0.4 mL of the filtered resultant solution was adequately diluted with mobile phase in order to yield a solution containing 12 μg of PZQ/mL. The assay was calculated by using calibration curve with a range of 1-14 μg/mL of PZQ.

Stability Indicating Method

A stability indicating RP-HPLC method [20] for quantitative determination of PZQ moiety in the bulk drug powder raw material and dosage form and its impurities is summarized below.

The chromatographic separation was carried out on a CaltrexAI® column. Chromatography was done using an isocratic binary mobile phase consisting of acetonitrile and 25 mM ammonium acetate in the ratio of 40:60 at flow rate of 1 mL/minute, 30°C column temperature and 210 nm wavelength for detection. The elution time of PZQ was found to be 6.15 ± 0.03 minutes. The method was validated for system suitability, linearity, precision, limit of detection, limit of quantitation, specificity, solution stability, and robustness. A high recovery value of 100.3% ±1.10 of PZQ was achieved.

Stability of Dosage Form

Biltricide® tablets are recommended to be stored below 30°C. The shelf-life of the product is 48 months.

Dosage Form Test Specifications

Test specifications for PZQ tablets as per USP 41 are summarized in Table 8.
Table 8. Test Specifications of PZQ Tablets

<table>
<thead>
<tr>
<th>Test</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Identification</td>
<td>Should pass</td>
</tr>
<tr>
<td>Dissolution test</td>
<td>Not less than 75% (Q) of the labeled amount of PZQ dissolved in 60 minutes</td>
</tr>
<tr>
<td>Uniformity of Dosage form</td>
<td>Should meet the requirements (90-110 % w/w of the label claim)</td>
</tr>
<tr>
<td>Residual Solvents</td>
<td>Methanol – Should be less than 3000 ppm</td>
</tr>
<tr>
<td></td>
<td>Dichloromethane – Should be less than 600 ppm</td>
</tr>
<tr>
<td>Assay</td>
<td>90-110 % w/w of the label claim</td>
</tr>
</tbody>
</table>
**Bioavailability and Pharmacokinetics**

PZQ is rapidly absorbed (80%) following oral administration with a $T_{\text{max}}$ of approximately 1 to 3 hours. When administered with food, the $C_{\text{max}}$ and AUC of PZQ are higher relative to the fasting state, although the variability is also increased. PZQ should always be taken with food. PZQ is rapidly and extensively metabolized (substantial first pass metabolism) into its main active metabolite (hydroxylated degradation product of praziquantel). The terminal elimination half-life of PZQ is approximately 0.8 to 3 hours when administered with food.

PZQ induces a rapid contraction of schistosomes by a specific effect on the permeability of the cell membrane [33]. The drug further causes vacuolization and disintegration of the schistosome tegument. However, the detailed mechanism of action is unknown.

**BCS Class of the Product**

According to FDA, APIs (as per BCS) have been classified into four categories [40]:

- **BCS class I**: “high” solubility - “high” permeability
- **BCS class II**: “low” solubility” - “high” permeability
- **BCS class III**: “high” solubility - “low” permeability
- **BCS class IV**: “low” solubility - “low” permeability

PZQ has low solubility and high permeability and therefore has been placed in BCS Class II.
Pharmacokinetics

Based on the FDA approved labeling of Biltricide® [33], the pharmacokinetic parameters are summarized below.

Absorption

After oral administration, 80% of administered PZQ dose is absorbed, subjected to a first pass effect. Maximal serum concentration is achieved 1 to 3 hours after dosing.

Elimination

Following oral administration of PZQ, the elimination half-life of PZQ in serum ranges between 0.8 to 1.5 hours.

Metabolism

PZQ is rapidly metabolized by the cytochrome P450 enzyme system and undergoes a first pass effect after oral administration of Biltricide®.

Excretion

Approximately 80% of an oral dose of Biltricide® is excreted in the kidneys, almost exclusively (greater than 99%) in the form of PZQ metabolites.

Special Populations

The pharmacokinetics of PZQ was studied in 40 patients with Schistosoma mansoni infections with varying degrees of hepatic dysfunction (Table 9). In patients with schistosomiasis, the pharmacokinetic parameters did not differ significantly between those with normal hepatic function (Group 1) and those with mild (Child-Pugh class A) hepatic impairment. However, in patients with moderate to severe hepatic dysfunction (Child-Pugh class B and C), PZQ half-life, $C_{\text{max}}$, and AUC increased progressively with the degree of hepatic impairment. In Child-Pugh class B, the increase in mean half-life, $C_{\text{max}}$, and AUC relative to Group 1 were 1.58, 1.76, and 3.55, respectively. The corresponding increase in Child-Pugh class C patients were 2.82-fold, 4.29, and 15 for half-life, $C_{\text{max}}$, and AUC.
Table 9. Pharmacokinetic Parameters of PZQ in Four Groups of Patients with Varying Degrees of Liver Function following Administration of 40 mg/kg under Fasting Conditions

<table>
<thead>
<tr>
<th>Patient Group</th>
<th>Half-life (hr)</th>
<th>T_max (hr)</th>
<th>C_max (μg/mL)</th>
<th>AUC (μg/mL* hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal hepatic function</td>
<td>2.99 ± 1.28</td>
<td>1.48 ± 0.74</td>
<td>0.83 ± 0.52</td>
<td>3.02 ± 0.59</td>
</tr>
<tr>
<td>(Group 1)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Child-Pugh A</td>
<td>4.66 ± 2.77</td>
<td>1.37 ± 0.61</td>
<td>0.93 ± 0.58</td>
<td>3.87 ± 2.44</td>
</tr>
<tr>
<td>(Group 2)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Child-Pugh B</td>
<td>4.74 ± 2.16(a)</td>
<td>2.21 ± 0.78(a,b)</td>
<td>1.47 ± 0.74(a,b)</td>
<td>10.72 ± 5.53(a,b)</td>
</tr>
<tr>
<td>(Group 3)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Child-Pugh C</td>
<td>8.45 ± 2.62(a,b,c)</td>
<td>3.2 ± 1.05(a,b,c)</td>
<td>3.57 ± 1.30(a,b,c)</td>
<td>45.35 ± 17.50(a,b,c)</td>
</tr>
<tr>
<td>(Group 4)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

A. p < 0.05 compared to Group 1
B. p < 0.05 compared to Group 2
C. p < 0.05 compared to Group 3

Dissolution Profile of the Reference Product

A dissolution test for the tablets is available in the monograph of PZQ tablets in USP 41[9]. No dissolution profile data are available for the reference product Biltricide®.

Food Effect on Pharmacokinetics

A study on nine healthy volunteers with mean weight of 72 kg and mean age of 33 years was performed[41] to evaluate the effect of food on bioavailability of PZQ. The subjects were divided into three groups. Group 1 was given 3 tablets of 600 mg (1800 mg) after 10 hours of fasting. Group 2 was given a high fat diet, and Group 3 was given a high carbohydrate diet immediately after administration of tablets. Lunch after 4 hours of administration was served to all the volunteers. Blood samples were collected at different time intervals and centrifuged. Plasma was separated and stored at −4°C until analysis.

The study suggested bioavailability of PZQ is significantly increased by food. The mechanism by which food increases the bioavailability of PZQ remains to be demonstrated. The influence of diet on bioavailability of PZQ was greater in the case of the
carbohydrate diet than the fatty diet and no diet. Figure 12 shows a comparison of the mean concentration of PZQ in plasma at different time points after dosing.

Figure 12. Mean Concentration of Plasma of PZQ in Volunteers

![Mean Concentration of Plasma of PZQ in Volunteers](image)

Mean concentration in plasma (± standard error of the mean) of praziquantel in healthy volunteers treated with a single oral dose of 1,800 mg (three tablets of 600 mg) during fasting (●) or immediately after a high-fat (△) or a high-carbohydrate (■) breakfast.

Table 10 shows $C_{\text{max}}$, $AUC_{0-8h}$, $T_{\text{max}}$, $t_{1/2}$ and mean residence time (MRT) of PZQ after dosing fasting and with different food conditions.
Table 10. Pharmacokinetic Parameters of PZQ Obtained after Different Treatments

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Parameter</th>
<th>C_{max} (ng/mL)</th>
<th>AUC_{0-8} (ng.h. mL^{-1})</th>
<th>T_{max} (h)</th>
<th>t_{1/2}^{b} (h)</th>
<th>MRT (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasting</td>
<td>Mean (SD)</td>
<td>318.81 (227.19)</td>
<td>882.33 (416.79)</td>
<td>1.39 (0.98)</td>
<td>2.03 (0.24)</td>
<td>4.39 (0.89)</td>
</tr>
<tr>
<td>High Fat Diet</td>
<td>Mean (SD)</td>
<td>1095.44 (779.91)</td>
<td>2474.95 (1165.99)</td>
<td>1.94 (1.10)</td>
<td>1.72 (0.18)</td>
<td>3.52 (0.54)</td>
</tr>
<tr>
<td></td>
<td>Statistical Comparison Ratio (90% CI)</td>
<td>NE</td>
<td>NE</td>
<td>NE</td>
<td>NE</td>
<td>NE</td>
</tr>
<tr>
<td>High Carbohydrate diet</td>
<td>Mean (SD)</td>
<td>1962.18 (779.76)</td>
<td>3276.20 (969.73)</td>
<td>1.47 (0.64)</td>
<td>1.66 (0.32)</td>
<td>2.91 (0.81)</td>
</tr>
<tr>
<td></td>
<td>Statistical Comparison Ratio (90% CI)</td>
<td>NE</td>
<td>NE</td>
<td>NE</td>
<td>NE</td>
<td>NE</td>
</tr>
</tbody>
</table>

a: NE, not equivalent; NS, no statistically significant difference; CI, confidence interval; b: t_{1/2}, half life

BE Study Protocol Guidance

As per the guidance document dated October 13, 2015, entitled “Notes on the Design of Bioequivalence Study: Praziquantel” [42], the following guidance with regard to the study design should be taken into account:

- **Dose:** A single oral dose of one tablet of PZQ 600 mg should be feasible. The bioanalytical method should be sufficiently sensitive to detect concentrations that are 5% of C_{max} in most profiles of each formulation (test or comparator).

- **Fasting/Fed:** The bioequivalence study should be conducted in the fed state, as PZQ is recommended to be taken with food. While specific requirements regarding the type of meal are not necessary, the variability is increased if the tablets are taken with a high-fat, high-calorie meal; hence, administration with a standard breakfast (not a high-fat, high-calorie meal) is recommended.

- **Subjects:** Healthy adult subjects should be utilized. It is not necessary to include patients in the bioequivalence study.

- **Power:** Currently available information indicates that intra-subject variability for PZQ is around 50%−60% for C_{max} and 35% for AUCT. These data will facilitate the calculation of sufficient power for the bioequivalence study.
• **Washout:** Taking into account the elimination half-life of PZQ in healthy volunteers (about 3 hours), a washout period of 7 days is considered sufficient to prevent carryover.

• **Blood Sampling:** Blood sampling for PZQ should be intensive in the first 4 hours after administration to properly characterize the PZQ $C_{\text{max}}$ but are not necessary after 12 hours.

• **Analytical Considerations:** Currently available information indicates that it is possible to measure PZQ in human plasma using LC-MS/MS analytical methodology. The bioanalytical method should be sufficiently sensitive to detect concentrations that are 5% of the $C_{\text{max}}$ in most profiles of each formulation (test or comparator).

• **Parent or Metabolite Data for Assessment of Bioequivalence:** The parent drug is considered to best reflect the biopharmaceutical quality of the product. The data for the parent compound are used to assess bioequivalence.

• **Statistical Considerations:** The data for PZQ should meet the following bioequivalence standards in a single-dose, crossover study design:
  - The 90% confidence interval of the relative mean $AUC_T$ of the test to reference product should be within 80%–125%.
  - The 90% confidence interval of the relative mean $C_{\text{max}}$ of the test to reference product should be within 80%–125%.

**Bioanalytical Methods**

A simple HPLC method to determine PZQ in human plasma was developed and validated [43]. The method recommends a drop-wise addition of 0.2 M zinc sulfate and acetonitrile to plasma sample for deproteinization. This method used a reversed-phase Spherisorb ODS2 column (5 µm), 250×4.6 mm i.d. as a stationary phase with a mobile phase of acetonitrile-methanol-water (36:10:54, v/v/v), a flow rate of 1.5 mL/minute and UV detection wavelength of 217 nm. Diazepam was used as internal standard. The validation parameters of the method are given in Table 11.
Another method for determination of PZQ in plasma and urine samples of patients with brain cysticercosis [44] is summarized below.

The instruments used were a Beckman (Fullerton, CA, USA) HPLC equipped with dual solvent delivery system with gradient control, an injection valve fitted with 20 µl sampling loop, a variable-wavelength UV detector, and a data module. Extractions were made using Sep-Pak C<sub>18</sub> cartridges (Waters-Millipore, Milford, MA, USA). Analysis was performed on an Ultrasphere ODSC<sub>18</sub> column (250 mm x 4.6 mm I.D., particle size 5 µm) (Beckman Instruments, San Ramon, CA, USA).

The mobile phase used for analysis was acetonitrile: water: 45:55 (for plasma and urine). The column was maintained at 20°C-24°C and with a flow rate of 1.5 mL/minute. The internal standard used was 2-cycloheptyl analogue of PZQ at a concentration of 10µg/mL. The analysis was performed at absorbance of 217 nm. The reported regression analysis data and summary of validation parameter for the bioanalytical method are given in Table 12.
## Table 12. Bioanalytical Method Parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linearity</td>
<td>0.125 - 4 µg/mL</td>
</tr>
<tr>
<td>Correlation Coefficient</td>
<td>0.9999</td>
</tr>
<tr>
<td>Limit of Detection (ng/mL)</td>
<td>31.2 ng/mL</td>
</tr>
<tr>
<td>Limit of Quantification (ng/mL)</td>
<td>102.3 ng/mL</td>
</tr>
<tr>
<td>Inter-day precision</td>
<td>8.83 % (plasma), 9.08% (urine)</td>
</tr>
<tr>
<td>Samples Stability</td>
<td>−4 °C (storage)</td>
</tr>
<tr>
<td>Interference</td>
<td>No interference of other excipients/metabolites</td>
</tr>
</tbody>
</table>
Toxicology Information

As per the literature review, no toxicity was identified with PZQ during pregnancy, and no major birth defects, miscarriages, or adverse maternal or fetal outcomes were reported. No adverse outcomes were observed with oral administration of PZQ during organogenesis. The estimated background risk of major birth defects and miscarriage for the indicated population are unknown. In a few clinical trials, it was observed that treatment with PZQ during pregnancy had no effect on birth weight, and there were no differences in rates of miscarriage, fetal death, and major birth defects between the PZQ-treated and control patients.

Mutagenicity studies of PZQ published in the scientific literature were inconclusive. Long-term oral carcinogenicity studies in rats and golden hamsters did not reveal any carcinogenic effect at doses up to 250 mg/kg/day [8]. PZQ had no effect on the fertility and general reproductive performance of male and female rats when given at oral doses ranging from 30 to 300 mg/kg body weight.

As PZQ can exacerbate central nervous system pathology due to schistosomiasis, paragonimiasis, or *Taenia solium* cysticercosis, as a general rule this medicine should not be administered to individuals reporting a history of epilepsy and/or other signs of potential central nervous system involvement, such as subcutaneous nodules suggestive of cysticercosis.

Neurocysticercosis is not an approved indication due to insufficient data. In animals, venous thrombosis and the development of granulomas at the site of worm attachment have been observed following treatment with PZQ. Patients treated with PZQ (for neurocysticercosis) have had a high incidence of severe headaches and seizures. Some patients also developed intracranial hypertension. Because of the potential for undiagnosed neurocysticercosis to be present in patients originating from endemic areas, extra care is necessary in managing such patients.

Since 80% of PZQ and its metabolites are excreted via the kidneys, excretion might be delayed in patients with impaired renal function. Nephrotoxic effects of PZQ are not known. Information on overdosage in humans is not available [33].

Activated charcoal may reduce absorption of the medicine if given within 1 to 2 hours after ingestion. In patients who are not fully conscious or have impaired gag reflex, consideration should be given to administering activated charcoal via a nasogastric tube, once the airway is protected.
Toxicity data of PZQ by administering through various routes in animals are as follows:

- **oral LD$_{50}$ (rat):** 2840 mg/kg
- **intraperitoneal LD$_{50}$ (rat):** 586 mg/kg
- **intramuscular LD$_{50}$ (rat):** >2 gm/kg
- **oral LD$_{50}$ (mouse):** 2454 mg/kg
- **intraperitoneal LD$_{50}$ (mouse):** 376 mg/kg
- **subcutaneous LD$_{50}$ (mouse):** 7172 mg/kg

**Occupational Exposure Limit Calculations**

The occupational exposure limit (OEL) of PZQ is 139 µg/m$^3$ [45].

Utilizing the uncertainty/safety factor method (now referred to as adjustment factor method) for determining OEL as presented by Sergent and Kirk, 1999 with consideration to the uncertainty factors discussed by Sargent, et al. [46], and as outlined in the new guidelines released from the ISPE Risk-Mapp$^®$ Baseline Guide [47], as occupational exposure limit for PZQ can be calculated as follows:

$$OEL = \frac{PoD \ (mg/day)}{(AF)(SS)(\alpha)(vol)}$$

$$OEL = \frac{250 \ (mg/day)}{(180)(1)(1) \ (10 \ m^3)} = 0.1388 \ mg/m^3 \ = 139 \ µg/m^3$$

Where:

- **AF** = adjustment factor (3 for low therapeutics dose to NOEL extrapolation, 5 for human variability, 2 for dog to human extrapolation, 3 for possible irreversible effects (possible carcinogenic effects from multiple exposures), and 2 for subchronic to chronic exposure)
- **SS** = steady state based on elimination half-life = 1
- **$\alpha$** = pharmacokinetic factor based on bioavailability = 1
- **Vol** = volume of air breathed in a shift = 10 m$^3$

This OEL is designed to be an 8-hour a day, 40-hour a week airborne concentration, which nearly all workers may be repeatedly exposed to day after day without adverse health effects, based on currently available information. It does not consider hypersen-
sitive or otherwise unusually responsive individuals or persons with hypersensitivity to PZQ, which may be exacerbated by exposure to this drug.

**Control Band Assignment**

Based on numerical OEL, PZQ is assigned as a Category 1 (Low) substance in the Affygility Solutions’ 5-band control banding system. This converts to a Category 2 in a traditional 4-band system.

**Table 13. Band System for Hazardous Chemicals**

<table>
<thead>
<tr>
<th>Band No</th>
<th>Target Range of Exposure Concentration</th>
<th>Hazard Group</th>
<th>Control</th>
</tr>
</thead>
</table>
| 1       | >1 to 10 mg/m³ dust
>5 to 500 ppm vapor | Skin and eye irritation | Use good industrial hygiene practice and general ventilation. |
| 2       | >0.1 to 1 mg/m³ dust
>5 to 50 ppm vapor | Harmful on single exposure | Use local exhaust ventilation |
| 3       | >0.01 to 0.1 mg/m³ dust
>0.5 to 5 ppm vapor | Severely irritating and corrosive | Enclose the process |
| 4       | <0.01 mg/m³ dust
<0.5 ppm vapour | Very toxic in single exposure, reproductive hazard, sensitizer | Seek expert advice |

**Industrial Hygiene Sampling and Analytical Methods**

Precautions for safe handling include the following: Avoid contact with skin and eyes. Avoid formation of dust and aerosols. Use adequate general or local exhaust ventilation to keep airborne concentrations below the permissible exposure limits. Normal measures for preventive fire protection.

**Acceptable Daily Exposure Calculations**

The Acceptable Daily Exposure (ADE) of PZQ is 694 µg/day [45].

Utilizing the uncertainty/modifying factor method (now referred to as adjustment factor method) for determining ADE values as presented in the revised ISPE Risk-Mapp® Baseline Guide [46, 48] with consideration to the methods discussed by
Sergant, et al. [46] and the European Medicines Agency [49], an ADE for PZQ can be calculated as follows:

$$ADE = \frac{(PoD \text{ mg/day})}{AFC \times MF \times PK}$$

$$ADE = \frac{250 \text{ mg day}}{360 \times 1 \times 1} = 0.6944 \text{ mg/day} = 694 \mu g/ \text{ day}$$

Where:

- **PoD** = Point of Departure
- **AFc** = Composite Adjustment Factor ($AF_A \times AF_H \times AF_S \times AF_L \times AF_D$)
- **AF_A** = Interspecies variability
- **AF_H** = Intraspecies variability
- **AF_S** = Study duration
- **AF_L** = Low dose extrapolation
- **AF_D** = Database completeness
- **MF** = Modifying Factor (severity)
- **PK** = Pharmacokinetic adjustment(s)

The ADE is the daily dose of a substance, below which no adverse effects are anticipated by any route, even if exposure occurs over a lifetime.

**Choice of Uncertainty and Modifying Factors**

In calculating the ADE value for PZQ, a composite **AF_c** of 360 was used. The choice was made to account for the following factors:

1. The low oral daily therapeutic dose was selected as the point of departure, and this dose is based on dog data; therefore, a factor of 2 was applied to $AF_A$.

2. In the absence of specific intraspecies variability of data, a conservative default factor of 10 is applied to $AF_H$ to extrapolate from general human population to sensitive subgroups, such as children and geriatrics.

3. The data reviewed were based on subchronic studies; therefore, to extrapolate to chronic exposure, a factor of 2 was applied to $AF_S$. 
4. A minimum daily therapeutic dose has been established, and an adjustment factor of 10 was already applied in $AF_H$ to protect sensitive subgroups. Therefore, to extrapolate from a low therapeutic dose to a probable no-observed-effect-level (NOEL), an adjustment factor of 3 is applied to $AF_L$.

5. The database of information was reasonably complete, and there have been reports that repeated exposure can increase cancer risk in humans; therefore, an adjustment factor of 3 was applied to $AF_D$.

6. There are no residual uncertainties not covered by other adjustment factors, therefore a modifying factor of 1 was applied.

7. A composite pharmacokinetic adjustment factor of 1 was used to account for variable human pharmacokinetics.

**Acute Toxicity**

The acute toxicity of Biltricide® (PZQ) is low, as demonstrated [33] in uninfected mice, rats, and rabbits after oral application and in mice and rats after subcutaneous, intraperitoneal, and intramuscular injection. The acute toxicity for dogs could not be evaluated owing to the emetic effect of higher doses of the compound in this species (as shown in Table 14).

**Table 14. Acute Toxicity of PZQ**

<table>
<thead>
<tr>
<th>Route of Administration</th>
<th>Species</th>
<th>LD50 in mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1 Day</td>
</tr>
<tr>
<td>Oral</td>
<td>Mouse</td>
<td>2454</td>
</tr>
<tr>
<td></td>
<td>Rat</td>
<td>2976</td>
</tr>
<tr>
<td></td>
<td>Rabbit</td>
<td>1100</td>
</tr>
<tr>
<td></td>
<td>Dog</td>
<td>&gt;200</td>
</tr>
<tr>
<td>Subcutaneous</td>
<td>Mouse</td>
<td>7268</td>
</tr>
<tr>
<td></td>
<td>Rat</td>
<td>&gt;16000</td>
</tr>
<tr>
<td>Intramuscular</td>
<td>Mouse</td>
<td>&gt;2000</td>
</tr>
<tr>
<td></td>
<td>Rat</td>
<td>&gt;1000</td>
</tr>
<tr>
<td>Intraperitoneal</td>
<td>Rat</td>
<td>796</td>
</tr>
</tbody>
</table>
In mice infected with *Schistosoma mansoni*, the acute toxicity of PZQ was within the same range as found in healthy animals.

PZQ proved to be well-tolerated in tests carried out in rabbits for primary skin tolerance and for mucosal tolerance in the eye. Furthermore, the substance showed no sensitizing effect in intracutaneous tests in guinea pigs and in epicutaneous tests in humans.

**Long-Term Toxicity**

In the 4-week study in rats and dogs and a 3-month study in dogs, the only consistent toxicities observed were enlarged liver and thyroid glands in rats (at 300 mg/kg/day and above), enlarged livers in dogs (180 mg/kg/day after 4 weeks of exposure), and increased absolute and relative liver weight (180 mg/kg/day after 3 months of exposure). These changes were not associated with abnormal findings in clinical chemistry or histopathological examination.

**Carcinogenicity**

Long-term carcinogenicity studies were conducted in Sprague-Dawley rats and golden hamsters. PZQ was not considered to be carcinogenic in rats. In hamsters, PZQ might be considered to be a weak carcinogen based on a slight increase in percent malignant tumors in the female.

**Reproductive Toxicology**

In reproduction tests with doses up to 40 times the human dose (300 mg/kg body weight/day), PZQ had no effect either on the fertility of male and female rats or on the embryonal and fetal development of the offspring. Even with daily oral administration during organogenesis, PZQ did not show any embryotoxic or teratogenic effects. An increase in the abortion rate was found in rats receiving three times the single human therapeutic dose. Reproduction studies in rabbits at doses up to 40 times the human dose revealed no evidence of impaired fertility or harm to the fetus due to PZQ.

**Mutagenesis**

Extensive studies in various test systems (both *in vitro* and *in vivo*) have yielded no evidence of mutagenicity. Mutagenic effects in Salmonella tests observed by one laboratory have not been confirmed in the same tested strain by other laboratories.
Manufacturing of Dosage Form

PZQ has good inherent stability and does not pose major challenges in the manufacturing of solid dosage form. However, to achieve the desired dissolution profile, efforts on particle size distribution of API, polymorphic form selection, and concentration of wetting agent must be optimized. The additional role of process parameters such as end point of wet granulation, strength of granules, mixing time of lubricant, and hardness of tablets should be carefully optimized. A dedicated area and equipment for fabricating PZQ tablets are not necessary. However, standard cleaning protocols and good manufacturing practices (GMP) should be strictly followed. The manufacturing facility should be maintained with optimum temperature and relative humidity conditions to achieve uniformity and batch-to-batch uniformity. Biltricide® has a shelf-life of 48 months, indicating the robust nature of drug product.

Facility Design and HVAC Requirements

The pharmaceutical facilities are closely inspected by WHO prequalification inspectors, which requires manufacturing companies to conform to current GMP. These regulations require that medicines manufacturers, processors, and packagers to take proactive steps to ensure their products are safe, pure, and effective. GMP regulations require a quality approach to manufacturing, enabling companies to minimize or eliminate instances of contamination, mix-ups, and errors.

The WHO guidance for HVAC services embraces a number of issues, starting with the selection of building materials and finishes; flow of equipment; personnel and products; determination of key parameters (e.g., temperature, humidity, pressure, filtration, and airflow); and classification of clean rooms. It also governs the level of control of various parameters for quality assurance, regulating the acceptance criteria, validation of the facility, and documentation for operation and maintenance.

HVAC system performs four basic functions [50]:

2. Maintain room pressure ($\Delta P$): Areas that must remain “cleaner” than surrounding areas must be kept under a “positive” pressurization, meaning that air flow must be from the “cleaner” area toward the adjoining space (through
doors or other openings) to reduce the chance of airborne contamination. This is achieved by the HVAC system providing more air into the “cleaner” space than is mechanically removed from that same space.

3. Maintain space moisture (RH). Humidity is controlled by cooling air to dew point temperatures or by using desiccant dehumidifiers. Humidity can affect the efficacy and stability of drugs and is sometimes important to effectively mold the tablets.

4. Maintain space temperature. Temperature can affect production directly or indirectly by fostering the growth of microbial contaminants on workers. The temperature also has to be maintained within the product’s labeled storage condition.

Manufacturing Process

The tablets for PZQ are recommended to be prepared using wet granulation process [5]. A wet granulation process is most commonly used to obtain granules for compaction. Major unit operations in wet granulation include milling, sieving, blending, granulation, drying, compaction, and coating. The first step may be the milling of raw material, but this is an optional step based on the particle size of the supplied raw material. Airjet mill or Quadro mill can be used for milling. Milled powder is then subjected to sieving to achieve uniform particle size. Bulk powder obtained after sieving is blended with appropriate inactive ingredients. A V blender, double cone blender, bin blender, or octagonal blender can be used for blending. A granulating fluid containing the binder is added to the dry powder blend in RMG to obtain wet dough mass and subsequently the granules, which are then dried using equipment such as a fluidized bed dryer. The dried granules are mixed with extra-granular inactive ingredients in a blender and then compressed in a tablet press to obtain the final formulation.

Process Controls

In-process quality controls (IPQCs) for manufacturing of dosage form of PZQ may include specifications of API like, polymorphic form, particle size distribution (D90 value using standard equipment like Malvern Mastersizer® - which is a laser diffraction technique).
Based on the properties of API, dissolution, chemical stability, and moisture content may be critical quality attributes (CQAs) for PZQ tablets. The pharmaceutical company should understand the role of formulation factors and process parameters on CQAs. A risk assessment using a standard tool (e.g., Failure Mode Effect Analysis) can be used to identify the most important parameters.

The CQA of dissolution may be affected by polymorphic form, PSD, specific surface area, grade, and concentration of magnesium stearate; strength of granules; granule size distribution; lubricant mixing time; and hardness of tablet (process parameter). The manufacturer should understand the contribution of these parameters and their interactions. A control strategy consisting of raw material specifications, process monitoring, IPQC, and finished product testing must be developed.

**Cleaning Validation**

The first step in cleaning validation (CV) involves risk assessment and mitigation strategies. Although the draft EMA Guideline based on Health Based Exposure Limits (HBEL) may be used to justify cleaning limits, traditional cleaning limits used by industry (e.g., 1/1000th of minimum therapeutic dose or 10 ppm of one product in another product) may apply for non-hazardous products such as PZQ.

This EMA draft position is an improvement over the Risk-MaPP Guide. This leeway allowed by EMA to justify using traditional cleaning limit approaches could allow manufacturers to leverage their existing CV work to meet the recent HBEL requirements. For products classed as highly hazardous (e.g., sensitizing, teratogenic, and mutagenic compounds), where a thorough risk assessment can justify manufacture in shared facilities, cleaning limits should include safety factors beyond the HBEL and should not be higher than the traditional cleaning limits approach.

One recommendation is selecting a worst-case product for matrix approach [51]. The following points may be considered for the approach:

- **Create a grouping of products or APIs manufactured on the same equipment train.**

- **Risk Identification:** The intent is to minimize the CV study to one product or API for each equipment train. Score the risk as high before the mitigation strategy in the protocol.
- **Mitigation strategy:** Identify the “worst case” product/API (considering the solubility), hardest to clean product/API, and most toxic candidate. Develop a detailed cleaning procedure for that product/API to be validated. Score the risk as theoretically low in the protocol if the CV strategy would be successful with the worst-case product/API. If the CV strategy meets the acceptance criteria, use of the risk-based approach was successful.

Options such as manual and automatic cleaning are available depending on the individual manufacturing facility. Based on available infrastructure and expertise, a suitable cleaning method may be adopted. Additionally, parameters such as dirty equipment hold time and limit of cleaning analytical method based on ADE value need to be adopted. The ADE value of PZQ has been reported as 694 µg/day [50].
Conclusion

This Product Information Report (PIR) summarizes the available literature and provides expert scientific analysis of the physicochemical, pharmaceutical, pharmacokinetic, and toxicological properties. The PIR also provides a summary of API synthesis, analytical methods, formulation development, and recommendations about manufacturing PZQ tablets. It is expected that the report will provide critical information and guidance to manufacturers and stakeholders concerned with access and supply of priority essential medicines.
Product Information Report: Praziquantel

References


