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Physicochemical and Pharmacokinetic Evaluation of Praziquantel Co-Crystals by Varying the Spacer Group of Co-Crystal Formers

(Penilaian Fizikokimia dan Farmakokinetik Gabungan Hablur Praziquantel dengan Mempelbagaikan Kumpulan Penjarak Pembentuk Gabungan Hablur)

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ABSTRACT

The research work focuses on investigating the effects of spacer group (varying aliphatic chain length=n) of co-crystal formers (oxalic acid (OA, n=0), (malonic acid (MA, n=1), (succinic acid (SA, n=2), (glutaric acid (GA, n=3), and (adipic acid (AA, n=4) on the physicochemical properties and oral bioavailability of praziquantel (PZQ) co-crystals. For this purpose, different co-crystals of PZQ with dicarboxylic acid co-crystal formers (OA, MA, SA, GA, and AA) were synthesized. These co-crystals were characterized by powder X-ray diffractometry (XRPD), differential scanning calorimetry (DSC), Fourier transform infrared spectroscopy (FT-IR), and thermogravimetry (TG) techniques. The *in-vitro* (solubility and dissolution) and *in-vivo* pharmacokinetic (P.K) studies were performed for PZQ co-crystals. Additionally, the effect of polymer hydroxypropyl cellulose (HPC) on the formation of PZQ co-crystals was also investigated. According to the study results, PZQ-SA co-crystal showed improved solubility, dissolution, and oral bioavailability. Overall, the solubility, dissolution, and oral bioavailability are consistent with each other. The order of improved solubility, dissolution, and oral bioavailability is observed as consistent like PZQ-SA > PZQ-AA > PZQ-GA > PZQ-OA > PZQ-MA > pure PZQ. Concerning HPC polymer effects, PZQ-OA, PZQ-MA, PZQ-GA, and PZQ-AA co-crystals were formed successfully in the presence of HPC polymer but the PZQ-SA co-crystal was inhibited.

Keywords: Co-crystal; pharmacokinetic parameters; physicochemical properties; spacer group

ABSTRAK

Penyelidikan ini memberi tumpuan kepada kajian kesan kumpulan penjarak (pelbagai panjang rantai alifatik=n) pembentuk gabungan hablur (asid oksalik (OA, n=0), (asid malonik (MA, n=1), (asid suksinik (SA, n=2), (asid glutarik (GA, n=3) dan (asid adipik (AA, n=4) pada sifat fizikokimia dan bioketersediaan oral bersama gabungan hablur praziquantel (PZQ). Untuk tujuan ini, gabungan hablur berbeza PZQ dengan pembentuk gabungan hablur asid dikarboksilik (OA, MA, SA, GA dan AA) telah disintesis. Gabungan hablur ini dicirikan oleh difraktometri sinar-X serbuk (XRPD), kalorimetri pengimbasan pembezaan (DSC), teknik spektroskopi inframerah transformasi Fourier (FT-IR) dan termogravimetri (TG). Kajian *in-vitro* (keterlarutan dan pelarutan) dan farmakokinetik *in-vivo* (P.K) telah dilakukan untuk gabungan hablur PZQ. Selain itu, kesan polimer hidroksipropil selulosa (HPC) pada pembentukan gabungan hablur PZQ turut dikaji. Menurut hasil kajian, gabungan hablur PZQ-SA menunjukkan kebolehgunaan sol yang lebih baik, pembubaran dan bioketersediaan oral. Secara keseluruhan, keterlarutan, pembubaran dan bioketersediaan oral adalah konsisten antara satu sama lain. Urutan keterlarutan, pelarutan dan bioketersediaan oral yang lebih baik diperhatikan sebagai konsisten seperti PZQ-SA > PZQ-AA > PZQ-GA > PZQ-OA > PZQ-MA > PZQ tulen. Mengenai kesan polimer HPC, gabungan hablur PZQ-OA, PZQ-MA, PZQ-GA dan PZQ-AA telah terbentuk dengan jayanya dengan kehadiran polimer HPC tetapi gabungan hablur PZQ-SA telah dihalang.

Kata kunci: Gabungan hablur; kumpulan penjarak; parameter farmakokinetik; sifat fizikokimia

Introduction

Co-crystals, a well-known crystalline solid, have drawn attention from researchers over the past decades and are presently an essential part of the pre-formulation research in drug discovery and development. The advantages of co-crystals include; enhanced stability of solid-state, flow property, compressibility, dissolution pattern, and oral bioavailability (Karagianni et al. 2018; McNamara et al. 2006; Perumalla & Sun 2013). Pharmaceutical co-crystals, without covalent modification of the molecule, can modify physicochemical properties (Duggirala et al. 2016). During drug development, design, and discovery, the main issue is selecting an appropriate API with optimum physicochemical properties (Aitipamula et al. 2012). Poor bioavailability is the most frequently encountered problem during product development. The most appropriate strategy for enhancing drug bioavailability is the use of a solid form of an API with higher aqueous solubility. Low aqueous-soluble drugs are more prevalent in the pharmaceutical industry, and increasing the solubility of low aqueous-soluble drugs is a primary challenge for pharmaceutical scientists (Savjani et al. 2012).

Praziquantel (PZQ) is an anthelmintic drug of choice in schistosomiasis and is also indicated in a variety of cestodes and trematodes infections (Costa et al. 2016; Li et al. 2007), and is commonly used in veterinary medicine (Dayan 2003). According to the biopharmaceutical classification system (BCS), PZQ is a BCS class-II drug with low aqueous solubility, so the dissolution process affects its efficiency distinctly (Dinora et al. 2005; Salazar-Rojas et al. 2020). The chemical structure of PZQ lacks the salt-forming groups, so the effective technique for optimization of physicochemical properties is co-crystallization (Cugovcan et al. 2017). PZQ and dicarboxylic acid co-crystal formers have the potential to be co-crystallized as the PZQ contains two hydrogen bond formation sites as shown in Figure 1(B) and 1(C) (Espinosa-Lara et al. 2013). As the chain length of co-crystal formers changes, the conformation also changes, so the crystal structure is changed (Kakkar 2018). So, the physicochemical properties and oral bioavailability may also be influenced by the varying chain length of dicarboxylic acid co-crystal formers. To the best of our knowledge, the solubility, dissolution behavior, and bioavailability of PZQ co-crystals with a

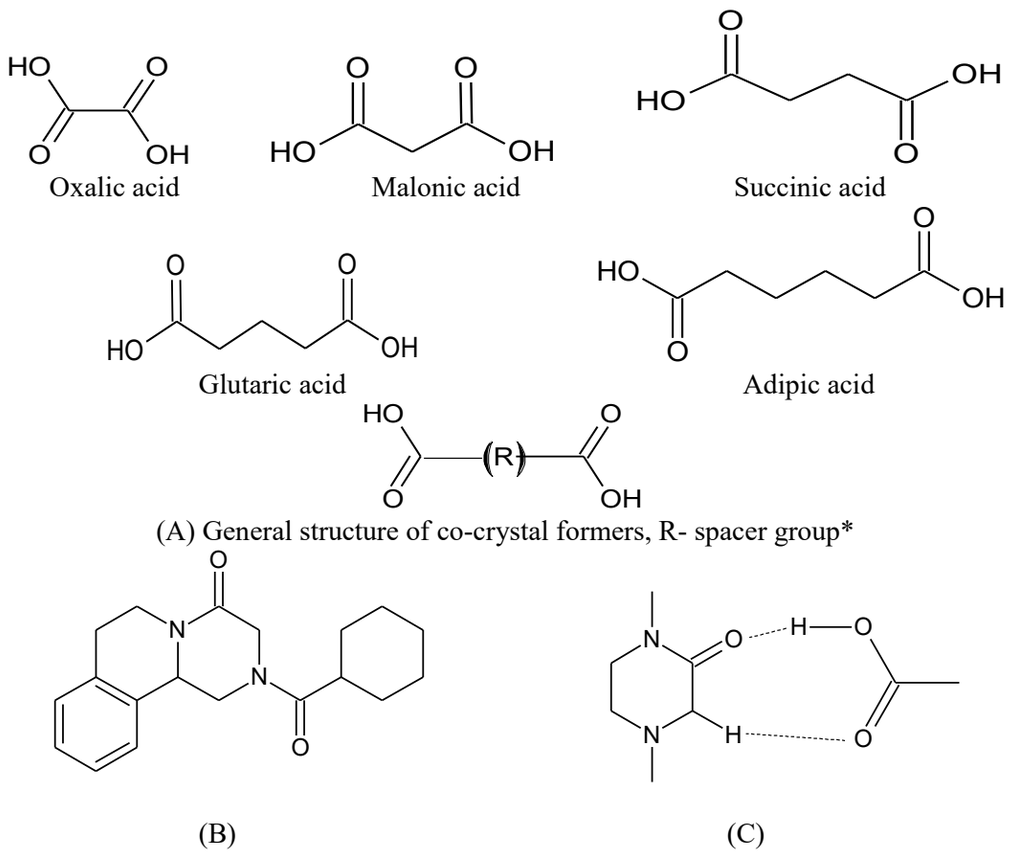

FIGURE 1. Dicarboxylic acid co-crystal formers used in the study (A), Praziquantel (B), Heterodimer synthon (C)

series of dicarboxylic acid co-crystal formers (COOH)–$(CH_2)_n$–(COOH) as shown in Figure 1(A): oxalic (OA, n = 0), malonic (MA, n = 1), succinic (SA, n = 2), glutaric (GA, n = 3), and adipic (AA, n = 4) have not been investigated. It is significant to investigate the relationship of physicochemical properties and pharmacokinetic parameters (C_{max}, T_{max}, and AUC) of PZQ co-crystals with varying chain lengths of dicarboxylic acid co-crystal formers because it would help in the selection of suitable co-crystals with improved physicochemical properties and oral bioavailability. Moreover, in this research work, we also attempted to investigate a polymer effect on PZQ co-crystal preparation using the slurry method.

Materials and Methods

PZQ was obtained from Alfa Aesar. The dicarboxylic acid co-crystal formers: OA, MA, SA, and AA were purchased from Acros Organics, while GA was procured from Sigma-Aldrich. Polymer hydroxypropyl cellulose (HPC) (average Mw of ~80,000; 20 mesh particle size) was also purchased from Sigma-Aldrich (Belgium). All analytical grade solvents were purchased from commercial sources.

Preparation of PZQ co-crystals with and without HPC polymer

PZQ was co-crystallized with dicarboxylic acid co-crystal formers, namely OA, MA, SA, GA, and AA. PZQ co-crystals were prepared by the slurry crystallization method. In this method, solvent acetone was added in screw-capped glass vials having solid, without reaching full dissolution. The mixtures were allowed to stir for 72 h on a magnetic stirrer at room temperature. For further characterization, the resulting powder was rapidly filtered and dried. The PZQ co-crystals were also tried to prepare in the presence of HPC polymer to investigate the outcome of polymer on their preparation. For this purpose, the same method was applied. The detail is given in Table 1.

TABLE 1. Detail of PZQ co-crystals preparation with and without HPC polymer

API	Co-crystal former	Polymer	Ratio	Code
PZQ	AA	HPC	1: 0.5: 1.2	PZQ-AA-HPC
PZQ	GA	HPC	1: 1: 1.2	PZQ-GA-HPC
PZQ	SA	HPC	1: 1: 1.2	PZQ-SA-HPC
PZQ	MA	HPC	1: 1: 1.2	PZQ-MA-HPC
PZQ	OA	HPC	1: 1: 1.2	PZQ-OA-HPC
PZQ	AA	-	1: 0.5	PZQ-AA
PZQ	GA	-	1: 1	PZQ-GA
PZQ	SA	-	1: 1	PZQ-SA
PZQ	MA	-	1: 1	PZQ-MA
PZQ	OA	-	1: 1	PZQ-OA

PZQ=praziquantel, OA=oxalic acid, MA=malonic acid, SA=succinic acid, GA=glutaric acid, AA=adipic acid, HPC=hydroxy propyl cellulose

CHARACTERIZATION OF PZQ CO-CRYSTALS

POWDER X-RAY DIFFRACTOMETRY

Samples were characterized on a diffractometer (Siemens D5000) using Cu as an X-ray source at a current of 40 mA and voltage of 40 kV. A monochromator was used to permit the selection of the Kα radiation of Cu (λ =1.5418 Å). The measurement of samples was performed with a scan rate of 0.01°/s from 2 to 50° at 2θ.

FOURIER-TRANSFORM INFRARED SPECTROSCOPY

Samples of approximately 3-7 mg were placed on a crystal surface (diamond) to get FT-IR spectra. The PerkinElmer FT-IR spectrophotometer was used for this purpose. The FT-IR spectral analysis of finely pulverized samples was done at a wavelength range of 450–4000 cm^{-1}.

DIFFERENTIAL SCANNING CALORIMETRY

DSC measurements of pure PZQ, co-crystal formers, and solid materials obtained from slurry crystallization were performed on a DSC1 from the Mettler Toledo (Switzerland). Before measurements, the DSC was calibrated using indium. Samples of around 8-10 mg were analyzed using perforated aluminum crucibles. The heating rate was set at 10 °C min^{-1} over a range of 30 to 250 °C, under a flow rate of about 50 mL min^{-1}.

THERMOGRAVIMETRIC ANALYSIS

The TGA scans of samples were obtained on the Mettler Toledo TGA-SDTA 851e. The TGA thermograms were measured at a temperature range between 30 and 450 °C with a scanning rate of 10 °C min^{-1} under a nitrogen purge of 50 mL min^{-1}. Eight to 10 mg solid samples were analyzed using an aluminum oxide crucible.

SOLUBILITY AND *in-vitro* DISSOLUTION STUDY OF PZQ CO-CRYSTALS

Concerning solubility, excess quantities of each co-crystal were added to the glass vials having water as a medium and stirred for 48 h. One mL supernatant was collected and filtered immediately and diluted suitably for UV analysis.

The dissolution study was conducted for pure PZQ and its co-crystals using dissolution apparatus USP-II. The medium used was 0.1N HCl having 0.2 mg sodium lauryl sulfate (SLS). The purpose of the SLS is to avoid the adherence of the API with the paddles as the PZQ is hydrophobic. Five mL of the solution was taken at 15, 30, 45, 60, and 90 min. The aliquot was immediately filtered and the concentration of PZQ at each time point was obtained by UV-visible spectrophotometer at λ_{max}=263 (USP 40−NF 35).

in-vivo PHARMACOKINETIC STUDIES OF PZQ CO-CRYSTALS

By using the protocols for *in-vivo* pharmacokinetic (P.K) studies with the approval of the 'Research Ethical Committee' Department of Pharmacy, COMSATS University Islamabad (CUI), Abbottabad Campus (ref. no PHM.Eth/ CS-M01-022-2901), *in-vivo* P.K parameters of samples were investigated after oral administration of PZQ and its co-crystals at a dose of 20 mg/kg. Capsules were filled manually for *in-vivo* P.K studies of PZQ co-crystals. All rabbits of group 1 (control group) were dosed with PZQ; while equivalent doses of PZQ-OA, PZQ-MA, PZQ-SA, PZQ-GA, and PZQ-AA were administered to treated groups (groups 2, 3, 4, 5, 6) via oral route with 2 mL of water using a syringe. The blood samples (0.3-0.5 mL) at different time intervals (0 to 12 h) were withdrawn and collected in Eppendorf tubes. Then, quickly each blood sample was centrifuged at 12,000 rpm for 8 to 10 min to separate out the plasma. The plasma samples were stored at -20 °C until analysis by high-performance liquid chromatography (HPLC).

PZQ was quantified in plasma samples as described in United States Pharmacopoeia (USP) with slight modification, using HPLC (series 200, PerkinElmer USA) technique (USP 40-NF 35). The mobile phase was freshly prepared daily and is composed of acetonitrile and water (70:30). The flow rate and retention time were 1 mL/min and 5.30 min, respectively. A 250 × 4.6 mm Supelco® C_{18} (5 µm particle size) was used as an analytical column. Acetonitrile was added to samples and centrifuged to precipitate proteins prior to HPLC analysis. A 20 µL supernatant was injected. PZQ concentration was measured at λ_{max} 210 nm by a UV detector. The detection and quantification limits were 0.015 and 0.02 µg/mL, respectively. P.K parameters like C_{max} and T_{max} were evaluated for the non-compartmental model using PK Solver 2.0 software. The trapezoidal rule was employed for the calculation of the area under the curve ($AUC_{0\rightarrow t}$) from the concentration−time curve. ANOVA (one-way analysis of variance) and *t*−test ($p < 0.05$) were used for comparison of P.K parameters and statistical analysis of data.

Results and Discussion

Co-crystals were prepared with and without HPC polymer using the slurry crystallization method. The crystalline nature of pure PZQ and PZQ co-crystals was assessed using powder X-ray diffractometry. The XRPD patterns of PZQ co-crystals are different from that of API and respective co-crystal formers, and in good agreement with the respective XRPD patterns of each co-crystal in CSD as shown in Figure 2. Table 2 enlists characteristic peaks of pure PZQ and its co-crystals with respective CSD codes. The perfect agreement of XRPD patterns of PZQ co-crystals with their XRPD patterns provided in CSD evidenced that PZQ co-crystals have successfully been prepared.

TABLE 2. XRPD characteristic peaks of pure PZQ and its prepared co-crystals

Sample	Characteristic peaks	CSD code
PZQ	3.8, 5, 6.7, 20	TELCEU
PZQ-OA	7.9, 17.8, 22	TELCOE
PZQ-MA	6.7, 12.1, 19.4	TELDEV
PZQ-SA	8.3, 9.5, 17.6, 20.7	TELDAR
PZQ-GA	7.6, 10.4, 18.6	TELDIZ
PZQ-AA	6.1, 21.1, 21.6	TELCAQ

The important XRPD peaks of pure PZQ that appeared at 2θ= 5º, 6.7º, and 20º are in good alignment with a study conducted by Qian et al. (2017) confirming the purity of the crystalline materials used in the present study. The XRPD pattern of PZQ-OA is arranged finely in accordance with the powder XRPD pattern of PZQ-OA co-crystal listed as TELCOE in CSD. Likewise, X-ray diffractograms of PZQ-MA, PZQ-SA, PZQ-GA, and PZQ-AA are absolutely overlapped with their X-ray diffractograms listed in CSD as TELDEV, TELDAR, TELDIZ, and TELCAQ, respectively.

Concerning FT-IR results, the spectra of PZQ co-crystals are different from their respective individual components (Figure 3). The amide bands (carbonyl stretching vibrations) are distinctive for the co-crystalline forms of PZQ with corresponding co-crystal formers as shown in Table 3. All peaks of PZQ that appeared in FT-IR spectra of prepared co-crystals indicate drug stability and the peaks of pure PZQ are in good alignment with that reported in the literature (Andrade et al. 2019).

The amide groups in PZQ mainly involve the carbonyl stretching vibrations (1645 and 1622 cm^{-1}), and the shifting in these values shows changes in H-bonding patterns in the resulting co-crystals. The two amide groups of PZQ involve in hydrogen bond formation and the oxygen atoms only act as hydrogen bond acceptors (Liu et al. 2006). Figure 1(B) and 1(C) shows that the appropriate moieties in PZQ are $R_2NC(O)CH_2N$ involve in the double-bridged heterodimeric synthons formation with dicarboxylic acids (Espinosa-Lara et al. 2013). In dicarboxylic acid co-crystal formers, the carbonyl stretching vibrations range from 1664 to 1697 cm^{-1} (Pavia et al. 2014). In co-crystals, these carbonyl stretching vibrations are shifted to a higher wavenumber (Arenas-Garcia et al. 2010). When co-crystal former is combined with a pure drug into co-crystal, the shifting of carbonyl stretching vibration to a larger wavenumber requires further energy, which is consistent with weaker hydrogen-bond formation (C−H···O and O−H···O) (Aakeroy & Sinha 2018). The co-crystal carbonyl stretching vibrations ranges from 1712 to 1742 cm^{-1} (Stuart 2004). The relevant FT-IR bands of co-crystal formers, PZQ, and corresponding co-crystals are listed in Table 3. The results are well supported by the study conducted previously about PZQ co-crystals with a series of dicarboxylic acid co-crystal formers (Espinosa-Lara et al. 2013).

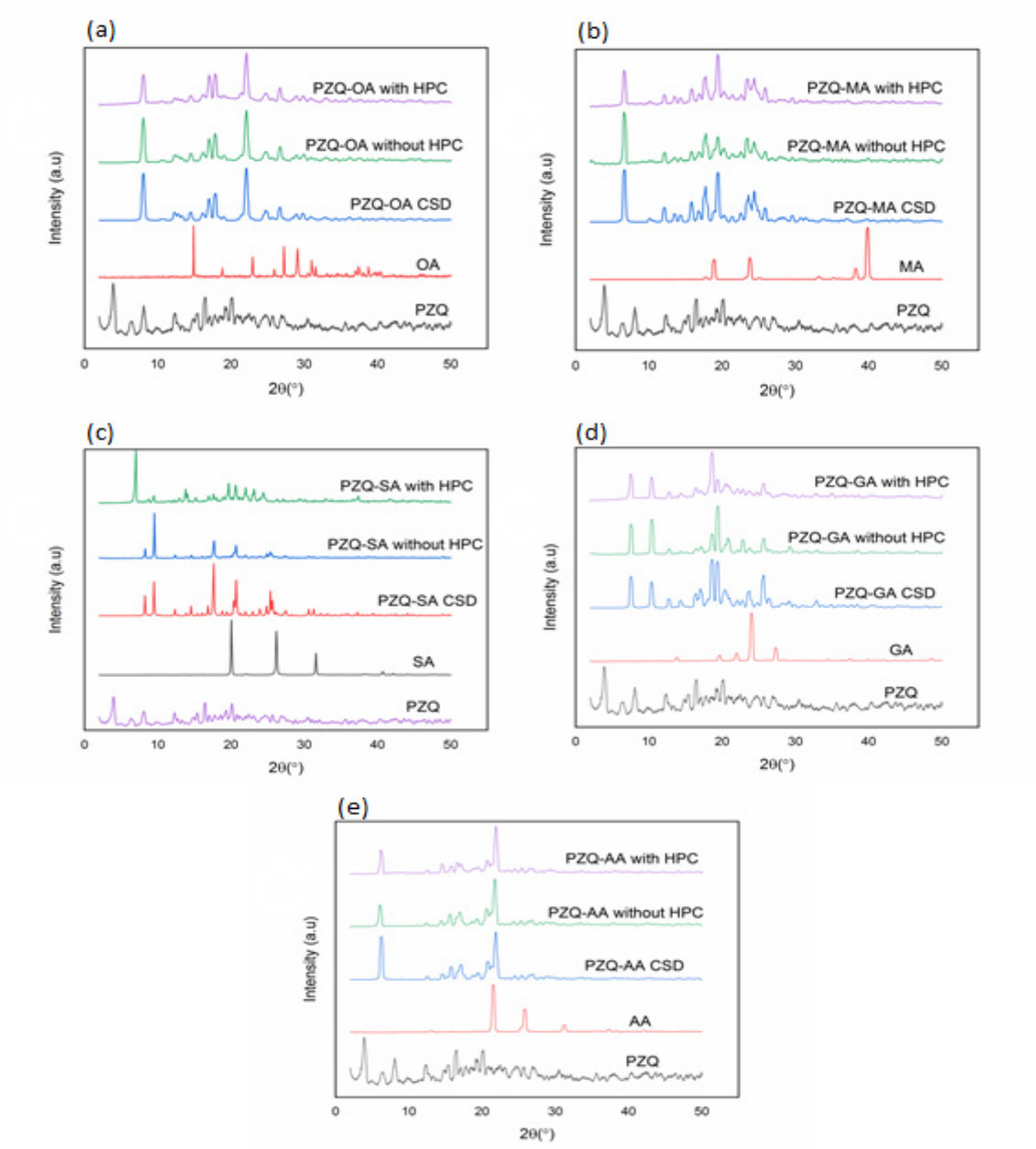

FIGURE 2. XRPD patterns of pure PZQ, respective co-crystal formers, and co-crystals with/without HPC polymer

TABLE 3. FT-IR spectral bands of PZQ and its co-crystals with their respective co-crystal formers

Samples	Frequencies (cm^{-1})			
	PZQ (amide) C=O	Co-crystals (amide) C=O	Co-crystal formers (COOH) C=O	Co-crystal (COOH) C=O
PZQ	1645/ 1622			
PZQ-OA		1593	1664	1719
PZQ-MA		1594	1697	1720/ 1742
PZQ-SA		1607	1682	1712
PZQ-GA		1615	1690	1713
PZQ-AA		1600/ 1630	1686	1712

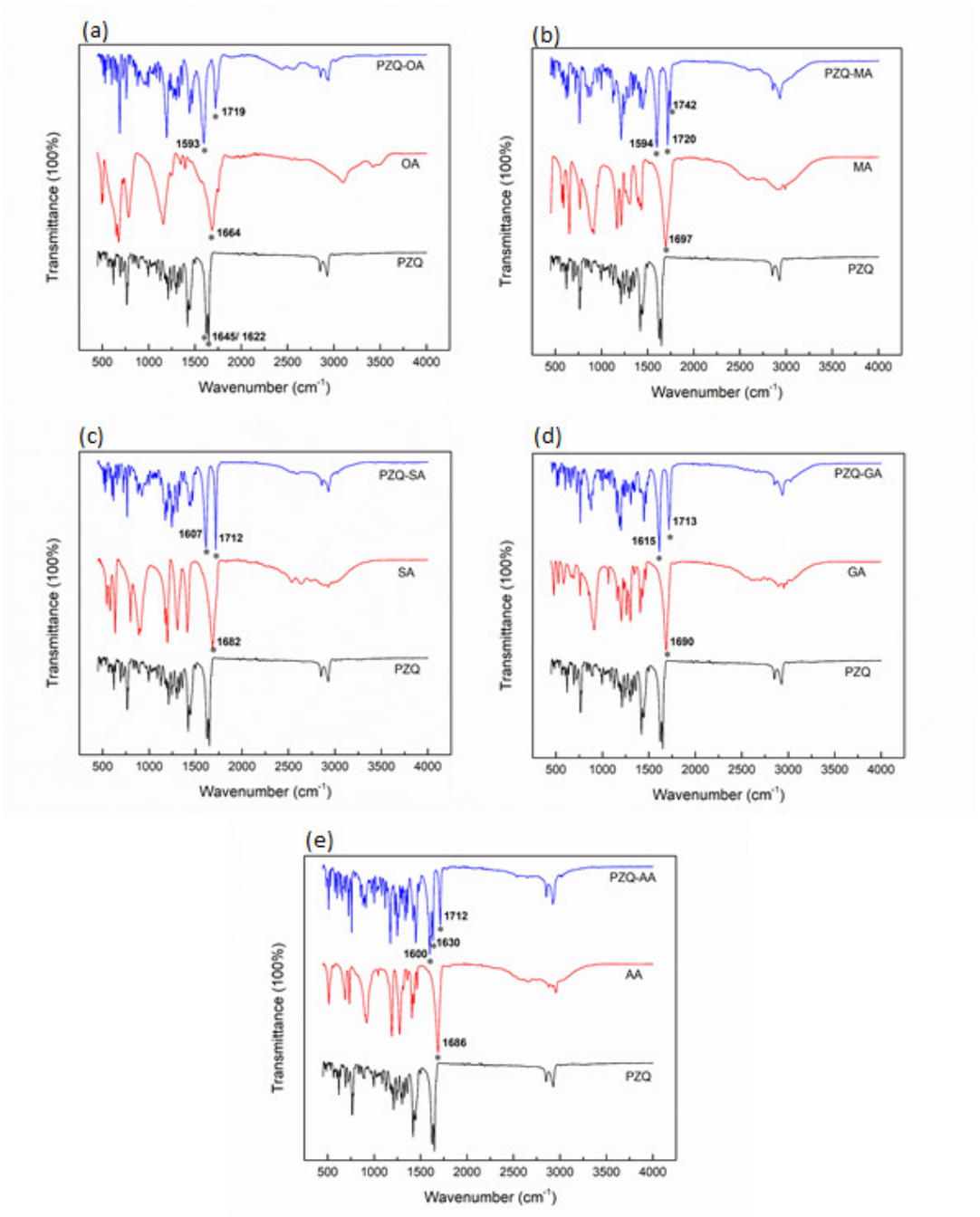

FIGURE 3. FT-IR spectra of PZQ, respective co-crystal formers, and co-crystals

Thermal stability assessment of drugs is quite important, especially melting point (M.P) and thermal degradation that can easily be altered by co-crystallization (Friscic & Jones 2010; Valdes-Negrin et al. 2022), because in a co-crystal, the molecules have non-covalent interactions that play a prime role in lattice energy and ultimately the thermal stability. According to the thermal analysis results, the M.P's of PZQ co-crystals are different from their respective individual components. The melting event of PZQ was observed at 142.7 °C as shown

in Figure 4(a) and the result is supported by reported literature (El-Arini et al. 1998; Mishra et al. 2014). In DSC analysis, no dehydration, as well as other thermal events, was seen after PZQ melting. The *T*peaks of co-crystal formers and co-crystals are enlisted in Table 4.

TABLE 4. Peak temperatures (*T*peak) of co-crystal formers, PZQ co-crystals acquired from DSC and literature

Co-crystal formers	Crystalline form[a]	*T*peak/ °C DSC	Literature data/ °C
Oxalic acid	Solid crystal	105 °C, 196 °C	113 °C, 199 °C / Fischer et al. (2015)
Malonic acid	Crystalline powder	138 °C	135.6 °C / Wicaksono et al. (2017)
Succinic acid	Crystalline powder	187.3 °C	188 °C / Saganowska & Wesolowski (2018)
Glutaric acid	Solid crystal	76.9 °C, 99 °C	76.6 °C, 98.2 °C / Saganowska & Wesolowski (2018)
Adipic acid	Crystalline powder	154.9 °C	155 °C / Butreddy et al. (2020)
PZQ	Crystalline powder	142.7 °C	Mishra et al. (2014)/ 142 °C
PZQ-OA	Solid crystal	159.9 °C	-
PZQ-MA	Solid crystal	147.7 °C	-
PZQ-SA	Solid crystal	141.2 °C	-
PZQ-GA	Solid crystal	126.2 °C	-
PZQ-AA	Solid crystal	122.9 °C	-

a= materials safety data sheet provided by producers

TGA study was performed to know about the % mass loss and phase transition of pure PZQ and its co-crystals. The results have shown in Figure 4(b) and 4(c). The first signal of oxalic acid dihydrate appeared at 105 °C and is further confirmed by 14% mass loss at the same temperature in TGA measurement. The second peak of oxalic acid in the DSC thermogram indicates the melting peak at 189 ℃ (Onset) and 196 ℃ (Peak). The result is supported by the study conducted by Fischer et al. (2015). Similarly, the melting events of all co-crystal formers used in the current study are consistent with the reported literature as shown in Table 4 and Figure 4(b). PZQ co-crystals especially PZQ-SA and PZQ-AA have lower M.P's i.e., 141.2 °C and 122.9 °C, respectively, compared to corresponding single components as displayed in Figure 4(a) and Table 4. The results are supported by the reported co-crystals in the literature like fluoxetine HCl−succinic acid (134 °C), and carbamazepine−(+)-camphoric acid (156 °C), and carbamazepine−benzoic acid (113 °C). These co-crystals have lower M.P's compared to their individual components i.e., API and respective co-crystal formers (Childs et al. 2008, 2004). Similarly, the M.P of PZQ-GA co-crystal (126.2 °C) was observed lower compared to the M.P of pure PZQ (142.7 °C) and higher than GA (99 °C). In the literature, many co-crystals e.g., carbamazepine−benzoquinone (170 °C), carbamazepine−terephthalaldehyde (124 °C), fluoxetine HCl–benzoic acid (132 °C), and norfloxacin−isonicotinamide (180-185 °C) further verified the results (Basavoju et al. 2006; Childs et al. 2004; Fleischman et al. 2003). In previous studies, various co-crystals have also been reported with higher M.P's compared to individual components, for example, ibuprofen–4,4′-bipyridine (117-120 °C) and flurbiprofen–4,4′-bipyridine (155-160 °C) (Walsh et al. 2003), similar results were observed by PZQ-MA co-crystal (147.7 °C) in the present study.

Likewise, piroxicam–saccharin (220 °C) (Bhatt et al. 2005) and indomethacin–saccharin (220 °C) (Basavoju et al. 2008) co-crystals have high M.P's than API and lower than co-crystal former further evidenced the M.P of co-crystal PZQ-OA (159.9 °C) the case herein. Therefore, it is evident from the discussion regarding the M.P's of the spacer group of co-crystal formers and co-crystal that a consistent effect between the spacer group of co-crystal formers and co-crystals was observed, as the number of carbons in spacer group (R) increases the M.P of co-crystal decreases.

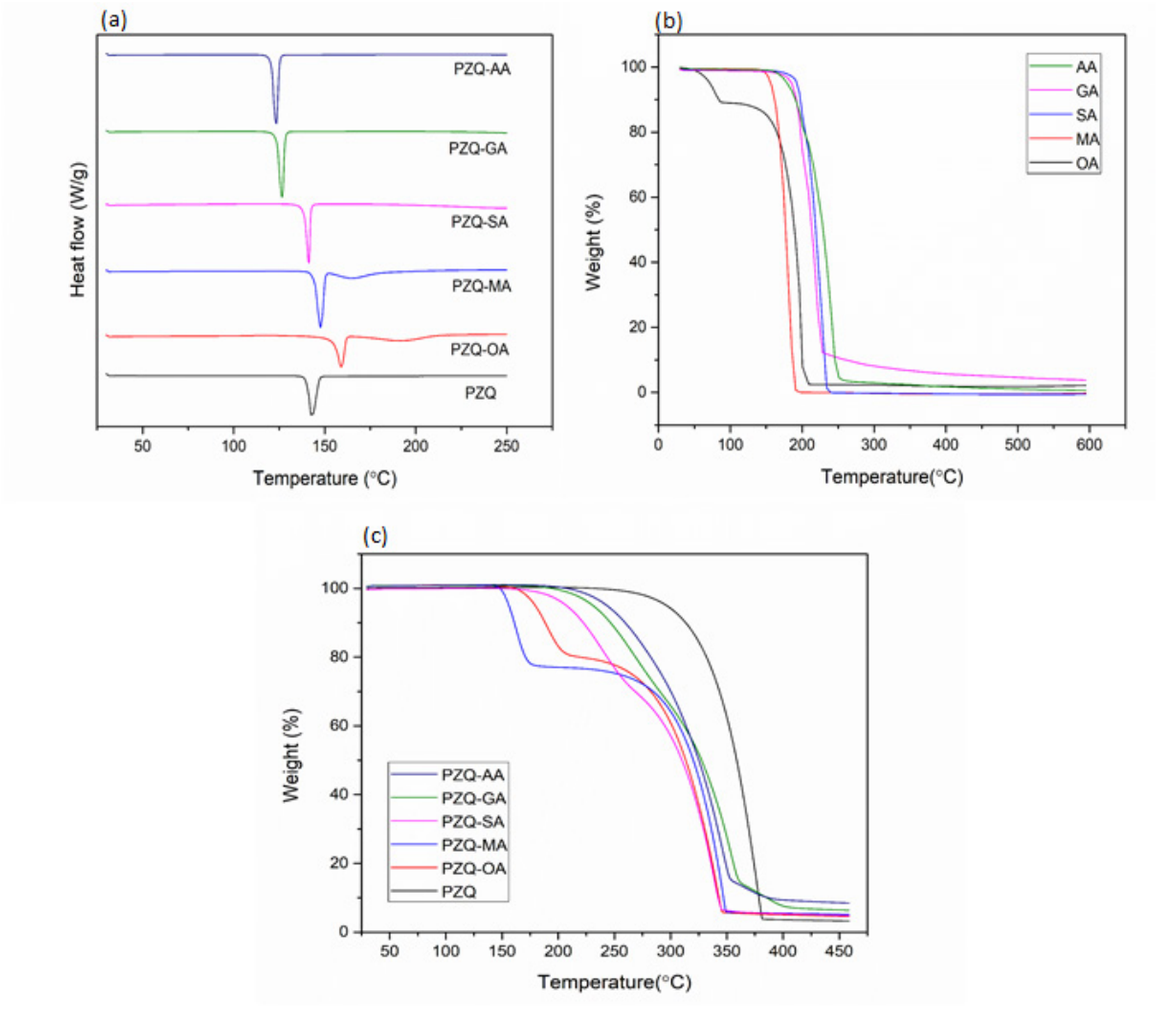

FIGURE 4. DSC thermograms of pure PZQ and its co-crystals (a), TGA thermograms of co-crystal formers (b), TGA thermograms of PZQ and its co-crystals (c)

Figure 4 shows the TGA results of PZQ and its co-crystals. The sample mass of PZQ-MA co-crystal begins to decrease at 147 °C, while at 178.6 °C the sample mass reduced to about 77.6% of the initial sample mass. Similarly, at 163 °C the mass of the PZQ-OA co-crystal begins to decrease, and at 212 °C the mass dropped to approximately 80% of the initial mass of the sample. Likewise, the PZQ-SA co-crystal mass started to decrease at 167 °C, and at 261 °C the mass is reduced to 72% of the initial sample mass. So, it is evidenced from the results that co-crystals PZQ-OA, PZQ-MA, and PZQ-SA start to disintegrate at about 163 °C, 147 °C, and 167 °C,

respectively. Whereas co-crystals PZQ-GA and PZQ-AA are thermally stable and start to decay at 199 °C and 208 °C, respectively. Moreover, pure PZQ starts to decay at 248 °C, and 97.8% mass is reduced at 383 °C. Overall, the thermal events of PZQ co-crystals in DSC and TGA are different from their respective individual components. The thermal analysis results further confirm the successful formation of co-crystals.

According to the solubility study results of PZQ co-crystals, the highest and lowest solubility observed was 10.5 ± 1.7 and 3.7 ± 1.08 mg/mL, respectively. PZQ co-crystals showed significantly high solubility compared to pure PZQ (0.392± 0.1 mg/mL) as shown in Table 5, and the result of pure PZQ is consistent with the reported data i.e., 0.381 mg/mL and 0.92 mmol/ L (El-Arini et al. 1998; USP 2017). PZQ is a hydrophobic drug with limited aqueous solubility and high permeability (Borrego-Sanchez et al. 2016). The PZQ solubility in different solvents is also reported as 3.001×10^{-2} (acetone), 5.964×10^{-2} (cyclohexanone), 4.379×10^{-2} (n-hexane), 2.896×10^{-2} (ethyl acetate), and 3.519×10^{-2} mole fraction (acetonitrile) (Li et al. 2020). The results of the present study showed a substantial improvement in the aqueous solubility of PZQ co-crystals. *In-vitro* dissolution study results showed improvement in dissolution by PZQ co-crystals compared to pure PZQ (24.05% at 90 min). The percent dissolution of pure PZQ with respect to time is in good agreement with that reported in the literature (Dametto et al. 2017). The intrinsic dissolution rate of pure PZQ is also reported as 31.2 ± 0.6 $\mu g.cm^{-2}$ min^{-1} at 37 °C in the literature (Zanolla et al. 2018). The descending order of improved dissolution was observed by co-crystals at 90 min as PZQ-SA (68%), PZQ-AA (57.96%), PZQ-GA (54.05%), PZQ-OA (51%), and PZQ-MA (46.05%) as shown in Figure 5(a).

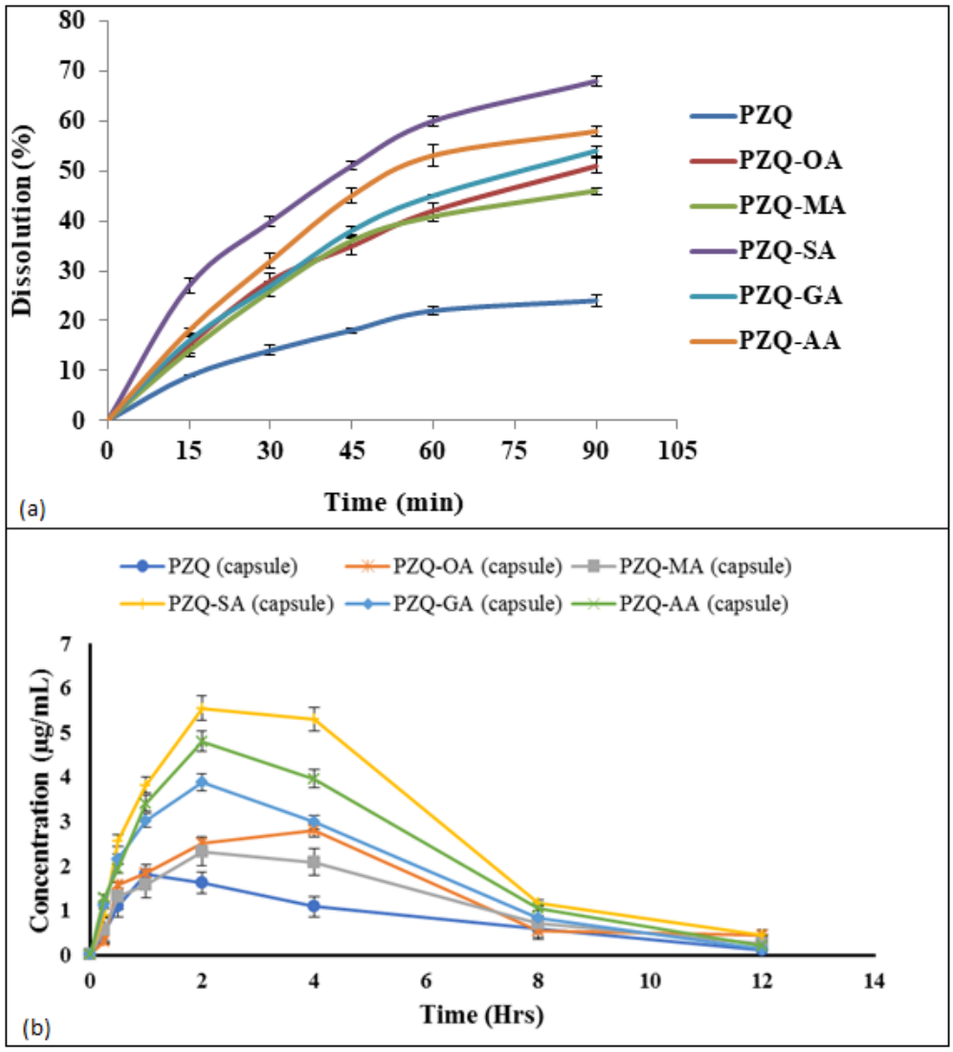

FIGURE 5. Dissolution profile of PZQ and its co-crystals (a), *In-vivo* drug release profile of pure PZQ and its five co-crystals (b)

TABLE 5. Solubility, % dissolution rate, and pharmacokinetic parameters of PZQ co-crystals

Samples	Solubility (mg/mL) MeanSD	% Dissolution rate (90 min)	AUC_{0-12} (µg h/mL)	C_{max} (µg/mL)	T_{max} (h)
PZQ	0.39 0.10	24.05	10.06 ± 2.51	1.80 ± 0.36	1 ± 0.57
PZQ-OA	4.20 1.10	51.00	17.16 ± 0.93	2.79 ± 0.16	4 ± 1.00
PZQ-MA	3.70 1.08	46.05	15.40 ± 3.65	2.30 ± 0.29	2 ± 1.00
PZQ-SA	10.50 1.70	68.00	33.84 ± 6.05	5.55 ± 1.06	2 ± 1.00
PZQ-GA	6.74 1.40	54.05	21.69 ± 7.15	3.88 ± 1.45	2 ± 1.00
PZQ-AA	7.00 1.05	57.96	27.23 ± 13.51	4.80 ± 2.47	2 ± 1.25

All p values of solubility and AUC are significant ($p< 0.05$) vs. PZQ

According to the P.K study results, enhanced oral bioavailability was observed by co-crystals compared to pure PZQ. PZQ-SA exhibited the highest oral bioavailability (33.84 ± 6.05 µg h/mL). While the lowest bioavailability showed by PZQ-MA (15.40 ± 3.65) among co-crystals. The oral bioavailability of all co-crystals was considerably better compared to pure PZQ (10.06 ± 2.51 µg h/mL) as shown in Figure 5(b). Table 5 enlists P.K parameters. The maximum enhanced bioavailability observed was approximately 3-fold compared to pure PZQ. The study result is consistent with the enhanced bioavailability observed in many studies, a 2.49 and 2-fold increase in bioavailability was observed by baicalein–nicotinamide and carbamazepine–succinic acid co-crystal, respectively (Huang et al. 2014; Ullah at al. 2016). The C_{max} is attained by pure PZQ quickly as compared to co-crystals. However, the C_{max} of co-crystals was higher than pure PZQ. In the case of PZQ-SA, the bioavailability is much higher than that of pure PZQ and the concentration reaches 5.55 µg/ mL in 2 h. The concentration achieved just in 1 h by all co-crystals is higher as compared to pure PZQ except for PZQ-OA and PZQ-MA. Attaining the high concentration quickly is quite interesting because, for many treatments, time is a critical factor. Higher plasma concentration can often be reached by increasing the drug dosage, but earlier bioavailability is much harder to achieve. This favorable property is shown by all co-crystals except PZQ-OA and PZQ-MA. In literature limited oral bioavailability study about PZQ has been performed, for example, the oral bioavailability of PZQ solid dispersion in comparison with a marketed product was observed as 456 ± 75 µg/L × h and 193 ± 19 µg/L × h, respectively (Liu et al. 2018). In another study, solid lipid nanoparticle of PZQ was investigated for an *in-vivo* study, and the *AUC* was observed as 14.03 ± 0.98 µg h/mL (Radwan et al. 2019). Improved bioavailability of low soluble drugs via co-crystallization technique has been reported in many studies, for instance, 168.7%, 10-fold, and 1.7-fold improved bioavailability was observed by 6-mercaptopurine–isonicotinamide (Wang et al. 2015), quercetin–theobromine dehydrate (Smith et al. 2012), and danazol–vanillin co-crystal, respectively (Childs et al. 2013).

The study results evidently exemplify that co-crystals have the ability to alter the physicochemical properties and oral bioavailability of low soluble drugs. Moreover, no correlation was observed between physicochemical properties/ P.K parameters and the spacer group of co-crystal formers.

Regarding the HPC polymer effect on PZQ co-crystal formation, the study results showed that the presence of HPC polymer did not inhibit the formation of PZQ co-crystals like PZQ-OA, PZQ-MA, PZQ-GA, and PZQ-AA whereas PZQ-SA was inhibited as shown in Figure 2. The absence of an important peak at 2θ= 13.52º and the presence of a prominent peak at 2θ= 6.98º represents the pure PZQ, evidenced by the inhibition of PZQ-SA co-crystal formation as shown in Figure 2(c). Quite limited studies have been performed about polymer's effect on co-crystal preparation, for example, the addition of PVP facilitates carbamazepine–saccharin, while inhibiting carbamazepine–nicotinamide co-crystal formation (Zhang et al. 2017), in another study,

carbamazepine co-crystals with SA and AA were not inhibited while carbamazepine co-crystals with MA and GA were inhibited at higher quantities of HPC polymer (Wasim et al. 2021).

Conclusion

The primary objective of the research work was to check the effects of the spacer group of co-crystal formers on the physicochemical properties and oral bioavailability of praziquantel (PZQ) co-crystals as well as to check the hydroxypropyl cellulose (HPC) polymer effect on co-crystal formation. The results of solubility, *in-vitro* dissolution, and oral bioavailability of PZQ co-crystals are consistent. In other words, the enhanced solubility is translated into improved *in-vitro* dissolution and oral bioavailability. No consistency of spacer group effects is seen in the physicochemical properties (solubility and dissolution) and oral bioavailability of PZQ co-crystals. In simple words, increasing hydrophobicity of co-crystal formers has no regular effect on physicochemical properties and oral bioavailability of co-crystals. An additional investigation is needed to expand and improve the conclusion about the effects of the spacer group of dicarboxylic acid co-crystal formers on the physicochemical and P.K behavior of low soluble drug co-crystals because the properties of co-crystals correspondingly change with different APIs and co-crystal formers as they are complex macromolecules. The study results about the effect of HPC polymer show that the formation of most of the co-crystals was not inhibited except one i.e. PZQ-SA. Based on the limited study we cannot draw the final conclusion about the polymer effect on co-crystal formation because the properties of polymers change with the monomer types, molecular weight, and degree of substitution and distribution. Therefore, further investigation is needed to support and improve the conclusion about the polymer effects on co-crystal formation.

In conclusion, better solubility, *in-vitro* dissolution, and oral bioavailability were observed by PZQ-SA followed by PZQ-AA, PZQ-GA, PZQ-OA, PZQ-MA, and PZQ pure. So the versatility of pharmaceutical co-crystal is reasonably clear about modifying the physicochemical and pharmacokinetic properties and effectively shows the importance of synthesizing 'co-crystals with a purpose'.

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References

Aakeroy, C.B. & Sinha, A.S. 2018. *Co-Crystals: Preparation, Characterization and Applications.* Vol. 24. Royal Society of Chemistry.

Aitipamula, S., Wong, A.B., Chow, P.S. & Tan, R.B. 2012. Pharmaceutical cocrystals of ethenzamide: Structural, solubility and dissolution studies. *CrystEngComm* 14: 8515-8524.

Andrade, L.N., Oliveira, D.M.L., Chaud, M.V., Alves, T.F.R., Nery, M., da Silva, C.F., Gonsalves, J.K.C., Nunes, R.S., Corrêa, C.B., Amaral, R.G., Sanchez-Lopez, E., Souto, E.B. & Severino, P. 2019. Praziquantel-solid lipid nanoparticles produced by supercritical carbon dioxide extraction: Physicochemical characterization, release profile, and cytotoxicity. *Molecules* 24(21): 3881.

Arenas-Garcia, J.I., Herrera-Ruiz, D., Mondragon-Vasquez, K., Morales-Rojas, H. & Hopfl, H. 2010. Co-crystals of active pharmaceutical ingredients-acetazolamide. *Crystal Growth & Design* 10: 3732-3742.

Basavoju, S., Bostrom, D. & Velaga, S.P. 2008. Indomethacin–saccharin cocrystal: Design, synthesis and preliminary pharmaceutical characterization. *Pharmaceutical Research* 25: 530-541.

Basavoju, S., Bostrom, D. & Velaga, S.P. 2006. Pharmaceutical cocrystal and salts of norfloxacin. *Crystal Growth & Design* 6(12): 2699-2708.

Bhatt, P.M., Ravindra, N.V., Banerjee, R. & Desiraju, G.R. 2005. Saccharin as a salt former. Enhanced solubilities of saccharinates of active pharmaceutical ingredients. *Chemical Communications* 28(8): 1073-1075.

Borrego-Sanchez, A., Viseras, C., Aguzzi, C. & Sainz-Díaz, C.I. 2016. Molecular and crystal structure of praziquantel. Spectroscopic properties and crystal polymorphism. *European Journal of Pharmaceutical Sciences* 92: 266-275.

Butreddy, A., Sarabu, S., Bandari, S., Dumpa, N., Zhang, F. & Repka, M.A. 2020. Polymer-assisted aripiprazole–adipic acid cocrystals produced by hot melt extrusion techniques. *Crystal Growth & Design* 20(7): 4335-4345.

Childs, S.L., Rodriguez-Hornedo, N., Reddy, L.S., Jayasankar, A., Maheshwari, C., McCausland, L., Shipplett, R. & Stahly, B.C. 2008. Screening strategies based on solubility and solution composition generate pharmaceutically acceptable cocrystals of carbamazepine. *CrystEngComm* 10(7): 856-864.

Childs, S.L., Chyall, L.J., Dunlap, J.T., Smolenskaya, V.N., Stahly, B.C. & Stahly, G.P. 2004. Crystal engineering approach to forming cocrystals of amine hydrochlorides with organic acids. Molecular complexes of fluoxetine hydrochloride with benzoic, succinic, and fumaric acids. *Journal of the American Chemical Society* 126(41): 13335-13342.

Childs, S.L., Kandi, P. & Lingireddy, S.R. 2013. Formulation of a danazol cocrystal with controlled supersaturation plays an essential role in improving bioavailability. *Molecular Pharmaceutics* 10(8): 3112-3127.

Costa, E.D., Priotti, J., Orlandi, S., Leonardi, D., Lamas, M.C., Nunes, T.G., Diogo, H.P., Salomon, C.J. & Ferreira, M.J. 2016. Unexpected solvent impact in the crystallinity of praziquantel/poly (vinylpyrrolidone) formulations. A solubility, DSC and solid-state NMR study. *International Journal of Pharmaceutics* 511(2): 983-993.

Cugovcan, M., Jablan, J., Lovric, J., Cincic, D., Galic, N. & Jug, M. 2017. Biopharmaceutical characterization of praziquantel cocrystals and cyclodextrin complexes prepared by grinding. *Journal of Pharmaceutical and Biomedical Analysis* 137: 42-53.

Dametto, P.R., Dametto, A.C., Polese, L., Ribeiro, C.A., Chorilli, M. & de Freitas, O. 2017. Development and physicochemical characterization of solid dispersions containing praziquantel for the treatment of schistosomiasis. *Journal of Thermal Analysis and Calorimetry* 127(2): 1693-1706.

Dayan, A.D. 2003. Albendazole, mebendazole and praziquantel. Review of non-clinical toxicity and pharmacokinetics. *Acta Tropica* 86(2-3): 141-159.

Dinora, G.E., Julio, R., Nelly, C., Lilian, Y.M. & Cook, H.J. 2005. *In vitro* characterization of some biopharmaceutical properties of praziquantel. *International Journal of Pharmaceutics* 295(1-2): 93-99.

Duggirala, N.K., Perry, M.L., Almarsson, O. & Zaworotko, M.J. 2016. Pharmaceutical cocrystals: Along the path to improved medicines. *Chemical Communications* 52(4): 640-655.

El-Arini, S.K., Giron, D. & Leuenberger, H. 1998. Solubility properties of racemic praziquantel and its enantiomers. *Pharmaceutical Development and Technology* 3(4): 557-564.

Espinosa-Lara, J.C., Guzman-Villanueva, D., Arenas-García, J.I., Herrera-Ruiz, D., Rivera-Islas, J., Roman-Bravo, P., Morales-Rojas, H. & Hopfl, H. 2013. Cocrystals of active pharmaceutical ingredients−Praziquantel in combination with oxalic, malonic, succinic, maleic, fumaric, glutaric, adipic, and pimelic acids. *Crystal Growth & Design* 13(1): 169-185.

Fischer, F., Scholz, G., Batzdorf, L., Wilke, M. & Emmerling, F. 2015. Synthesis, structure determination, and formation of a theobromine: Oxalic acid 2: 1 cocrystal. *CrystEngComm* 17(4): 824-829.

Fleischman, S.G., Kuduva, S.S., McMahon, J.A., Moulton, B., Bailey Walsh, R.D., Rodriguez-Hornedo, N. & Zaworotko, M.J. 2003. Crystal engineering of the composition of pharmaceutical phases: Multiple-component crystalline solids involving carbamazepine. *Crystal Growth & Design* 3(6): 909-919.

Friscic, T. & Jones, W. 2010. Benefits of cocrystallisation in pharmaceutical materials science: An update. *Journal of Pharmacy and Pharmacology* 62(11): 1547-1559.

Huang, Y., Zhang, B., Gao, Y., Zhang, J. & Shi, L. 2014. Baicalein–nicotinamide cocrystal with enhanced solubility, dissolution, and oral bioavailability. *Journal of Pharmaceutical Sciences* 103(8): 2330-2337.

Kakkar, S., Bhattacharya, B., Reddy, C.M. & Ghosh, S. 2018. Tuning mechanical behaviour by controlling the structure of a series of theophylline co-crystals. *CrystEngComm* 20(8): 1101-1109.

Karagianni, A., Malamatari, M. & Kachrimanis, K. 2018. Pharmaceutical cocrystals: New solid phase modification approaches for the formulation of APIs. *Pharmaceutics* 10(1): 18.

Li, R., Chen, X., He, G., Wu, C., Gan, Z., He, Z., Zhao, J. & Han, D. 2020. The dissolution behaviour and thermodynamic properties calculation of praziquantel in pure and mixed organic solvents. *The Journal of Chemical Thermodynamics* 144: 106062.

Li, J., Wang, Y., Fenwick, A., Clayton, T.A., Lau, Y.Y., Legido-Quigley, C., Lindon, J.C., Utzinger, J. & Holmes, E. 2007. A high-performance liquid chromatography and nuclear magnetic resonance spectroscopy-based analysis of commercially available praziquantel tablets. *Journal of Pharmaceutical and Biomedical Analysis* 45(2): 263-267.

Liu, Y., Wang, T., Ding, W., Dong, C., Wang, X., Chen, J. & Li, Y. 2018. Dissolution and oral bioavailability enhancement of praziquantel by solid dispersions. *Drug Delivery and Translational Research* 8(3): 580-590.

Liu, Y., Wang, X., Wang, J.K. & Ching, C.B. 2006. Investigation of the phase diagrams of chiral praziquantel. *Chirality: The Pharmacological, Biological, and Chemical Consequences of Molecular Asymmetry* 18(4): 259-264.

McNamara, D.P., Childs, S.L., Giordano, J., Iarriccio, A., Cassidy, J., Shet, M.S., Mannion, R., O'Donnell, E. & Park, A. 2006. Use of a glutaric acid cocrystal to improve oral bioavailability of a low solubility API. *Pharmaceutical Research* 23(8): 1888-1897.

Mishra, A., Vuddanda, P.R. & Singh, S. 2014. Intestinal lymphatic delivery of praziquantel by solid lipid nanoparticles: Formulation design, *in vitro* and *in vivo* studies. *Journal of Nanotechnology* 2014: Article ID. 351693. doi.org/10.1155/2014/351693

Pavia, D.L., Lampman, G.M., Kriz, G.S. & Vyvyan, J.A. 2014. *Introduction to Spectroscopy*. Cengage Learning.

Perumalla, S.R. & Sun, C.C. 2013. Improved solid-state stability of salts by cocrystallization between conjugate acid–base pairs. *CrystEngComm* 15(29): 5756-5759.

Pharmacopoeia, U.S. 2017. USP 40−NF 35. *The USP Convention.* Rockville. pp. 5803-5805.

Qian, M., Ho, R.J., Qiao, C. & Shi, J. 2017. U.S. Patent No. 9,657,017. Washington, DC: U.S. Patent and Trademark Office.

Radwan, A., El-Lakkany, N.M., William, S., El-Feky, G.S., Al-Shorbagy, M.Y., Saleh, S. & Botros, S. 2019. A novel praziquantel solid lipid nanoparticle formulation shows enhanced bioavailability and antischistosomal efficacy against murine *S. mansoni* infection. *Parasites & Vectors* 12(1): 1-12.

Saganowska, P. & Wesolowski, M. 2018. DSC as a screening tool for rapid co-crystal detection in binary mixtures of benzodiazepines with co-formers. *Journal of Thermal Analysis and Calorimetry* 133(1): 785-795.

Salazar-Rojas, D., Maggio, R.M. & Kaufman, T.S. 2020. Preparation and characterization of a new solid form of praziquantel, an essential anthelmintic drug. Praziquantel racemic monohydrate. *European Journal of Pharmaceutical Sciences* 146: 105267.

Savjani, K.T., Gajjar, A.K. & Savjani, J.K. 2012. Drug solubility: Importance and enhancement techniques. *International Scholarly Research Notices* 2012: 195727. doi:10.5402/2012/195727

Smith, A.J., Kavuru, P., Wojtas, L., Zaworotko, M.J. & Shytle, R.D. 2012. Cocrystals of quercetin with improved solubility and oral bioavailability. *Molecular Pharmaceutics* 8(5): 1867-1876.

Stuart, B.H. 2004. *Infrared Spectroscopy: Fundamentals and Applications*. New York: John Wiley & Sons.

Ullah, M., Hussain, I. & Sun, C.C. 2016. The development of carbamazepine-succinic acid cocrystal tablet formulations with improved *in vitro* and *in vivo* performance. *Drug Development and Industrial Pharmacy* 42(6): 969-976.

Valdes-Negrin, H.L., Alvarez, O., Perez-Rodriguez, Z., Rodriguez-Negrin, Z. & Perez-Rodriguez, M. 2022. Analyzing thermal degradation of furvina drug using a stability indicating spectrophotometric method and characterization studies. *Chemistry Africa* 5: 305-312.

Walsh, R.B., Bradner, M.W., Fleischman, S., Morales, L.A., Moulton, B., Rodriguez-Hornedo, N. & Zaworotko, M.J. 2003. Crystal engineering of the composition of pharmaceutical phases. *Chemical Communications* 2: 186-187.

Wasim, M., Mannan, A., Asad, M.H.H.B., Amirzada, M.I., Shafique, M. & Hussain, I. 2021. Fabrication of carbamazepine cocrystals: Characterization, *in vitro* and comparative *in vivo* evaluation. *BioMed Research International* 2021: 6685806.

Wang, J.R., Yu, X., Zhou, C., Lin, Y., Chen, C., Pan, G. & Mei, X. 2015. Improving the dissolution and bioavailability of 6-mercaptopurine via co-crystallization with isonicotinamide. *Bioorganic & Medicinal Chemistry Letters* 25(5): 1036-1039.

Wicaksono, Y., Wisudyaningsih, B. & Siswoyo, T.A. 2017. Cocrystal of atorvastatin calcium–malonic acid. *UNEJ e-Proceeding*. pp. 75-78.

Zanolla, D., Perissutti, B., Passerini, N., Chierotti, M.R., Hasa, D., Voinovich, D., Gigli, L., Demitri, N., Geremia, S., Keiser, J. & Albertini, B. 2018. A new soluble and bioactive polymorph of praziquantel. *European Journal of Pharmaceutics and Biopharmaceutics* 127: 19-28.

Zhang, H., Zhu, Y., Qiao, N., Chen, Y. & Gao, L. 2017. Preparation and characterization of carbamazepine cocrystal in polymer solution. *Pharmaceutics* 9(4): 54.

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