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ORIGINAL ARTICLE

Preparation of inclusion complex of praziquantel with 2-hydroxypropyl-β-cyclodextrin and pharmacokinetic property improvement

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KEYWORDS

Inclusion complex;
Praziquantel;
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Pharmacokinetic property evaluation

Abstract An inclusion complex of praziquantel with 2-hydroxypropyl-β-cyclodextrin was designed and prepared by an optimized method and the inclusion complex was characterized by infrared absorption spectral, proton NMR spectral and scanning electron image studies. It was shown that the aqueous solubility of praziquantel has increased (~104 fold) in comparison with praziquantel alone, which is the best result so far for praziquantel solubility study. The *in vivo* pharmacokinetic properties of praziquantel in dogs such as C_{max}, T_{max}, AUC_0-∞, and $t_{1/2}$ have been improved significantly after complexation. The increased water solubility of the praziquantel in the complex resulted in the improved values of C_{max} (4.82 μg/mL vs 0.51 μg/mL, 9.45 fold higher) and AUC_0-∞ (98.06 μg h/mL vs 20.63 μg h/mL, 4.75 fold higher) in dog pharmacokinetic studies, which is useful to find a new praziquantel formulation as an anti-schistosomal agent.

1. Introduction

Praziquantel (Fig. 1) is a broad-spectrum worm drug (Da Silva and Campos, 2017) and considered as a most effective anti-schistosomal drug so far (Chai, 2013). However, its poor physical characteristics such as low water solubility (0.26 mg/mL), limited bioavailability, bitter taste and strong odor (Erko et al., 2012) restrict its clinical applications (Mourão et al., 2005). Further, the large doses of praziquantel are required to overcome first pass metabolism as it's well absorbed across the gastrointestinal tract to achieve adequate concentrations at the target sites by the oral route (Cioli and Pica-Mattocia, 2003), which leads to a series of side effects (Couland and Charmot, 1993). It's reasoned that if water solubility of praziquantel is increased, it may be administered through parenteral formulations as alternative means of delivery.

Several strategies to increase a drug's water solubility have been reported in the literature (Khadka et al., 2014), and one

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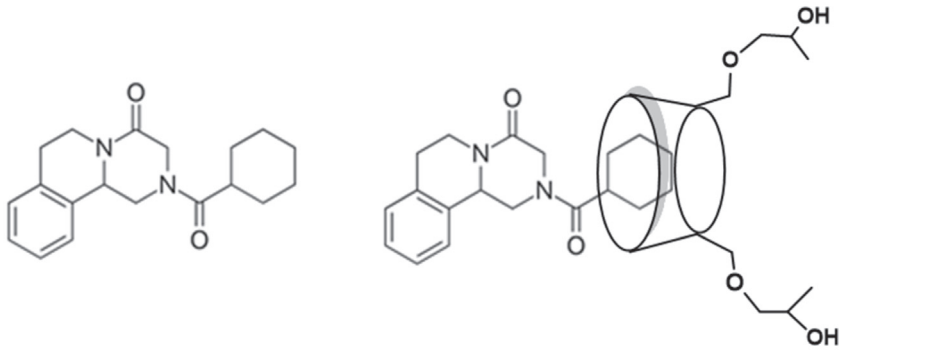


Praziquantel **Inclusion complex**

Fig. 1 The structures of praziquantel and its inclusion complex with HP-β-CD.

of them is the formation of inclusion complexes with cyclodextrins. Cyclodextrins and their derivatives are a class of cyclic oligosaccharides with outer hydrophilic surface and a central lipophilic cavity. They readily form inclusion complexes with different kinds of substrates, which enable to improve substrate's water solubility for the poorly soluble drugs, which in turn increase drug's bioavailability and stability (Loftsson, 2002).

The praziquantel inclusion complexes with α-, β- and γ-cyclodextrins were reported to have shown improved solubility by 2.6, 5 and 8-fold respectively in comparison to that of the pure drug. Further, only the β-cyclodextrin complex of praziquantel had a stability constant in the optimum range for pharmaceutical use (El-Arini and Leuenberger, 1996). It is obvious, these complexes did not increase praziquantel water solubility significantly.

2-Hydroxypropyl-*β*-cyclodextrin, a synthetic analog of natural cyclodextrins, is widely used as a drug carrier due to its high aqueous solubility (1200 g/L) and stability to heat, it has no nephrotoxicity, is almost non-irritating on muscles and the mucosa. Over the last decade, several methods had been reported for the preparation of praziquantel/HP-β-CD inclusion complex. Feng reported the preparation of praziquantel/HP-β-CD complex through ultrasonic method, and the solubility of praziquantel was increased by 27 times (Feng et al., 2007). With heating and sonication, Maragos (Maragos et al., 2009) prepared the praziquantel/HP-β-CD complex, however, the praziquantel's water solubility was increased only 6-fold. Interestingly, Cugovčan (Cugovčan et al., 2017) made the same complex by neat grinding, and praziquantel's aqueous solubility was improved by 12 times only. These results indicated that different inclusion complex preparation methods will generate different results for praziquantel's water solubility. Since none of them could improve the praziquantel's water solubility significantly, and it is necessary to find a good procedure to prepare the praziquantel/HP-β-CD complex with excellent water solubility.

Because of our continued interest in using cyclodextrins as drug carriers (Ding et al., 2018), the above results piqued our curiosity to study the inclusion complex preparation procedures in detail to further improve praziquantel's water solubility significantly.

In this report, we like to present an efficient and consistent synthetic procedure for preparation of the inclusion complex (Fig. 1) of praziquantel with HP-β-CD, along with its characterization. Further, enhanced water solubility, dissolution determination, and *in vivo* pharmacokinetic properties evaluation of the inclusion complex are also discussed in detail.

2. Results and Discussion

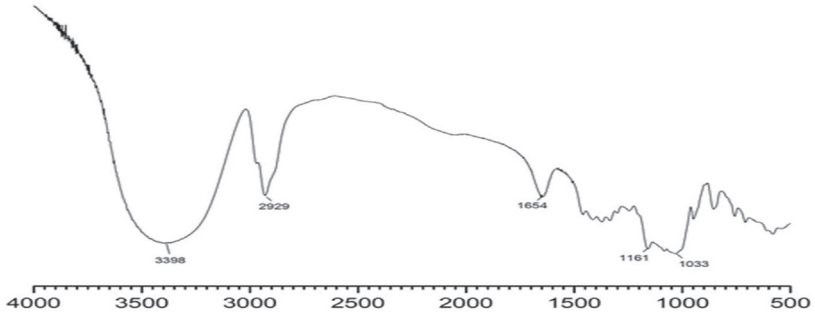
It's a well known fact in the art of formation of inclusion complexes that the inclusion effect of substrate is affected by the reaction physical parameters such as temperature, stirring speed, and reaction duration. To arrive at the optimal conditions in the formation of inclusion complex of praziquantel with HP-β-CD, several conditions were tried as expected. For example, the ratio between praziquantel and HP-β-CD were evaluated between 1:1 and 1:3, while stirring speeds of reaction ranged between 400 and 600 rpm, and the reaction temperatures lied in a range of 55 to 65 °C.

In essence more than fifty conditions were tried to optimize the preparation process, and the inclusion ratio and the yield of the resulting inclusion complex was determined by HPLC. The best condition was found as follows: the ratio of praziquantel and HP-β-CD should be 1:3, the stirring speed should be 600 rpm, the reaction temperature should be 65 °C, and the reaction time should be 5 h. These conditions lead to an optimum yield of 93.26% with the ratio of 92.64% for the resultant praziquantel and HP-β-CD inclusion complex.

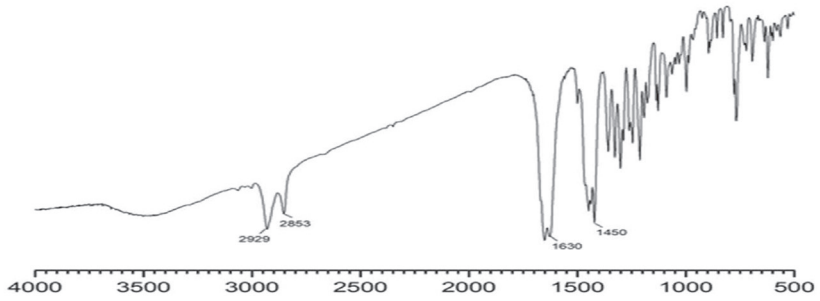
The UV spectra evaluation of praziquantel and HP-β-CD found that praziquantel has the maximum absorption at 210 nm, and in this range, hydroxypropyl-β-cyclodextrin has no absorption. tTherefore, HPLC with UV detector at 210 nm was used to analyze the praziquantel/HP-β-CD inclusion complex.

The formation of the inclusion complex was confirmed by Fourier-transform infrared spectroscopic studies (Fig. 2). The non-covalent interactions such as hydrophobic interactions, van der Waals interactions, and hydrogen bonding between the HP-β-CD and praziquantel in the inclusion complex play an important role in lowering the energy of the praziquantel entrapped part in the inclusion complex. Accordingly, it's anticipated to observe the changes in absorbance due to inclusion effect and the absorption peak's size may decrease or shift their absorption position or may disappear altogether with respect to the peak intensities of the corresponding frequencies for that part of the praziquantel.

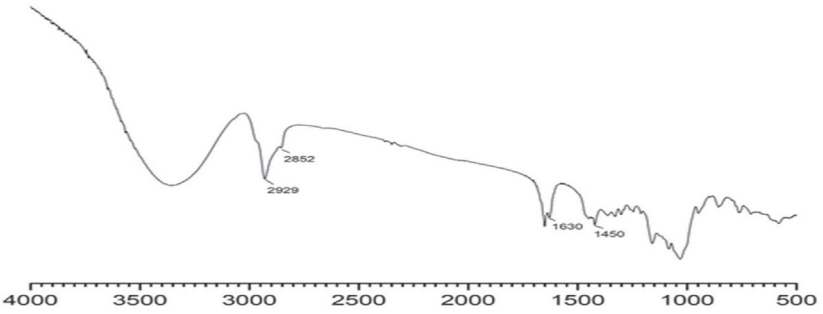
The characteristic peaks in FTIR spectrum of praziquantel appearing at 2929 cm^{-1} and 2853 cm^{-1} belong to CH_2 and CH



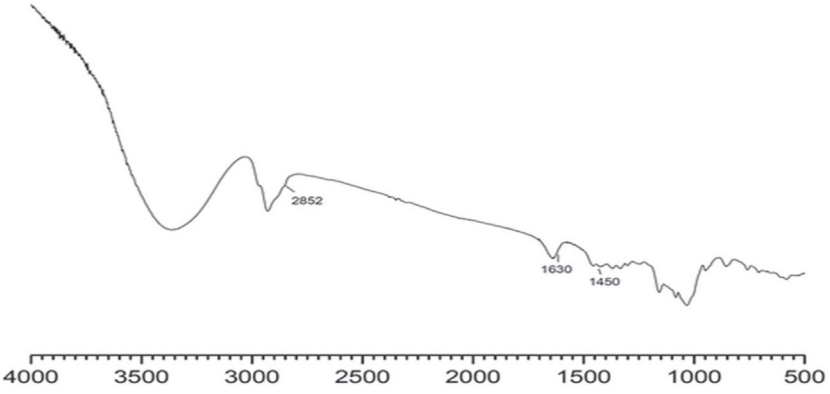
HP-β-CD



Praziquantel



The mixture of praziquantel and HP-β-CD



The inclusion complex of praziquantel and HP-β-CD

Fig. 2 Fourier-transform infrared spectroscopy spectra of HP-β-CD, praziquantel, and the mixture of praziquantel and HP-β-CD, and their inclusion complex.

vibrations, while the peak at 1630 cm^{-1} corresponds to carbonyl stretching. The peak appearing at 1450 cm^{-1} confirms to C-C stretches in the aromatic ring. The formation of the inclusion complex was indicated in the FTIR spectrum of inclusion complex wherein the peaks at 1630 cm^{-1} and 1450 cm^{-1} appeared with reduced intensities, while the peak at 2852 cm^{-1} was disappeared altogether. These changes are due to the formation of inclusion complex in which the cyclohexyl moiety in praziquantel is entrapped in the HP-β-CD cavity which resulted in the changes seen in the FTIR spectrum.

The surface morphology of praziquantel and the subsequent changes in its morphology due to inclusion complex are discerned from their scanning electron micrographs (Fig. 3). The scanning electron micrograph of praziquantel in its pure form shows it as granular crystals (Fig. 3a), while the HP-β-CD was shown as ball shape crystals (Fig. 3b), and the micrograph of the mixture of praziquantel and HP-β-CD appears (Fig. 3c) as an heterogenous mixture of granular and ball shape crystals reflecting their compositional nature, while the micrograph of inclusion complex shows it as the compound spherical structures (Fig. 3d). The surface changes in the micrographs clearly confirm praziquantel/ HP-β-CD inclusion complex formation.

The ^{1}H NMR spectra of HP-β-CD and its inclusion complex with praziquantel in D_2O were recorded and displayed in Fig. 4. A ratio of the aromatic protons at 7.30 ppm (4H) to the anomeric protons at 5.03 ppm and 5.20 ppm (7H) in inclusion complex is 2.50, which is close to 1,75, and it's believed that the inclusion complex contains one praziquantel molecule and one HP-β-CD molecule. The higher ratio seen may be due to some free HP-β-CD in the product. The NMR spectra of HP-β-CD, praziquantel and their inclusion complex in $DMSOd_6$ were also recorded. A careful comparison of the peaks from 1.40 ppm to 1.75 ppm (11*H*) from praziquantel in inclusion complex indicated shift in their values in comparison to that of praziquantel alone, Especially, the shift in ppm values of cyclohexane part in praziquantel in the inclusion complex is more pronounced as this part was entrapped in the HP-β-CD. The ^{1}H NMR data is supported by H-H COSY and NOESY studies of the inclusion complex in D_2O (Fig. 5).

In the H-H noesy spectrum, the signal at 1.75 ppm from the proton in cyclohexane part in praziquantel showed an interaction with the signal at 3.75 ppm from cyclodextrin part which supports the conclusion obtained from the proton NMR spectra.

The Fig. 6 displays the graphs of dissolution rates of praziquantel, the mixture of praziquantel and HP-β-CD, and praziquantel/HP-β-CD inclusion complex. From these graphs, it was observed that the dissolution rate of praziquantel/HP-β-CD inclusion complex was remarkably superior to that of praziquantel, and the mixture of praziquantel and HP-β-CD. At 5 min., the additive solubility of the inclusion complex

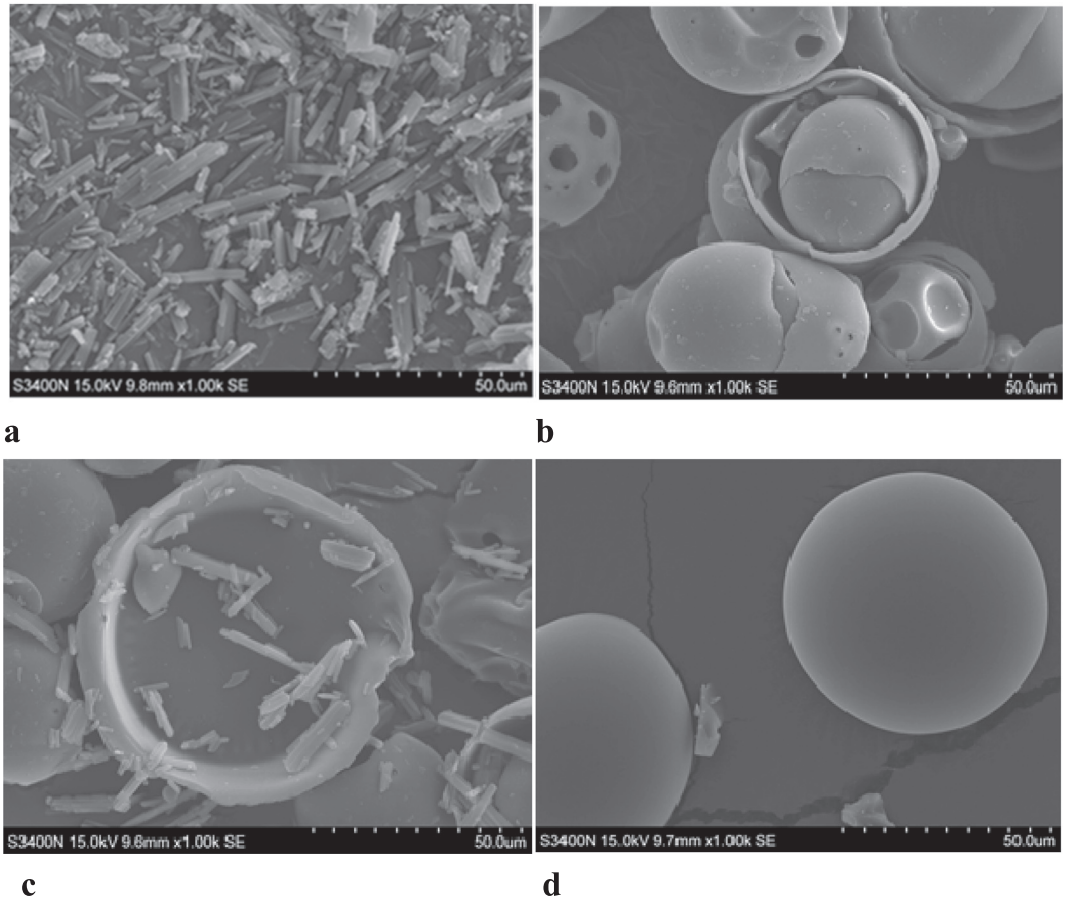
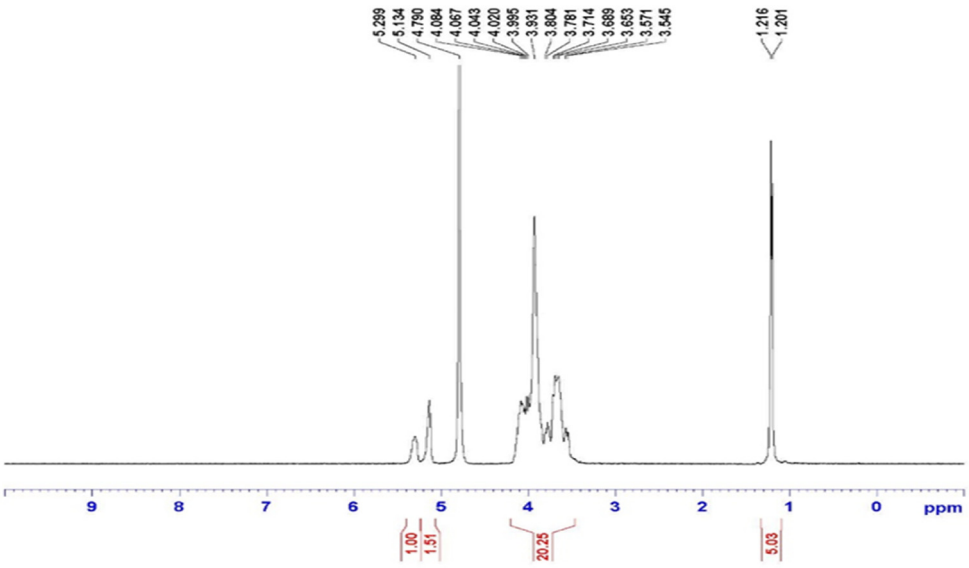
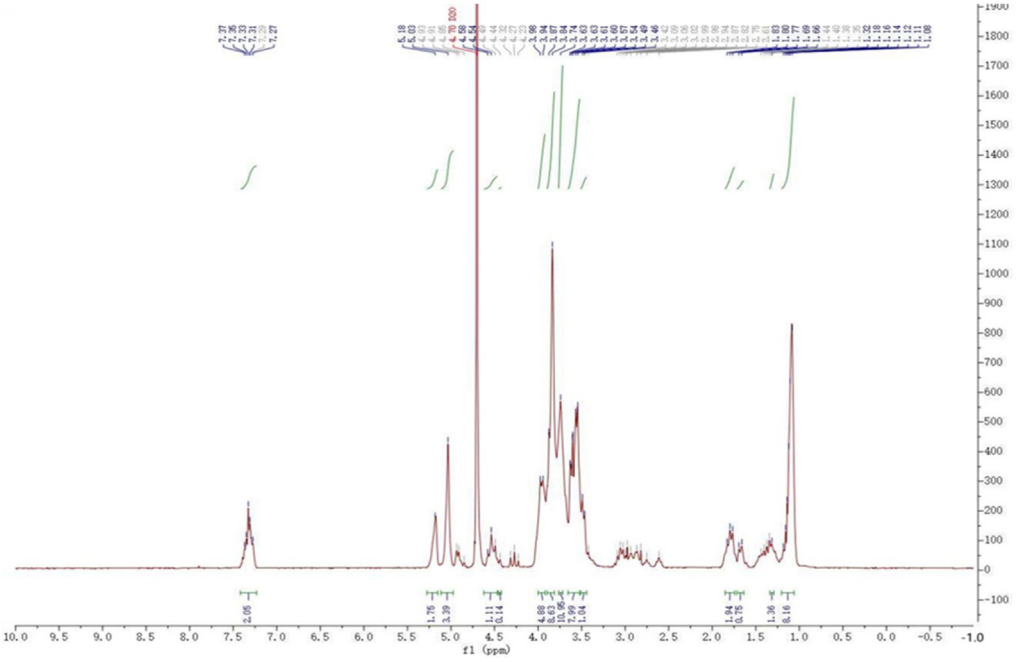


Fig. 3 The scanning electron microscopy imagines of the praziquantel (**3a**), HP-β-CD (**3b**), the mixture of praziquantel and HP- β-CD (**3c**), and the praziquantel/HP-β-CD inclusion complex (**3d**).



Proton NMR spectrum of HP-β-CD in D_2O



Proton NMR spectrum of the inclusion complex of praziquantel with HP-β-CD in D_2O

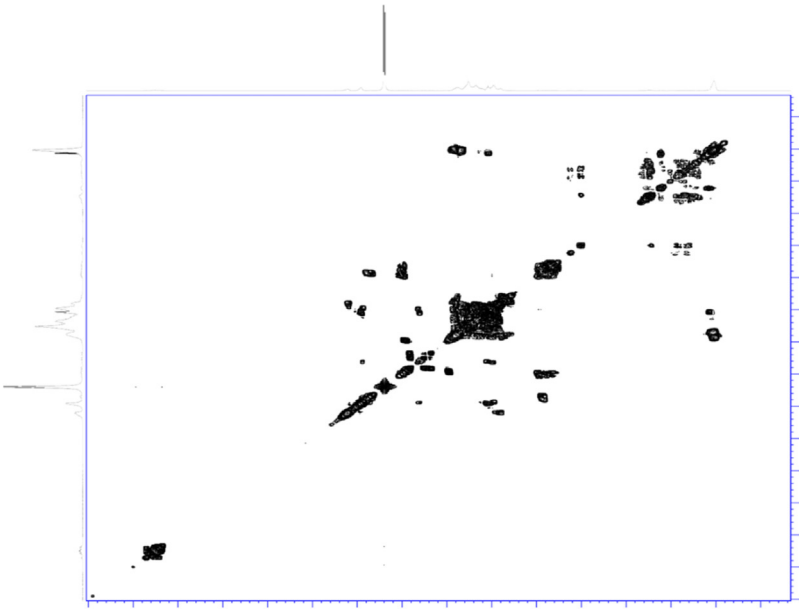
Fig. 4 Proton NMR spectra of HP-β-CD, praziquantel and their inclusion complex (This product was prepared by using 1:1 ratio of HP-β-CD and praziquantel).

was close to 87%, while the praziquantel had not even reached the detection limit level. At 60 min., the inclusion complex solubility reached 90% surpassing that of the praziquantel by 15 folds.

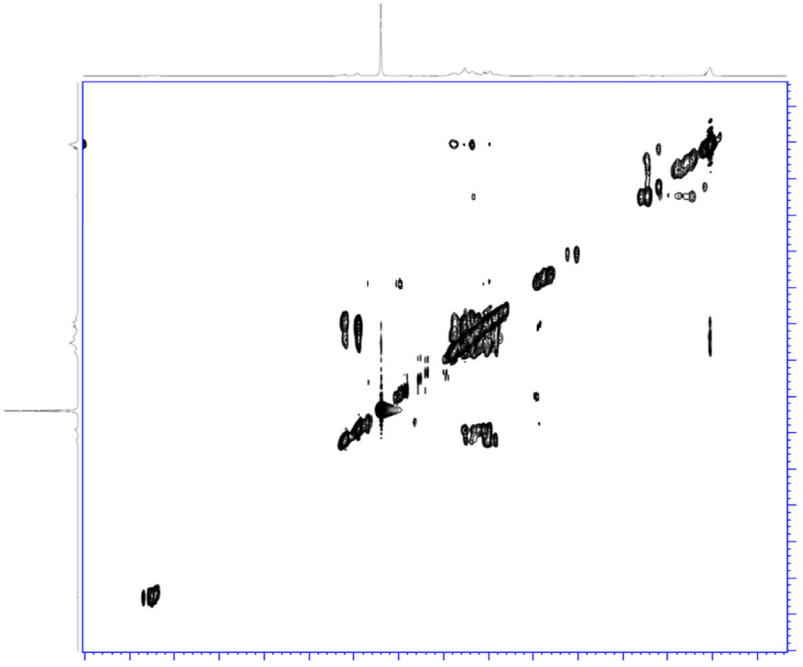
The aqueous solubility (Feng et al., 2007) of praziquantel was reported as low as 0.26 mg/mL, while the quantity of praziquantel in the saturated aqueous solution of praziquantel/HP-β-CD inclusion complex was obtained based on the HPLC analysis.The water solubility of praziquantel in the inclusion complex was 27 mg/mL which is nearly 104 folds more than the solubility of praziquantel alone, and it is the best result among the praziquantel solubility studies done so far.

The praziquantel/HP-β-CD inclusion complex's shelf stability was assessed as follows. The inclusion complex in a sealed tube was refrigerated at 4 °C for about six months, and the HPLC analysis results indicated that there was no change in the praziquantel content in the inclusion complex.

Additionally, it is believed that due to the hydrophilic outer surface of the inclusion complexes, the praziquantel is ensconced in the cyclodextrin cylinder, the direct contact between the praziquantel and taste sensors is inhibited, and because of this,



H-H Cosy of the inclusion complex



H-H Noesy of the inclusion complex

Fig. 5 H-H Cosy and H-H Noesy spectra of the inclusion complex of praziquantel with HP-β-CD.

the nauseating bad taste and odor sensation of praziquantel were eliminated.

The pharmacokinetic properties of praziquantel inclusion complex were studied by fitting a double-compartment model to the individual concentration–time data for plasma. Thus, the C_{max}, T_{max}, $AUC_{0-\infty}$ and $t_{1/2}$ values of praziquantel and its inclusion complex in dog plasma were calculated after oral administration, and the standard working curve obtained for praziquantel was shown in Fig. 7. Accordingly, the concentration of praziquantel in plasma was ranged from 0.2 to 12 μg/mL, and a linear relationship was observed between the peak areas in HPLC and concentrations of praziquantel in plasma. The corresponding linear regression equation and correlation coefficient are obtained as $Y = 10^{-5}X\text{-}0.0858$ ($R^2 = 0.9996$; Y: peak area; X: concentration), LOQ was 0.1 μg/mL, and LOD was 0.05 μg/mL.

The Fig. 8 displays the HPLC traces of pure praziquantel, the blank dog plasma, the mixture of the dog plasma and praz-

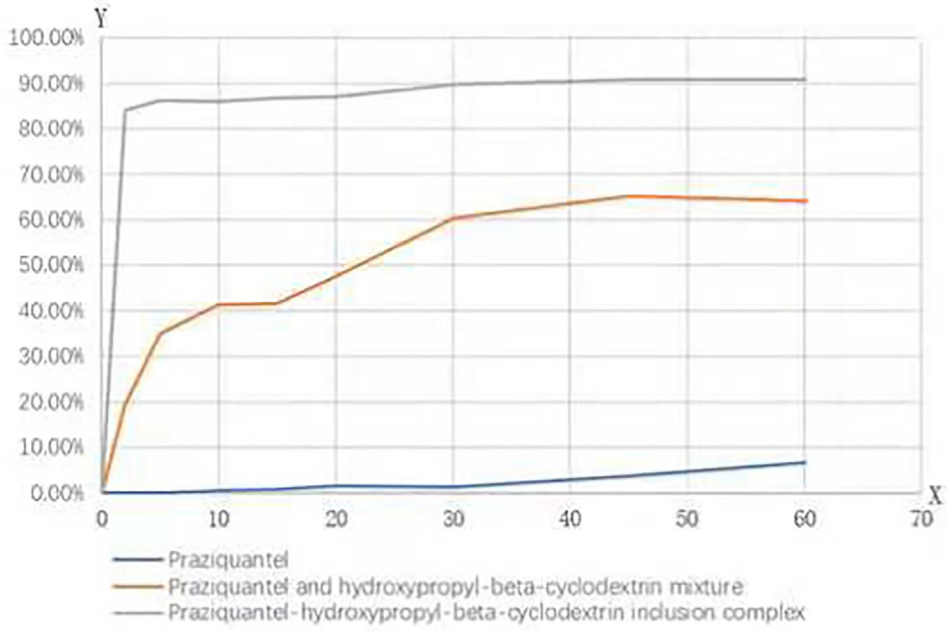


Fig. 6 The dissolution rates of praziquantel, the mixture of praziquantel and HP-β-CD, and their inclusion complex.

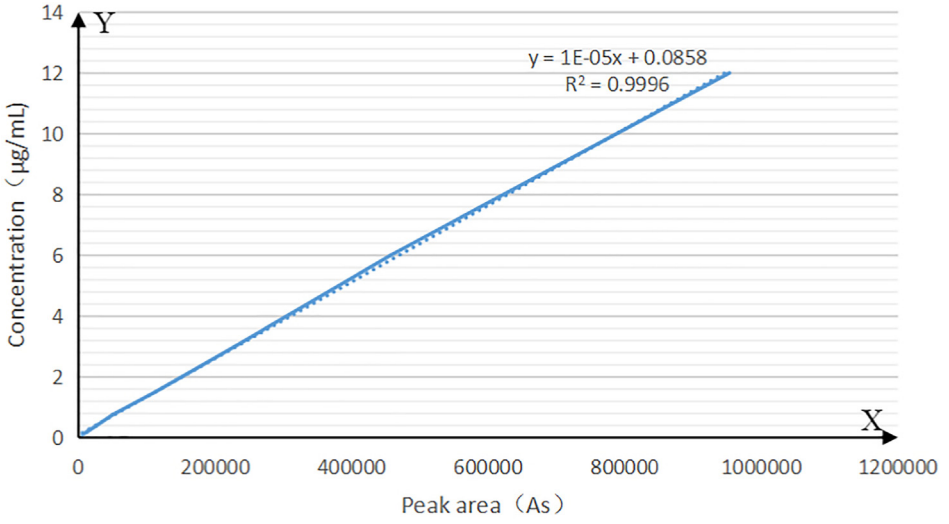


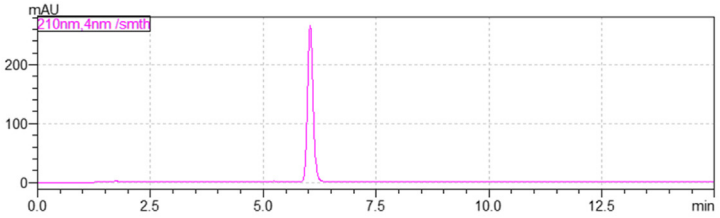
Fig. 7 The standard curve of praziquantel in dog blank plasma.

iquantel, the dog plasma from the dogs treated with praziquantel, and the dog plasma from the dogs dosed with praziquantel inclusion complex in a sequential manner.

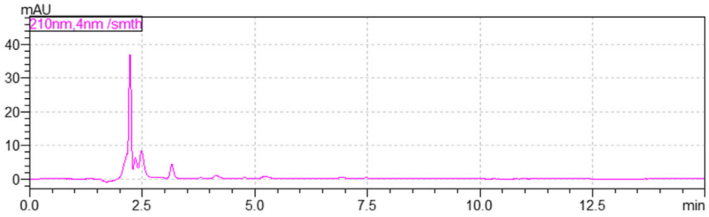
The examination of HPLC traces shown above in Fig. 8 demonstrated that praziquantel and the dog blank plasma peaks are quite apart under the study conditions. Therefore, these HPLC conditions were employed to estimate praziquantel in the dog plasma samples and the results were shown in Fig. 9.

The pharmacokinetic properties data of praziquantel and its HP-β-CD inclusion complex in healthy dogs conformed to the first-order absorption two-compartment model (following WinNonlin 5.2.1) and is presented in Table 1.

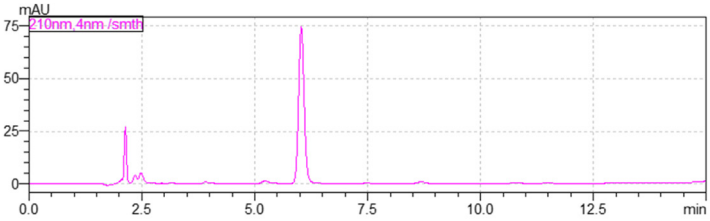
After oral administration, interestingly, in the praziquantel inclusion complex group, C_{max} (4.82 μg/mL, at 1.57 h) was considerably higher (9.45 folds) than that in the praziquantel group (C_{max}: 0.51 μg/mL, at 2.05 h), whereas T_{max} in the inclusion complex group is slightly smaller than that in the praziquantel group while $t_{1/2}$ has increased by 1.4 folds for inclusion complex group compared to that of praziquantel group. However, the AUC_0-∞ of inclusion complex was determined as 98.06 μg h/mL which was 4.75 folds more than that of praziquantel (20.63 μg h/mL) alone, while clearance has decreased (4.75 folds) by the same order for inclusion complex group compared to that of praziquantel group.



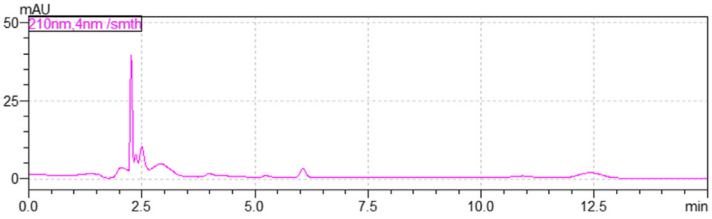
HPLC trace of praziquantel



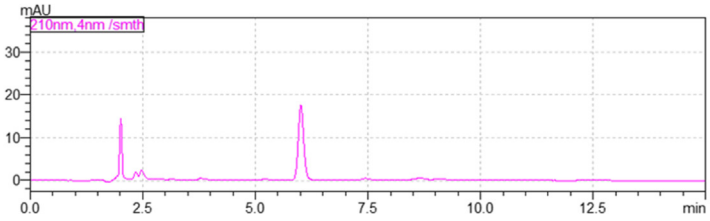
HPLC trace of the blank dog plasma



HPLC trace of the mixture of the dog plasma and praziquantel



HPLC trace of the dog plasma from the dogs dosed with praziquantel



HPLC trace of the dog plasma from the dogs dosed with praziquantel inclusion complex

Fig. 8 HPLC traces of praziquantel, dog plasma, mixture of dog plasma and praziquantel, and dog plasmas from the dogs dosed with praziquantel or its inclusion complex.

The C_{max}, AUC_0-∞, $t_{1/2}$ and clearance values of praziquantel and its inclusion complex shown in the Table 1 were quite remarkably distinct. It's believed that the increased values of C_{max} and AUC_0-∞ of praziquantel/HP-β-CD inclusion complex versus the free praziquantel are a result of increased water solubility of the praziquantel (~104 fold) in complexation with HP-β-CD.

3. Conclusion

The inclusion complex of praziquantel/HP-β-CD was synthesized by a consistent and reproducible procedure which resulted in the increased (~104 fold) aqueous solubility of praziquantel against that of praziquantel alone, which is the best result so far for praziquantel solubility study. The nauseating

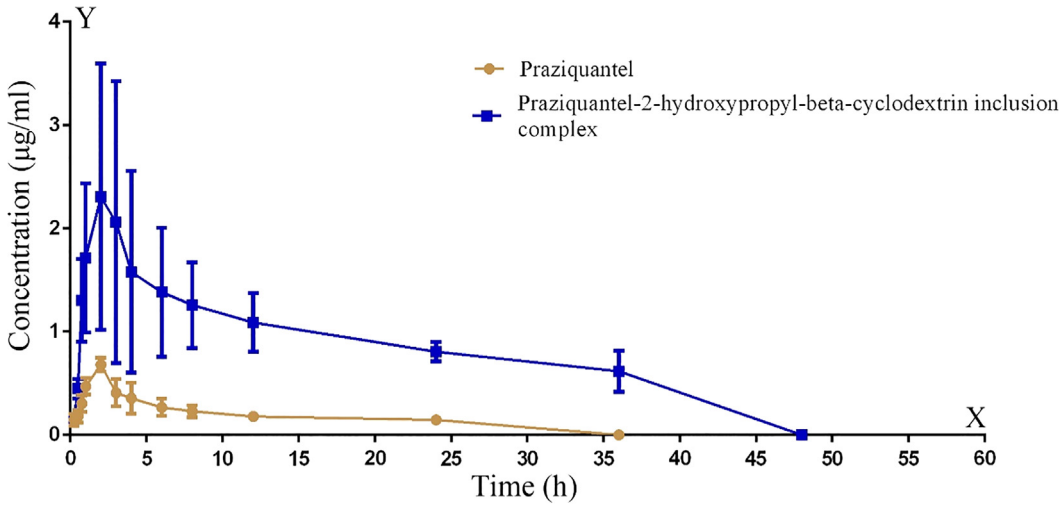


Fig. 9 Curve of drug concentrations in plasmas from dogs dosed with praziquantel or its inclusion complex.

Table 1 Pharmacokinetic parameters of praziquantel and its inclusion complex.

Parameters	Unit	Value	
		Praziquantel	Praziquantel/ 2-hydroxypropyl-β-cyclodextrin
T_{max}	h	2.05	1.57
C_{max}	μg/mL	0.51	4.82
AUC	μg·h/mL	20.63	98.06
$t_{1/2}$	h	13.32	18.82
CL	L/kg/h	242.37	50.99

bad taste and odor sensation of praziquantel were eliminated. The inclusion complex has been characterized by various analytical techniques such as scanning electron microscopy, ^{1}H NMR, FT-IR etc. The praziquantel and HP-β-CD ratio in the complex was determined by NMR studies as 1:2. Evaluation of the pharmacokinetic properties of praziquantel/HP-β-CD inclusion complex in dogs was done following an uneventful oral dosing of the praziquantel/HP-β-CD inclusion complex in dogs, an improved pharmacokinetic profile (C_{max}, T_{max}, $AUC_{0-\infty}$ and $t_{1/2}$) of praziquantel was observed in the dog *in vivo* pharmacokinetic study. These improvements in the pharmacokinetic properties of praziquantel/HP-β-CD inclusion complex strongly suggest that it can be used as a broad-spectrum worm drug formulation in a clinical study.

4. Experimental

Materials and instruments: Praziquantel (99.9% pure) was obtained from Jiangsu Hengsheng Pharmaceutical Co., while HP-β-CD (Molecular weight: 1431–1806; degree of substitution: 4.81–4.87) was purchased from Yunan County Yongguang Ring Dextrin Co. The anhydrous solvents such as ethanol, methanol, acetonitrile, and acetic acid (analytical grade) were procured from Tianjin Guangcheng. The blank dog plasma was acquired from Guangzhou Ruite Co. Ltd. Bruker spectrometer operating at 400 MHz was employed for recording ^{1}H NMR spectra, D_2O and DMSO-d_6 were used as lock solvents, while AVATAR360 Fourier transform infrared spectrometer (Nicolet, USA) was used for recording the FTIR spectra, Lambda 25 UV–vis spectrophotometer (PerkinElmer, USA) was employed for recording UV spectra, while Shimadzu LC-20A high-performance liquid chromatography instrument was used for HPLC analysis (C-18 column, 250 mm × 4.6 mm, 5 μm), the acetonitrile and water mixture (60:40) were used as the mobile phase with UV detector at 210 nm wavelength at 40 °C with 1.0 mL/min. flow rate, S-3700 N Scanning Electron Microscope (Hitachi, Japan) was employed for recording the scanning electron microscope images, RC-3 Dissolution tester (Tianjin Xintianguang Analytical Instrument Technology) was used for evaluating the dissolution rate, HN1012 ultrasonic cleaning machine (Guangzhou, South China Ultrasound Equipment Co.) was utilized for the ultrasonication of liquids.

Praziquantel and HP-β-CD inclusion complex preparation: To distilled water (5 mL), HP-β-CD (4.5 g) was added, and the mixture was heated at 65 °C for 30 min with stirring at 600 rpm. To the clear HP-β-CD solution was added an ethanolic solution of praziquantel (0.32 g of praziquantel in 20 mL of ethanol) drop by drop. The mixture was stirred with heating at 55 °C for another 5 h, the solution was evaporated under reduced pressure, and the residue was dissolved into water. After filtration and lyophilization, the product (4.50 g) was obtained as a white solid.

Fourier transform infrared spectroscopic study of praziquantel and its inclusion complex with HP-β-CD: the praziquantel (5 mg), HP-β-CD (5 mg), the mixture of praziquantel and HP-β-CD, and their inclusion complex (5 mg) were pressed with KBr respectively, and their infrared absorption spectra were measured in the absorbance range of 500 cm^{-1} to 4000 cm^{-1}.

Proton NMR study of praziquantel and its inclusion complex with hydroxypropyl-β-cyclodextrin: the proton nmr spectra of praziquantel, HP-β-CD and their inclusion complex in DMSO d_6, HP-β-CD and their inclusion complex in D_2O, the H-H COSY and H-H NOESY of the inclusion complex in D_2O were recorded at room temperature at 400 MHz.

Scanning electron microscope image study: the HP-β-CD, inclusion complex, praziquantel, and the mixture of HP-β-CD and praziquantel were scanned on a scanning electron microscope (S-3700 N, Hitachi, Japan). The solid sample upon gold spray treatment for 30 min was placed on the magnetic block, then the sample rod was loaded with the sample. For scanning, the acceleration voltage applied was10 kV, and the image magnification was 500x.

UV spectroscopic study: praziquantel and HP-β-CD were used for recording their UV spectra 200–400 nm in wavelength range by using the distilled water as a blank. The praziquantel had the maximum absorption at 210 nm, while HP-β-CD had no absorption in the above wavelength range. Consequently, 210 nm was selected as the screening wavelength in HPLC analysis.

Method establishment for determination of praziquantel in the inclusion complex: To the solution of praziquantel (10 mg) in acetonitrile (4 mL) was added distilled water (6 mL) with shaking to obtain a solution with a concentration of 1 mg/mL. A series of solutions with different concentrations (from 1 μg/mL to 5000 μg/mL) of praziquantel in the mixture of water and acetonitrile (60:40) were prepared from the above stock solution. Each solution was analyzed on HPLC over octadecyl silane bonded silica gel column by using acetonitrile–water (60:40) as the mobile phase with UV detector at 210 nm. A good linear relationship between the absorptions and concentrations of praziquantel in HPLC analysis was seen in the ranges of 15.625 ~ 1000.0 μg/mL. Based on the standard curve, the regression equation was obtained as $Y = 10^{-7}X + 0.0937$ (where Y: the absorption, X: concentration).

Determination of praziquantel in the inclusion complex: the inclusion complex (25 mg) was dissolved in deionized water (100 mL), and then diluted to the ranges of 15.625 ~ 1000.0 μg/mL. Its absorbance in HPLC at 210 nm wavelength was measured by using the deionized water as blank, the concentration was obtained based on the regression equation to get the content of praziquantel in the inclusion complex.

Determination of solubility of praziquantel in water: the saturated water solution of the inclusion complex (500 mg) in water (1 mL) was analyzed by HPLC with a UV detector at 210 nm.

Determination of inclusion ratio and inclusion yield: to evaluate the effect of inclusion by the inclusion compound, the inclusion ratio and the yield of inclusion compound were used. The inclusion ratio and the yield of praziquantel inclusion compound are calculated by using the following formulas:

Inclusion yield (%) = praziquantel inclusion complex (mg)/[praziquantel (mg) + (HP-β-CD (mg)] × 100%

Inclusion ratio (%) = praziquantel in inclusion complex (mg)/praziquantel (mg) × 100%

The inclusion complex (10 mg) from the product was dissolved in water (10 mL), after HPLC analysis, and the amount of praziquantel in 10 mg of complex was determined. Based the amount of the product, the inclusion ratio was obtained by using above formula. The inclusion yield can be obtained from the preparation process directly.

Dissolution determination: the dissolution tests are done over the USP Apparatus 2 (paddle). The degassed deionized water (900 mL) is used as the medium of dissolution, and the testing temperature is 37 ± 0.3 °C at the rate of 100 rpm stirring speed. The pure praziquantel (100 mg), inclusion complex (containing 100 mg of praziquantel), and the mixture of praziquantel (100 mg) and hydroxypropyl-β-cyclodextrin (1500 mg) were used. Samples of the above solutions was collected at 2, 5, 10, 15, 20, 30, 45, and 60 min. respectively for HPLC analysis, and the same amount (5 mL) of isothermal medium was supplemented after each collection of the solution. The collected solutions were purified by filtration through 0.22 μm microporous membrane and were analyzed by HPLC at 210 nm as the wavelength with UV detector while using deionized water as the blank. From the standard curve and regression equation, the dissolution rate was obtained. Each testing was repeated for three times.

5. Pharmacokinetic study:

5.1. Sample preparation

Six healthy adult Chinese idyllic dogs purchased from Guangzhou Yuansheng Pharmaceutical Technology Co., Ltd were randomly divided into two groups as praziquantel group and inclusion complex group. Before administration of the drug, the dogs underwent one week of adaptation in the new environment. The dogs were fasted for 12 h before the blank blood was collected from them. and, There after a single dose of 5 mg/kg of praziquantel and its inclusion complex (measured by the effective content of praziquantel) was administered via mixing with oral feed material to the dogs, and the blood (3 mL) was collected with a syringe (5 mL) from the medial cephalic vein of the forelimb of each dog at 0.25, 0.5, 0.75, 1, 2, 3, 4, 6, 8, 12, 24, 36, 48 and 72 h intervals. The dogs were returned to normal diet after 2 h of oral administration of the sample with whole value feedstuff and drinking water.

The collected blood sample was treated with heparin sodium and placed in a 5 mL centrifugal tube, then centrifuged for 10 min at 3500 r/min speed, the supernatant (0.5 mL) was extracted with acetonitrile (1 mL) after transferring and by centrifuging (13000 r/min for 10 minutesx2). The combined supernatant was dried in a 10 mL sterilized centrifugal tube by nitrogen blower at 37 °C. To the dried residue, a mixture of acetonitrile and water (6:4, 0.5 mL) was added, and the solution was shaken on vortex mixer for 4 min., the sample solution after filtration through 0.22 μm filter membrane was used for HPLC analysis.

5.2. Establishment of the HPLC standard curve for pharmacokinetic study

A standard solution (1000 μg/mL) of praziquantel (10 mg) was prepared by adding 10 mg of praziquantel to the mobile phase (5 mL) and further diluted with mobile phase to the desired concentration. The blank dog plasma was diluted with the above standard solution to get a series of sample solutions with different drug concentrations from 0.05, 0.1, 0.25, 0.5, 1, 2.5, 5, to 10 μg/mL. After work up based on the standard methods, the sample was analyzed by HPLC, the concentrations were used as abscissa, and the peak areas were used as ordinate. Based on these results, the linear regression equation $Y = 10^{-5}X + 0.0858$ (Y: concentration, X: peak area), correlation coefficient (0.9996), LOQ (0.1 μg/mL), and LOD (0.05 μg/mL) were obtained.

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