Microsampling with dried blood spots and mass spectrometry enables PK/PD profiling of responses to praziquantel in a Schistosoma haematobium-exposed Zimbabwean population

Supplementary Methods

Supplementary Table 1: Demographic characteristics of the 38 Zimbabwean subjects recruited to assess the pharmacokinetics of praziquantel and its metabolites. This included the division of demographics by the matching performed for this study by age category, baseline infection intensity, treatment outcome and sex.

Age Category	Baseline *S. haematobium* Intensity	Treatment Outcome	Sex		Total
			Male	Female	
School-age children (6-17 years)	Heavy (≥50 eggs/10mL)	Cleared	2	3	5
		Total	2	3	4
	Light (<50 eggs/10mL)	Cleared	12	10	22
		Not cleared	0	1	1
		Total	12	11	23
	Total	Cleared	14	13	27
		Not cleared	0	1	1
		Total	14	14	28
Adults (≥18 years)	Heavy (≥50 eggs/10mL)	Cleared	1	0	1
		Not cleared	1	0	1
		Total	2	0	2
	Light (<50 eggs/10mL)	Cleared	8	0	0
		Total	8	0	8
	Total	Cleared	9	0	0
		Not cleared	1	0	1
		Total	10	0	10
Total participants		Cleared	23	13	36
		Not cleared	1	1	2
		Total	24	14	38

Liquid chromatography-Mass Spectrometry

Hydrophilic interaction liquid chromatography (HILIC) was carried out on a Dionex UltiMate 3000 RSLC system (Thermo Fisher Scientific, Hemel Hempstead, United Kingdom) using a ZIC-pHILIC column (150mm × 4.6mm, 5µm column, Merck Sequant, Gillingham, United Kingdom). The column elutes the samples with a linear gradient (20mM ammonium carbonate in H_2O [A] and ACN [B]) over 26 minutes at a flow rate of 0.3mL/min (**Supplementary Table 2**). The column was maintained at 25°C, and 10µL of each sample was injected into the LC-MS/MS system for analysis, with samples maintained at 5°C before injection. A Thermo Scientific Q Exactive Orbitrap MS was used for the MS analysis, running in positive/negative switching mode, with the specific settings listed in **Supplementary Table 3**. For positive mode ionisation: source voltage +3.8kV, S-Lens RF Level 30.00, S-Lens Voltage -25.00 (V), Skimmer Voltage -15.00 (V), Inject Flatopole Offset -8.00 (V), Bent Flatapole DC -6.00 (V). For negative mode ionisation: source voltage-3.8kV. The raw data was converted from a Thermo-specific file format to the open format mzXML. The unique signals were then extracted using the centwave algorithm and matched across biological replicates based on the mass-to-charge ratio (m/z) and retention time (RT). The grouped peaks were then filtered based on relative standard deviation and combined into a single file. The combined datasets were then filtered on signal-to-noise score, minimum intensity and minimum detections. The final peak set was then gap-filled and converted to text for use with IDEOM v18.[1]

Quality control (QC) of the detected MS peaks was conducted to ensure the capture of the complete peak area, with no overlapping or cut-off peaks. After QC, a calibration curve was plotted using the known standards across the d11-PZQ (IS) concentrations. The d11-PZQ standards were 0.1uM, 1uM, and 10uM, and from the average of a triplicate of each of these standards, a line of best fit through the origin was plotted. The equation of the line was then used to calculate the concentration (uM) in each sample. Using y=x+c, the concentration (uM) was found to be equal to the peak area/$6x10^8$. To ensure this equation had an acceptable accuracy, a comparison of the known d11-PZQ standards versus the concentration extracted from the peak area of MS was also conducted, as seen in **Supplementary Table 5**. The recovery of d11-PZQ from the DBS cards was deemed within an acceptable range (>100%) and therefore no further changes to analyte concentration were made. Hence, the peak area output was used to calculate the concentration of PZQ and its metabolites using the above equation.

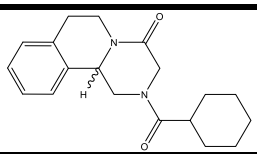
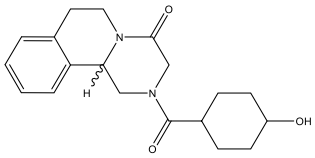
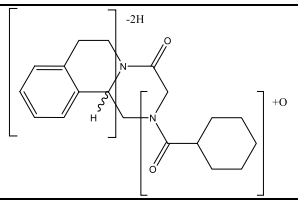
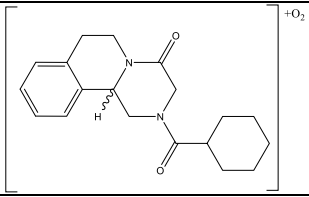
Supplementary Table 2: Column flow rate for ZIC-pHILIC column

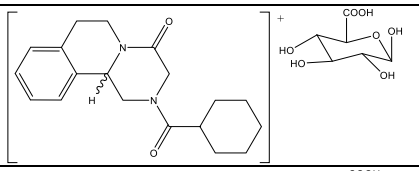
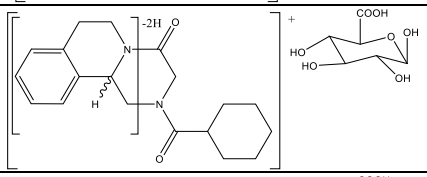
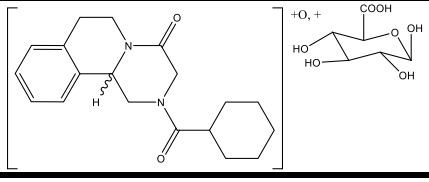
Time (minutes)	%A	%B
0	20	80
15	80	20
15	95	5
17	95	5
17	20	80
24	20	80

Supplementary Table 3: Mass spectrometry (MS) settings for the Thermo Orbitrap QExactive (Thermo Fisher Scientific) used for the analysis of dried blood spot (DBS) extracts.

Setting	Value
Resolution	70,000
AGC	1e6
m/z range	70–1050
Sheath gas	40
Auxiliary gas	5
Sweep gas	1
Probe temperature	150°C
Capillary temperature	320

Supplementary Table 4: Analyte identification for praziquantel (PZQ) and its metabolites, in addition to the key fragments required to identify the PZQ metabolites.

Analyte	Reported $[M+H]^+$	Exact Mass	Structure	Key Fragment Ions (m/z)	Published Information
PZQ	313.18	312.18		-	-
4-OH-PZQ	329.18	328.18		203 174 146 132	Major human metabolite. Has been detected as *trans*-4-OH-PZQ and *cis*-4-OH-PZQ for both of the (R)/(S)-PZQ enantiomers.
(-2H)-O-PZQ	327.173	326.173		144 130	Detected in the urine and faeces of mice
O2-PZQ	345.182	344.182		219 201	Detected in the urine and faeces of mice

O-PZQ-Glucuronide	505.218	504.218		395 329 219	Detected in the urine of mice
(-2H)-O-PZQ-Glucuronide	503.204	502.204		393 327 217	Detected in the urine of mice
O2-PZQ-Glucuronide	521.215	520.215		393 345 327 217	Detected in the urine of mice

Acronyms: 4-OH: 4-monohydroxy, (-2H)-O: dehydro-mono-oxidised, O2: di-oxidised, min: minute, m/z: mass-to-charge ratio.

Supplementary Table 5: Comparison of the known d11-PZQ internal standard (IS) concentration versus the concentration extracted from the peak area from the mass spectrometer (MS).

Samples	**Average Recovered Concentration (ng/mL)**	**Percentage Accuracy (%)**
Pooled	720.98 ± 230.15	120.16 ± 38.36
Individual	713.83 ± 95.06	118.97 ± 15.84

d11-PZQ: (deuterated-praziquantel)

DNA extraction and sequencing

Genomic DNA was extracted from each participant's whole blood sample using QIAamp DNA MicroKit (Qiagen, GmbH, Germany), according to the manufacturer's protocol. The DNA samples were shipped on dry ice for library preparation and whole exome sequencing at the Beijing Genomics Institute (BGI, Shenzhen, China). After the quantification of the DNA samples, the integrity and purity of DNA were assessed by a 1% agarose gel electrophoresis. Qualified DNA was then randomly fragmented and end repair of DNA fragments was performed with an "A" base added to the 3'-end of each strand. Adapters were then ligated to both ends of the end-repaired/dA-tailed DNA fragments. The size-selected DNA fragments were hybridised to an exome array for enrichment, including custom fragments covering rs2069514 and rs762551 in *CYP1A2*. Non-hybridised fragments which did not adhere to the array were then washed out, and captured fragments were amplified. Each captured library was loaded on the Illumina Hiseq 4000 platform (Illumina, San Diego, USA) and the raw image files were processed by DNBseq base calling software with default parameters. The sequence data of each individual was generated as paired-end reads and stored in FASTQ format. The raw reads were initially filtered by BGI, removing the adaptor sequences, contamination, and low-quality reads from the raw reads. If the sequence read i) matched ≥25% of the adapter sequence ii) had bases with a quality value ≤10 that accounted for ≥50% of the entire read, or iii) the N content accounted for ≥0.1%, the entire read was deleted. To filter for clean reads, the Phred threshold score was also set at ≥33. After this, the raw FASTQ format sequences from each sample were further processed using FASTQC v0.11.9 (https://www.bioinformatics.babraham.ac.uk/projects/fastqc/). The number of read pairs generated per sample ranged from 20,315,195 to 27,956,273 in the sequencing run. The reads had an output quality Phred threshold score of >33 and a read length of 150 bp.

References:

1. Creek, D. J., Jankevics, A., Burgess, K. E. V., Breitling, R. & Barrett, M. P. IDEOM: an Excel interface for analysis of LC-MS-based metabolomics data. *Bioinformatics* **28**, 1048–1049 (2012).