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Sensitive method for the quantitative determination of proguanil and its metabolites in rat blood and plasma by liquid chromatography–mass spectrometry

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Abstract

A sensitive, simple and fast liquid chromatography tandem mass spectrometry (HPLC-MS/MS) method for the determination of proguanil (PG) and its metabolites, cycloguanil (CG) and 1-(4-chlorophenyl)biguanide (4CPB), was developed and validated over a concentration range of 1–2000 ng/mL using only 50 μL of blood or plasma. After a simple solvent precipitation procedure, the supernatant was analysed directly by HPLC-MS/MS. Separation was achieved using an ethyl-linked phenyl reverse phase column with polar endcapping with an acetonitrile–water–formic acid gradient. Mass spectrometry was performed using a triple quadrupole mass spectrometer operating in positive electrospray ionization mode. The elution of PG (254.07 → 169.99), CG (252.12 → 195.02) and 4CPB (212.06 → 153.06) was monitored using selected reaction monitoring. The three compounds and the internal standard (chloroproguanil) were well separated by HPLC and no interfering peaks were detected at the usual concentrations found in blood and plasma. The limit of quantification of PG and CG was 1 ng/mL and 5 ng/mL for 4CPB in rat blood and plasma. The extraction efficiency of PG, CG and 4CPB from rat blood and plasma was higher than 73%. The intra- and inter-assay variability of PG, CG and 4CPB were within 12% and the accuracy within ±5%. This new assay offers higher sensitivity and a much shorter run time over earlier methods.
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Keywords: Proguanil; Cycloguanil; Antimalarial; LC–MS; Blood and plasma assay

1. Introduction

Proguanil (PG) is an antimalarial drug used for prophylaxis and treatment of the infection caused by *Plasmodium falciparum*. PG is a prodrug which is converted by the hepatic cytochrome P-450 isoenzyme to its active metabolite, cycloguanil (CG) [1] and the inactive metabolite, 1-(4-chlorophenyl)biguanide (4CPB) [2] (Scheme 1). Differences in metabolism of drugs can lead to therapeutic failure due to poor metabolism to the active metabolite. PG is activated by the phase I enzyme CYP2C19 in the liver to CG and has been found to show genetic polymorphism [3]. The prevalence of poor metabolizers of PG was observed in 3–6% of Caucasian [4] and African [5] and 13–23% of Oriental populations [6]. Few data are reported on the relationships between phenotype, PG and metabolite concentration and antimalaria response [7,8].

Antimalarials such as PG and CG exert antimalarial activity in erythrocytes. Determination of the drug concentration in plasma alone may not reveal the effective concentration at the site of action. In our current program of antimalarial drug candidate screening, it was decided to re-examine the pharmacokinetic properties of the well-known drug, PG, and its active metabolite, CG, in both blood and plasma in a laboratory rodent model. It is very important to have a simple and robust analytical method with high sensitive, low sampling volume and short analytical run-times to allow better definition of pharmacokinetic profiles and accommodate the throughput necessary for a large pharmacokinetic studies.

Several analytical techniques for quantifying PG and its metabolites in biological fluids have been reported, including micellar electrokinetic chromatography [9], high performance liquid chromatography (HPLC) [10,11] and capillary

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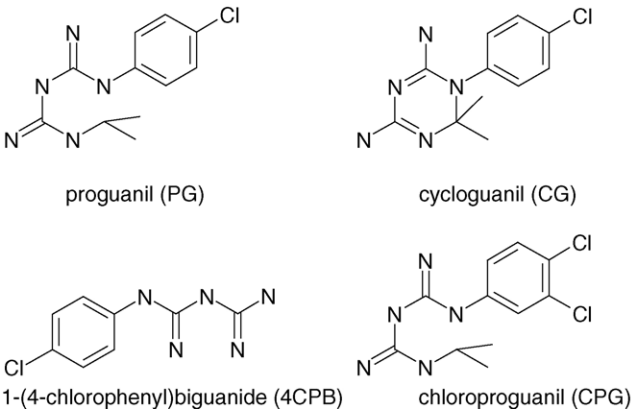

Scheme 1. Chemical structures of the analytes and internal standard.

electrophoresis [12]. The lower limit of quantification for these methods ranged from 1 to 110 ng/mL. The best sensitivity was reported for an HPLC method, but the method was limited by long chromatographic run-time, large sample volumes (1–2 mL) and the laborious extraction procedures [11].

This work describes a procedure employing liquid chromatography tandem mass spectrometry detection for a more sensitive and higher throughput determination of PG, CG and 4CPB applicable for both rat blood and plasma.

2. Experimental

2.1. Chemicals

Proguanil (PG) and chloroproguanil (CPG) were kindly donated by GSK (Brentford, UK). Cycloguanil (CG) and 1-(4-chlorophenyl)biguanide (4CPB) were purchased from Sigma-Aldrich (Milwaukee, WI, USA). The purity of the reference compounds are more than 97% and were used without further purification. Acetonitrile (UV grade) was obtained from Merck (Darmstadt, Germany) and formic acid from BDH (Poole, UK). De-ionized water was prepared using Micropore Milli-Q plus ultra pure system (Millipore, Billerica, Massachusetts, USA).

2.2. LC–MS/MS instrumentation and analytical conditions

Mass spectrometry was performed on a Micromass Quattro Ultima Pt triple quadrupole instrument coupled with a Waters 2795 HPLC (Waters Corp., Milford, MA, USA). Analytical separations were performed on a 50 mm × 2 mm, 5 μm Phenomenex Synergi Polar RP reversed phase column equipped with a pre-column of the same material (Torrance, CA, USA). Compounds were eluted using a ternary gradient solvent system consisting of Milli-Q water (solvent A), acetonitrile (solvent B) and 1% formic acid in water (solvent C). Solvent C is kept at 5% throughout the entire gradient. The gradient profile is as follows: 0–0.8 min, 1% B; 0.8–1.0 min, 1–10% B; 1–2.5 min, 10% B; 2.5–5 min, 10–50% B; 5–5.5 min, 50–95% B; 5.5–6.5 min, 95% B; 6.5–7 min, 95–1% B and a 2 min equilibration at the initial conditions. The total cycle time is 9 min. The peaks of interest eluted between 2.9 and 6.2 min at a flow rate of 0.4 mL/min.

Sensitivity of the multiple reaction monitoring (MRM) was optimized for each analyte by testing with an infusion of standard solutions (1 μmol/L) in a mixture of acetonitrile and 1% formic acid (1:1, v/v). Mass spectrometry was performed with positive mode electrospray ionisation and MS–MS conditions with a capillary voltage of 3.2 kV, source cone voltage of 35 V and collision energy of 20 V. Source block and desolvation temperature of 90 °C and 390 °C, respectively and a desolvation gas flow of 450 L/min were maintained. The collision gas (Ar) pressure was set to 3.0 mTorr (1 Torr = 133.322 Pa). The MS–MS transitions monitored were m/z 254.07 → 169.99 for PG, 252.12 → 195.02 for CG, 212.06 → 153.06 for 4CPB and 288.12 → 204.00 for CPG, the internal standard. The dwell time was 300 ms for each transition. Both quadrupoles were maintained at unit resolutions. Data acquisition, peak integration and calculations were performed using Micromasss Masslynx version 4.0 software.

2.3. Preparations of stock solutions, calibration standards and quality control samples

Individual stock solutions of PG (1 mg/mL), CG (1 mg/mL), 4CPB (1 mg/mL) and CPG (1 mg/mL) were prepared in absolute ethanol. A combined stock solution of PG, CG and 4CPB at 25 μg/mL was prepared in acetonitrile-water (50:50, v/v). Working standard solutions were prepared by serial dilutions of the combined stock solution in acetonitrile–water (20:80, v/v). The internal standard working solution was prepared similarly at 10 μg/mL.

Blank blood and plasma were obtained from heparinised blood collected from untreated healthy Sprague–Dawley rats. Blood and plasma standards of PG, CG and 4CPB were prepared fresh daily by spiking 10 μL of the appropriate working solutions into 50 μL of rat control blood or plasma to yield calibration concentrations 2000, 1000, 500, 100, 50, 10, 5 and 1 ng/mL. QC working solutions at concentrations of 25, 250 and 2500 ng/mL were prepared by successively diluting the 25,000 ng/mL stock solutions of the mixture PG, CG and 4CPB with acetonitrile-water (20:80, v/v). Blood and plasma QC samples were prepared by adding 10 μL of the appropriate QC working solutions to 50 μL of control rat blood or plasma to yield concentrations of 5, 50 and 500 ng/mL of PG, CG and 4CPB.

2.4. Blood and plasma extraction

All standard, QC and pharmacokinetic samples were treated in identical manner except 10 μL of drug-free acetonitrile–water (20:80, v/v) was added to the pharmacokinetic samples to compensate for the volume of the standard solution in the standard samples. An aliquot of CPG (20 μL, 10 μg/mL) internal standard was added to 50 μL of blood or plasma samples followed by acetonitrile (120 μL) to precipitate the proteins. After centrifugation (3 min, 14,850 × g), a 10 μL aliquot of the supernatant was injected directly onto the analytical column.

2.5. Assay validation

2.5.1. Linearity

Calibration standards of eight concentrations of PG, CG and 4CPB ranged 1–2000 ng/mL were analysed. A least square second order curve fitting regression model with inversed concentration weighting factor was used to determine the blood and plasma concentration from the peak area ratio data. Linearity was assessed over three independent determinations.

2.5.2. Assay specificity

Assay specificity was assessed by analyzing drug-free pooled blood and blood from three individual rats and checking for peaks that interfered with the target compounds. A similar assay was done with plasma instead of blood. The chromatograms were visually inspected for interfering chromatographic peaks from endogenous substances.

2.5.3. Extraction recovery

To determine the extraction recovery from the biological matrix, the recovery test samples were prepared as described for the calibration standards. The control samples were prepared by spiking the standard solutions into processed blank samples. The recovery is defined as the concentration of the test sample relative to the control sample. The recovery of PG, CG and 4CPB was determined at three QC concentration levels (5, 50 and 500 ng/mL) in both plasma and blood using the same procedure. The recovery of the internal standard was also assessed at 500 and 5000 ng/mL.

2.5.4. Precision and accuracy

The precision of the assay was determined at the low, medium and high QC blood and plasma samples by replicate analyses of three concentrations of PG, CG and 4CPB (5, 50 and 500 ng/mL). Intra-day precision and accuracy was determined by replicate analysis of the group of standards on 1 day ($n = 3$), and inter-day precision and accuracy was determined by replicate analysis over 3 days ($n = 3$). The concentration of each sample was determined using calibration standards prepared on the same day. Accuracy is defined as the percentage deviation of the measured concentration (M) of a standard from the spiked concentration (S) calculated using the following formula, % R.E. $= 100 \times (M - S)/S$. Assay precision was defined as the percentage deviation from the mean (% R.S.D.).

2.5.5. Storage stability

The stability of PG, CG and 4CPB in blood and plasma was evaluated at $-80\,^{\circ}$C for 3 weeks to support sample storage and at room temperature for 2 h to support sample processing. The autosampler storage stability after sample processing was assessed at $10\,^{\circ}$C for 24 h. The stability was tested by the analysis of standard plasma and blood samples in triplicate at three concentrations of PG, CG and 4CPB (5, 50 and 500 ng/mL).

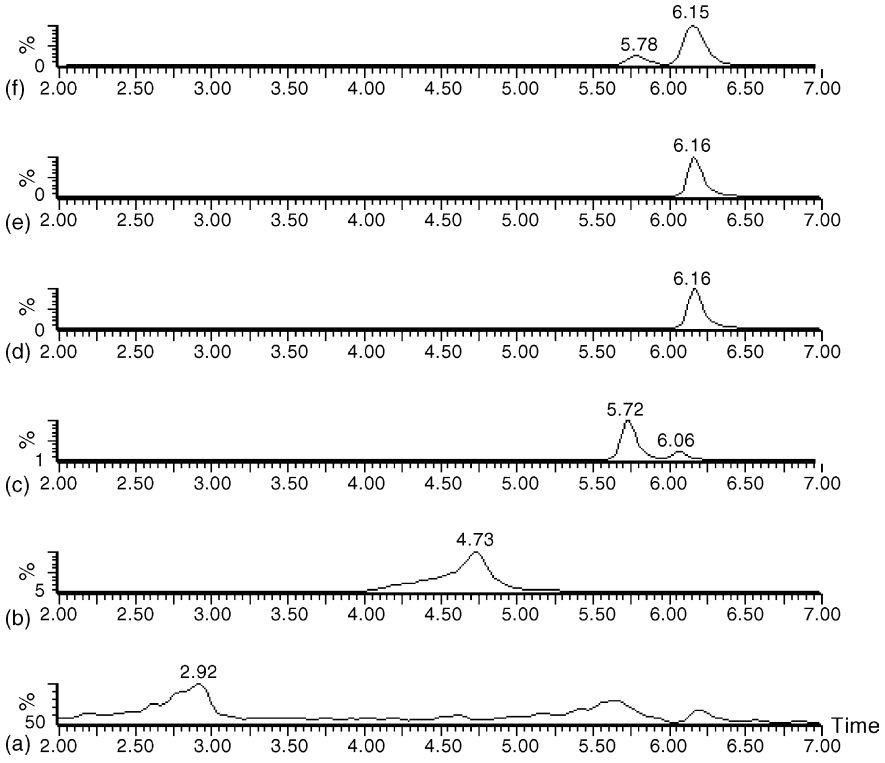

Fig. 1. MRM chromatograms of proguanil (c), cycloguanil (b), 1-(4 chlorophenyl)biguanide (a) and internal standard, chloroproguanil (d) in spiked rat plasma standard at 100 ng/mL. TIC chromatogram of blank rat plasma spiked with internal standard CPG (e) and TIC chromatogram of a rat plasma sample taken 1 h after oral administration of proguanil at 10 mg/kg. The measured plasma concentration of PG was 47.5 ng/mL and the metabolites, CG and 4CPB were below detection limit (f).

Table 1
Linearity data for PG, CG and 4CPB from rat blood and plasma

Compound	Nominal concentration (ng/mL)	Blood		Plasma	
		Measured concentration (ng/mL)	% deviation	Measured concentration (ng/mL)	% deviation
PG	1	0.8	−19	0.98	−2.2
	5	5.3	5.2	5.0	0.1
	10	10.3	2.8	10.6	5.6
	50	49.8	−0.5	55.6	11
	100	98.9	−1.1	110.5	11
	500	583.4	17	525.1	5.0
	1000	1064.9	6.5	995.4	−0.5
	2000	1920.1	−4.0	1932.4	−3.4
CG	1	1.1	14	0.86	−14
	5	4.8	−4.8	5.3	5.2
	10	10.2	2.3	9.6	−4.0
	50	48.6	−2.9	54.0	8.0
	100	102.2	2.2	104.7	4.7
	500	433.1	−13	497.6	−0.5
	1000	999.5	−0.1	1016.6	1.7
	2000	2026.0	1.3	1965.4	−1.7
4CPB	5	4.8	−4.2	4.2	−16
	10	9.5	−4.8	10.5	5
	50	50.6	1.2	53.1	6.3
	100	108.2	8.2	107.3	7.3
	500	513.5	2.7	484.9	−3
	1000	957.8	−4.2	1001.4	0.1

2.5.6. Matrix effect

The matrix effect on the ionization of the analytes was investigated using a post-column infusion procedure [13]. A solution containing a mixture of PG, CG, 4CPB and CPG at 500 ng/mL in 50% acetonitrile–water was delivered at 10 μL/min via a syringe pump (Harvard Apparatus, South Natick, MA, USA) into the post-column stream of eluant via a zero dead volume tee before the mass spectrometer inlet. The signals of the four compounds were acquired while blank aqueous acetonitrile or drug-free blood or plasma samples prepared as described above was injected under the LC analytical conditions.

3. Results and discussion

3.1. Optimization of LC–MS/MS conditions

The selection of mobile phase components was a critical factor in achieving good chromatographic peak shape and resolution. Acetonitrile was selected and formic acid and ammonium acetate were evaluated as buffer. The ionization of PG, CG, 4CPB and CPG was about the same in either buffer. However, formic acid exhibited better selectivity than ammonium acetate with regards to optimizing the separation of the target compounds. Due to the large difference in polarity, in order to obtain good separation of the target compounds and to achieve as short a run time as practical, a gradient elution system was developed. Representative chromatograms are shown in Fig. 1 in which the retention time were 5.8 min (PG), 4.7 min (CG), 2.9 min (4CPB) and 6.1 min (CPG). Care was taken to optimize the gradient profile such that the target compounds were separated from blood or plasma components which interfere with ionization of the analytes.

3.2. Linearity

Table 1 shows the calibration data for PG, CG and 4CPB. Calibration was performed using a second order curve fitting method

Table 2
Extraction efficiency of PG, CG, 4CPB from rat blood

Compound	Control sample (ng/mL)	Recovery sample (ng/mL) ($n = 3$)	Extraction recovery (%)	R.S.D. (%)
PG	7.9	6.2 ± 0.2	77.7	2.8
	76.4	58.0 ± 3.9	75.9	5.1
	597.0	439.6 ± 7.8	73.6	1.3
CG	4.4	4.2 ± 0.3	96.6	6.6
	49.2	41.1 ± 2.8	83.6	5.7
	605.3	492.2 ± 17	81.4	2.8
4CPB	6.5	5.8 ± 0.5	89.7	7.3
	55.5	48.4 ± 1.6	87.2	2.8
	514.5	381.5 ± 14	74.2	2.8

Table 3
Extraction efficiency of PG, CG, 4CPB from rat plasma

Compound	Control sample (ng/mL)	Recovery sample (ng/mL) ($n = 3$)	Extraction recovery (%)	R.S.D. (%)
PG	8.5	6.9 ± 0.0	80.5	0.3
	74.1	64.9 ± 5.1	87.6	6.8
	506.0	505.8 ± 22	99.9	4.3
CG	7.5	6.2 ± 0.4	82.9	5.9
	72.1	61.2 ± 0.9	84.8	1.3
	509.2	475.3 ± 45	93.4	8.8
4CPB	4.2	4.1 ± 0.6	97.6	14
	60.5	55.6 ± 4.4	91.9	7.4
	501.2	469.7 ± 18	93.7	3.6

Table 4
Precision and accuracy of the assay for the determination of PG, CG and 4CPB in blood ($n = 3$)

Compound	Nominal concentration (ng/mL)	Intra-assay			Inter-assay		
		Mean calculated concentration (ng/mL)	R.S.D. (%)	R.E. (%)	Mean calculated concentration (ng/mL)	R.S.D. (%)	R.E. (%)
PG	5	5.2 ± 0.2	4.2	4.1	5.2 ± 0.1	1.8	4.2
	50	51.3 ± 2.0	4.0	2.5	50.1 ± 1.0	2.0	0.2
	500	489.6 ± 7.8	1.6	−2.1	482.8 ± 6.1	1.3	−3.6
CG	5	4.9 ± 0.5	11	−2.0	5.0 ± 0.1	1.7	−0.1
	50	51.2 ± 2.8	5.5	2.2	51.6 ± 1.4	2.8	3.0
	500	492.9 ± 17	3.5	−1.4	492.2 ± 7.5	1.5	−1.6
4CPB	5	5.1 ± 0.4	8.3	2.6	4.9 ± 0.2	4.6	−1.6
	50	48.5 ± 1.6	3.3	−3.2	50.6 ± 2.7	5.2	1.2
	500	481.5 ± 14	3.0	−3.8	487.7 ± 6.7	1.4	−2.5

weighted ($1/x$) to compensate for the wide range of concentration values covered. The calibration curves in spiked blood or plasma were curvilinear within the range 1–2000 ng/mL for PG and CG and within the range of 5–1000 ng/mL for 4CPB with typical correlation coefficients greater than 0.99 and the deviation of the back-calculated values from the nominal standard concentrations were less than 15%.

3.3. Extraction recovery

Tables 2 and 3 show the mean extraction recovery of PG, CG and 4CPB, calculated as the average recoveries from the three QC levels, 5, 50 and 500 ng/mL (and $n = 3$ for each QC levels). The recovery of the internal standard CPG was found to be 70% at the working concentration and was independent of spiked concentration at both 500 and 5000 ng/mL.

3.4. Specificity

There were no interfering peaks observed at the retention times of the analyte, its metabolites or internal standard in any of blood or plasma samples.

3.5. Precision and accuracy

Assay precision and accuracy were assessed by extracting and analyzing blood and plasma standards of PG, CG and 4CPB at low, medium and high concentration levels (5, 50 and 500 ng/mL) in triplicates. The intra-assay accuracy in blood and plasma (% R.E.) ranged from −4.5 to 4.4% and the inter-assay accuracy ranged from −3.6 to 4.7%. The intra- and inter-assay precision of PG, CG and 4CPB, in blood and plasma (% R.S.D.) lied within 11% and 12%, respectively (Tables 4 and 5).

3.6. Storage stability

The stability data of PG, CG and 4CPB are presented in Tables 6 and 7. PG, CG and 4CPB were found to be stable in rat blood and plasma while stored at −80 °C for at least 3 weeks. It was also found to be stable in blood and plasma for at least 2 h at room temperature. The solvent precipitated samples were stable for at least 24 h under autosampler conditions (10 °C).

3.7. Sensitivity

The LLOQ for this assay was defined as the lowest concentration with better than 20% accuracy and precision and a signal

Table 5
Precision and accuracy of the assay for the determination of PG, CG and 4CPB in plasma ($n = 3$)

Compounds	Nominal concentration (ng/mL)	Intra-assay			Inter-assay		
		Mean calculated concentration (ng/mL)	R.S.D. (%)	R.E. (%)	Mean calculated concentration (ng/mL)	R.S.D. (%)	R.E. (%)
PG	5	4.9 ± 0.1	0.5	−2.9	5.0 ± 0.1	3.1	−1.1
	50	48.2 ± 0.7	1.5	−3.7	49.3 ± 0.9	1.9	−1.5
	500	505.8 ± 21	4.3	1.1	507.9 ± 11	2.3	1.6
CG	5	5.2 ± 0.4	8.5	4.1	5.1 ± 0.1	2.6	1.5
	50	51.2 ± 0.9	1.8	2.3	51.2 ± 0.2	0.4	2.4
	500	495.3 ± 29	5.8	−0.9	498.3 ± 3.0	0.6	−0.3
4CPB	5	4.8 ± 0.3	7.0	−4.5	5.1 ± 0.2	4.7	0.9
	50	52.3 ± 2.4	4.5	4.4	52.2 ± 0.3	0.7	4.3
	500	493.0 ± 2.9	0.6	−1.4	498.3 ± 4.7	0.9	−0.3

Table 6
The stability of PG, CG and 4CPB under a variety of conditions in blood

Nominal concentration (ng/mL)	−80 °C storage stability (3 weeks)			Autosampler storage stability at 10 °C (24 h)			Benchtop storage stability at 20 °C (2 h)		
	PG	CG	4CPB	PG	CG	4CPB	PG	CG	4CPB
5									
Mean	5.1	5.1	4.9	5.1	5.0	4.9	5.0	5.1	5.1
SD	0.3	0.1	0.1	0.1	0.2	0.7	0.1	0.1	0.2
% R.S.D.	4.9	2.7	2.6	2.6	3.6	1.2	1.7	1.6	4.8
% accuracy	0.8	1.8	−2.1	1.1	0.5	−2.1	0.5	−2.7	2.6
50									
Mean	49.7	49.8	51.2	49.9	49.6	49.0	52.1	46.3	49.3
S.D.	1.9	1.6	1.4	1.0	1.2	1.4	3.5	0.1	1.3
% R.S.D.	3.8	3.1	2.8	1.9	2.4	2.8	6.7	3.1	2.6
% accuracy	−0.7	−0.4	2.3	−0.2	−0.8	−1.9	4.1	−7.9	−1.3
500									
Mean	497.7	500.4	499.9	500.4	498.2	499.4	500.1	501.9	501.6
S.D.	2.4	3.5	3.3	2.1	4.4	0.9	10.8	7.1	3.9
% R.S.D.	0.5	0.7	0.7	0.4	0.9	0.2	2.2	1.4	0.8
% accuracy	−0.5	0.1	−0.0	0.1	−0.4	−0.1	0.0	0.4	0.3

to noise ratio of >10. A LLOQ of 1 ng/mL for PG and CG and 5 ng/mL for 4CPB were determined. The sensitivity of the current method (Table 8) is a vast improvement to those previously reported for sweeping technique in micellar electrokinetic chromatography method (sweeping-MEKC) [9] and capillary zone electrophoresis using field-amplified sample injection (FASI-CZE) [12]. A comparable sensitivity was reported by Taylor et al. [11], however, it is important to note that a large sample size (1–2 mL) and extensive sample preparation procedure was necessary to achieve similar results.

3.8. Matrix effect

Using the post-column infusion procedure [13], the HPLC eluant with drug-free samples of aqueous acetonitrile or precipitated blood or plasma matrix was found to have insignificant effect on the intensity of the four analytes delivered via an infusion pump into the effluent of the HPLC throughout the whole LC gradient. Thus, the current method was not adversely affected by the biological matrix in blood and plasma.

3.9. Application

This method has been applied in the study of both blood and plasma pharmacokinetics for proguanil and cycloguanil in rats following IV or oral administration. Blood samples (250 μL) were collected into heparinised tubes via an in-dwelled carotid arterial catheter. Aliquot of blood and plasma (50 μL) were kept at −80 °C until analysis. Typical blood and plasma profiles after oral dosing of PG in rats were shown in Fig. 2. Contrary to literature data, neither the active metabolite, CG, nor the inactive metabolite, 4CPB, was detected in blood and plasma samples after intravenous or oral administration of PG. This observation

Table 7
The stability of PG, CG and 4CPB under a variety of conditions in plasma

Nominal concentration (ng/mL)	−80 °C storage stability (3 weeks)			Autosampler storage Stability at 10 °C (24 h)			Benchtop storage stability at 20 °C (2 h)		
	PG	CG	4CPB	PG	CG	4CPB	PG	CG	4CPB
5									
Mean	5.1	5.0	5.0	5.0	5.0	5.1	5.4	5.2	4.9
S.D.	0.2	0.2	0.2	0.3	0.5	0.4	0.2	0.3	0.1
% R.S.D.	3.7	3.1	3.0	5.1	10.0	6.8	4.1	6.2	2.1
% accuracy	1.1	−0.1	0.3	0.3	−0.3	2.7	6.5	4.0	−2.3
50									
Mean	50.1	50.2	48.9	49.3	50.3	50.7	52.0	52.2	50.4
S.D.	1.5	1.5	1.3	1.6	1.1	1.5	3.0	1.4	2.4
% R.S.D.	3.0	3.0	2.7	3.2	2.1	2.9	5.7	2.7	4.8
% accuracy	0.2	0.4	−2.3	−1.5	0.5	1.4	3.9	4.2	0.7
500									
Mean	502.3	499.0	502.1	501.3	502.4	499.4	498.6	498.8	506.9
S.D.	2.2	3.0	3.1	2.4	2.8	6.5	8.0	9.9	4.9
% R.S.D.	0.4	0.6	0.6	0.5	0.6	1.3	1.6	2.8	0.1
% accuracy	0.5	−0.2	0.4	0.3	0.5	−0.1	−0.3	−0.3	1.4

Table 8
Comparison of the lower limit of quantitation (ng/mL) for proguanil (PG), cycloguanil (CG) and 4-chlorophenylbiguanide (4CPB) in plasma using different techniques

Injection method	Proguanil	Cycloguanil	1-(4-Chlorophenyl) biguanide
LC/MS–MS (this study)	1	1	5
Sweeping-MEKC [9]	10	20	20
LC [10]	30	10	10
LC [11]	1	0.5	0.5
CZE [12]	110	90	80

is consistent with in vitro metabolism studies in rat liver microsomes conducted in our laboratory that the extent of conversion of PG to CG is insignificant. Also, the conversion of PG to CG was found to be species dependent which led us to review the choice of antimalarial animal model and the suitability of the prodrug approach in our antimalarial project. This study also revealed that PG has high affinity for erythrocytes. The blood-to-plasma ratio of PG was found to be around 5 throughout the study period. Results from these studies will be reported in detail in the future.

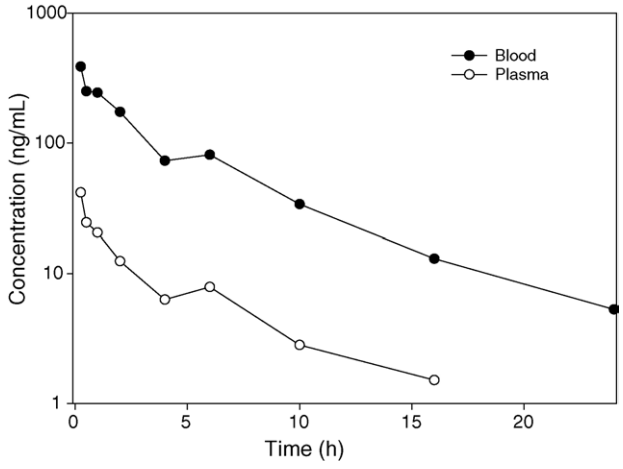

Fig. 2. Representative blood and plasma profiles of proguanil in a single rat after oral administration of proguanil (ca. 3 mg/animal or 10 mg/kg) by oral gavage. CG and 4CPB concentrations were below detection limits.

4. Conclusion

A simple, sensitive and reliable method for the determination of proguanil and two of its metabolites (cycloguanil and 4-chlorophenyl biguanide) in rat blood and plasma using LC–MS–MS was developed and validated. This method has an LLOQ of 1 ng/mL for PG and CG and 5 ng/mL for 4CPB using 50 μL of blood or plasma and has a calibration range from 1 to 2000 ng/mL. The current method provided a simple and rapid sample preparation procedure, a short analytical run time, and improved sensitivity for the determination of PG and its metabolites in rat blood and plasma. The sensitivity and sample throughput of current method are sufficient for the conduct of small animal pharmacokinetics studies. It has been successfully applied in the study of PG in rats.

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