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Research Article

A RAPID AND SENSITIVE HPLC METHOD FOR THE ANALYSIS OF PROGUNAIL AND CYCLOGUANIL IN PLASMA: APPLICATION TO SINGLE DOSE PHARMACOKINETIC STUDIES

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ABSTRACT

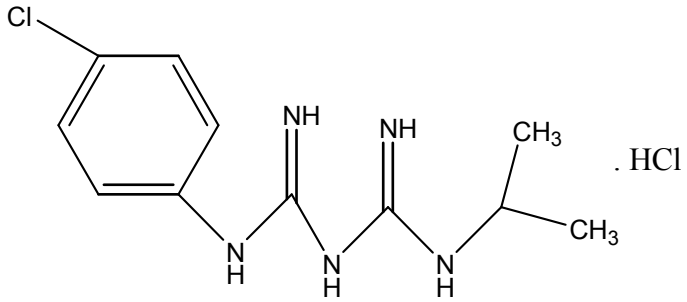
A simple, sensitive cost-effective and reproducible reverse phase high performance liquid chromatographic (HPLC) method was developed to quantitate plasma levels of proguanil (PGN) and its active metabolite, cycloguanil (CGN) in order to conduct single dose pharmacokinetic studies. The drug and the internal standard were added to plasma samples, vortexed and rendered alkaline with 2 M NaoH and the samples extracted with ether, evaporated to dryness and the residue was reconstituted in methanol, whirlmixed before injecting an aliquot onto the HPLC system. The calibration plots were linear over the concentration range up to 4.0 µg /ml. The correlation coefficients (r) were of the order of 0.99 and above for both PGN and CGN. The ion pair method was carried out on a 5 µ reverse phase C-18 column, using perchlorate ion as the counter ion and ultra violet detection at 254

nm. The method was reproducible with coefficient of variation for PGN and CGN, being less than 4.0 %. PGN was well resolved from its active metabolite, CGN, and the internal standard, pyrimethamine. The limit of detection of PGN was 10 ng /ml and the recovery was greater than 95% in plasma. The analytical method therefore, exhibits good precision and sensitivity in detecting and quantifying PGN and CGN and has been demonstrated to be suitable for the pharmacokinetic studies of proguanil. The clinical applicability of the method was assessed by the preliminary pharmacokinetic study of PGN and CGN, in fifteen healthy volunteers. The in vivo study was carried out according to a single dose randomized design.

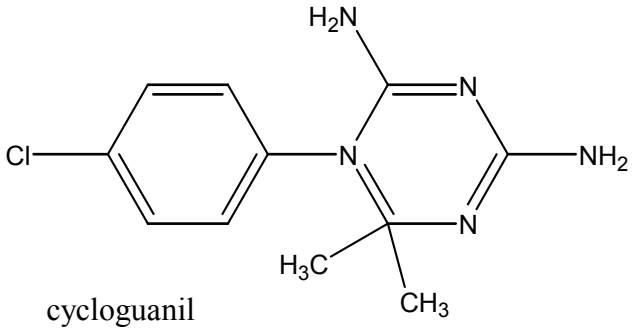
Keywords: Proguanil; Cycloguanil; Liquid chromatography; Pharmacokinetic studies.

INTRODUCTION

Proguanil is a synthetic biguanide derivative of pyrimidine. It is widely used in chemoprophylaxis of malaria. It is chronically administered for malaria prophylaxis in sickle cell patients and in pregnant women in Nigeria (1). Proguanil has found use in combination with other drugs such as atovaquone and dapsone in the treatment of resistant cases of falciparum malaria (1).



proguanil



cycloguanil

Fig 1: Structures of Proguanil and Cycloguanil

Proguanil (Fig. 1) is well absorbed, achieving C_{max} within 2 to 5 hours (2, 3). The drug is considered a prodrug, since it is metabolized in the liver to the dihydrofolate reductase (DHFR) inhibitor cycloguanil (Fig. 1), but there are also recent indications that proguanil itself enhances the activity of atovaquone (4). Nevertheless, transformation to cycloguanil is rapid, its C_{max} occurs 1 hour after the C_{max} of proguanil, and C_{max} of the inactive metabolite, 4-chlorophenylbigunanide occurs a further 1 hour later. The metabolism of proguanil is mediated partly by CYP 3A4 but mainly by CYP 2C19 (5). There is considerable genetic polymorphism of CYP 2C19 enzyme, with up to 20 % poor metabolisers in Asian and African populations (6,7). Poor metabolisers have very low or undetectable plasma concentrations of cycloguanil during prophylaxis. This polymorphism may be the cause of failure of prophylaxis by poor metabolisers, but due to large variability in data a clear association between CYP 2C19 activity and efficaciousness of prophylaxis has not yet been demonstrated (6,7). The half-life of proguanil is 12 to 20 hours in patients with malaria and healthy volunteers (2,4), but longer in poor metabolisers (2). The half life of cycloguanil is approximately 12 hours.

Due to the renewed interest in the use of proguanil in prophylactic treatment of malaria it is worthwhile to develop an HPLC method suitable for its pharmacokinetic studies, hence, this study. There are various methods for the determination of proguanil and its metabolites in the body ranging from colorimetric, microbiological and chromatographic methods. The colorimetric and bioassay method of analysis as described by (8, 9) lacked specificity and sensitivity. They are, therefore, of little value in pharmacokinetic studies. Moody et al. (1980) (10) reported a chromatographic method for the analysis of proguanil in the biological fluid using a reversed phase, ion-pair high performance liquid chromatographic (HPLC) technique.

Specific quantitation was obtained but the assay sensitivity was inadequate for the determination of the metabolites. The methods of Bergqvisty et al (1998) (11) and Kusaka et al (1996)(12) were less sensitive and expensive.

The method developed by Paci et al (2002) (13) for the simultaneous determination of proguanil and chloroquine was not applicable in biological fluid but for the determination of the drugs in a dosage form. A very sensitive method with a short analysis time involving a combination with mass spectrometer was developed by Leveque et al (2006) (14). This method may not be suitable in most developing countries where a mass spectrometer is not affordable. Due to some of these inconsistencies on the analytical data of proguanil and inter laboratory differences, the report of WHO informal consultation on the use of antimalarial drugs (2001) (15) adjudged proguanil to have limited pharmacokinetic data in literature. The method described here is simple, sensitive, selective, and cost-effective and allows for routine analysis of proguanil in biological fluids.

EXPERIMENTAL PROCEDURE:

Chemicals and reagents:

Proguanil and cycloguanil were obtained from Imperial Chemical Industries Ltd, Great Britain, while pyrimethamine was obtained from Swiss Pharma Nigeria (Lagos, Nigeria), Proguanil hydrochloride tablets, Paludrine^{R} (Astra Zeneca, Germany) were purchased from a retail pharmacy in Nigeria. HPLC grade acetonitrile and methanol, and analytical grade

diethylether, perchloric acid, sodium hydroxide and hydrochloric acid were purchased from Sigma (Sigma-Aldrich chemical company, Germany).

Subjects

Fifteen healthy volunteers (10 males and 5 females) between the ages of 22 and 30 years weighing 56 to 71 Kg were enrolled into the study after giving written informed consent. They were judged healthy by a physician on the basis of history, clinical examination, biochemical, hematological and electrocardiographic screening prior to entry into the study. Approval was obtained from the Obafemi Awolowo University Teaching Hospitals Research Ethics Board and safety committee.

Sample collection

In period 1 blood samples (5 ml) were withdrawn by venipuncture from the forearm of each subject prior to and at 1,2,4,6,8,12,24,36, and 48 hours after drug administration into heparinized tubes. They were immediately centrifuged (1500 g at 20 ^{O}C for ten minutes) to separate plasma. The plasma aliquots were stored at -20 ^{O}C until analyzed.

In period 2 blood samples (5 ml) were withdrawn from the forearm of each volunteer by venipuncture into heparinised tubes immediately before the 9th dose of efavirenz and co administration with single oral dose of 300 mg proguanil, and at 1,2,4,6,8,12,24,36 and 48 hrs after drug administration. They were centrifuged to separate plasma and stored at -20 ^{o}C until analyzed.

Analysis of Samples

The plasma samples were analyzed for proguanil and cycloguanil to obtain proguanil baseline pharmacokinetics in period 1, and analyzed for proguanil and its metabolite in the

presence of efavirenz in period 2. The HPLC method used for the analysis was a modification of that described by Ebeshi *et al*., (2005). The column used was a Hypersil ODS (C-18) 5µm particle size with dimension of 250 x 4.6 mm I.D. A mobile phase consisting of methanol: acetonitrile: 0.5 % ammonium acetate (40:5:55) containing 75 mM/L perchloric acid was pumped through the column at a flow rate of 1.2 ml/min. The pH of the mobile phase was 2.9 and the chromatogram was run at ambient temperature.

Analytical procedure

Calibration curve in plasma:

1 ml of blank plasma samples were placed in extraction tubes; and varying amounts of the stock solutions (100 µg/mL) of proguanil and cycloguanil were added to give concentration range between 0.1 – 3 µg/ml for the two compounds. 20 µL of the internal standard, pyrimethamine (100µg/ml) was added to each tube. The stock solutions (1 mg/ml each) of proguanil and cycloguanil were prepared in methanol, while that of pyrimethamine was prepared in acetonitrile. The plasma samples were rendered alkaline with 2 M NaOH (0.5 ml) and whirlmixed for 1 min. Then 3 ml of ether was added to each of the samples and whirlmixed for 1 min after which the tubes were centrifuged at 1500 g for 10 min. The upper organic layer was aspirated into another tube. The extraction with ether was repeated twice and pooled extract was evaporated to dryness in a water bath at 40^{o}C. The residue was reconstituted in 100 µL of methanol and whirlmixed before injecting 50 µL onto the HPLC. The peak area ratio was plotted against the concentration of each of the compounds injected. The regression analysis was carried out with the aid of a computer.

Analysis of test plasma samples:

To 1 ml of plasma sample in extraction tube, 20 µL of the internal standard was added. The extraction and reconstitution followed as described above before injecting 50 µL onto the HPLC.

Precision studies in plasma:

Intra-day Precision Studies: Two sets, each set consisting of six centrifuge tubes were used. Each tube in the first set contained 1 ml of blank plasma spiked with the stock solution of proguanil and cycloguanil to give a concentration of 0.5 µg/ mL.The second set also contained 1 ml of blank plasma spiked with stock solutions of the two compounds to give a concentration of 2µg/mL of each. All the samples were then spiked with 20 µL of the internal standard solution. Extraction followed under alkaline conditions as earlier described. The residue was reconstituted in 100 µL methanol, whirlmixed before 50 µL was injected onto the HPLC. The coefficient of variation of each set was computed.

Inter-day Precision Studies:

The procedure above was followed but two samples for each set were analyzed daily for three days.

Recovery Studies in Plasma:

The procedure described for precision studies was followed but coefficients of variation of replicate samples were not determined. Rather the stock solutions were diluted in such a way as to give concentrations equivalent to extracted 0.5 µg /mL and 2.0 µg /mL for the two compounds. The solutions were injected directly onto the chromatograph. To determine the recovery, the peak areas obtained with the extraction and direct injection methods were compared.

Accuracy of method:

The accuracy of the analytical method for each of proguanil and cycloguanil was evaluated from the percentage ratio of the experimentally determined concentration to that of the actual drug concentration.

Chromatographic conditions and instrumentation

The HPLC equipment was an AKTA system (Amersham Pharmacia Biotech, Uppsala Sweden) consisting of binary pumps (P-900) fitted with a gradient mixer and a variable wavelength (200–800 nm) ultraviolet–visible detector (model UV-900). Sample injection was through a model INV-907 valve fitted with a 50µL loop. The detector output was linked to a computer via a brain box interphase (AKTA instrument), which transforms signals from the detector to the computer that eventually records the chromatograms. Chromatographic separation was achieved at ambient temperature on Eclipse – XDB (C-18) (Agilent Technologies, Palo Alto, CA, USA), a 5µm particle size C-18 column (200mm×4.6mm I.D.). A mobile phase consisting of methanol: acetonitrile: 0.5 % ammonium acetate (40:5:55) containing 75 mM/L perchloric acid was pumped through the column at a flow rate of 1.2 ml/min. The pH of the mobile phase was 2.9 and the chromatogram was run at ambient temperature. The column effluent was monitored with the detector set at 254 nm. Vortex mixer (Gallenkamp, London, UK) and centrifuge (Gallenkamp) were used in the extraction procedure.

Selectivity

Various antimalarial drugs and other drugs commonly co-administered with antimalarials such as amodiaquine, chloroquine, quinine, primaquine, cycloguanil, paracetamol, chlopheniramine and promethazine were evaluated for interference with the assay. Drug-free

plasma was spiked with therapeutic concentrations of the drugs followed by extraction and analysis as described.

Application of the analytic method

Fifteen healthy volunteer who had not been taking any other drug, received a single oral dose of 300 mg proguanil. Thereafter, venous blood samples (5 ml) were collected into heparinised tubes just before and at 1, 2, 4, 6, 8, 12, 24, 36 and 48 h after drug administration. The blood samples were centrifuged at 2000 x g for 15 min to obtain the plasma which was analyzed for PGN and CGN concentrations using 1 ml aliquots of plasma samples and following the procedures described.

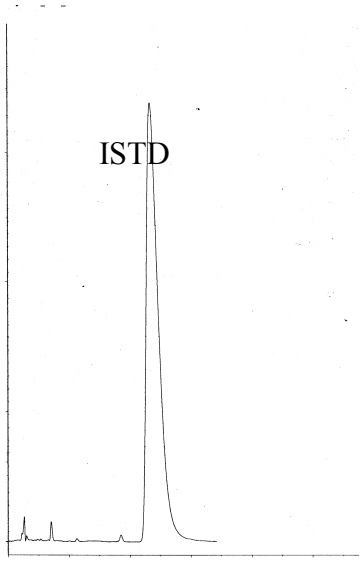
The maximum plasma drug concentration (C_{max}) was estimated by visual inspection of the concentration – time data. The total area under plasma concentration vs. time curve (AUC_T) was obtained from a sum of AUC_{0-t} and Ct/β where AUC_{0-t} was derived using the linear trapezoid method up to the last time point concentration (Ct). β is the elimination rate constant obtained by linear regression analysis of the terminal phase of the curve.

RESULTS

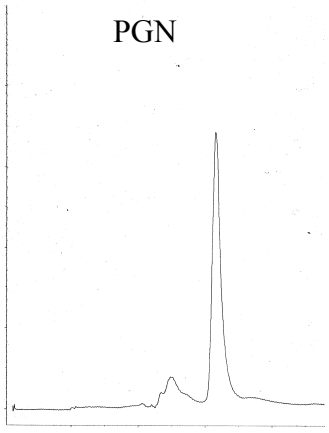
Typical chromatograms obtained from the described HPLC method are shown in Fig. 2. These demonstrate that the peak of PGN was well resolved from the internal standard as well as from CGN. The retention times (t_R) of cycloguanil, ISTD and proguanil were 6.9, 8.6, and 10.7 min respectively. There was no interference from endogenous compounds. Also no interference with the peaks of the compounds was found from chloroquine, primaquine, paracetamol, chlopheniramine, and promethazine. The minimum detectable concentrations, taken as a concentration giving a peak three times the baseline noise was 5 ng/ml for PGN and CGN and 30

ng/ml for pyrimethamine. Linear curves were obtained for proguanil and cycloguanil in plasma with correlation coefficients of not less than 0.99 for each of the curves. (Fig. 3). The results of the precision as well as the recovery and accuracy of the analytical method for both PGN and CGN are shown in Table1. While the pharmacokinetic parameters derived from the plasma concentration-time profiles are presented in Table 2 a and b. The mean T_{max} of proguanil was 2.8 ± 0.99 h, while the peak plasma concentrations (C_{max}) ranged from 2.20 to 3.00 mg/L with a mean of 2.55 ± 0.24 mg/L. The drug had an elimination half-life of 16.50 ± 4.55 h, a total clearance (Cl/F) of 7.08 ± 1.97 L/h (range 4.14 – 10.98 L/h) and an apparent volume of distribution (V_d/F) of 160.54 ± 36.06 L (range 109.89 – 234.09 L). The AUC_T varied between 27.33 to 72.50 mg/L.h (mean 45.58 ± 12.75 mg/L.h)

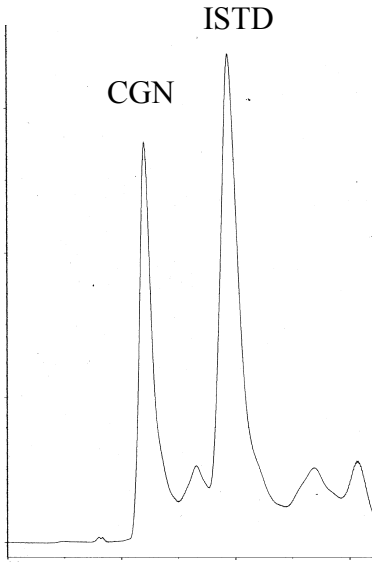
A)



B)



C)



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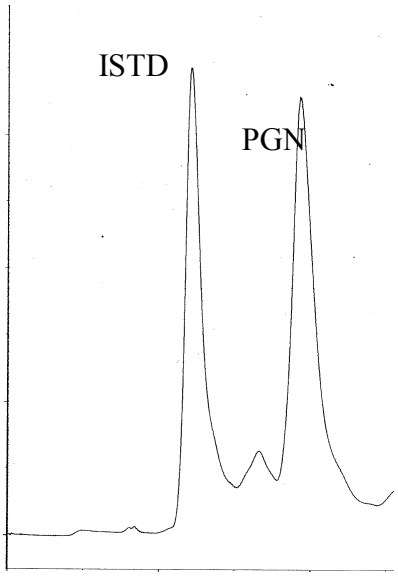

Fig. 2a HPLC chromatograms: A) extracted spiked plasma containing ISTD, B) extracted spiked plasma containing PGN, C) extracted spiked plasma containing CGN and ISTD, D) extracted spiked plasma containing PGN and ISTD.

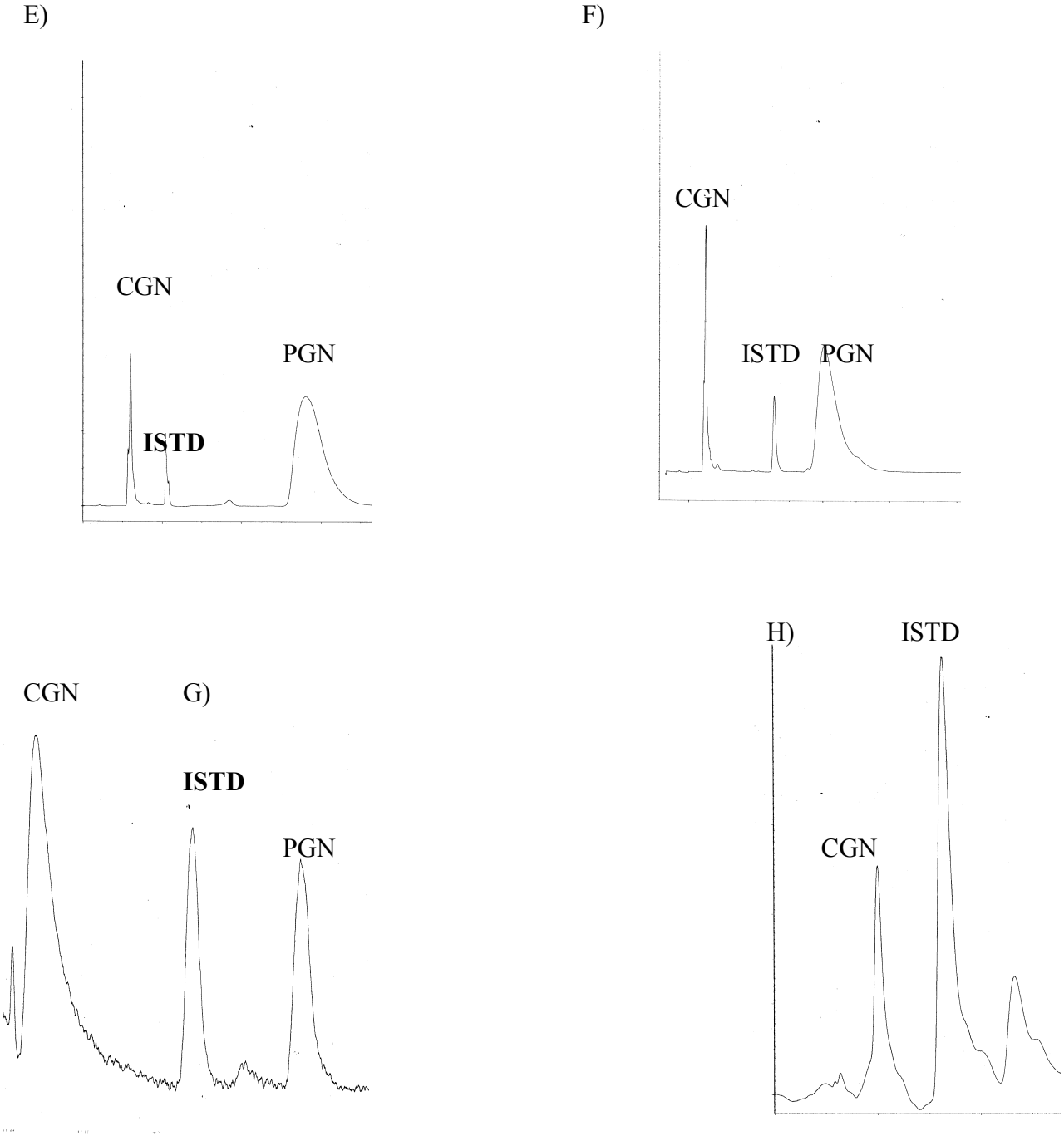

Fig 2b: HPLC chromatograms: E) 2h plasma extract from a volunteer, F) 4 h plasma extract from a volunteer, G) extracted spiked plasma containing ISTD, PGN and CGN, H) 6 h plasma extract from a volunteer.

Table 1a

Result of precision study in plasma.

Sample	Concentration (μgml^{-1})	Coefficient of variation %	n
Intraday			
Proguanil	0.5	3.86	6
	2.0	3.57	6
Cycloguanil	0.5	3.75	6
	2.0	3.64	6
Interday			
Proguanil	0.5	3.62	6
	2.0	3.48	6
Cycloguanil	0.5	3.59	6
	2.0	3.40	6

Table 1b:

Result of Recovery in Plasma

Sample	Concentration (μgml^{-1})	% Recovered ±SD	n
Proguanil	0.5	95.2±4.8	6
	2.0	96.5±3.3	6
Cycloguanil	0.5	85.4±2.6	6
	2.0	80.7±3.8	6

Table 1c:

Accuracy of Analytical Method

Sample	Concentration (μgml^{-1})	% Mean ± SD	n
Proguanil	0.5	90.0 ± 6.0	6
	2.0	92.2 ± 2.5	6
Cycloguanil	0.5	92.8 ± 5.0	6
	2.0	92.8 ± 2.8	6

Table 2a

Derived pharmacokinetic parameters of proguanil following single oral administration of 300 mg dose of proguanil hydrochloride to each of fifteen healthy volunteers

Volunteer	Wt (Kg)	T_{max} (h)	C_{max} mg/L	AUC_T (mg/L.h)	$T_{1/2}$ (h)	Cl/F (L/h)	Vd/F (L)
BK	64	2	2.60	57.74	20.51	5.20	153.77
AO	59	2	2.40	38.37	15.14	7.82	170.80
TF	66	2	2.80	47.26	12.00	6.35	109.89
IP	70	4	3.00	54.94	16.97	5.46	133.61
OS	65	2	2.40	45.30	19.63	6.62	187.50
FE	68	4	2.40	47.06	12.00	6.37	110.38
BF	62	4	2.80	72.50	24.95	4.14	148.97
YF	58	2	2.20	28.99	10.34	10.35	154.30
EO	65	4	2.60	54.60	15.14	5.49	120.02
AY	60	2	2.40	38.37	15.14	7.82	170.80
BS	56	4	2.80	36.68	12.00	8.18	141.59
FI	67	2	2.20	27.33	12.00	10.98	190.02
FT	54	2	2.40	36.02	19.48	8.32	234.09
OK	64	2	2.60	36.64	18.15	8.19	214.47
CF	62	4	2.70	61.90	24.00	4.85	167.81
Mean	62.67	2.80	2.55	45.58	16.50	7.08	160.54
SD	4.55	1.00	0.24	12.75	4.55	1.97	36.06

Table 2b

Pharmacokinetic parameters of proguanil metabolite (cycloguanil) following single oral administration of 300mg proguanil hydrochloride to each of fifteen volunteers

Volunteer	Wt (Kg)	T_{max} (h)	C_{max} (mg/L)	AUC mg/L.h	MRT (h.)	AUC_{0-48}(met)/ AUC_{0-48}(drug)
BK	64	4.0	0.48	9.52	16.75	0.18
AO	59	4.0	0.56	12.74	16.71	0.29
TF	66	4.0	0.70	16.82	16.80	0.32
IP	70	6.0	0.72	23.07	19.60	0.43
OS	65	4.0	0.40	7.42	16.78	0.19
FE	68	4.0	0.68	18.76	18.55	0.38
BF	62	6.0	0.76	22.71	19.30	0.38
YF	58	4.0	0.70	22.15	19.67	0.69
EO	65	4.0	0.74	25.38	20.51	0.44
AY	60	4.0	0.39	6.91	16.74	0.20
BS	56	6.0	0.56	15.87	19.22	0.40
FI	67	4.0	0.69	16.09	17.39	0.56
FT	54	4.0	0.48	9.08	15.91	0.29
OK	64	4.0	0.74	20.59	18.57	0.60
CF	62	4.0	0.62	15.80	17.97	0.31
Mean	62.67	4.40	0.61	16.19	18.03	0.38
SD	4.55	0.80	0.13	6.01	1.41	0.15

DISCUSSION

The extraction procedure employed in this study produced clean and clear supernatants from plasma as there was no interference from endogenous compounds. All the three compounds (proguanil, cycloguanil and ISTD) were completely resolved to baseline and samples could be injected at 12-min intervals. Sulphadoxine–pyrimethamine is not used in combination with proguanil in malaria chemotherapy; therefore, the possibility of interference from the internal standard is remote. Results of the assessments of precision, recovery and accuracy given in Table 1(a,b,c) show that the method has a high degree of precision as the intraday and inter-day coefficients of variation were not greater than 9% at low and high concentrations of the three compounds. The recovery of over 90% for PGN and CGN by the analytical method shows that the sample preparation and extraction procedure was efficient for the compounds. Evidence of accuracy of the method is demonstrated in the results which ranged between 92 and 97% for the compounds at low and high concentrations. It was necessary to precipitate the protein so as to release more of the drugs since proguanil (16,17) is highly bound to plasma proteins. This was achieved by adding 200µL of perchloric acid which was enough to denature the proteins before proceeding with the extraction of the drug.

The method reported here retains the sensitivity and precision of other previous methods for the analysis of the drug but it also has the advantage of being simple, cost-effective and devoid of any cumbersome extraction procedure. The composition of the mobile phase also proves the simplicity of the method. The mobile phase consisted mainly (75%) of potassium dihydrogen phosphate solution and small amounts of methanol and acetonitrile which are commonly

available, thus, making the method cost-effective and affordable. The rapidity of the method is underlined by the relatively short analysis time. The maximum time required for a sample treatment prior to injection was 10 min, which is much shorter than the analysis time of 62 and 40 min reported by other workers (10,12) for the analysis of proguanil in plasma.

To evaluate the application of this method in pharmacokinetic studies, concentrations of PGN and CGN were measured in plasma of fifteen volunteers after a single oral dose of PGN (300mg). The results from this present study indicate that proguanil is rapidly absorbed after oral administration in all subjects with a T_{max} in the range of 2 to 4 h. The pharmacokinetic parameters obtained for proguanil and cycloguanil (Table 2a and 2b) such as T_{max}, elimination $T_{½}$, Cl/F, AUC_T and V_d/F are generally in agreement with the findings of other workers (3,18).

CONCLUSION

In conclusion, the HPLC method described is very simple, reproducible, sensitive and rapid. The method is also accurate, selective and cost-effective. It will facilitate the conducting of pharmacokinetic studies on proguanil.

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REFERENCES

[1] Looareesuwan, S., Viravan, C., Webster, H. K., Kule, D. E. Hutchinson, D. B., Canfield, C. J. (1996). Clinical studies of atovaquone alone or in combination with other antimalarial drugs in the treatment of acute uncomplicated malaria in Thailand . *Am J Trop Med Hyg.* **54**: 62-68

[2] Edstein, M. D. Yeo, A. E. T., K. H. Rieckmann, K. H. (1997) Antimalarial activity of the triple combination of proguanil, atovaquone and dapsone. *Acta Tropica* **67** (3):207-214

[3] Wattanagoon, Y., Taylor, R. B. Moody, R.R. (1987) Single dose pharmacokinetics of proguanil and its metabolites in healthy subjects. *Br J Clin Pharmacol*, **24**(6): 775-780.

[4] Srivastava, I. K. Vaidya, A. B. (1999). A mechanism for the synergistic antimalarial action of atovaquone and proguanil. *Antimicrob Agents Chemother*, **43**(6): 1334-1339.

[5] Rasmussen, B. B., Nielsen, T.L., Brosen, K. (1998). Fluvoxamine inhibits the CYP2C19-catalyzed metabolism of proguanil in vitro. *Eur J Clin Pharmacol*, **54**(9-10): 735-740.

[6] Kaneko A, Lum JK, Yaviong L, (1999). High and variable frequencies of CYP2C19 mutations: medical consequences of poor drug metabolism in Vanuatu and other Pacific islands. *Pharmacogenetics.* ;**9**:581–590.

[7] Helsby, N.A., Ward, S. A., Edwards, I. G. and Breckenrige, A. M. (1993). The multiple dose pharmacokinetics of proguanil. . *Br. J. Clin. Pharmac*., **35**: 653-656.

[8] Maegreath BG, Tottey MM, Adam ARD, Andrews WH, King JD (1946). the absorption and extraction of proguanil in human subject. Ann. Trop. Med. Parasitol. 40: 493-506.

[9] Watkins WM, Chulay JD, Sixsmith DG, Spencer HC, Howells RE (1987). A preliminary pharmacokinetic study of the antimalarialdrugs, proguanil and chlorproguanil. J. Pharm. Pharmacol. 39: 261-265.

[10] Moody RR, Selkirk AB, Taylor RB (1980). High Performance Liquid Chromatography of proguanil, cycloguanil and 4-chlorophenylbiguanide using a hydrophobic pairing ion and its application to serum assay. J. Chromatogr. 182: 359-367.

[11] Bergqvist Y, Funding L, Krysèn B, Leek T, Yvell K. (1998). Improved validated assay for the determination of proguanil and its metabolites in plasma, whole blood, and urine using solid-phase extraction and high-performance liquid chromatography. Ther drug monit 20(3):325-30.

[12] Kusaka, M, Setiabudy, R, Chiba, K , Ishizaki **,** T**.** (1996) Simultaneous Measurement of Proguanil and Its Metabolites in Human Plasma and Urine by Reversed-Phase High-Performance Liquid Chromatography, and Its Preliminary Application in Relation to Genetically Determined S-Mephenytoin 4'-Hydroxylation Status. Am. J. Trop. Med. Hyg., 54(2), 1996, pp. 189-196.

[13] Paci A.M., Caire-Maurisier, A., Rieutor, F. B, Clair, P (2002). Dual-mode gradient HPLC procedure for the simultaneous determination of chloroquine and proguanil. Journal of Pharmaceutical and Biomedical Analysis. 27, (1-2), 1-7

[14] Leveque, N.L, William N. C, Francis C.K. (2006). Sensitive method for the quantitative determination of proguanil and its metabolites in rat blood and plasma by liquid chromatography–mass spectrometry. Journal of Chromatography B, 830, (2), 314-321

[15] World Health Organization (2001). Antimalarial drug combination therapy WHO/CDS/RBM/2001.35.

[16] Setiabudy, R, Kusaka, M, Chiba, K, Darmansjah, I, Ishizaki, T. (1995). Metabolic disposition of proguanil in extensive and poor metabolizers of S-mephenytoin 4-hydroxylation recruited from an Indonesian population. *Br. J. Clin. Pharmacol*. **39**: 297-303.

[17] World Health Organization. WHO expert committee on malaria, 18th report. World Health Organization Technical Report Series, 735, 1986, 57.

[18] Brentano, C. F., Becquemont, L., Leneveu, A., Roux, A., Jaillon, P., and Beaune, P. (1997) Inhibition by Omeprazole of Proguanil Metabolism: Mechanism of the interaction in vitro and Prediction of in vivo Results from the in vitro Experiments. *The Journal of Pharmacology and Experimental Therapeutics.* **280**: 730-738.

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