Full Length Research Paper

Development and validation of a thin layer chromatographic method for the determination of artesunate and amodiaquine in tablet formulations

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Artemisinin-based combination therapies (ACTs) are recommended for the treatment of uncomplicated falciparum malaria. Artemisinin derivatives are potent, rapidly acting antimalarias that reduce gametocyte carriage and patient infectivity; the sustained use of artesunate – amodiaquine reduced falciparum malaria transmission and progression of drug resistance. High efficacy of artemisinin-based combinations (artesunate plus amodiaquine) was observed in areas where malaria is endemic. This paper describes a routine, simple, precise, economical and reproducible thin layer chromatographic technique for the detection of artesunate and amodiaquine in tablet dosage form. Chromatographic separation was performed on glass silica gel plates (20 × 20 cm), paraffin – n-hexane (2:3 v/v) and ethylacetate – toluene (2.5:47.5 V/V) as mobile phases. Artesunate exhibited a detection limit of 0.001 mg/ml, while that of amodiaquine was 0.05 mg/ml. The two drugs were satisfactorily resolved with mean Rf values of 0.04 ± 0.03 and 0.06 ± 0.07 for artesunate and amodiaquine, respectively. The accuracy and reliability of the method was assessed by evaluation of linearity (0.001 – 6.0 and 0.05 – 6.0 mg/ml for artesunate and amodiaquine), precision (intraday RSD 10.68 – 25.78% and interday RSD 10.68 – 20.17 for artesunate, and intraday RSD 8.25 – 37.26% and inter day RSD 8.25 – 19.74% for amodiaquine) and specificity, in accordance with International Conference for Harmonization (ICH) guidelines. The method developed can be used for the analysis of ten or more formulation on a single plate and is a rapid and cost-effective quality-control tool for routine analysis of artesunate and amodiaquine as the parent drug and in tablet formulations.

Key words: Artemisin based combination therapy (ACT), malaria, falciparum, artesunate, amodiaquine.

INTRODUCTION

More than 600 million people worldwide are infected with malaria (WHO, 2003) and an average of 1 to 2 million die every year, most of them are below the age of five. Several non-ACTs (artemisinin-based combination therapies) have been used to fight this infectious disease, but only ACTs have continued to show efficacy with no or minimal reported cases of resistance (Bonifacio, 2004.).

Malaria is one of the leading causes of morbidity and mortality worldwide (Snow et al., 2005). The spread of parasite resistance to first-line drugs adds to the burden of the disease (Greenwood et al., 2005). A contributing factor has been the continued use of failing drugs, partly because of the difficulty to assess their efficacy *in vivo*. Methodological issues still being debated include how long patients should be monitored after treatment and whether clinical or parasitological outcomes should be given greater weight. Methods used so far differ greatly, making comparison and synthesis of data very difficult (Talisuma et al., 2004). The WHO has issued different sets of guidelines, shifting emphasis with time from parasitological to clinical assessment and more recently recommending that patients be assessed both parasitologically (WHO, 1973) and clinically (WHO, 1996) and monitored for 28 days if true failures are to be distinguished from new infections; it also makes provision for the use of life table analysis of results (WHO, 2003).

Malaria is one of the three major infectious diseases along with tuberculosis and AIDS (Greenwood et al.,

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2005). After World War II, the successful use of chloroquine as an efficient antimalaria drug against the 4 strains of human *Plasmodia (vivax*, *malariae*, *ovale* and *falciparum*) and the cheap DDT insecticide significantly reduced the importance of this tropical disease up to 1960. Unfortunately, the emergence of parasite strains resistant to chloroquine and other classical drugs was at the origin of the comeback of malaria (>200 million people are infected each year and there are >1 million deaths) (White et al., 1999). Resistance of mosquitoes to some insecticides has been documented just within a few years after the insecticides were introduced. There are over 125 mosquito species with documented resistance to one or more insecticides. The development of resistance to insecticides used for indoor residual spraying was a major impediment during the Global Malaria Eradication Campaign. Judicious use of insecticides for mosquito control can limit the development and spread of resistance. However, use of insecticides in agriculture has often been implicated as contributing to resistance in mosquito populations (CDC, 2004).

Fortunately, antimalarial drug research is active again after a decline period of several decades (Nwaka and Ridley, 2003). The renewal of activity in this field is caused mainly by the design of new small molecules active against chloroquine-resistant (CQR) strains of *Plasmodium falciparum*. With a chemical structure significantly different from that of quinoline-based drugs, the natural product artemisinin and its derivatives have attracted the attention of many different groups regarding the mechanism of action of these potent antimalarias devoid of significant clinical resistance up to now in Africa (Meshnick et al., 1996; Robert et al., 2005; Olliaro et al., 2001; Wu, 2002; Eckstein-Ludwig et al., 2003). Artemisinin, with its 1,2,4-trioxane as active motif, has served as a source of motivation for the design of synthetic peroxide-containing drugs (Jefford, 2007; Posner et al., 2008; O'Neill and Posner, 2004; Vennerstrom et al., 2004; Singh et al., 2006). Artemisinin (qinghaosu) has been used for centuries in traditional Chinese medicine for the treatment of fever. In 1972, Chinese scientists isolated the active principle from the plant *Artemisia annua*. Artemisinin is a sesquiterpene lactone charac-terized by the presence of an endo-peroxide that is associated with its potent antimalarial activity. Because artemisinin is chemically unstable and poorly soluble in water or oil, the carbonyl group at C-10 of the parent compound is often reduced to obtain dihydroartemisinin. Several derivatives have been developed by adding ether, ester or other substituents to the hydroxyl group of dihydroartemisinin. These semi-synthetic derivatives include water soluble derivatives, sodium artesunate and artelinic acid and the oil-soluble derivatives, artemether and arteether. With the exception of arteether and artelinic acid, these compounds are used to treat malarial infections in endemic countries (Ringwald et al., 1999). Artemisinin derivatives are available in different formulations for oral, parenteral and rectal administration. Clinical studies have shown that artemisinin derivatives are highly potent, rapidly acting and well-tolerated blood schizontocides, resulting in short parasite clearance times as compared to other antimalarial drugs. Artemisinin derivatives are highly effective against *P. falciparum* isolates that are resistant to other drugs. These derivatives are indicated for the emergency treatment of severe and complicated falciparum malarial by parenteral administration and for the oral treatment of uncomplicated multidrug-resistant malaria. All the artemisinin derivatives are metabolized rapidly to the active metabolite dihydroartemisinin, which is more active than the other artemisinin derivatives. The use of dihydroartemisinin instead of the substitute compounds (example, artesunate or artemether) has advantages. The drug is easy to produce with less synthetic steps, and thus a lower cost (http:/www.Rdi. gpo.or.th/htmls/dihyro.html). Dihydroartemisinin is thermally labile, lacks ultraviolet absorbence or fluorescent chromophore and does not possess functional groups for derivatization. Therefore, the development of sensitive and specific analytical methods for determination of dihydroar-temisinin is a challenging problem. Several techniques have been used to address this problem, such as gas chromatography (GC), and gas chromatography combined with mass spectrophotometry, e.t.c. However, presently, there is no cheap, simple and reliable analytical method available, which can be used to follow up the history of people undergoing a treatment as well as to identify counterfeit and substandard anti-malarial drugs in circulation. Furthermore, there is no single simple semi-quantitative method, which can be used to detect more than one anti-malarial drug using the same test system and conditions. This means that there is a shortage of simple and convenient analytical methods for monitoring antimalarial drug quality, usage, adequate absorption and excretion (Bojang et al., 2005). The purpose of this research was to establish and validate, in accordance with International Conference on Harmoni-zation (ICH) guidelines, a routine, simple, precise, economical and reproducible thin layer chroma-tographic technique for the detection of artesunate and amodiaquine in commercial antimalarial drugs.

MATERIALS AND METHODS

Sample collection

The antimalarial drugs- artesunate and amodiaquine, were generous gift from the Department of Paediatrics, University College Hospital, Ibadan.

Preparation of stock solution of artesunate and amodiaquine

One tablet of commercial artesunate tablet containing 5 mg of artesunate was dissolved in 50 ml of dimethyl sulfoxide (DMSO), to

Table 1a. Data for artesunate retention factor (Rf) values (Day 1).

Concentration (µg/ml)	Distance moved by mobile phase solvent front	Distance moved by sample	Retention factor (Rf)
0.001	10.4	0.1	0.009
0.00125		0.1	0.009
0.0025		0.1	0.009
0.005		0.1	0.009
0.01		0.1	0.009
0.0125		0.1	0.009
0.025		0.2	0.019
0.05		0.2	0.019
0.1		0.3	0.029
0.125		0.4	0.038
0.25		0.5	0.048
0.5		0.7	0.067
1.0		0.5	0.048
2.0		0.6	0.058
3.0		0.6	0.058
4.0		0.7	0.067
5.0		0.4	0.038
6.0		0.5	0.048

obtain a stock containing 50 mg/50 ml of artesunate in DMSO. One tablet of commercial base amodiaquine tablet containing 200 mg of amodiaquine in the form of a base was dissolved in 50 ml of dimethylsulfoxide (DMSO), to give a stock containing 200 mg/50 ml of amodiaquine in DMSO.

Preparation of calibration curves

To prepare the calibration curve for the artesunate, accurately measured aliquots were taken from the stock solution, each aliquot corresponding to 0.001, 0.00125, 0.0025, 0.005, 0.01, 0.0125, 0.025, 0.05, 0.1, 0.125, 0.25, 0.5, 1.0, 2.0, 3.0, 4.0, 5.0, 6.0 mg/ml, respectively. Different solvents and solvent combinations were tested for their abilities to separate the drugs from excipients in their formulations. Some of the tested solvent combinations include, ethyl acetate: toluene, ethyl acetate: toluene: isopropanol, hexane: toluene: paraffin mixtures. However, the plates were developed with 50 ml of paraffin- n hexane mixture 2:3 (v/v).

To prepare the calibration curve for amodiaquine, accurately measured aliquots was taken from the stock solution, each aliquot corresponding to 0.05, 0.1, 0.125, 0.25, 0.5, 1.0, 2.0, 3.0, 4.0 and 5.0 mg/ml, respectively. Different solvents and solvent combinations were tested. Some of the tested solvent combinations include: isopropanol-n hexane, cyclohexanol-toluene, methylamine- n hexane, ethyacetate-toluene-isopropanol, hexane-toluene-paraffin mixtures, tetrahydrofuran-paraffin-cyclohexanol-water and tetrahydrofuran-paraffin-isopropanol-cyclohexanol. However, the plates were developed with 50 ml of ethyacetate-toluene mixture (2.5: 47.5) (v/v).

Spotting of the TLC plates

A 20 × 20 cm prepared glass TLC plates were used for the separations. Samples were spotted on the TLC plates at a distance of 1 cm apart and a distance of 1 cm from the edge and bottom of

the plate.

Development of the spotted TLC plates

The spotted plate was developed in a chromatographic tank with paraffin- n hexane mixture, 2:3 (v/v) as mobile phase for artesunate and ethyacetate-toluene mixture, 2.5: 47.5 (v/v) as mobile phase for amodiaquine. The plate was allowed to develop for 20, 22 and 25 min at a development distance of 10.0, 10.4 and 10.8 cm, respectively, for artesunate and 9.4, 12.5 and 12.8 cm, respectively, for amodiaquine, for both intraday and interday determinations. After separations, the plates were allowed to dry at ambient temperature for 5 min and then transferred to the iodine tank for colour development. The development time was 25 min. The retention factor (Rf) values were determined for each concentration of the sample solution as presented in Table 1.

The procedure was repeated twice on the same day and repeated also on three different days in a space of two weeks.

Method validation

The method was validated for linearity, specificity, intra-day and inter-day precision, repeatability of measurement of peak area, and repeatability of sample application, in accordance with International Conference on Harmonisation (ICH) guidelines (ICH, 1994). The robustness of the method was studied, during method development, by determining the effects of small variations of mobile phase composition (+2%), development chamber saturation period and development distance as shown in Table 3. Intra-day precision was determined by analysis of solutions of artesunate and amodiaquine in the range of 0.1 to 6.0 mg/ml, three times on the same day (Tables 2a and 4a and b). Inter-day precision was determined by analysis of similar standards on three different days over a period of two weeks. Relative standard deviation (RSD) was calculated for both series of analyses.

Table 1b. Data for artesunate retention factor (Rf) values (Day 2).

Concentration (μg/ml)	Distance moved by mobile phase solvent front	Distance moved by sample	Retention factor (Rf)
0.001	10.8	0.3	0.03
0.00125	10.8	0.4	0.04
0.0025	10.8	0.4	0.04
0.005	10.8	0.4	0.04
0.01	10.8	0.4	0.04
0.0125	10.8	0.4	0.04
0.025	10.8	0.4	0.04
0.05	10.8	0.4	0.04
0.1	10.8	0.5	0.05
0.125	10.8	0.5	0.05
0.25	10.8	0.7	0.06
0.5	10.8	0.9	0.08
1.0	10.8	1.0	0.09
2.0	10.8	1.1	0.10
3.0	10.8	1.0	0.09
4.0	10.8	1.0	0.09
5.0	10.8	1.2	0.11
6.0	10.8	1.3	0.12

Table 1c. Data for artesunate retention factor (Rf) values (within Day 2).

Concentration (μg/ml)	Distance moved by mobile phase(solvent front	Distance moved by sample	Retention factor (Rf)
0.001	10.0	0.0	0.00
0.00125	10.0	0.0	0.00
0.0025	10.0	0.0	0.00
0.005	10.0	0.0	0.00
0.01	10.0	0.0	0.00
0.0125	10.0	0.0	0.00
0.025	10.0	0.0	0.00
0.05	10.0	0.0	0.00
0.1	10.0	0.2	0.02
0.125	10.0	0.3	0.03
0.25	10.0	0.2	0.03
0.5	10.0	0.3	0.04
1.0	10.0	0.4	0.05
2.0	10.0	0.5	0.06
3.0	10.0	0.6	0.07
4.0	10.0	0.8	0.08
5.0	10.0	0.8	0.08
6.0	10.0	0.7	0.07

RESULTS AND DISCUSSION

Tablet powder of artesunate and amodiaquine were pulverized and dissolved in dimethylsulfoxide. Several solvents for example, ethyl acetate-toluene, ethyl acetate-toluene-isopropanol, hexane-toluene-paraffin mixtures and Isopropanol-n hexane, cyclohexanol-toluene, methylamine-n hexane, ethyl acetate-toluene-isopropanol, hexane-toluene-paraffin mixtures, tetrahydrofuran-paraffin-cyclohexanol-water, tetrahydrofuran-paraffin-isopropanol-cyclohexanol were investigated for the separation of artesunate and amodiaquine, respectively from excipients in their formulation. Paraffin-n hexane 2:3 (v/v) and ethyl acetate–toluene 2.5:47.5

Table 2a. Data for amodiaquine retention factor (Rf) values (Day 1).

Concentration (µg/ml)	Distance moved by mobile phase(solvent front	Distance moved by sample	Retention factor (Rf)
0.05	12.8	0.0	0.0000
0.10	12.8	0.1	0.0078
0.125	12.8	0.1	0.0078
0.25	12.8	0.2	0.0016
0.50	12.8	0.3	0.0234
1.0	12.8	0.3	0.0234
2.0	12.8	0.4	0.0313
3.0	12.8	0.4	0.0313
4.0	12.8	0.4	0.0313
5.0	12.8	0.5	0.0391

Table 2b. Data for amodiaquine retention factor (Rf) values (Day 2).

Concentration (µg/ml)	Distance moved by mobile phase(solvent front	Distance moved by sample	Retention factor (Rf)
0.05	12.5	0.0	0.000
0.10		0.1	0.008
0.125		0.1	0.008
0.25		0.1	0.008
0.50		0.2	0.016
1.0		0.3	0.024
2.0		0.3	0.024
3.0		0.4	0.032
4.0		0.4	0.032
5.0		0.6	0.048

Table 2c. Data for amodiaquine retention factor (Rf) values (within Day 2).

| Concentration (µg/ml)	Distance moved by mobile phase(solvent front	Distance moved by sample	Retention factor (Rf)
0.05	9.4	0.5	0.05
0.10		0.7	0.07
0.125		0.8	0.09
0.25		1.5	0.16
0.50		1.6	0.17
1.0		1.6	0.17
2.0		1.7	0.18
3.0		1.8	0.19
4.0		1.9	0.20
5.0		2.0	0.21

(v/v) were found to result in best peak shapes for artesunate and amodiaquine, respectively. Artesunate and amodiaquine were satisfactorily resolved with retention factor (Rf) values ranging from 0.03-0.06 ± 0.02-0.03 and 0.02-0.1 ± 0.01-0.07, respectively (Figures 1 and 2). Pre-saturation of the TLC chamber and iodine chamber with mobile phase vapour and iodine vapour,

respectively for 20 to 30 min ensured more reproducible migration of the drugs and better resolution.

As recommended by International Conference on Harmonization (ICH, 1994), calibration plots were established for artesunate and amodiaquine standards using seventeen concentrations (0.001, 0.00125, 0.0025, 0.005, 0.01, 0.0125, 0.025, 0.05, 0.1, 0.125, 0.25, 0.5,

Table 3. Summary of the method validation data for artesunate and amodiaquine.

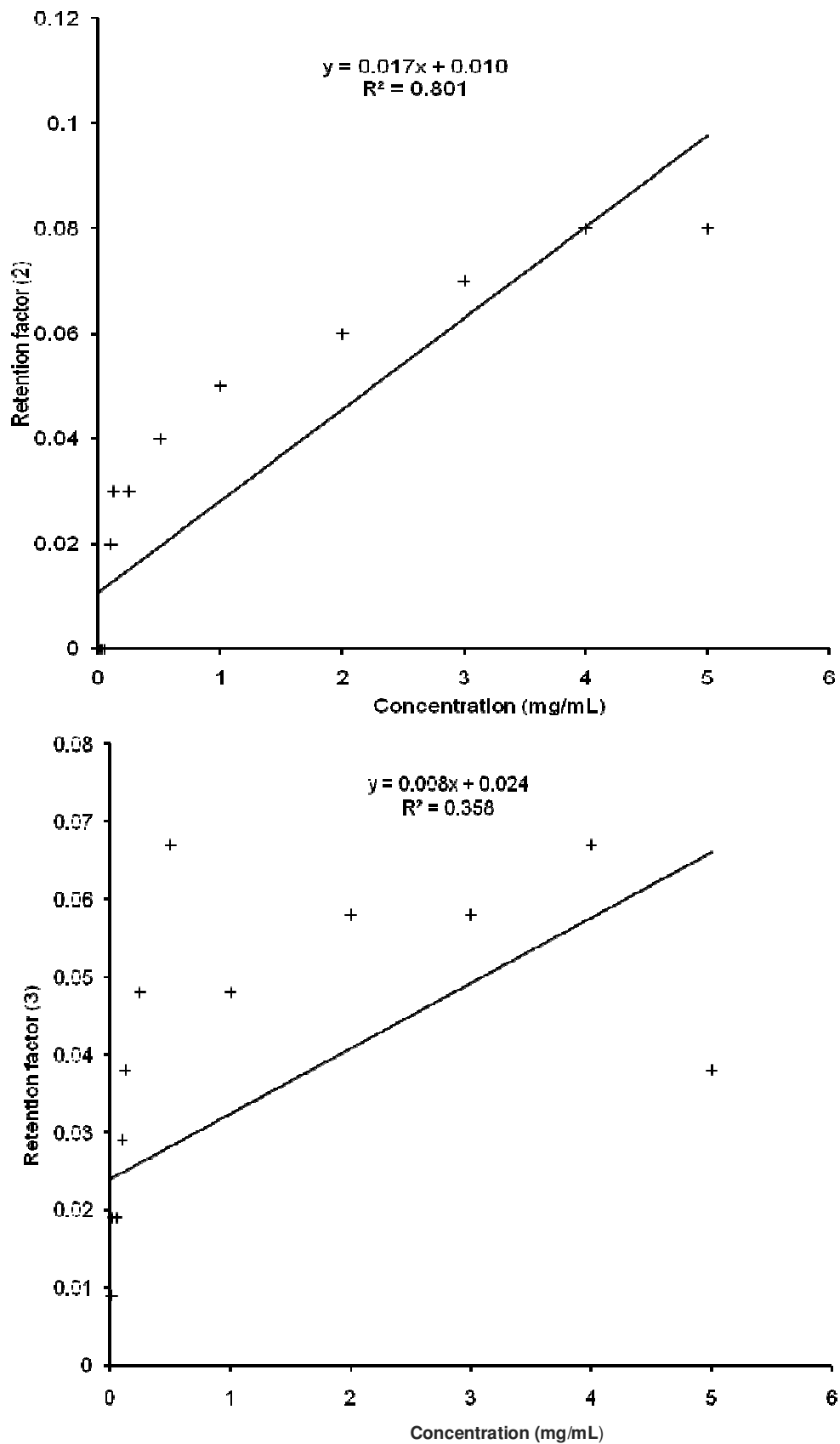| Method characteristic	Artesunate	Amodiaquine
Linear range (mg/ml)	0.001 – 5.0	0.05 – 5.0
Correlation coefficient (r) P = 0.01	0.873 – 0.895	0.859 – 0.945
Repeatability of measurement of peak area (RSD, %, n = 3)	12.9	8.74
Precision (RSD, %) Intraday (n = 6)	10.7 – 25.8	19.74 – 37.3
Inter-day (n = 6)	10.68 – 20.2	19.74 – 8.2
Specificity	Specific	Specific
Standard deviation	0.02 – 0.03 (n = 17)	0.01 – 0.05 (n = 10)
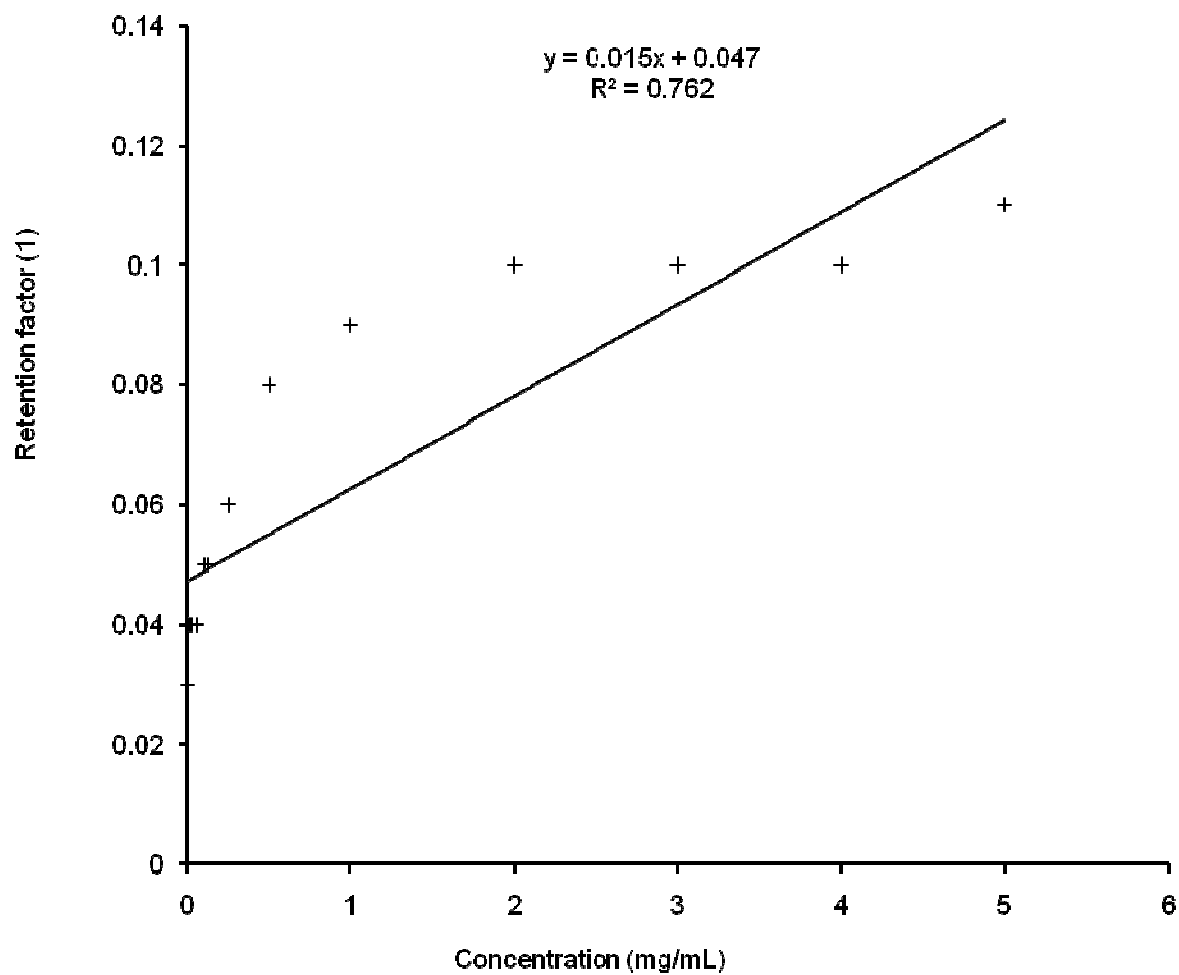
Figure 1. Plots of artesunate retention factor values against concentrations.

Table 4a. Mean, standard deviations, number of samples and relative standard deviations (%RSD) of artesunate tablet.

Variable	Mean	Standard deviation	N	Relative standard deviation (%RSD)
Rf1	0.032	0.022	17	68.8
Rf2	0.063	0.029	17	46.5
Rf3	0.027	0.029	17	107.4

Table 4b. Mean, standard deviations, number of samples and relative standard deviations (%RSD) of amodiaquine tablet.

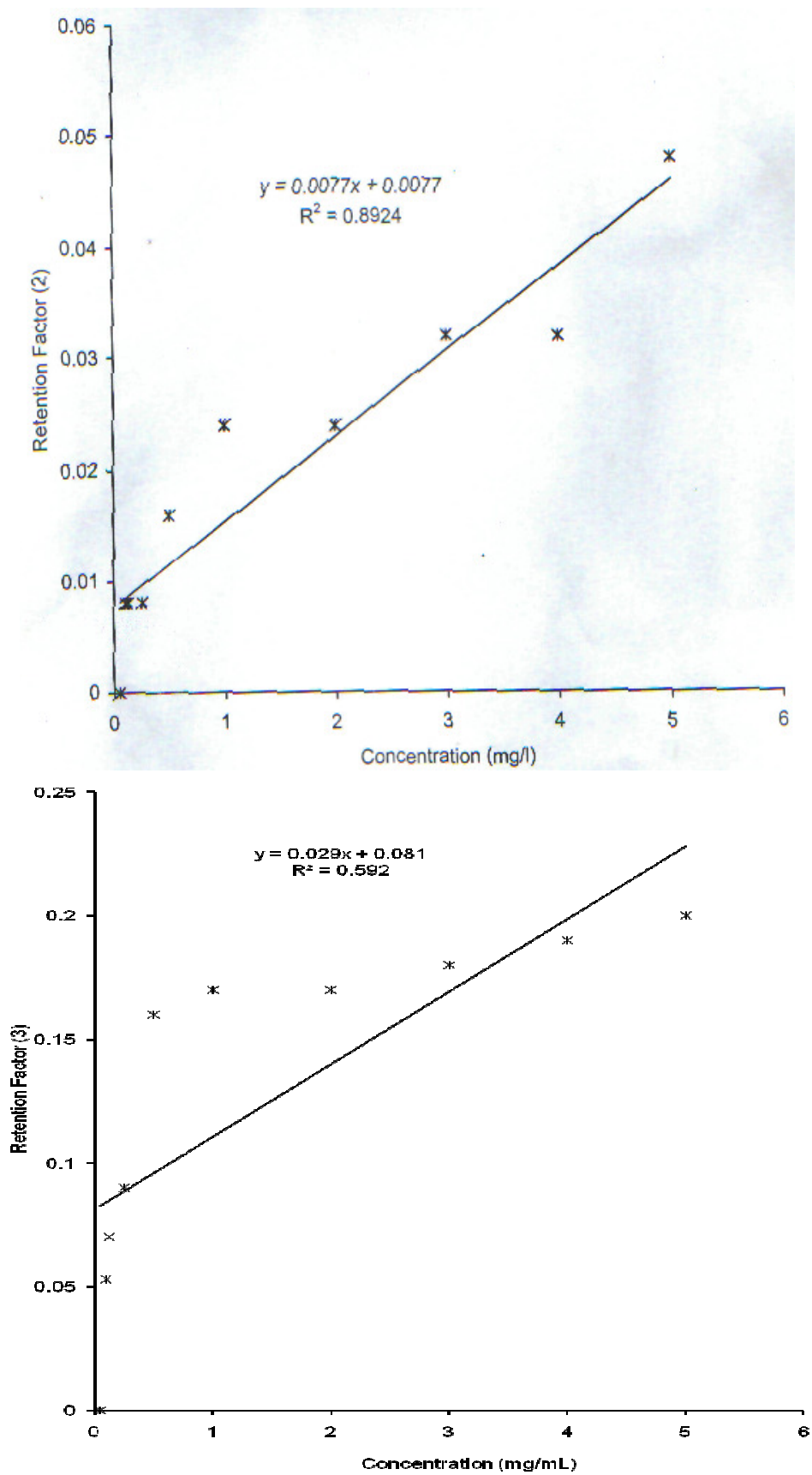| Variable	Mean	Standard deviation	N	Relative standard deviation (%RSD)
Rf1	0.0197	0.0134	10	68.0
Rf2	0.0200	0.0139	10	69.5
Rf3	0.1490	0.0543	10	36.4
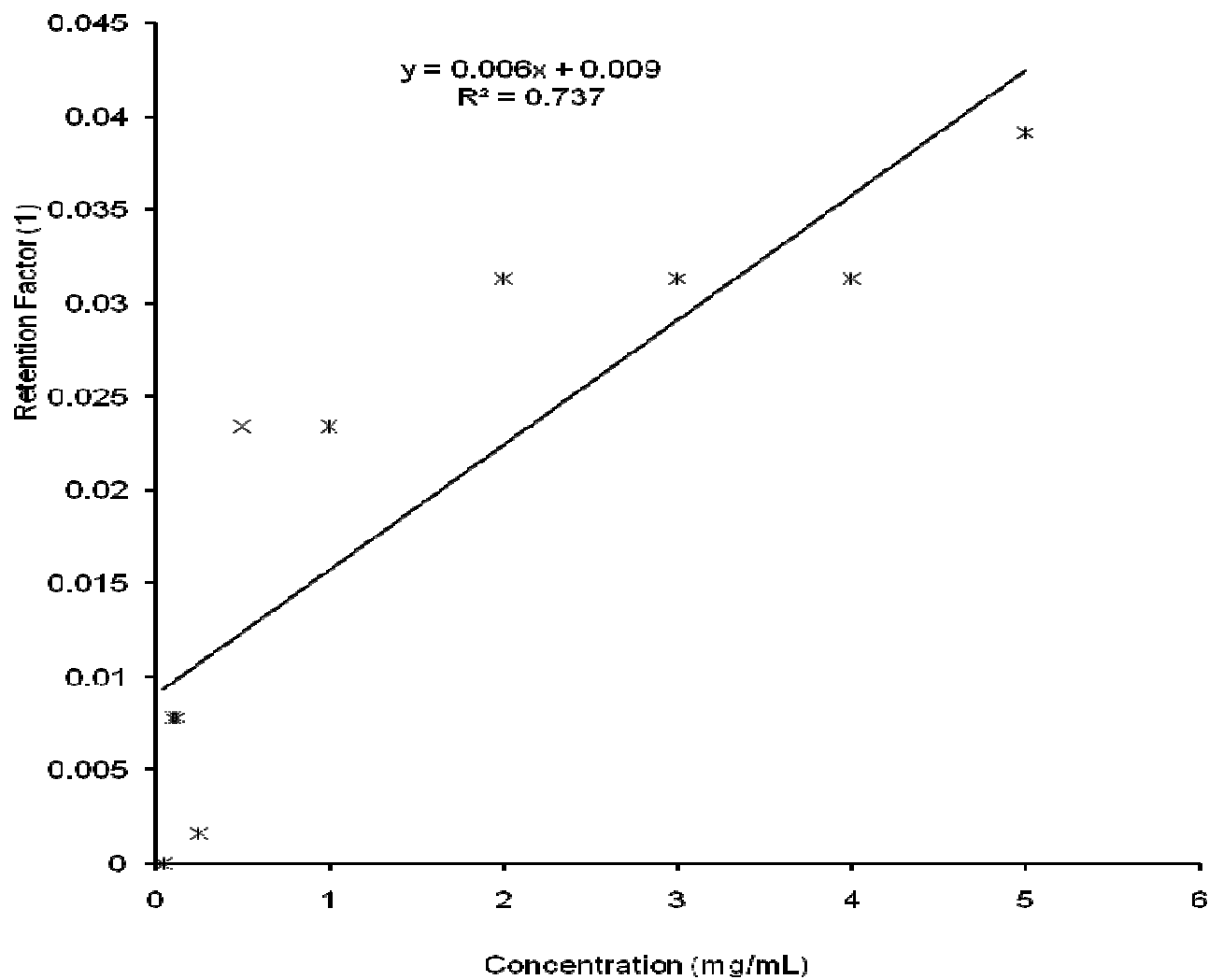
Figure 2. Plots of amodiaquine retention factor values against its concentration.

Table 5a. Correlation data for artesunate tablet.

Parameter		Concentration	Rf1	Rf2	Rf3
Concentration	Pearson correlation	1	0.873**	0.895**	0.599*
	Sig. (2-tailed)		0.000	0.000	0.011
	N	17	17	17	17
Rf1	Pearson correlation	0.873**	1	0.975**	0.851**
	Sig. (2-tailed)	0.000		0.000	0.000
	N	17	17	17	17
Rf2	Pearson correlation	0.895**	0.975**	1	0.867**
	Sig. (2-tailed)	0.000	0.000		0.000
	N	17	17	17	17
Rf3	Pearson correlation	0.599*	0.851**	0.851**	1
	Sig. (2-tailed)	0.011	0.000	0.000	
	N	17	17	17	17

Table 5b. Correlation data for amodiaquine.

Parameter		Concentration	Rf1	Rf2	Rf3
Concentration	Pearson correlation	1	0.858**	0.945**	0.770**
	Sig. (2-tailed)		0.001	00.000	00.009
	N	10	10	10	10
Rf1	Pearson correlation	0.859**	1	0.940**	0.938**
	Sig. (2-tailed)	0.001		0.000	0.000
	N	10	10	10	10
Rf2	Pearson correlation	0.945**	0.940**	1	0.894**
	Sig. (2-tailed)	0.009	0.000		0.000
	N	10	10	10	10
Rf3	Pearson correlation	0.770**	0.938**	0.894**	1
	Sig. (2-tailed)	0.009	0.000	0.000	
	N	10	10	10	10

1.0, 2.0 3.0, 4.0 and 5.0 mg/ml) and ten concentrations (0.05, 0.1, 0.125, 0.25, 0.5, 1.0, 2.0 3.0, 4.0 and 5.0 mg/ml) for artesunate and amodiaquine, respectively. The correlation coefficients for the plots range within 0.873 and 0.895 for artesunate and 0.859 and 0.945 for amodiaquine (intra-day and inter-day correlation co-efficients) as shown in Tables 5a and b. To test the specificity of the method, certain concentrations of artesunate and amodiaquine were spotted on the TLC plates and developed separately; this was repeated thrice for the same concentration. Excipients present in both formulations did not interfere with the peaks of artesunate and amodiaquine. When small changes were made to the method conditions, there were no marked changes in chromatographic behaviour, indicating that the method is robust. Correlations were fairly good. A relatively weak to fairly good linear association (R^2 = 0.36 to 0.80) was observed between artesunate concentrations and their retention factors (Rf) and a fairly good linear association (R^2 = 0.59 − 0.89) was observed for amodiaquine concentrations and their retention factors (Rf) values. The intraday and interday relative standard deviations (RSD) were in the ranges of 10.7 to 25.8% and 10.7 to 20.2% for artesunate and 8.2 to 19.74% and 8.2 to 37.3% for amodiaquine. RSD for measurement of peak area was 12.9 and 8.74% for artesunate and amodiaquine, respectively. The detection limit was measured as the lowest concentration resulting to the lowest observable TLC peak height. According to this rule, artesunate and amodiaquine exhibited a detection limit of 0.001 and 0.05

mg/ml, respectively. These results were reproducible and the precisions were negligible.

Conclusion

It is shown that our new TLC method achieved reproducibility, repeatability, linearity and selectivity that compares favourably with those of GC, GC-MS, HPLC, HPTLC, spectrophotometry and other methods reported regularly in literatures. The results also meet ICH guidelines (ICH, Q2A and ICH, Q2B) for validation of pharmaceutical TLC methods. The proposed TLC method is simpler, less expensive, routine, more rapid, and more flexible than GC–MS and HPLC.

REFERENCES

Bonifacio VDB (2004). Design and synthesis of antimalarial endoperoxides. Org. Chem. Highlights, October 25.
Eckstein-Ludwig U, Webb RJ, Van Goethem ID, East JM, Lee AG, Kimura M, O'Neill PM, Bray PG, Ward SA, Krishna S (2003). Artemisinins target the SERCA of *Plasmodium falciparum*. Nature, 424: 957–962.
Greenwood BM, Bojang K, Whitty CJ, Targett GA (2005). Malaria. Lancet, 365: 1487–1498.
(http:/www.Rdi.gpo.or.th/htmls/dihyro.html).
ICH, Q2A Validation of Analytical Procedures: Methodology International Conference on Harmonization, Geneva, October, 1994.
ICH, Q2B Validation of Analytical Procedures: Methodology International Conference on Harmonization, Geneva, March, 1996.
Jefford CW (2007). New development in synthetic drugs as artemisinin mimics. Drug Discov. Today, 12: 487–495.
Meshnick SR, Traylor TE, Kamchonwongpaisan S (1996). Artemisinin and the antimalarial endoperoxides: From herbal remedy to targeted chemotherapy. Microbiol Rev. 60: 301–315.
Nwaka S, Ridley RG (2003). Virtual drug discovery and development for neglected diseases through public- private partnerships. Nat. Rev. Drug Discov. 2: 919–928.
Oduola AMJ, Sowunmi A, Milhous WK, Kyle DE, Martin RK, Walker O, Olliaro PL, Haynes RK, Meunier B. and Yuthavong Y (2001). Possible modes of action of the artemisinin-type compounds. Trends Parasitol. 17: 122–126.
Olliaro PL, Haynes RK, Meunier B, Yuthavong Y (2001). Possible modes of action of the artemisinin-type compounds. Trends Parasitol. 17: 122–126.

O'Neill PM, Posner GH (2004). A medicinal chemistry perspective on artemisinin and related endoperoxides. J. Med. Chem. 47: 2945–2964.
Posner GH, Paik I-H, Sur S, McRiner AJ, Borstnik K, Xie S, Shapiro TA (2008). Malaria-infected mice are cured by oral administration of new artemisin derivatives. J. Med. Chem. 51: 1035–1042.
Ringwald P, Bickii J, Basco LK (1999). *In vitro* activity of Dihydroartemisinin against Clinical Isolates of Plasmodium Falciparum in Vaounde, Cameroon. Am. J. Trop. Med. Hyg. 61: 187–192.
Robert A, Cazelles J, Dechy-Cabaret O, Meunier B (2002). From mechanistic studies on artemisinin derivatives to new modular antimalarial drugs. Acc Chem. Res. 35: 167–174.
Robert A, Benoit-Vical F, Claparols C, Meunier B (2005). The antimalarial drug artemisinin alkylates heme in infected mice. Proc. Natl. Acad. Sci. USA., 102: 13676–13680.
Salako LA (1992). Innate resistance to new antimalarial drugs in plasmodium falciparum from Nigeria. Trans. R. Soc. Trop. Med. Hyg. 86: 123 –126.
Singh C, Malik H, Puri SK (2006). Orally active 1,2,4-trioxanes: Synthesis and antimalarial assessment of a new series of 9-functionalized 3-(1-arylvinyl)-1,2,5 trioxaspiro[5.5]undecanes against multi-drug-resistant *Plasmodium yoelii* nigerensis in mice. J. Med. Chem. 49: 2794–2803.
Snow RW, Guerra CA, Noor AM, Myint HY, Hay SI (2005). The global distribution of clinical episodes of *Plasmodium falciparum* malaria. Nature, 434: 214-217.
Talisuna AO, Bloland P, D'Alessandro U (2004). History, dynamics and public health importance of malaria parasite resistance. Clin. Microbiol. Rev. 17: 235-254.
Vennerstrom JL, Arbe-Barnes S, Brun R, Charman SA, Chiu FCK, Charman Net W, Porter CJH, Kaukonen AM, Shackleford DM, Fassen WA, Houwing N, Lass H, Edwards GA (2004). Identification of an antimalarial synthetic trioxolane drug development candidate. Nature, 430: 900–904.
White NJ (1999). Averting a malaria disaster. Lancet, 353: 1965–1967.
WHO (2003). Assessment and monitoring of antimalarial drug efficacy for the treatment of uncomplicated falciparum malaria. Document WHO/HTM/RBM/2003.50. World Health Organization, Geneva, Switzerland.
WHO (1996). Assessment of therapeutic efficacy antimalarial drug for uncomplicated falciparum malaria in areas with intense transmission. Document WHO/MAL/96.1077. World Health Organization, Geneva, Switzerland.
WHO (1973). Chemotherapy of malaria and resistance to antimalarials. Report of a WHO Scientific Group. WHO Tech. Rep. Ser. 529: 1-21.
Wu Y (2002). How might Qinghaosu (artemisinin) and related compounds kill the intraerythrocytic malaria parasite? A chemist's view. Acc Chem Res. 35: 255–259.