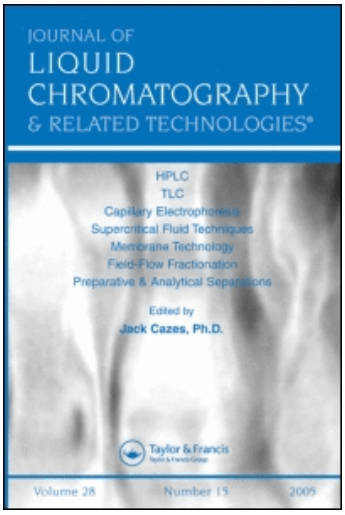

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LIQUID CHROMATOGRAPHIC ASSAY FOR AMODIAQUINE IN TABLETS AND BIOLOGICAL FLUIDS

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ABSTRACT

A high performance liquid chromatographic assay for quantitating amodiaquine (A) in tablets, urine, plasma, bile and saliva is described. The method involved acid extraction of the drug from tablets and chloroform extraction of its base from the biological fluids after alkalinization with ammonia. Quinidine was used as the internal standard. A μ-Bondapak phenyl column was used for separation together with a mobile phase made of methanol, water and glacial acetic acid (pH 2.3). Good chromatograms with efficient separation of drug and internal standard peaks were observed. Retention times of 5.2 and 7.1 min. were obtained for the drug and the internal standard respectively. Correlation between areas under the curve and drug concentration was high. The mean percentage recovery of A from tablets was 102.03%, while from the biological fluids, it ranged from 85.2 to 104.61%. Urine and saliva obtained from volunteers and bile obtained from animals administering amodiaquine showed chromatograms similar to those obtained for blank samples spiked with A. Interference from tablet excipients of biological fluids was undetectable or negligible. The method was found to be precise and simple.

INTRODUCTION

Amodiaquine is 4-(7-chloro-4-quinolylamino)-2-(diethylamino-methyl) phenol. Tablets and suspensions of the dihydrochloride salt of the drug as well as suspensions of the base have been used for their antimalarial effect. Until recently, A was considered equivalent to chloroquine in its activity against plasmodium falciparum (1). Recent studies (2-4), however, indicate that amodiaquine is superior to chloroquine against strains of the same parasite. Inspite of the relatively long duration during which the drug has been used, very few pharmacokinetic studies were performed in man. This is believed to be due to the limited clinical interest in A as well as due to the lack of a suitable method of analysis for its determination in biological media. The spectrophotometric method of analysis (5) and the complexation methods (6,7) were found to be of limited sensitivity and inadequate for A determination in biological media. A recently developed HPLC method (8) was used to determine A in plasma. Good selectivity and high sensitivity was claimed with a 60% recovery of A from plasma.

In this report, a modified HPLC method is described. The method was tested and found precise and sensitive for determination of A in tablets as well as in urine, plasma, bile and saliva.

MATERIALS

Instrumentation:

A Waters Associates (Milford, MA) liquid chromatograph was used for analysis. The instrument was equipped with a 6000 A pump,

U6K injector, Lambda Max 481 detector and a M 730 data module. A reversed phase column was obtained prepacked (μ-Bondapak-Phenyl, 3.9 mmX30cm, Waters Associates) and used for separation.

Chemicals:

Amodiaquine tablets were obtained from the local market (Camoquine®, Park-Davis, Pontypool, Gwent, UK). An authentic sample of the drug was obtained from the same source. The chemicals used for drug extraction and in mobile phase were chloroform, glacial acetic acid, ammonia and hydrochloric acid, B.D.H. Chemicals Ltd. (Poole, England) and methanol, Merck (Darmstadt, Germany). The internal standard, quinidine, was also obtained from Merck. The mobile phase was prepared by mixing methanol, water and glacial acetic acid in a ratio of 25:25:1 by volume. The mixture which had a pH of 2.3 was then filtered through a 0.45 μm pore size membrane filter obtained from Millipore Corporation (Bedford, Massachusetts) before degassing. LC quality water was freshly prepared by double distilling deionized water. Glasswares containing drug solutions were protected from light by wrapping in aluminium foil.

METHODS

Development of HPLC conditions:

Several preliminary trials on HPLC conditions including the one already published (8) were carried out to find one condition which provides us with good resolution for all systems tested. This, would eliminate the need for adjustments required to be carried in many other methods upon changing the medium in which the

drug is assayed. For this purpose, a μ-Bondapak-phenyl column and a mobile phase made of methanol, water and glacial acetic acid (25:25:1) with a pH of 2.3 flowing at a rate of 1.5 ml per minute, were found to be adequate. A retention time of 5.2 min was observed. Examination of the integrated areas under the peak for six different concentrations of drug solution showed proper correlation between peak areas and drug concentration. Ultraviolet absorption at a wave length of 343 nm was used for detection. A pure sample of quinidine was used as the internal standard. Under the chromatographic conditions used, quinidine had a well defined peak separated from that of the drug with a retention time of 7.1 min.

Assay calibration:

A number of standard solutions of amodiaquine were prepared by serial dilution from a stock solution of A containing 50 μg/ml internal standard in 0.1 N hydrochloric acid solution. Dilutions were made with 50 μg/ml internal standard solution in 0.1 N hydrochloric acid and concentrations of A base in the range of 0.25-20 μg/ml were obtained. A 10 μl aliquot of each solution was injected in the HPLC. A second injection of a different volume was made for confirmation. The calibration was repeated several times during experiments.

Determination of Amodiaquine in Tablets:

Two tablets containing 400 mg of A were crushed. After thorough mixing, a fraction of the powder was weighed and directly

extracted with three successive 10 ml volumes of 0.1 N hydrochloric acid containing 50 µg/ml internal standard. The combined filtrates were adjusted to volume and adequately diluted with the same vehicle before injection. Duplicate injections for two different volumes were used for drug assay. A second determination was conducted on two new tablets.

In order to evaluate the efficiency of the extraction process, powdered tablets were pooled, then divided into five fractions. Four of these fractions were spiked with different amounts of pure drug. After thorough mixing, total A was determined in each fraction as before.

Determination of Amodiaquine in Urine:

Urine samples were collected from a volunteer immediately before and two hours after administering 600 mg (3 tablets) amodiaquine. Portions of the blank urine sample were spiked with known concentrations of A and the internal standard. A 5 ml aliquot of each urine specimen was alkalinized in a separating funnel with 0.5 ml concentrated ammonia and then extracted with 20 ml chloroform. The chloroform layer was separated and evaporated to dryness in a rota-vapor at 40°C. The residue was reconstituted in 0.5-1 ml mobile phase. Injections of 10 and 20 µl volumes were made in duplicates. A second determination was carried following the same extraction procedure.

Determination of Amodiaquine in Plasma:

Blood samples were collected before and two and four hours after administering 600 mg amodiaquine to a volunteer. The plasma

was immediately separated. Two blank plasma samples were spiked with two different concentrations of A in presence of the internal standard. Duplicate extractions were carried on each sample by following the procedure described for the determination of A in urine and using 1 ml sample volume.

Determination of Amodiaquine in Bile:

Bile samples were collected from rats by bile duct cannulation. To two rats, amodiaquine hydrochloride solution in saline was slowly injected into the jugular vein just before bile collection. A total dose of 20 mg A/Kg body weight was injected into each rat. Three blank bile samples were spiked with known concentrations of A and the internal standard. The extraction and determination proceeded as for urine using 1 ml sample volume.

Determination of Amodiaquine in Saliva:

Saliva was collected from a volunteer before and four hours after administering 600 mg A. Three samples of the blank saliva were spiked with known concentrations of A and the internal standard. Extraction was then carried on 1 ml samples and determination proceeded as described for the urine samples.

RESULTS AND DISCUSSION

Figure 1 shows representative HPLC chromatograms of standard solutions of A and A with the internal standard used for assay calibration. As can be seen, well identified peaks with distinct separation between A and the internal standard were obtained. Calibration curves were linear ($r \geq 0.99$) in the range 0.25-20 µg/

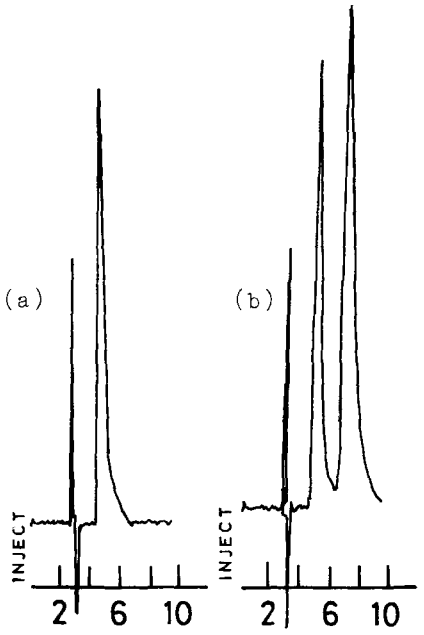

Figure 1: Typical Amodiaquine Chromatogram.
a, amodiaquine in mobile phase;
b, amodiaquine and internal standard in mobile phase.

ml with zero intercept. Lower concentrations were found to show slight positive deviation from linearity. Concentrations as low as 0.1 µg/ml were detectable. Quantitation of the results was carried based upon the ratios of the areas under A curves to those areas under the internal standard curves.

The determination of A in tablets indicated the presence of a slight over dosage. The results of two determinations showed that the average drug content was 106.0% of the amount labelled on the tablets. The chromatograms obtained showed no interference from

TABLE 1

Amodiaquine Recovery From Tablets

% A spiked to A label %	% A recovered to A label (average of two runs)	% A recovered to A spiked
0	106.0	------
20	126.2	101.0
40	148.8	107.0
60	163.0	95.0
80	190.1	105.1
		(Mean, S.D.=102.03, 5.31)

the excipients. Further extraction of A with chloroform form an alkalinized portion of the acid extract and evaporation yielded a product which matched the authentic A sample in its thin layer chromatogram and melting point. Amodiaquine recovery from tablets (Table 1) ranged from 95 to 107% with a mean of 102.03%. Deviation from ideality was not related to concentration spiked. This indicates the adequacy and accuracy of the method used for A extraction from tablets.

Figures 2-5 show the chromatograms obtained for blank and spiked biological fluids. Except for plasma, biological fluid samples collected from volunteers and animals administering A, showed chromatograms similar to those obtained with the spiked fluids. Under the present conditions, it was not possible to measure A in 1.0 ml plasma collected from volunteers 2 or 4 hours after administering the drug. Experiments with whole blood did not

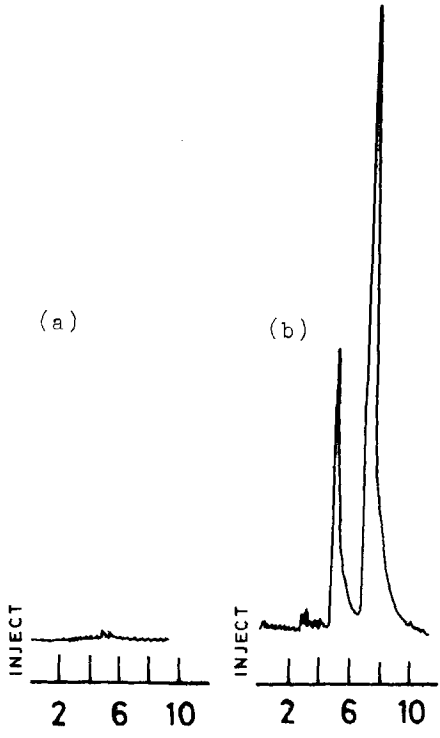

Figure 2: Chromatograms of urine extracts. a, blank; b, spiked with amodiaquine and internal standard.

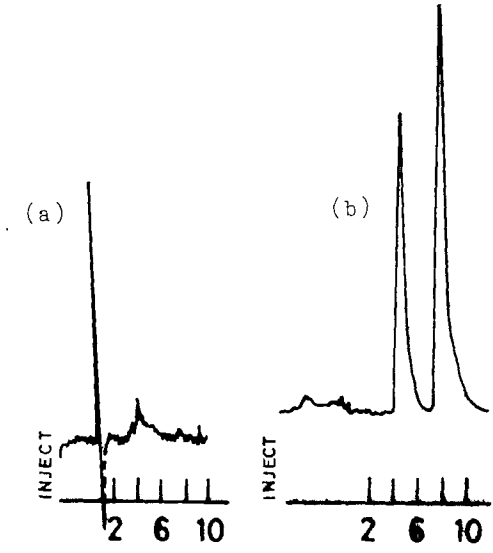

Figure 3: Chromatograms of plasma extracts. a, blank; b, spiked with amodiaquine and internal standard.

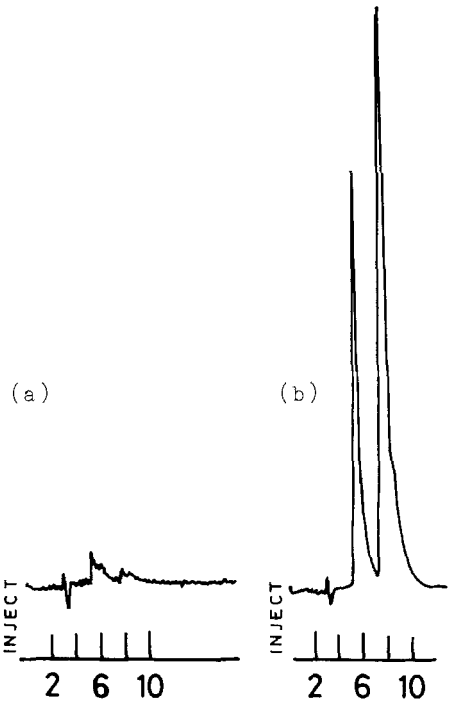

Figure 4: Chromatograms of bile extracts. a, blank; b, spiked with amodiaquine and internal standard.

improve the results. A single experiment carried on a rat which involved administering the drug (20 mg/Kg body weight) orally and sacrificing the animal after one hour showed that A was mostly bound to intestine, heart and liver tissues with very small amount detectable in plasma. No interference was observed from urine or saliva with the A or the internal standard peaks (Figs. 2 and 5). A slight baseline noise was observed with plasma and bile (Figs. 3 and 4). However, at the highest detector sensitivity used (.005 a.u.f.s.), and for the minimum determinable concentration of A, the interference was less than 10% of the area under the curve.

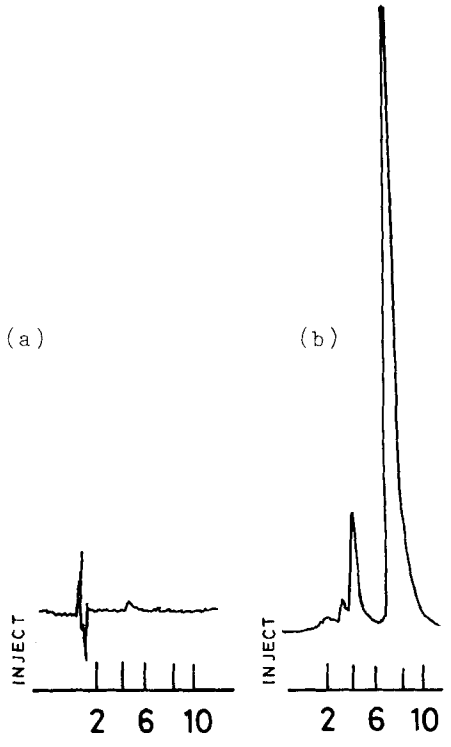

Figure 5: Chromatograms of saliva extracts. a, blank; b, spiked with amodiaquine and internal standard.

Table 2 presents the summary of the results of the recovery experiments performed on the different biological fluids. Individual amounts recovered from each sample were first determined. From the data obtained for every biological system, the mean percentage recovered as well as the standard deviation were calculated. As can be seen, A recovery ranged from 85.2 to 104.61%. Good correlation was observed between the concentrations spiked and recovered in all systems which indicates the high sensitivity and linearity of the assay method.

TABLE 2

Amodiaquine Recovery From Spiked Biological Fluids

Biological Fluid	Concentration of A spiked (µg/ml)	Number of extractions	Percent A recovered (Mean, S.D.)
Urine	1,3,5,10,20,50.	12	95.82, 2.75
Plasma	2.5,15.	4	97.85, 4.44
Bile	5,10,20.	6	104.61, 7.77
Saliva	1,5,10.	6	85.20, 6.98

The coefficient of variation (CV, %) for A extraction at the 5 µg/ml level from urine, bile and saliva and at the 2.5 µg/ml level from plasma was found to be 4.3, 2.9, 4.0 and 1.7 respectively. Thus, this method of extraction is precise in measuring A in the biological fluids examined.

During these experiments, protection from light was found to be important to prevent A losses attributable to photodegradation. Adsorption of A to glass surfaces was tested and proved to be negligible.

The chromatographic method discussed in this communication is sensitive and selective for determining amodiaquine in tablets and in biological fluids. Drug extraction was simple and the assay was sensitive and reproducible. We believe that the difficulty we met in determining A in human plasma samples was due to the high and fast binding of the drug to the other tissues. This view is supported by the results of the recovery experiments carried on spiked

human plasma (Fig. 3 and Table 2). While more experiments are required to improve testing A in human plasma, this method presents a valuable base for clinical pharmacokinetic studies.

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REFERENCES

1. Martindale, The Extra Pharmacopoeia, 28th Edition, Reynolds, J., ed., The Pharmaceutical Press, London, 1982, p. 397.

2. Spencer, H.C.; Oloo, A.J.; Watkins, W.W.; Sixmith, D.G.; Churchill, F.C. and Koech, D.K., Amodiaquine is More Effective than Chloroquine Against Plasmodium falciparum Malaria on Kenya Cost, Lancet, 1, 956, 1984.

3. Watkins, W.M.; Sixmith, D.G.; Spencer, H.C.; Boriga, D.A.; Karluki, D.M.; Kipingor, T. and Koech, D.K., Effectiveness of Amodiaquine as Treatment For Chloroquine-Resistant Plasmodium falciparum Infections in Kenya, Lancet, 1, 357, 1984.

4. Deleron, P.; LeBras, J. and Ramanamirija, J.A., Amodiaquine and Chloroquine Efficacy Against Plasmodium falciparum in Madgascar, Lancet, 1, 1303, 1984.

5. Rao, G.R.; Rao, Y.P. and Raju, I.R.K., Spectrophotometric Determination of Amodiaquine Hydrochloride in Pharmaceutical Dosage Forms, Analyst, 107, 776, 1982.

6. Hassan, S.M.; Metwally, M.E.S. and Abou Ouf, A.A., Determination of Certain Aminoquinoline Antimalarials Through Ternary Complex Formation, Analyst, 107, 1235, 1982.

7. Katakwar, T.K. and Kacchawaha, M.S., Microdetermination of Amodiaquine by Complexation Method, East.Pharm. 26, 129 (1983).

8. Mihaly, G.W.; Nicholl, D.D.; Edwards, G.; Ward, S.A.; Orme, M. L'E.; Warrell, D.A. and Breckenridge, A.M., High Performance Liquid Chromatographic Analysis of Amodiaquine in Human Plasma, J. of Chromatography, 337, 166, 1985.