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Comparative determination of halofantrine tablets by titrimetry, spectrophotometry and liquid chromatography

YT Kolade, OM Adegbolagun, OS Idowu, CP Babalola and AA Olaniyi
Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Ibadan, Ibadan, Nigeria

Summary
Comparative determination of halofantrine tablets by titrimetry, ultraviolet spectrophotometry and liquid chromatography (LC) is described. Non-aqueous titrimetry on halofantrine hydrochloride tablets was carried out using glacial acetic acid as solvent, perchloric acid as titrant and crystal violet as indicator. Simultaneous potentiometric monitoring of end point delineated an exact color shade of indicator at the end point. Direct measurement of methanol solution, at 254nm, was adopted for UV spectrophotometric method while reversed-phase liquid chromatographic (LC) method employed a C8 column (4.6mm x 25cm) with mobile phase consisting of methanol / 0.05 M NaH_2PO_4 (76:24, v/v) containing 55mmol/L perchloric acid (pH 3.4) at a flow rate of 1ml / min. The three methods gave precise and accurate results. Mean percentage recovery were obtained respectively as 100.73± 0.41, 100.36±0.79 and 99.93±3.74 % while coefficient of variation were 0.41, 1.36 and 3.74 % for non-aqueous, UV spectrophotometry and LC. The three methods were successfully applied to analysis of halofantrine tablets (Halfan®) and showed no statistically significant difference in accuracy ($P>0.05$, ANOVA). Validated assay methods for halofantrine tablets have been developed. The titrimetric and spectrophotometric methods are of equivalent accuracy with the liquid chromatographic method and could be used for routine quality control of halofantrine tablets where LC method is not readily available.

Keywords: *Halofantrine hydrochloride tablets, non-aqueous titrimetry, spectrophotometry, liquid chromatography.*

Résumé
Cette étude comparative décrit l analyse des comprimés d halofantrine par titrimétrie, spectophotométrie a ultraviolet et par chromotographie liquide.La titrimétrie non acqueuse des comprimés d hydrochloride d halofantrine était faite utilisant l acide glacial comme solvent, l acide perchlorique comme titrant et le crystal violet comme indicateur.La surveillance simultanée potentiométrique était a base du point final de déliénation à une couleur précise de l indicateur. Les mesures directe de la solution méthanol à 254 nm était adopté pour la méthode de spectophotométrique alors que la phase reverse de chromotographie liquide(CL) utilisait la colone C8 (46mm x 25mm) avec la phase mobile consistant du méthanol/0.05 NaOH2PO4(76.24.v/v) contenant 5.5 mmol/L d acide perchlorique(PH= 3.4) avec une coulée de 1ml/min. les 3 méthodes étaient bien appliquées pour analyser ces comprimés et donnaient des résultats précise et juste sans difference statistiquement ($P>0.05$, Anova). Les pourcentages de recouvrement du product étaient de 100.73 ±0.41, 100.36 ± 0.76 et 99.9 ± 3.74% respectivement avec un coefficient de variation de 0.4, 1.36 et 3.74 pour la solution non-acqueuse, uv-spectophotométrie et LC. Les méthodes de validation des comprimés d halofantrine ont été developés et peuvent être utilisées en routine pour le contrôle de la qualité des comprimés ou la technique de chromotographie liquide n est pas disponsible.

Introduction

Halofantrine (Hf) is a synthetic phenanthrene-methanol antimalarial [1], currently marketed as the hydrochloride, under the trade name Halfan®. Although its use as an antimalarial is banned in certain countries, on account of reported cardiotoxicity, [2-3], it is a highly effective drug for the treatment of malaria. The drug has proven efficacy against infection with chloroquine and or pyrimethamine resistant strains of *P. falciparum* [4, 5]. Moreover, there is on-going mechanistic delivery research, with the view to developing alternative formulation strategies that could alter the biopharmaceutical outcomes, and hence the safety profile of the drug [6]. These facts require accurate and precise assay methods for evaluating the quality of the delivery systems. High performance liquid chromatography (HPLC) has been used for the analysis of Hf in solid dispersions [7], whole blood [8] and also for the determination of Hf and its metabolites in plasma [9-11], packed red cell and urine [10].

Although no titrimetric or ultraviolet spectrophotometric method has been reported for the assay of Hf tablets, these methods are still relevant in the assay of pharmaceutical substances. A spectrophotometric method was recently reported for the analysis of ascorbic acid with reported superiority of performance over the official (B.P) assay method [12]. The discovery of widespread fake artesunate tablets in Cambodia generated a serious public health concern. This was informed by the poor-resource economy of Cambodia and the unaffordability of the sophisticated methods of analysis commonly reported in the analytical literature for its analysis. The lack of local ca-

Correspondence: Mrs. Y. T.Kolade, Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Ibadan, Ibadan, Nieria. E-mail: yomyetkol@yahoo.com

pacity to effectively carry out market surveillance of quality warranted the combined efforts of USAID and Center for Disease Control and Prevention, Atlanta, USA, to develop a simple method for detection of artesunate in a tablet [13].

The control of the quality of pharmaceutical preparations that requires the use of sophisticated equipment such as HPLC often presents difficulties in many developing countries where such instrument are not readily accessible. This often necessitates the use of alternative methods [14-16]. In order to effectively control the quality of halofantrine hydrochloride in developing countries such as most countries in sub-Saharan Africa where HPLC is not readily available, there is need for the development of simple inexpensive and readily available analytical methods that are of equivalent accuracy with HPLC method. In this paper, we report a comparative evaluation of non-aqueous titrimetric, UV spectrophotometric and liquid chromatographic methods of determining halofantrine in tablet dosage form.

Materials and methods

Chemicals and Reagents

Pure Halofantrine.HCl reference substance (Smithkline Beecham pharmaceutical SKF 102886-A, Batch No 3). Halofantrine hydrochloride tablets, (Halfan®, 250mg, Smithkline and French lab. Batch No 743 and 748). Concentrated $HClO_4$ (70%), Glacial acetic acid, (BDH, Analar, Poole, UK). Acetic anhydride, (BDH, Analar, Poole, UK). Potassium hydrogen phthalate (BDH, Analar, Poole, UK). Crystal violet, (BDH, UK). Mercuric acetate (99.9%) (BDH, UK). Methanol (Fischer's, HPLC grade). NaH_2PO_4 (BDH, Analar,Poole, UK)

Equipment

pH meter (model no 7020, Electronic Instrument Ltd, England). UV Visible scanning spectrophotometer (Unicam, Aurora, Helio Scan Software, v 1.1 Cambridge, UK). HPLC CE 1100 series 1000 liquid chromatography pump (Cecil, England). HPLC CE 1200 variable wavelength monitor (Cecil, England). C-8 column (5 µm, 250 x 4.6mm I.D.).

Non-aqueous titrimetry

Acetous perchloric acid (0.01M) was prepared and standardized using potassium hydrogen phthalate crystals. 0.0500g of pure Hf.HCl was dissolved in 15ml of glacial acetic acid with addition of about 1ml of mercuric acetate solution [17]. The solution was then titrated with the standardized 0.01M $HClO_4$ using crystal violet as indicator until a blue colour end point was obtained (colour at end point was determined potentiometrically in the presence of crystal violet indicator) [17]. The same procedure was carried out using 0.0100g and 0.1000g of pure Hf.HCl with addition of 0.2ml and 2ml of mercuric acetate solution respectively.

The assay depends on equation 1

$$C_{26}H_{30}ClF_3NOHCl + Hg(OAc)_2 + 2HClO_4 \rightarrow 2C_{26}H_{30}Cl_2F_3NOH^+ + HgCl_2 + 2CH_3COOH + 2ClO_4^- \quad \ldots\ldots(1)$$

Millequivalent of titrant 0.005162g of Hf.HCl

Ultraviolet spectrophotometry (UV)

0.0200g of pure Hf.HCl was weighed into a 50ml volumetric flask and dissolved with methanol and made up to volume. 1ml of the solution was taken with pipette into a 20ml volumetric flask and made to volume with methanol to obtain 20µg/ml stock solution. 1,1.5,2,2.5,3,3.5,4 and 5ml of the stock solution were each transferred into a 10ml volumetric flask and diluted to volume to prepare solutions with concentration 2,3,4,5,6,7,8 and 10µg/ml respectively. Absorption spectrum of methanol solution of Hf.HCl (8µg/ml) was recorded to determine the absorption maxima. Calibration line was plotted using methanol as blank and standard solutions of 0, 2,4,6,8, and 10µg /ml of Hf.HCl in methanol. Linear regression analysis was used to compute the slope, intercept and correlation coefficient (r) of the calibration lines.

High performance liquid chromatography (HPLC)

HPLC assay method was carried out on a C-8 column (5 µm, 250 x 4.6mm I.D.). The mobile phase consists of methanol / 0.05 M NaH_2PO_4 (76:24, v/v) containing 55mmol/L perchloric acid (pH 3.4). An ultraviolet detector was used to monitor eluates at 254 nm. Calibration line was plotted by using standard solutions of 200, 500, 2000, 4000, and 8000 ng/ml of pure Hf.HCl in the mobile phase. Linear regression analysis was used to compute the slope, intercept and correlation coefficient of the calibration line. 50µl aliquot of the samples was routinely injected into the liquid chromatograph which was equipped with a 20µl sample loop.

Analytical recovery, accuracy and assay precision of the three methods:

Analytical recovery using titrimetric method was estimated by comparing amount found by experiment with amount taken while the precision was assessed by replicate analysis of known amount of the compound. The analytical recovery for the UV spectrophotometric method was determined by replicate analysis of sample solutions from pure Hf.HCl such as to contain 3, 5 and 7 µg/ml and comparing concentration determined with concentration taken for the experiment. Analytical recovery for liquid chromatographic method was estimated by analysing samples prepared from pure Hf.HCl such as to contain 1000, 2500 and 4000 ng/ml and comparing concentration determined with concentration taken for the experiment. The precision for UV and HPLC methods were assessed by replicate analysis of known concentrations of the compound. These determinations were carried out as previously described [18]

Assay of Tablet Dosage form (Halfan®, batch 743 and 748)

Twenty tablets (Halfan®, 250mg) were powdered and the amount of powder equivalent to 0.0500g of Hf.HCl was weighed and dissolved in 15ml-neutralized glacial acetic acid and titrated against the standardized perchloric acid in replicates.

Powdered tablets equivalent to 20mg Hf.HCl were weighed into a 50 ml volumetric flask to get the stock solution. 5µg/ml solution was prepared from the stock solution and absorbance was measured at 254nm. Content of Hf.HCl in the tablet was then determined by interpolation from calibration line. 2000ng/ml solution of powdered tablets was prepared from the stock solution. Appropriate aliquot was injected into the liquid chromatograph and monitored as described above. Content of Hf.HCl in the tablet was then determined by interpolation from calibration line.

Statistical analysis

The relative merit of the three methods was evaluated by one-way analysis of variance (ANOVA) and P value less than 0.05 was taken as significant. ANOVA was performed by GraphPad Prism Version 4.01 for Windows [19]

Results

The indicator colour at end point which was monitored potentiometrically corresponded to blue. Mean percentage recovery of varying sample sizes of Hf.HCl is 100.73± 0.41% with a coefficient of variation of 0.41% (Table 1) The limit of quantitation was 0.0100g below which the method was not reproducible. Assay of the dosage form by this titrimetric method gave 100.77± 0.28% for batch 743 and 100.66± 0.89% for batch 748 (Table 2). Titration was carried out in the presence of, and also in the absence of undissolved particles of powdered Hf.HCl tablet and the undissolved particles did not interfere with the end point titre values.

Halofantrine has a fairly extensive chromophore, due to the phenanthrene nucleus (Figure 1). Absorption spectrum of Hf in methanol indicated that it has absorption maxima at 254nm and 310nm (Figure 2). Assay regression equation for the calibration line is $y = 0.1014x - 0.0066$ with r^2 of 0.9995. The overall recovery is 100.36± 0.79% and the coefficient of variation is 0.79%. The limit of quatitation was found to be 1i g/ml. Assay of dosage form gave 100.21± 0.72% for batch 743 and 100.62± 0.81 for batch 748 (Table2).

Hf had a retention time of 13 minutes in the chromatographic system employed. Assay regression equation for the calibration line is $y = 0.0004x + 0.0299$ with r^2 of

Table 1: Three-day assessment of accuracy and precision of the three assay methods for halofantrine

	Day 1 Mean±SD* RSD,%	Day 2 Mean±SD* RSD,%	Day 3 Mean±SD* RSD, %
Non aqueous titrimetry[a]			
Wt. of Hf.HCl (g)			
0.01	100.40±1.55 1.54	100.4±1.55 1.54	101 30±0 0
0.05	100.07±0.63 0.63	100.61±1.40 1.39	100.97±1.14 1.13
0.10	100.70±0.16 1.59	101.22±1.33 1.33	100.89±1.23 1.22
UV spectrophotometry[b]			
Conc. ((µg/ml)			
3	99.20±1.97 1.99	99.97±4.89 4.89	99.70±2.53 2.54
5	101.80±4.09 4.02	101.32±0.30 0.30	100.38±1 82 1 81
7	100.36±3.26 3.26	100.28±0.37 0.37	100.23±1.96 1.96
Reversed phase liquid chromatography[c]			
Conc. (ng/ml)			
1000	98.51±5.91 5.91	93.97±1.00 1.06	94.98±1.31 1.38
2500	101.79±1.32 1.30	105.08±0.80 0.76	103.22±0.72 0.75
4000	99.69±0.87 0.87	102.8±0 0	98.93±1.20 1.21

**n=3*

a *Between day statistics = 100.73±0.41%(mean±s.e.m), RSD(of s.e.m) = 0.41%*

b *Between day statistics = 100.36±0.79% (mean±s.e.m.), RSD (of s.e.m) = 1.36%*
regression equation y=0.1014 x-0.0666, r^2 =0.9995

c *Between day statistics =99.93±3.74% (mean±s.e.m), RSD (of s.e.m) = 3.74%*
regression equation y=0.0004 x+0.0299, r^2 =0.9995

0.9995. The overall recovery is 99.93± 3.74% and a coefficient of variation of 3.74%. The limit of quantitation is 200ng. Assay of dosage form by this method gave 101.08± 2.65% for batch 743 and 101.61± 2.67% for batch 748 (Table 2).

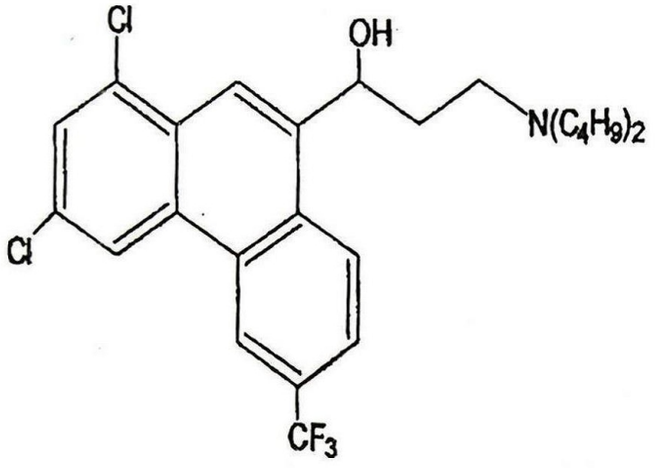

Fig. 1: Chemical structure of halofantrine

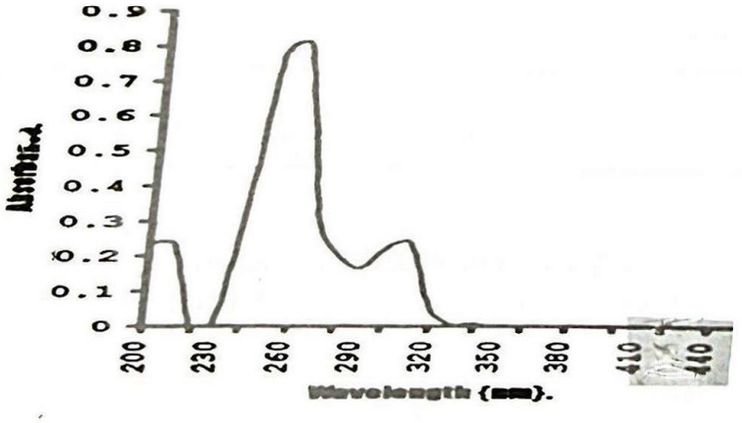

Fig. 2: Absorption spectrum of halofantrine in methanol.

Table 2: Assay of halofantrine tablets by the three assay methods

Batch of Halfan®	Mean ±SD*		
	Titrimetry	UVspectrophotometry	Reverse phase liquid chromatography
743	100.77±0.28%	100.21±0.72	101.08±2.65%
748	100.66±0.29%	100.62±0.81%	101.61±2.67%

*$n = 5$

One-way Analysis of variance indicates that the relative performance of the three methods show no statistically significant difference in their accuracy ($p>0.05$, ANOVA) (Table 3).

Table 3: One-way analysis of variance (ANOVA) of the mean percentage content of Halofantrine tablets obtained by the three methods.

Halfan® Batch	P value*	P value summary
743	0.6900	Not significant
748	0.5652	Not significant

*P 0.05 is taken as significant.

Discussion

Non-aqueous titrimetric assay method was considered because of the weak amino group in Halofantrine. Hf is a weak base and not very soluble in water. It dissolves in acetic acid readily and it acts as a relatively stronger base in acetic acid than in water. The solvent used was neutralized to avoid interference of titritable impurities with assay results. The colour at end point using crystal violet as indicator was determined potentiometrically in the presence of indicator because there is no established end point colour for Hf.HCl. with this indicator [17]. Filtration of excipients from dissolved powdered tablet before titration was found unnecessary, as titre values in the presence of and in the absence of undissolved particles were the same. From the results obtained (Table 2), titrimetric method developed is simple, accurate and precise in the determination of Hf.HCl.

Spectrophotometric determinations were carried out at 254nm, an absorption maximum, as shown in Figure 1. In measuring the absorbance of a sample from the tablets, methanol was used as blank solvent. Liquid chromatographic method has been used in the determination of Hf in pharmaceuticals [7] and plasma [9-11], it is also increasingly been adopted in compendial assay methods for routine quality control. Hence, the application of HPLC was found relevant for the assay of Hf.HCl tablets in this study.

All the three methods investigated were reasonably precise and of equivalent accuracy in the assay of Hf.HCl. This relative performance was assessed by one-way analysis of variance (ANOVA) of the percentage content of the two batches of tablets evaluated by the three methods. However, titrimetry was more precise than UV spectrophotometric method and this in turn more precise than liquid chromatographic method. This is typical of classical methods as they are usually more precise than instrumental methods [20]. UV spectrophotometric assay method

developed in this study is however faster in the determination of Hf.HCl than titrimetry and liquid chromatography. The titrimetric method requires painstaking standardization of volumetric solutions. Liquid chromatographic method takes the longest time as each sample has a retention time of 13 minutes. it however has the distinct advantage over the other assay methods in requiring less sample size. Titrimetry requires the largest sample size for effective quantitation on account of its much higher limit of detection. but it requires no reference standard for routine application. Overall. titrimetry is more affordable than UV spectrophotometric and this in turn more affordable than liquid chromatographic method. which requires sophisticated instrumentation. Acquisition and maintenance cost of LC chromatograph often put routine use of methods based on this technique at a disadvantage especially in poor resource economies.

Conclusion

Validated titrimetric. spectrophotometric and liquid chromatographic methods have been developed for the assay of halofantrine tablets. The titrimetric and spectrophotometric methods are of equivalent accuracy with the liquid chromatographic method. These alternative methods could therefore be reliably employed for routine quality control of halofantrine tablets especially in poor resource economies where liquid chromatography is not generally available.

References

1. Colwell WT. Brown V. Christie P. lange J. Reece. L. Yanamoko K and Henry DW. Anti malarial amino propanol JMed Chem. 1972;15 (7): 771-775.
2. Matson PA. Luby SP. Redd SC. Rolka HR and Meriwether RA. Cardiac effects of standard dose halofantrine therapy. Am. J. Med. Hyg. 1996; 54(3): 229-231.
3. Monlun E. Le Metayer. P. Szwandt S. Neau D. Longy-Boursier M. Horton J and LeBras M. Cardiac complications of halofantrine: a prospective study of 20 patients. Transactions of the Royal Society of Tropical Medicine and Hygiene 1995; 89: 430-433.
4. Watkins W M. Lury JD. Kariuki D. Koech D K. Oloo JA. Mosoba M. Mjomba M and Gilles HM. Efficacy of multi-dose Halofantrine in the treatment of chloroquine resistant falciparum malaria in children in Kenya. Lancet 332 (1988) 247-250.
5. Terkuile F.O.. Dolar G. Nostar F. Halofantrine versus mefloquine in the treatment of multi drug resistant falciparum malaria. Lancet 1998: I: 1044-1049.
6. Lim. LY. and Go. ML. Caffeine and nicotinamide enhances the aqueous solubility of the antimalarial agent halofantrine. European Journal of Pharmaceutical Science. 2000: 10 (1): 17-28.
7. Ahmad M.. Abdul F and Hridaya NB. .A new high performance liquid chromatographic method for the analysis of halofantrine in pharmaceuticals. Journal of Pharmaceutical and Biomedical Analysis 2002; 29 (5): 901-908.
8. Gawienowski M. Benet LZ. Fleckenstein L and Lin ET Ion paired liquid chromatography method for the analysis of blood and plasma for the antimalarial drug halofantrine and its putative monodesbutylated metabolite. Journal of Chromatography. 1988; 430: 412-419.
9. Bassi PU. Onyeji CO. Otas E and Ukponwan E Effects of tetracycline on the pharmacokinetics of halofantrine in healthy volunteers. British Journal of Clinical Pharmacology. 58 (1): 52.
10. Milton KA. Ward SA and Edward G. Determination of Halofantrine and its principal metabolite desbutyl Halofantrine in biological fluids by reverse phase high performance liquid chromatography. Journal of chromatograraphy. 1988; 433: 339-344.
11. Kolade YT Babalola CP and Scriba G.K. Analysis of the antimalarial drug halofantrine and its major metabolite N-desbutyl halofantrine in human plasma by high performance liquid chromatography. Journal of Pharmaceutical and Biomedical Analysis 2005: Dec 5 (Epub ahead of print)
12. Wenming Z.. Frank M and Alexander M. Development and application of a novel UV method for the analysis of ascorbic acid . Journal of Pharmaceutical and Biomedical Analysis. 2005; 36: 1107-1111.
13. A field Method for the Assay of Artesunate in Tablets; http://www.paho.org/english/ad/dpc/cd/mal-fake-artesunate-who.htm
14. Kilic F. Koseoglu F and Akay MA. The non-aqueous titrimetric assay of selected antibiotics using tetra-n-butyl ammonium hydroxide as titrant. Journal of Pharmaceutical and Biomedical Analysis. 1994; 12 (3): 347-352.
15. Olaniyi AA (Ed). Towards better quality assurance of drugs and food. Proceeding of a National Workshop. Department of Pharmaceutical Chemistry. University of Ibadan. 1993.
16. British pharmacopoeia. Her Majesty's stationery office. London. 1998; Vol.1: 1793.
17. Pifer CW and Wolish EG Non-aqueous determination of hydro halide salt of amine using mercuric acetate. Anal. Chem. 1952:24: 24-30
18. Validation of Compendial Methods. 2000: United States Pharmacopoeia 24/NF 19. 2149-2152. The United States Pharmacopoeial Convention Inc.. 12601 Twinbrook Parkway. Rockville MD. USA.

19. GraphPad Prism Version 4.01 for Windows (2004). GraphPad Software. San Diego. California USA. www.graphpad.com
20. Williams HH. Marritt LL (Jr). Dean JA. Seltle F.A and (Jr). An introduction to instrumental method of analysis. In: Instrumental method of Analysis. Ed.7. Belmont California USA. Wordsworth Publishing Company.1988: 6-7.

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