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Research Article

Development and Validation of Novel Hydrotropic Solubilization Method for Spectrophotometric Determination of Halofantrine in Pure and Solid Dosage Form

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Abstract: Simple spectrophotometric method for assay of halofantrine, a poorly aqueous soluble drug, in pure and tablet dosage forms by the principles of single hydrotropic solubilization using 4 M sodium acetate and 5 M sodium citrate was developed and validated. Solubility of halofantrine in distilled water and each of the hydrotropes were determined. The percentage label claim of halofantrine in bulk and tablet forms was estimated spectrophotometrically. The method was validated for accuracy, linearity, precision, specificity and robustness. Findings showed that solubility of halofantrine was enhanced by a factor of 11 and 18 in 4 M sodium acetate and 5 M sodium citrate respectively. Beer-Lambert's calibration curves were linear at concentration of 2-20 µg/mL (r^2=0.999 and % RSD < 2 both intra- and inter-day). Analysis of commercial tablets were found to contain 99.6 – 101.6 % of halofantrine while % recoveries in both hydrotropes range from 99.9 to 101.4, % RSD and % CV were < 2 with low standard error. Intra- and inter-day CV were <0.40, LOQ and LOD were 0.925, 0.742 and 0.627, 0.420 in both hydrotropes respectively. The developed method was simple, specific, selective, reproducible and robust for routine analysis of halofantrine in pure and tablet dosage forms.
Keywords: halofantrine, validation, spectrophotometry, recovery, label claim, hydrotrope.

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INTRODUCTION

Solubilization of drugs involves spontaneous breaking of intermolecular bonds in solutes and separation of solvent to create spaces for solvent-solute interaction [1]. The importance of solubility in drug formulation and development cannot be over emphasized due to the relationship between drug solubility and drug bioavailability. Various techniques have been employed to enhance solubilization of poorly water-soluble drugs such as hydrotropic solubilization [2-6], chemical [7-11] and physical [12-16] modifications and other techniques [17-21]. Such modifications have resulted in improved pharmacokinetics and pharmacodynamics profile of drug molecules in many instances. Solubilization of poorly water-soluble by hydrotropy has attracted researchers' interests in the recent times because more than 40 % of hydrophobic new chemical entities, with good pharmacodynamic activity, do not get to the market due to problems associated with solubility. Hydrotropy, which involves solubilization of poorly water-soluble drugs by addition of large amount of another solute, has several advantages over other solubilization techniques because the solvent character is independent of pH or ionization characters of the drug, it is highly selective and does not require emulsification process rather it involves simple mixing with hydrotrope in distilled water. The technique is also devoid of any chemical modification of the hydrophobic molecule or use of any organic solvents [22]. Apart from the application of hydrotropy in quantitative estimation of poorly water-soluble drugs in bulk and dosage forms, which this study intends to achieve, the principle has been successfully applied in development of injection dosage forms of poorly water-soluble drugs using mixed-hydrotropes, preparation of topical solution of poorly water-soluble drugs using sodium benzoate and sodium citrate as permeation enhancers, preparation of dry syrup and solid dispersions of poorly water-soluble drugs using polyethylene glycol 6000, PEG 6000 and polyvinyl alcohol, PVA, and in quantitative estimations of poorly water-soluble drugs by tritrimetric analysis using sodium benzoate [23].

MATERIALS AND METHODS

Equipment, Chemicals and Reagents

The pure halofantrine powder used for the study was obtained from GSK Pharma,, South Africa. Tablet dosage forms of halofantrine (Halfan®, Halofantrin® and Adfantrin®) were randomly purchased from an open drug market in Nigeria. UV-Vis spectrophotometer (Jeenway Model 6305, England), methanol (Sigma-Aldrich, Germany), sodium citrate and sodium acetate (May & Baker, England) were also used. All chemicals/reagents and equipment were used as received without further purification or re-calibration.

Determination of Minimum Hydrotrope Concentration (MHC)

Excess halofantrine was dispersed in 5 mL of different molar concentration (1.0, 1.5, 2.0, 2.5, 3.0, 4.0, 5.0, 7.5 and 10.0 M) of each of sodium acetate and sodium citrate in a 10 mL boiling tube fitted with a mercury seal. The tubes were shaken with a fabricated shaking machine for 1 h to attain equilibrium. The equilibrated solution was filtered through a Whatman filter No. 41 and the absorbances recorded at 290 nm against sodium acetate and citrate blanks. A plot of absorbance against molar concentration of hydrotrope was done to determine the MHC.

Standard Calibration of Halofantrine

The standard stock solution was prepared by dissolving 10 mg of pure halofantrine in 50 mL sodium acetate. Six working solution was prepared by serially diluting aliquot of the sample with sodium acetate to prepare 1, 2, 4, 5, 10 and 20 µg/mL. The absorbances were recorded in triplicate at 290 nm using sodium acetate solution as blank. The procedure was repeated in sodium citrate. The calibration curves (absorbance verses halofantrine concentration) were constructed by measuring standard solutions of halofantrine for every series of samples. Validation of the method was performed to ensure that the calibration curves between 1 and 20 µg/mL were in the linearity range of the assay and the coefficients of variation were less than 2 % both intra-day and inter-day.

Preliminary Solubility of Halofantrine in Hydrotropes

The solubility of halofantrine was determined by saturation aqueous solubility technique [24] in 4 M sodium acetate, 5 M sodium citrate and double distilled water maintained at 28 ºC in a water bath equilibrated for 24 h. The temperature uniformity within the water bath was maintained at ±0.5 ºC to eliminate the effect of temperature on solubility of halofantrine. The solutions were filtered through Whatman filter No. 41 and filtrates diluted appropriately and assayed for halofantrine spectrophotometrically at 290 nm against solvent blank [25].

Analysis of Halofantrine in Tablets Dosage Forms

Twenty halofantrine tablets from each brand were powdered. A powder equivalent to 250 mg halofantrine was dissolved in 50 mL of each of the hydrotrope in a 100 mL volumetric flask and shaken for 10 minutes to dissolve. The solutions were filtered through Whatman filter No. 41. One part of each of the filtrates was diluted and assayed spectrophotometrically at 290 nm for halofantrine while the other parts were similarly assayed after 48 hours.

Proposed Method Validation

Recovery Studies (Accuracy)

Tablet powder equivalent to 100 mg halofantrine was dissolved in three 50 mL of each of the hydrotropic solution in a 100 mL volumetric flasks and each spiked with 20, 40 and 80 mg of pure halofantrine. The flasks were shaken to dissolve the drug and filtered through Whatman filter No. 41 and assayed spectrophotometrically to calculate the drug contents and percentage recoveries.

Linearity

Five independent serially diluted volumes of stock solution of halofantrine each corresponding to 2-20 µg/mL was prepared in each of the hydrotrope. Each solution was analyzed in triplicate to obtain the linear calibration curve.

Precision

Precision of the analytical method was determined and expressed in relative standard deviation (% RSD) of series of measurements. In inter-day variation, the absorbances of standard solutions of halofantrine (2-20 µg/mL) were measured on three consecutive days; while in intra-day variation the absorbances were measured three times in a day. In repeatability study, six independent determinations of the fixed amount of halofantrine were analyzed separately.

Limit of Detection (LOD) and Limit of Quantitation (LOQ)

LOD is the smallest concentration of analytes that produces measurable responses while LOQ is the smallest concentration of analytes which gives response that can quantified accurately. They are calculated from $3.3\delta/S$ and $10\delta/S$ respectively; δ where is the standard deviation of the response, S is the slope of the calibration curve.

Specificity

The specificity of the proposed method was determined by comparing the UV spectra obtained from 10 µg/mL solution of halofantrine in pure and commercial samples in order to assess the level of interference from excipients and additives used in making commercial dosage forms of the drug.

RESULTS AND DISCUSSION

The results of solubility of halofantrine in distilled water and hydrotropic agents show that the solubility enhancement was found to be more than 11 in sodium acetate and 18 in sodium citrate. Table 1 shows the results of analysis of halofantrine in commercial formulations using the developed method. The method was able to obtain nearly 100 % of halofantrine in the dosage forms. Table 2 shows the results of recovery of halofantrine from tablets using the proposed method. The recovery of halofantrine from tablets ranges from 99.9 % to 101.4 % which lies within the acceptable range while Table 3 shows the results of validation of the proposed method. The method is precise, rugged, and robust and can be repeated to obtain accurate data by analyst to analyst on same or different days. Figure 1 shows the chemical structure of halofantrine. Figure 2

shows the minimum hydrotrope concentration (MHC) of the hydrotropic agents used for the study. This is a measure of the smallest molar concentration of the hydrotrope that can increase the aqueous solubility of hydrophobic molecules without altering the chemical functionality of either the solubilizate or the solubilizer. Figure 3 shows the calibration curves of halofantrine in different hydrotropes. The calibration curves are linear within 2-20 µg/mL of halofantrine with high correlation values (r^2>0.9) and y=ax±c, where c=0 in both hydrotropes. Figure 4 are overlain spectra of pure halofantrine and halofantrine in tablet formulations. The appearances of similar spectra (ʎ max 290±1.5 nm) of pure and tablet dosage forms in both hydrotropes show that the excipients used in formulation of the halofantrine dosage forms do not interfere significantly with the developed method.

Table 1: Analysis of Halofantrine in Tablet Formulation

Hydrotrope	4 M Sodium Acetate			5 M Sodium Citrate		
Tablet Formulation	I	II	III	I	II	III
Label claim (mg)	250	250	250	250	250	250
% Label estimate	99.8	100.4	100.8	101.6	100.4	99.6
% Coefficient of variation	0.96	0.63	1.02	0.94	1.09	1.01
Standard error (n=6)	0.84	0.72	0.86	0.63	0.72	0.80

Table 2: Results of Recovery Studies of Halofantrine

Hydrotrope	Formulation	Label claim (mg)	Spiked qty (mg) SE	% Recovery*±SD
4M Sodium Acetate	I	250	20.0 0.287	100.9±0.972
	II	250	40.0 0.835	101.4±1.002
	III	250	80.0 1.002	100.6±0.168
5M Sodium Citrate	I	250	20.0 0.483	99.9±1.482
	II	250	40.0 0.972	100.1±0.396
	III	250	80.0 0.845	100.7±0.593

*Mean of six determinations.

Table 3: Results of Validation Parameters and Optical Characteristics of Proposed Method

Parameters	4M Sodium Acetate	5M Sodium Citrate
Established $ʎ_{max}$ (nm)	290	291
Beer's Limit (µg/mL)	2-20	2-20
Molar Absorptivity	4.35 x 10^4	3.80 x 10^4
Correlation Coefficient*	0.999	0.999
Intercept*	0.00	0.00
Slope*	0.043	0.037
LOD* (µg/mL)	0.627	0.420
LOQ* (µg/mL)	0.925	0.742
Intra-day* (CV)	0.278	0.348
Inter-day* (CV)	0.275	0.252
Robustness	Robust (%RSD<2)	% RSD <2
Ruggedness	Rugged	Rugged

*Mean of 6 determinations. CV = coefficient of variation, RSD=relative standard deviation.

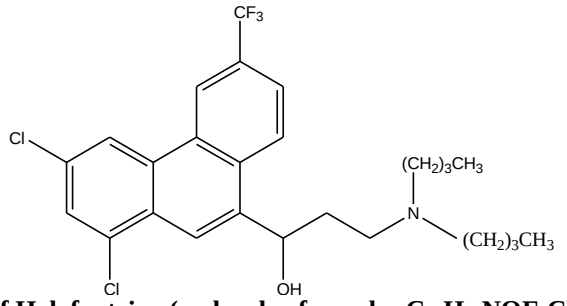

Figure 1: Chemical Structure of Halofantrine (molecular formula: $C_{26}H_{30}NOF_3Cl_2$, molar weight: 536.89 $gmol^{-1}$, IUPAC name: 1, 3-dichloro-α-[2-(dibutylamino) ethyl]-6-trifluoromethyl-9-phenanthrene methanol)

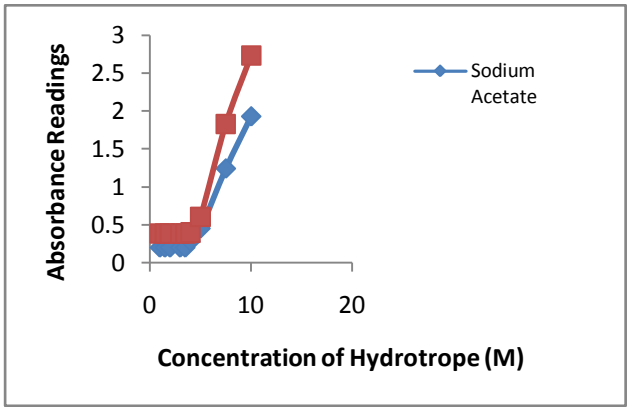

Figure 2: Minimum Hydrotrope Concentration (MHC) of Sodium Acetate (4M) and Sodium Citrate (5M)

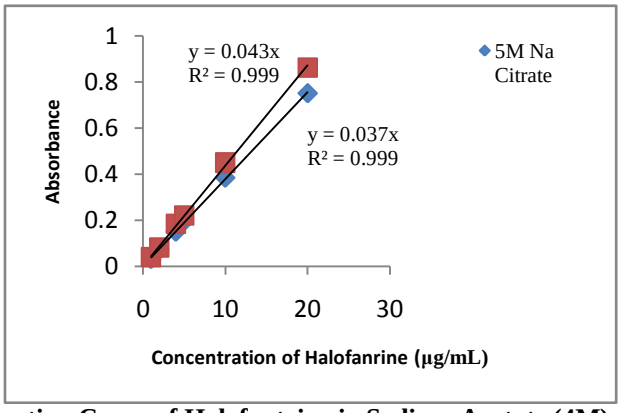

Figure 3: Standard Calibration Curve of Halofantrine in Sodium Acetate (4M) and Sodium Citrate (5M)

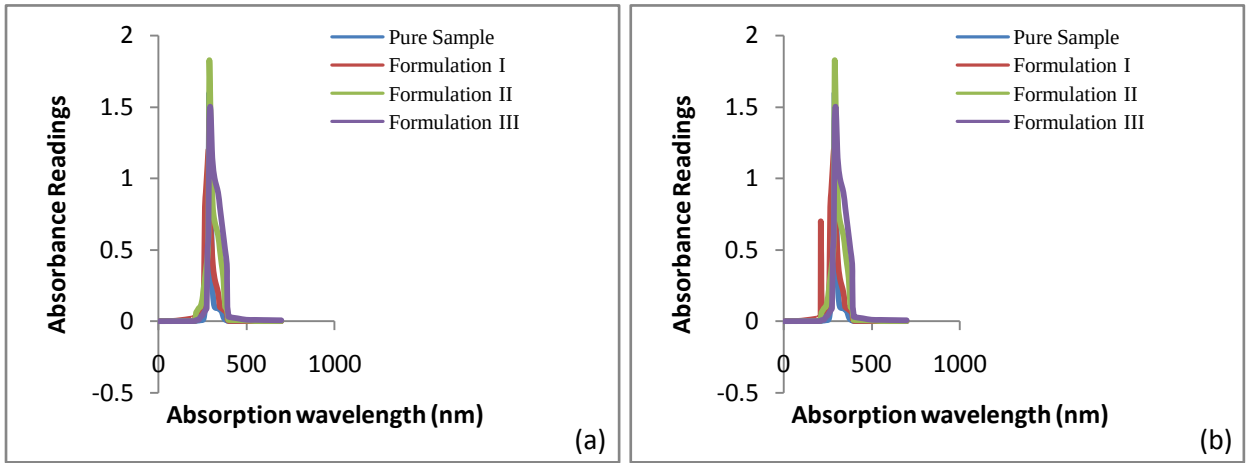

Figure 4: Overlain Spectra of Pure and Tablet Formulations of Halofantrine in: (a) 4M Sodium Acetate (b) 5M Sodium Citrate.

The solution of the drug analyzed after 48 h did not show any sign of precipitation which shows that degradation did not occur in the solution and this method of analysis can be performed within such period with acceptable level of accuracy and precision. The development of this method was considered significant since hydrotropes, like surfactants, contain both hydrophilic and lipophilic parts but do not have critical micelle concentration (CMC) and thus do not self-aggregate in solution with the drugs. This method can be selectively applied in the routine analysis of halofantrine in commercial formulation as the spectra of the pure drug and its tablet formulations remain unchanged in both hydrotropic solution.

CONCLUSION

The developed method is novel, simple, selective, cheap, precise, and accurate and can be conveniently applied in routine analysis of halofantrine in solid dosage forms of the drug. The method is also eco-friendly because the method does not involve the use organic solvents which can be hazardous to the analyst and environment.

Acknowledgement/Authors' Contributions

Authors appreciate Chinenye, Chidera and Chibundo Charles-Nnadi for their technical supports. Mrs. Ngozi Justina Nwodo (Ph.D) designed and supervised the research; edited and approved the manuscript for publication. Nnadi, Kenneth Ifeanyi carried out the bench work while Nnadi Charles Okeke analyzed the data generated and put up the manuscript for publication.

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