British Journal of Pharmaceutical Research
6(5): 313-321, 2015, Article no.BJPR.2015.073
ISSN: 2231-2919

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Extractive Ion-Pair Spectrophotometric Assay of Amodiaquine

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Authors' contributions

This work was carried out in collaboration between all authors. Author SAA proposed the study, using ion-pair as a model. Author FAO carried out the research work under the supervision of authors SAA and CJE (who modified the concept). Author FAO wrote the protocol, wrote the first draft of the manuscript; managed the literature searches, analyses of the study and performed the spectroscopy analysis. Authors CJE and SAA advised, managed the experimental process and result interpretations. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/BJPR/2015/15084
Editor(s):
(1) Rafik Karaman, Bioorganic Chemistry, College of Pharmacy, Al-Quds University, USA.
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Complete Peer review History: http://www.sciencedomain.org/review-history.php?iid=986&id=14&aid=8844

Original Research Article

Received 5th November 2014
Accepted 28th March 2015
Published 16th April 2015

ABSTRACT

The quality of antimalaria drugs is a concern in malaria treatment and management. The quality of these drugs can be monitored and assured if simple, sensitive and accurate methods are available for routine use. Thus this extractive ion-pair spectrophotometric method was developed. Ion-pair was formed from equimolar concentration (10^{-4} M) of amodiaquine (5 ml) and bromocresol green (10 ml) in phthalate buffer (pH 5, 10 ml). The ion-pair was extracted into chloroform; re-extracted into 0.1 M sodium hydroxide; and determined spectrophotometrically. International Standard Requirements validation parameters were determined to evaluate the method applicability. The recovery data of the method was statistically compared with those obtained using nonaqueous titration. The extract absorbed maximally at 620 nm. The extract showed a stoichiometry of 1:2. Beer's law was obeyed in the concentration range of 1-50x 10^{-4} M of amodiaquine with good linearity (R> 0.99). A good accuracy was obtained from recovery studies. The extract was stable

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over 48 hours. The method complied with International Standard Requirements. The method compared positively with nonaqueous titration. Thus the method is good for amodiaquine analysis, with increased sensitivity from re-extraction into 0.1M sodium hydroxide. The method is simple; equipment and materials are readily available and no significant interference from common excipients.

Keywords: Amodiaquine analysis; ion-pair extraction; ion-pair formation; bromocresol green; drug-dye complex.

1. INTRODUCTION

Malaria is endemic in Africa. It is the leading cause of mortality in Africa [1,2]. The treatment of malaria has been approached in many ways, with resistance to drug therapy leaving little to desire [1,2]. One of the leading causes of drug resistance & drug failure is poor quality of drugs and poor quality control [3]. The quality of antimalaria drugs can be routinely monitored and assured if simple, sensitive and accurate methods are available for routine use

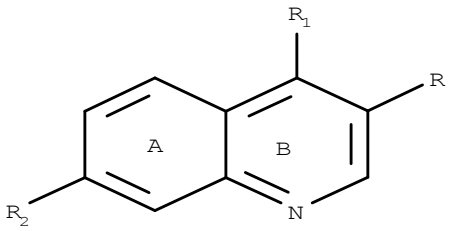
Ion-pair is an outer sphere complex, which possesses some unique properties that are different from those of its constituents. An ion-pair behaves as one unit in determining certain properties. The use of ion- pair in drug analysis dates back into time. Its uses can be seen more in chromatography, where it is used to selectively manipulate the retention time of analyte for the desired separation, and also in some spectrophotometric methods of analysis, where a coloured extractible ion-pair is form and determined spectrophotometrically [4-9]. Most of these reported extractive ion- pair spectrophotometric methods have the challenge of absorbing at similar or close wavelengths with the drug. Amodiaquine solution, for instance, has same colour with the organic extract of the ion-pair and absorbed at similar wavelength with the organic extract of the ion-pair; thus the need to have a higher wavelength of absorbance measurement, which made a possible re-extraction to come handy here. Also, there is the challenge of volatility of the organic solvent. This challenge could be taken care of by re- extraction into an aqueous phase, with practically no volatility problems. Thus this research work sought to fill these research gaps, by developing a method for amodiaquine analysis that would address these challenges.

Basic drugs in acid medium can form cations that can interact with anions of dyes in ion associate complex called ion- pairs. These ion- pairs are extractible and have unique properties different from their constituents [10]. The availability of the unshared electron pair on nitrogen and the relative stabilities of the ammonium ion define the basicity.

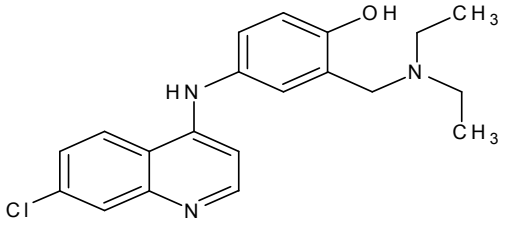
$$N^+ + D^- \leftrightarrow [ND]$$

Where N^+ = the protonated basic drug; D^- = the anion of the dye and [ND] = the ion- pair of the protonated drug and anion of the dye.

Amodiaquine is a basic drug with quinolines nucleus. Other examples of antimalaria drugs with quinolines nucleus include chloroquine, mefloquine and quinine.



Quinoline nucleus



Amodiaquine, 4-[(7-chloroquinolin-4-yl)amino]-2-[(diethyl amino)methyl]phenol

2. METHODS

The developed method involved amodiaquine analysis through the formation of ion- pair by mixing of equimolar concentrations of amodiaquine (5 ml) and bromocresol green (10 ml) in phosphate buffer (10 ml) at buffer pH of 5. The mixture was shaken vigorously to allow the reaction to equilibrate. The ion-pair so formed was isolated through a two- step extraction of the formed ion-pair: into organic phase of chloroform and subsequently into aqueous phase of 0.1 M sodium hydroxide.

The absorbance reading of the 0.1 M sodium hydroxide extract of the ion-pair was read at wavelength of maximum absorbance of 620 nm, which was previously determined. The drug concentration of the amodiaquine was determined from the calibration plot by extrapolation of the absorbance reading. The colourless blank in all the cases considered has practically negligible absorbance

The following International Standard Requirements validation parameters were carried out, to ascertain the reliability of the developed method: The effect of the buffer pH on absorbance of the 0.1 M sodium hydroxide extract of ion- pair. The linearity of the analytical method was determined. The stoichiometry of the ion- pair formed was determined using Job's method [11]. The stability of 0.1 M sodium hydroxide extract was estimated, by measuring the absorbance as a function of time. The identity of constituent of sodium hydroxide extract was determined, using comparison of UV- visible spectrum scan of the 0.1 M sodium hydroxide extract of control with those of the 0.1 M sodium hydroxide extract of drugs. The recoveries of the drugs from standard solutions, in presence of common excipients, from tablets and suspensions were also determined [12-17]. Limit of detection and limit of quantitation were also carried out [18,19]. Amodiaquine was also assayed with classical nonaqueous titration as a parallel determination and the results compared with those of the developed method. The results obtained were statistically treated, using standard error of mean, student t-test (paired- t-test) where necessary.

3. RESULTS

Chloroform blank was near colourless, not absolutely colourless, in most buffer pH range. 0.1 M sodium hydroxide blank was colourless from buffer pH 5- 14. This means that at lower pH (< 5) the organic solvent extracted the ionic bromocresol green. This was evident due to re-extraction into 0.1 M sodium hydroxide solution. This re-extraction greatly enhanced the selectivity and sensitivity of the developed method. Reported ion-pair analytical works based on determination of the organic extract have inherent errors, in addition to the problem of extract evaporation due to solvent volatility. These inherent errors and complications were taken care of working with an ideal buffer pH and with the two-step extraction.

The colour of the 0.1 M sodium hydroxide extracts of ion- pair complex at buffer pH 1- 14 was blue. In the first extraction into chloroform, the blue aqueous layer faded with increasing concentration of drug. Also, increased intensity of the yellow- wine coloration of the chloroform extracts as the drug concentration increased. The blue coloration of the 0.1 M sodium hydroxide extract increased with increased drug concentration.

3.1 Wavelength of Maximum Absorbance

As earlier noted, most of the reported extractive ion- pair spectrophotometric methods have the challenge of absorbing at similar or close wavelengths with the drug and the challenge of volatility of the organic solvent ion-pair extract. The re-extraction into 0.1 M sodium hydroxide (aqueous phase) offered a better wavelength of maximum absorbance and also addressed the volatility problem associated with the organic phase. The 0.1 M sodium hydroxide ion-pair extract absorbed best at 620 nm as shown in Fig. 1. Amodiaquine absorbed maximally at about 420 nm and bromocresol green at about 620 nm. This higher wavelength of maximum absorption enhanced the specificity and selectivity of the developed method, as it excluded all irrelevant absorptions that might undermine the method accuracy, specificity and selectivity.

3.2 The Effect of pH

The developed method ideal pH of ion-pair formation, extraction and analysis was pH 5 as illustrated in Fig. 2. This was the pH at which maximum ionic components necessary for maximum ion-pair formation and extraction was best guaranteed. Also, at this pH the organic solvent, chloroform extracted only the neutral species, ion-pair without extracting the dye, bromocresol green. The blank determination was actually colourless from pH 5.

3.3 Drug-dye Stoichiometry

The drug-dye stoichiometry was determined at 1:2 ratio combinations as shown in Fig. 3, with a mole fraction of about 0.6. This was in tandem with number of effective electron donor atoms present in the structure- the basic centers.

3.4 Beer's Law Linearity Plot

The linearity or calibration plot shown in Fig. 4 showed a good linearity, with a linear regression of 0.9972. This means that Beer's was obeyed, with a good relationship between the drug concentration and absorbance of the ion-pair extract; showing that the method is analytical. Beer's law was obeyed in the concentration range of 1-50 x 10^{-4} M of amodiaquine.

3.5 Chemical Reaction between Bromocresol Green and Amodiaquine

The proposed equation of reaction of the amodiaquine cation with the anion of bromocresol green is illustrated in Fig. 5 below. This is in agreement with the stoichiometric information in the Job's plot in Fig. 3 and in tandem with the number of effective electron donor atoms present in amodiaquine.

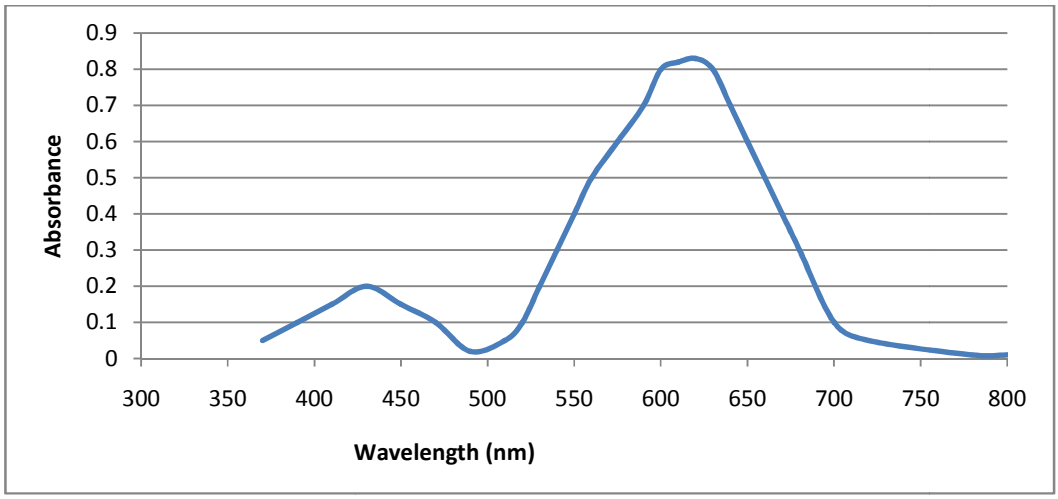


Fig. 1. Spectra of ion-pair extracts showing the wavelength of maximum absorbance of ion-pair extract at 620 nm

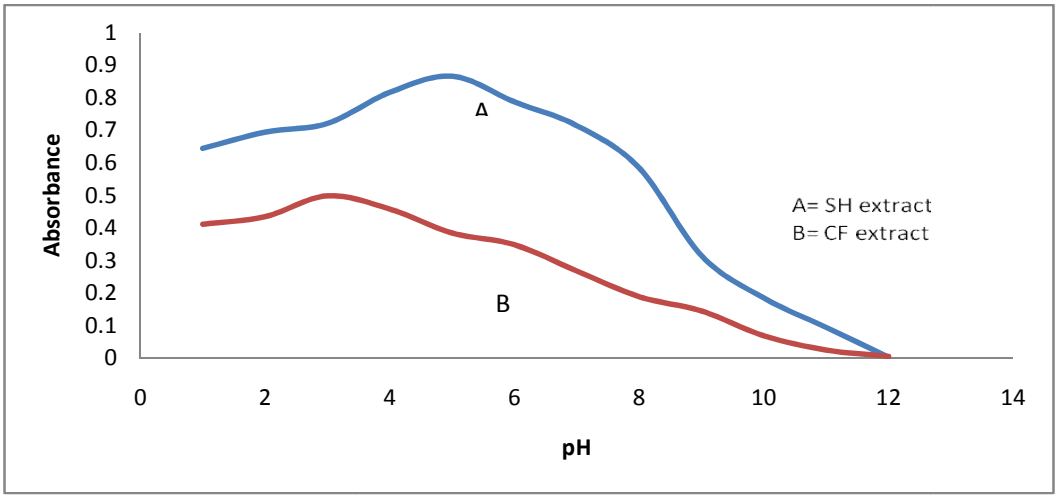


Fig. 2. Effects of pH of buffer on extraction and absorbances of chloroform (B) and sodium hydroxide (A) extracts of AQ-BG Ion-pair

Fig. 3. AQ-Dye stoichiometric relationship plot, with Inflection at 0.66

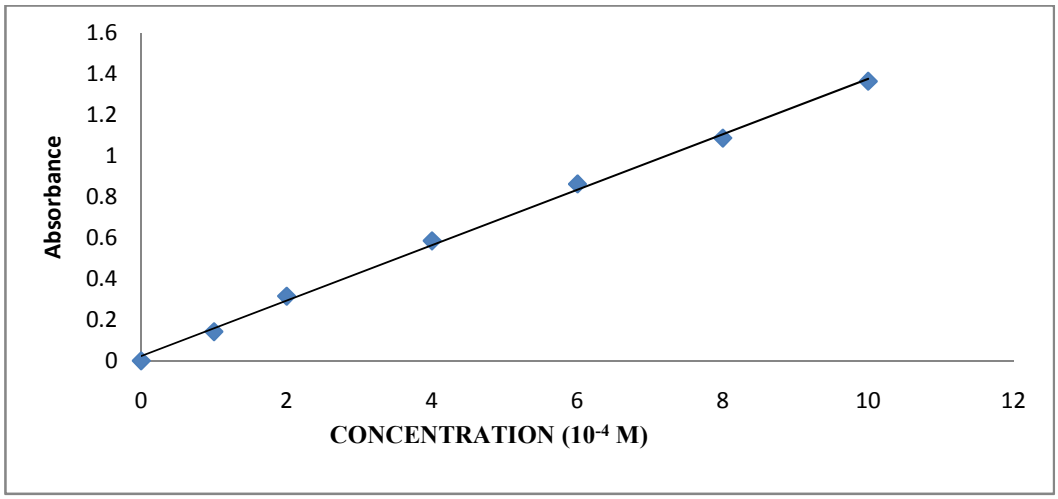


Fig. 4. Showing the calibration plot of AQ- BG ion- pair complex

3.6 Stability of Extract over Time

The 0.1 M sodium hydroxide extract is very stable over 48 hours as shown in Fig. 6. This stability window or duration is enough to allow for repeat determination of the 0.1 M sodium hydroxide extract where ambiguity arises or exists.

3.7 Limits of Detection and Quantitation

The low limits of detection and quantitation; and the clear separation of the limits detection and quantitation (Table 1) underscored the good sensitivity of the developed method.

Table 1. Limit of detection and limit of quantitation

Drug	LoD (10^{-4}) M	LoQ (10^{-4}) M
AQ	0.46	1.4

LoD= Fxb; b=slope; F=3.3 for LoD and 10 for LoQ [18,19]

3.8 Accuracy of Method from Recovery Data

The recovery studies from standard solutions, in the presence of common excipients, tablets and suspension (Tables 2-5) showed a very good accuracy of determination from method; with the least recovery being 98%.

3.9 Comparison of Method with Parallel Determination

Parallel non-aqueous titration used in assaying amodiaquine recorded good recovery (Table 6). The statistical comparison of these recovery data from amodiaquine tablet determination (Table 7) showed no statistical difference, using two sample hypotheses (paired-t-test). The method is very reliable for amodiaquine determination.

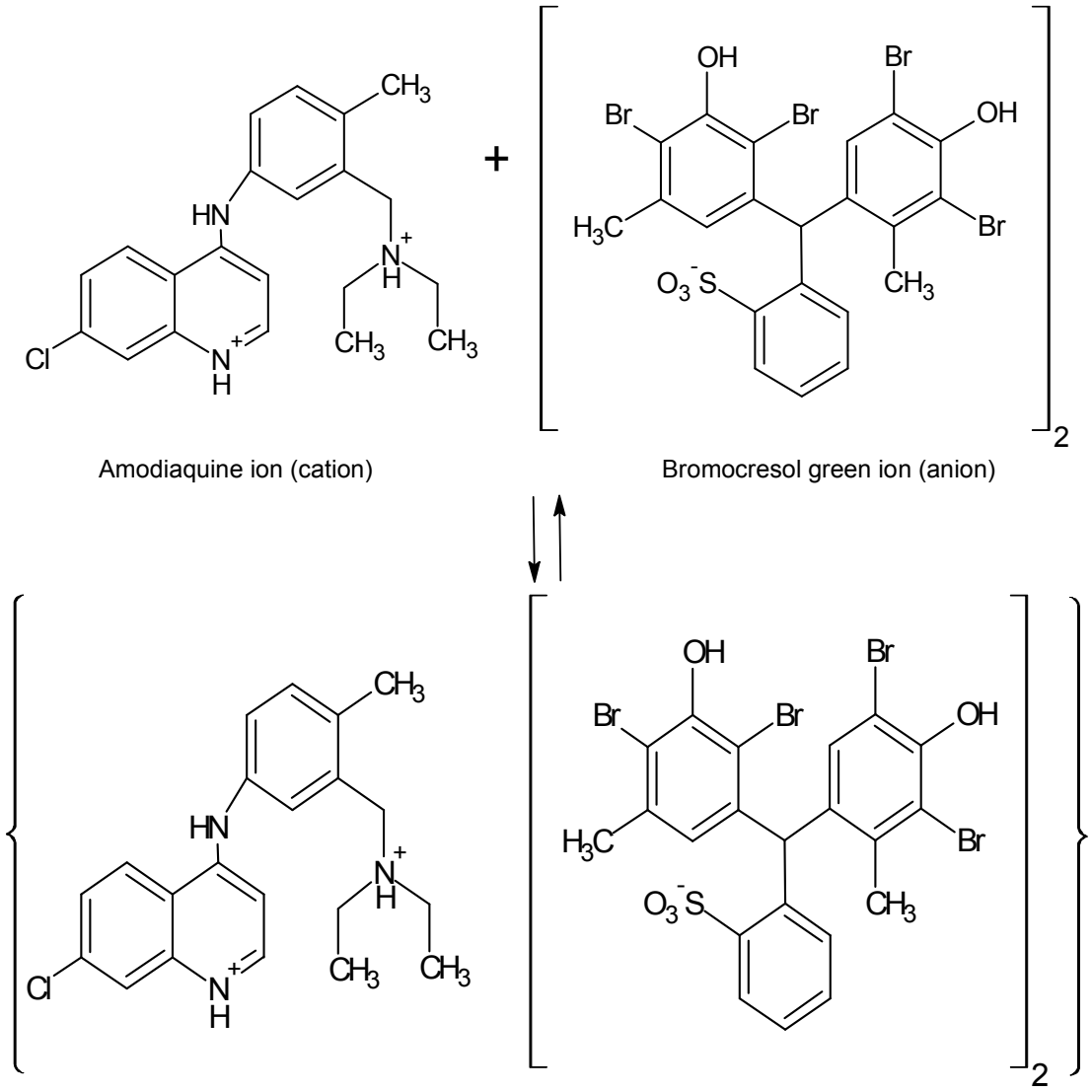


Fig. 5. Schematic reaction showing the reaction of amodiaquine cation (A) with bromocresol green anion (B) to form the neutral ion-pair

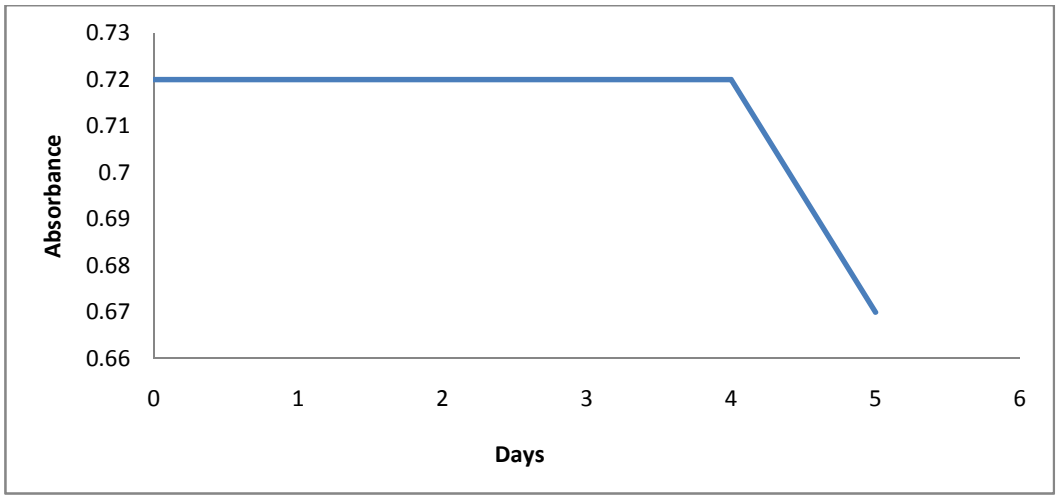


Fig. 6. Stability study of ion-pair extracts of AQ-BG in 0.1 M NaOH at 620 nm, showing the absorption pattern of extract with time

Table 2. Recovery of amodiaquine from standard solutions

S/N	Concn taken X (10^{-4} M)	AQ % recovery (MEAN ±SEM)
1	2.00	98±0.41
2	4.00	98±0.23
3	8.00	99±0.33
4	10.00	101±0.15
5	12.00	99±0.24

Table 3. Recovery of drugs from common excipients

Excipients	Concn spiked (10^{-4} M)	AQ % recovery (MEAN±SEM)
Corn Starch	5.0	99±0.55
Glucose	5.0	98±0.20
Talc	5.0	99±0.40
Sucrose	5.0	98±0.49
Ca Stearate	5.0	100±0.40
Lactose	5.0	100±0.49
Mg Carbonate	5.0	100±0.45

Table 4. Recovery of drugs from tablet formulations

Brand codes	AQ (% mean recovery ± SEM)
AA	98.44±0.41
AB	99.14±0.28
AC	100.68±0.54
AD	99.66±0.46
AE	100.54±0.25

Table 5. Recovery of drugs from suspensions

Brand code	AQ (% recovery mean± SEM)
AX	98.80± 0.30

Table 6. Recovery from nonaqueous titration method

S/N	AQ Sample	Recovery (%)
1	AA	98.62± 11
2	AB	98.62± 13
3	AC	97.55± 23
4	AD	98.62± 13
5	AE	97.55± 18

Table 7. Statistical comparison of extractive and non-aqueous methods

Sample	$AQ_{Extractive}$	$AQ_{Nonaqueous}$
1	98.44	98.62
2	99.14	98.62
3	100.6	97.55
4	99.66	98.62
5	100.5	97.55

4. CONCLUSION

The developed extractive spectrophotometric method was successfully applied in the determination of amodiaquine in pure form, tablets and suspensions. No significant interference was observed from excipients commonly used as pharmaceutical aids with the developed method.

The 0.1M sodium hydroxide extract was stable over 48 hours, which is a sufficient time for the analysis to be carried out. The developed and validated analytical method, showed good statistical and analytical results, and can be recommended for use in routine analysis of amodiaquine in pure form, tablets and suspensions. The method is cost- effective, easy to use, accurate, sensitive and precise.

The study successfully applied the theory of ion-pair formation and its characteristics in the analysis of the amodiaquine, with increased sensitivity when re- extracted into 0.1M sodium hydroxide. An ideal pH, in which only the ion-pair was extracted into the organic phase, was achieved. An increase in wavelength of maximum absorbance was realized with enhanced specificity. The method is simple, cheap, sensitive, and precise. It can be applied routinely for analysis of these drugs, even in developing countries.

CONSENT

This is not applicable to this research work, as it did not involve any human subject.

ETHICAL APPROVAL

This is not applicable to this research work, as it did not involve any human nor animal subject.

ACKNOWLEDGEMENTS

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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