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Extractive ion- pair spectrophotometric assay of quinine

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Abstract
Quality control and assurance of antimalarials is vital in malaria treatment success. This research developed an extractive spectrophotometric method for routine assay of quinine, for enhanced quality control and assurance. The developed method is based on the formation of coloured ion-pair complex between quinine and bromocresol green in phthalate buffer, pH 5. The ion-pair was extracted into chloroform and re-extracted into 0.1M sodium hydroxide. The 0.1M sodium hydroxide extract was determined at 620nm. Relevant method validation requirements were determined, in line with International Standards Requirements. The ion-pair extract stoichiometry was 1:1. Beer's law was obeyed in the concentration range of 1-50 x 10^{-4} M, with good linearity (R> 0.99). The extract showed good stability over 48 hours. A good accuracy was obtained from recovery studies in standard solutions, in the presence of common excipients, in tablets and suspension. The developed method complied with International Standards Requirements for analytical method development. The recovery data of the developed method were not statistically different from recovery data obtained using non-aqueous titration. The developed method was successfully applied in the determination of quinine in bulk drug, tablets and suspension. No significant interference was observed from excipients commonly used as pharmaceutical aids with the developed method.

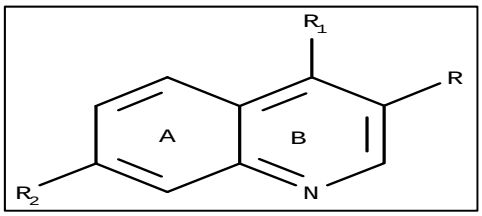
Keywords: Quinine, extractive, ion-pair, bromocresol, anti-malaria, chloroform

Introduction
Malaria is a concern in Africa and other developing countries. It is the leading cause of mortality in Africa. Malaria treatment has been approached in many ways, with resistance to drug therapy leaving little to desire. One of the leading causes of drug resistance & drug failure is poor drug quality and poor quality control [1]. The quality of these drugs can be routinely monitored and assured with simple, sensitive and accurate methods.
Ion-pair is a type of complex. A complex possesses some properties that are different from those of its constituents [2]. There are two types of complex- the coordination or inner sphere complex and the molecular or outer sphere complex [2]. An ion- pair is an outer sphere complex, which 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 techniques, where it is used to selectively manipulate the retention time of analyte for the desired separation, and also in some colorimetric and spectrophotometric methods of analysis, where a coloured extractible ion-pair is formed and determined colorimetrically and spectrophotometrically respectively.
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 [3]. The availability of the unshared electron pair on nitrogen and the relative stabilities of the ammonium ion define the basicity.

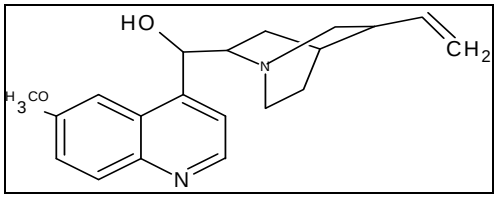
$N^+ + D^- \leftrightarrow [ND]$
Where N^+ is the basic drug ion, acidic D^- is the dye ion and [ND] is the neutral ion-pair.

Quinine is a quinoline anti-malarial drug, which are basic drugs. Many literatures have reported works with applications of ion-pair in the analysis of quinine and quinoline anti-malarials; in which ion-pair was formed, extracted and determined, at pH 2.5- 3.0. [3-9]. These determinations have inherent errors. At this pH range, the extracting organic solvent extracts some quantities of the dye, which may be zeroed out fairly with honest blank determination. But this is not analytical enough. Also, the organic extract is quite volatile and present some challenges that may and do render such methods un- analytical.

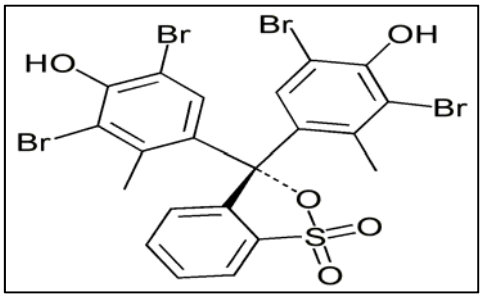
Thus, this research work sought to exploit the theory of ion-pairs to develop and validate a simple, sensitive and accurate spectrophotometric method; that addressed these shortcomings in reported works and offered good alternative for the routine analysis of quinine in bulk drugs and pharmaceutical dosage form.



Quinoline nucleus



Quinine, 5-ethenyl-1-azabicyclo [2.2.2] octan-7-yl-(6-methoxy quinoline-4-) methanol.



Bromocresol green (adapted from Wikimedia.org)

Method

Quinine was analyzed through the formation of ion-pair by mixing equimolar concentrations of quinine and bromocresol green in buffered medium, at buffer pH of 5, using phthalate buffer. 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 0.1M sodium hydroxide.

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

The following method validation procedures, in line with international standard requirement, were carried out, to ascertain the reliability of the developed method. The effect of the buffer pH on absorbance of the 0.1M 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 [10]. The stability of 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 0.1M sodium hydroxide extract of control with those of the 0.1M 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, in line with international standard requirement. [11-16] The limit of detection and limit of quantitation were also carried out. [17]

The results obtained were subjected to statistical treatments, using standard error of mean and student t-test (paired- t- test) where necessary.

Results and Discussion

Preliminary observations: Chloroform blank was colourless in most buffer pH range. 0.1M sodium hydroxide blank was colourless from buffer pH 5- 14. Colour of ion- pair extracts at different buffer pH of reaction and extraction: The colour of the 0.1M 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, increasing intensity of the yellow- wine coloration of the chloroform extracts as the drug concentration increases.

Wavelength of measurement determination: The wavelength of maximum absorbance was obtained to be 620 nm as shown in Figure 1. This higher wavelength further from that obtained with the organic extract (420 nm) excludes irrelevant absorptions, thus imparting sensitive of the method.

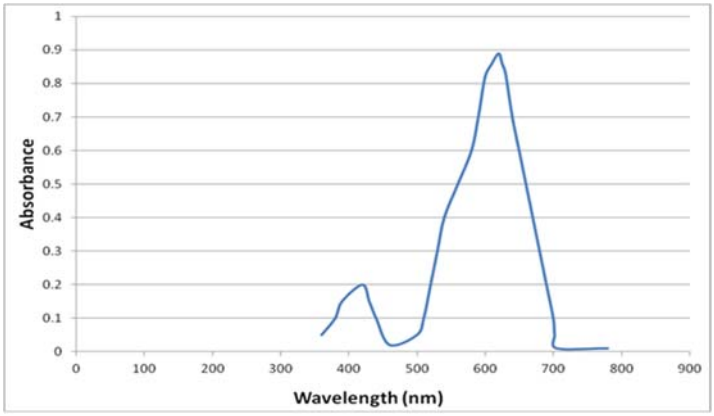


Fig 1: showing the determination of wavelength of maximum absorbance of extract

The effect of pH of buffer solution: The effect of pH of buffer solution used was such that maximum absorbance was recorded at pH 5 as against the chloroform extract in which maximum absorbance was recorded at pH 3, as shown in Figure 2. It was at pH 5 that optimum complex formation and extraction was observed. At this pH the un-reacted dye was not extractible into the organic phase and hence the aqueous phase of 0.1M sodium hydroxide.

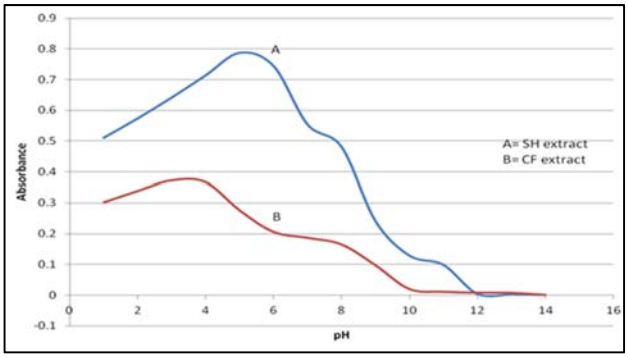


Fig 2: showing the effects of pH of buffer on extraction and absorbances of chloroform (CF) and sodium hydroxide (SH) extracts of the ion-pair

Drug- Dye Stoichiometric Relationship- Job's Method: The result of the Job's plot showed a peak at 0.5 mole fraction as shown in Figure 3. This indicated a 1:1 combination ratio of the quinine: bromocresol green. The anion is mono-cation and quinine has one of the nitrogen atoms relatively available for pairing.

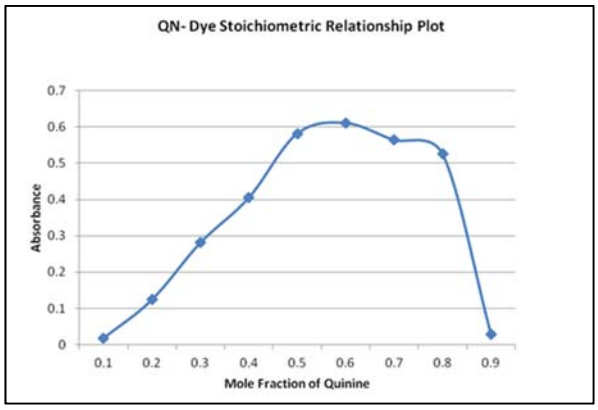


FIG 3: showing the determination of the mole fraction of quinine-bromocresol green ion- pair with peak at 0.5, using Job's method

Linearity Tests of Extracts: The Beer's plot gave a straight line with a R^2 value of 0.9886, showing that Beer's law was obeyed as shown in Figure 4. This shows that the method developed is analytical for assay of quinine.

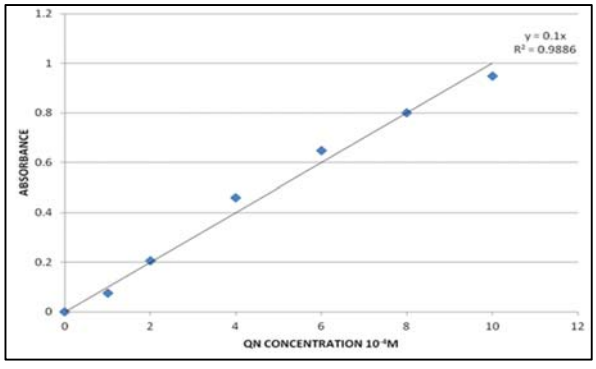


Fig 4: showing the calibration plot of quinine- bromocresol green ion- pair complex

Stability Study of Ion-pair Extract in 0.1 M NaOH at 620 nm: The 0.1M sodium hydroxide was stable over 48 hours as shown in Figure 5. This has great advantage in analysis, because has an ample time to take the absorbance and even repeat absorbance reading within 48 hours.

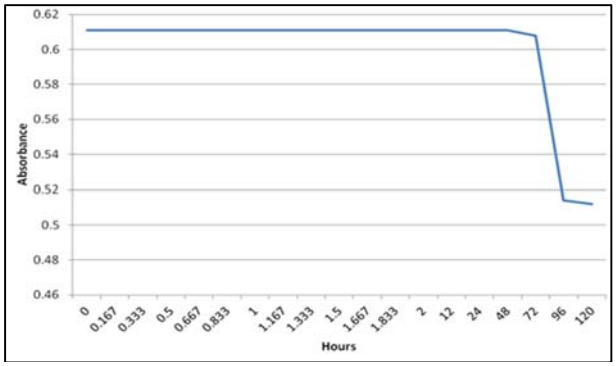


Fig 5: showing the stability of sodium hydroxide extract over time

Limit of Detection and Limit of Quantitation: The limits of detection and quantitation of the developed method as shown in Table 1 below is reasonably low and was taken into cognizance in subsequent procedures in the developed method.

Table 1: Limit of Detection and Limit of Quantitation

DRUG	LoD (10^{-4})	LoQ (10^{-4})
QN	0.33	1.0
LoD= Fxb; b=slope; F=3.3 for LoD and 10 for LoQ		

Recovery Studies: The recovery studies from standard solutions, in the presence of common excipients and pharmaceutical dosage forms showed good results (Tables 2, 3, 4, 5). This underpinned good accuracy of the developed method.

Table 2: Recovery of Drugs from Standard Solutions

S/N	Amount Taken X (10^{-4} M)	Quinine % Recovery
1	2.00	99
2	4.00	98
3	8.00	101
4	10.00	99
5	12.00	99
		99.20 ± 0.50

Table 3: Recovery of Quinine from Common Excipients

Excipients	Amount Spiked (10^{-4}m)	% Quinine Recovery (Mean ± SEM)
Corn Starch	5.0	98 ± 0.51
Glucose	5.0	99 ± 0.80
Talc	5.0	100 ± 0.66
Sucrose	5.0	99 ± 0.90
Ca Stearate	5.0	98 ± 0.40
Gelatin	5.0	99 ± 0.66
Stearic Acid	5.0	98 ± 0.51
MEAN		**98.71**

Table 4: Recovery of Quinine from Tablet Formulations

Brand Codes	Quinine (% Mean Recovery ± SEM)
QA	98.62 ± 0.52
QB	99.96 ± 0.44
QC	97.08 ± 0.24
QD	99.14 ± 0.36
QE	99.24 ± 0.43
QF	98.68 ± 0.62

Table 5: Recovery of Quinine from Suspensions

Brand Code	Quinine (% Recovery Mean ± SEM)
QX	98.40 ± 0.40

Nonaqueous titration method (parallel method): The recovery study from non- aqueous titrimetric assay quinine showed good result as shown in table 6 below.

Table 6: Recovery from Nonaqueous titration method

S/N	Quinine Sample	Recovery (%)
1	QA	100.49
2	QB	101.79
3	QC	99.18
4	QD	99.18
5	QE	101.79
6	QF	97.88

Statistical Comparison of Results of Extractive and Non-aqueous Methods: The recovery study data from the developed method when compared with data from non-aqueous titrimetric assay of quinine, using two sample hypothesis (paired-t-test), showed no statistical difference (Table 7). There is no significant difference between the percent recovery data of the two methods (P>0.05).

Table 7: Statistical Comparison of Results of Extractive and Non-aqueous Methods

Sample	Quinine $_{Extractive}$	Quinine $_{Nonaqueous}$
1	98.62	100.49
2	99.96	101.79
3	97.08	99.18
4	99.14	99.18
5	99.24	101.79
6	98.68	97.88

Conclusion

The developed extractive visible spectrophotometric method was successfully applied in the determination of quinine in bulk drug, 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 quinine 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 quinine, 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 quinine, even in developing countries.

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