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High Performance Liquid Chromatography Assay of Anti-Malarial Quinine Sulfate Utilizing Isocratic Solvent Conditions

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Abstract: A method to assay quinine sulfate, which is utilized for the treatment of Plasmodium falciparum, is presented using isocratic high performance liquid chromatography (HPLC). Elution of the analyteis detected with ultraviolet light detector, set at 222 nm. The stock solution of quinine sulfate was prepared in solvent conditions that consisted of 64 % ethanol (v/v) and 36 % water (v/v), at a concentration of 1.3155×10^{-2} molar. The test samples of quinine sulfate that were injected into HPLC instrument were mainly in a solvent that consisted of 95% (v/v) water and 5 % ethanol (v/v). The solvent utilized for column of the HPLC instrument was 5 % ethanol, 1 % glacial acetic acid (v/v), and 94 % water (v/v). A limit of detection (LOD) was found to be 2.2628×10^{-5} molar and the limit of quantitation (LOQ) was found to be 7.542×10^{-5} molar. A standard curve presented showed a coefficient of determination of $R^2 = 0.9972$, which indicates that the model describes 99.72% variance in the dependent variable (peak area) that is predictable from the independent variable (molar concentration). The Pearson r correlation coefficient of this standard curve is 0.9986, indicating very high positive correlation. Quinine sulfate is assayed from various matrixes that are utilized in the formulation of this important drug, including5% glucose, 0.9% sodium chloride, water, and cellulose for tablet formulation. The determination of the drug quinine sulfate is consistent and accurate utilizing isocratic conditions with HPLC.

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I. INTRODUCTION

Quinine sulfate is an alkaloid derived from the bark of the cinchona tree. This compound derived from the Cinchona family of South American trees has been shown to have considerable antimalarial activity. The compound quinine has been shown to have anticholinergic activity (a substance that blocks the neurotransmitter acetylcholine in the central and the peripheral nervous system), as well as a very strong activity for suppressing the protozoan parasite *Plasmodium falciparum* [1].*Plasmodium falciparum* (*P. falciparum*) causes the most severe form of human malaria and has been found to be responsible for 85% of all malaria cases[1] .Quinine sulfate is a very important antimalarial that has been shown to be effective in geographical regions where resistance to chloroquine has been observed. Fully ninety percent of all diagnosed malaria cases occur in the African continent, in which *P. falciparum* is known to be the most prevalent of the *Plasmodium*species[1] .

The unicellular and protozoan parasite *P. falciparum,* is transmitted through the bite of the female *Anopheles* mosquitos[2].This protozoan infection has been known to be the cause of the most dangerous form of malaria, a form referred to as *falciparum* malaria[3, 4]. This *P. falciparum* form of malaria ultimately causes approximately one million deaths per year[5]. Clinically, the drug quinine is utilized for the treatment of pregnant females, which as a group are particularly susceptible to malaria infection with side effects of stillbirth and premature delivery[6]. Severe incidents of malaria infection can be treated with a combination of clindamycin, quinine, and artesunate[7]. Quinine is also effective in the treatment of malarial induced vision impairment (retinopathy) when it occurs simultaneously with infection[8]. Quinine and a corticosteroid combinational therapy is effective for treatment of malaria infection accompanied by thrombocytopenia[9]. Quinine administered by intravenous infusion is also known to cause glycemic effects in otherwise healthy individuals[10].

Quinine sulfate dihydrate when prepared in capsules is known as qualaquin, and in this form is an antiprotozoal and antiparasitic pharmaceutical that has the appearance of white crystalline powder that is also light sensitive[11, 12]. When quinine sulfate is prepared in capsule form, it is then referred to as qualaquin, but still utilized in clinical application for treatment of malaria infection and nocturnal leg cramps[12]. Quinine sulfate is a very important antimalarial that shown to be effective in geographical regions where resistance to chloroquine has been observed. The known pharmacological activity of quinine sulfate includes substantial antimalarial activity, potassium channel blocker, and anticholinergic activity[12].

Quinine has been assayed from human plasma by use of high performance liquid chromatography (HPLC), which utilized reversed-phase C-18 column as well as an isocratic mobile phase of acetonitrile-aqueous phosphate buffer[13]. The drug quinine sulfate can be determined after dissolving in acetic anhydride with malachite green TS, followed by titration with perchloric acid[14]. In addition, other HPLC assay method had utilized isocratic solvent conditions with reversed-phase column followed with ultraviolet detection of eluted quinine at 254 nm[15]. Additional methods to assay this very important antimalarial drug are necessary for quality control monitoring in manufacturing production and processes, as well as patient compliance. This study describes a sensitive and effective methodology for the determination of quinine hemi-sulfate salt dehydrate that uses isocratic HPLC conditions and ultraviolet detection at 222 nm.

II. MATERIAL AND METHODS

Chemical Reagents

Analytical grade solvent reagents were obtained from Sigma-Aldrich (St. Louis MO 63178 USA). The quinine hemi-sulfate salt dihydrate (referred to as qualaquin when in tablet form) drug to be used in the accomplishment of standards and preparation of test samples was obtained from Matheson Coleman & Bell (East Rutherford, New Jersey USA) or from Sigma-Aldrich (St. Louis MO 63178 USA). Distilled water is utilized wherever the use of aqueous solvent is noted.

Instrumentation Description

An Alltech 426 HPLC Pump and Linear UVS 200 detector were utilized to accomplish high performance liquid chromatography analysis (Deerfield, Illinois 60015-1899). Reversed-phase isocratic conditions (unchanging column solvent) are applied for all types of samples. The HPLC Column consists of 5µ packing, having a length of 150 millimeters, and an internal diameter of 4.6 millimeters. Ultraviolet detection is applied for analyte elution and at a wavelength of 222 nm.

HPLC Instrument Settings and Components

For this HPLC analysis, a reversed-phase C-18 octadecylsilyl (C18H37) bonded phase column packing was utilized throughout the duration of the study. Detection of eluted species was accomplished by use of ultraviolet detector set to 222 nm, rise time 0.1, and with range AUFS set to 1.0. The HPLC pump was set to 1300 psig with one milliliter per minute flow rate. The actual volume injected into the column is known to be 20 microliters. The dead time for the elution of non-retained species is 1.5 minutes and is calculated by the relationship, dead time= volume/flow rate = 1.5 mL/1.0 mL/min. The column solvent used throughout the study is as follows: 52.6 mL of 95% ethanol, 10 mL of glacial acetic acid, and 937.4 mL of distilled water.

Preparation of Sample Types

Column solvent that was utilized throughout the project was made as follows: total volume of 1000 mL prepared by adding 52.6 mL of 95% ethanol, 937.4 ml of distilled water, and 10 mL of glacial acetic acid (stock of glacial acetic acid at 17.4 molar). Therefore, the working concentrations will be 5% ethanol, 0.174 molar acetic acid, and 93.7% water (v/v). Sample solvent used for solubilizing quinine hemi-sulfate salt dihydrate for assay by HPLC: 95% distilled water with 5% ethanol. Stock standard of quinine hemi-sulfate salt dihydrate (molecular weight 782.96 grams/mole) was prepared by dissolving 2.2711 grams of the compound into 250 mL volumetric flask of distilled water, making a mixture of 1.1603×10^{-2} molar. If any sample required clarification prior to HPLC analysis, this was accomplished by Whatman 6900-2502 GD/X Sterile Syringe Filter, 25 mm, 0.2 Micron, PVDF Filtration Medium, with a suitable plastic syringe. Samples for HPLC analysis were in sample solvent, which works very well for this purpose. Ampoule type samples, used in clinical application, were quinine sulfate drug prepared in normal saline (0.9%) or 5% glucose or water. Tablet/solid samples were the drug quinine hemi-sulfate salt dihydrate prepared in various known percentage of combinations of excipient cellulose.

Statistical Analysis, Properties Determination, Molecular Modeling

Where indicated the numerical analysis utilizing Paired tests, F and T test, Kruskal-Wallis, and correlation between sets of data is accomplished by PAST version 2.06 (copyright Hammer and Harper 1999-2011). Microsoft EXCEL (copyright 2010 Microsoft Corporation, Microsoft Office Professional Plus 2010) and PAST v. 2.06 also performed summary statistical analysis. Detection of numerical outliers was accomplished by use of the Grubb's test for outliers (or extreme studentized deviate) was performed by Graph Pad InStat version 3.00 (Copyright 1992-1998 Graph Pad Software Inc. (www.graphpad.com) for Windows 95, San Diego California USA). EXCEL and PAST v. 2.06 accomplished linear regression. Molecular properties of quinine hemi-sulfate salt dihydrate were determined utilizing Molinspiration cheminformatics http://www.molinspiration.com/ (Molinspiration Cheminformatics, Nova ulica, SK-900 26 SlovenskyGrob,

Slovak Republic). Passing-Bablok analysis was accomplished by ACOMED statistik: (www.acomed-statistik.de, copyright: Dr. Thomas Keller). The Bland-Altman plot (also referred to as difference plot) was accomplished by use of Method Validator (www.multiqc.com).

III. RESULTS AND DISCUSSION

Quinine hemi-sulfate salt dihydrate (Qualaquin dispensed in capsules), that was utilized for this study, is a very important antimalarial compound that is still an effective treatment for uncomplicated malaria caused by the parasite *Plasmodium falciparum*[1]. The molecular scaffolding with constituents of this compound is shown in Figure 1. The molecular structure consists of a quinoline ring in addition to various substituents that are attached to the ring. This drug consists as a white, crystalline powder that is sensitive to light, and will darken upon exposure to light. Quinine hemi-sulfate salt dihydrate is odorless and slightly soluble in water, alcohol, chloroform, and ether. For this study, a combination of ethyl alcohol and water was utilized for solubilization of this drug as well as for the column solvent utilized in this HPLC determination.

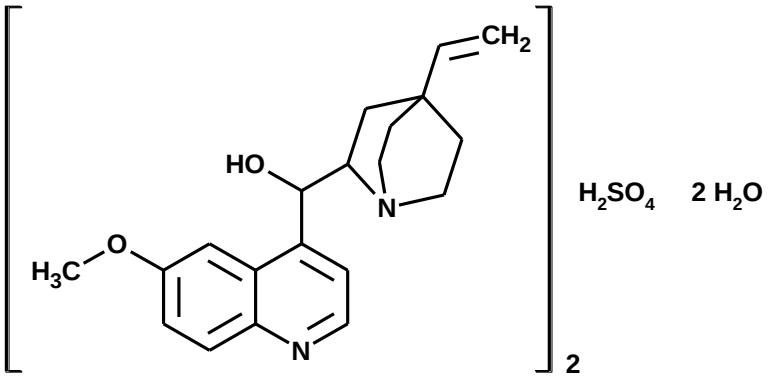

Fig. 1 The molecular structure of quinine sulfate (quinine hemi-sulfate salt dihydrate). It has the molecular formula of $(C_{20}H_{24}N_2O_2)_2 \bullet H_2SO_4 \bullet 2H_2O$ and a molecular weight of 782.96 grams/mole.

For this HPLC determination, isocratic solvent conditions were utilized. When utilizing isocratic conditions, the mobile phase (column solvent) has a composition that remains constant. The isocratic conditions for HPLC determination involves having the column equilibrated during the assay run and this accomplished without rapid chemical changes. Isocratic conditions are generally more preferred when test samples contain less than 10 weakly retained components and the gradient baseline would impede trace analysis[15,16,17].

For this study, the column solvent for reversed phase HPLC was distilled water combined with the organic modifier ethyl alcohol. Since the water component is most polar, it repels any hydrophobic analytes into the stationary phase more than any other component of the solvent, with the consequence of longer retention times. An organic modifier is added to allow the analyte to spend less time in the stationary phase, with shorter elution times. Other common organic modifiers are simple alcohols; with ethyl alcohol utilized in this study[18,19].

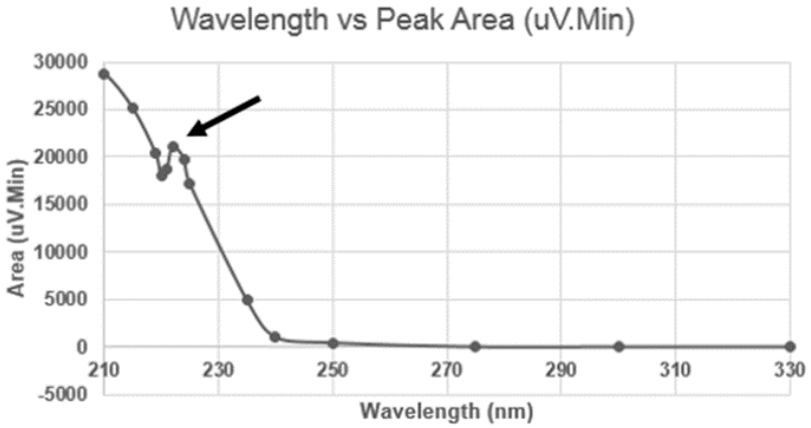

Fig. 2 Absorbance peak of quinine hemi-sulfate salt dihydrate located at 222 nm is utilized for detection by HPLC after elution from C-18 reversed phase column.

The ultraviolet light HPLC detector was consistently set to 222 nm (see Figure 2), allowing consistent and sensitive detection of quinine sulfate elution. Upon completion of each injection, the chromatogram is shownwithin the software windows of the program, followed by printout. The standard curve established for injected quinine sulfate samples injected, is presented in Figure 3. The plot of peak area (dependent variable) was found to be very highly linear with the molar concentration (independent variable). The equation for the standard curve line (1) is as follows:

y = 234,380,231.9(x) (1)

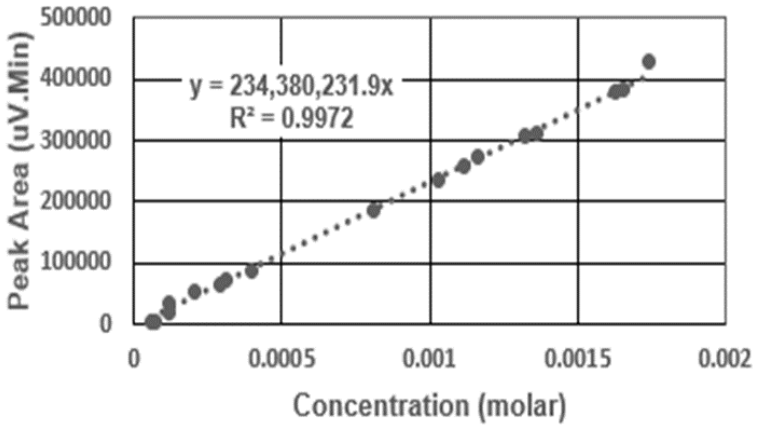

Fig. 3 Standard curve having coefficient of determination R2 = 0.9972, Pearson r correlation is 0.9986, indicating a very strong positive correlation between concentration (molar) and peak area. Slope of line is 234,380,231.9 Uv.Min/molar.

The coefficient of determination R^2 is 0.9972, indicating the 99.72 % of the proportion of the variance in the dependent variable (peak area) that is predictable from the independent variable (molar concentration) [20, 21]. The Pearson r correlation coefficient is 0.9986, which is a very high positive correlation [21]. The slope of the line is 234,380,231 Uv.Min/molar. The span of molar concentration in the standard curve is from 7.5420×10^{-5} molar to 1.7405×10^{-3} molar.

The percent recovery accomplished for samples injected into HPLC under isocratic conditions is presented in Table 1. Comparing actual molar concentration of injected samples to measured molar concentrations determined by HPLC and using standard curve Figure 3. The percent recovery is the concentration of the quinine sulfate determined by HPLC, compared to the actual molar concentration (i.e. percent recovery = amount measured/expected amount).

Percent recovery of quinine sulfate ranges from 95.4 % to 105 %, with the median equal to 99.4 %. The average percent recovery is 99.9 % of quinine sulfate, with a standard deviation of 3.3 %. The skewness of the percent recovery values is +0.2443, which indicates that the numerical values are fairly symmetrical[20, 21]. Percent recovery values analyzed by Grubb's Test for outliers (also referred to as extreme studentized deviate), showed no outliers among the range of values.

Statistical analysis of actual molar concentration to measured molar concentration determined by HPLC shows consistency and reproducibility. The correlation of "actual" to "measured" molar values is a very high positive value of 0.9909. The paired tests for mean (t test) showed that both populations have the same mean (P=.92) and have the same median (Wilcoxon test, P=.69) [20, 21]. This was further confirmed by applying the F and T test, showing the two groups of values have the same mean (P=.99) and the same variance (P=.95) [20, 21]. In addition, the Kruskal-Wallis test comparing "actual" and "measured" molarity indicates the two groups have equal medians (P=.93)[20].

To further show the efficiency of the percent recovery of the drug quinine sulfate by HPLC analysis, there is the Passing-Bablok method[22]. This is a statistical procedure that allows estimation of an analytical method's agreement with actual concentrations and identifies possible systematic bias between them[22]. Analysis of percent recovery values (Table 1), by comparing actual molar values to measured molar values as a Passing-Bablok plot shows an excellent relationship (see Figure 3).

The Passing-Bablok procedure fits the parameters a and b of the linear equation y = a + b x using non-parametric methods, with a being the y-axis intercept and b the slope of the line[22]. The results are interpreted as follows: If 0 is in the confidence interval of a, and 1 is in the confidence interval of b, the two methods are comparable within the investigated concentration range. If 0 is not in the confidence interval of a, then there is a

systematic difference and if 1 is not in the confidence interval of b then there is a proportional difference between the two methods[22].

Table 1. Percent Recovery of Quinine Sulfate Following HPLC Assay

Run	Actual Molar Concentration	Molar Concentration Measured By HPLC	Percent Recovery
1	1.1007×10^{-3}	9.6617×10^{-4}	96.0
2	1.0704×10^{-3}	1.0767×10^{-3}	101
3	3.1038×10^{-4}	3.1202×10^{-4}	100
4	2.3496×10^{-4}	2.4694×10^{-4}	105
5	1.0820×10^{-3}	1.1186×10^{-3}	103
6	1.3430×10^{-3}	1.2960×10^{-3}	96.5
7	1.3140×10^{-3}	1.2864×10^{-3}	97.9
8	1.4301×10^{-3}	1.4966×10^{-3}	104
9	1.6041×10^{-3}	1.6124×10^{-3}	101
10	1.6679×10^{-3}	1.5916×10^{-3}	95.4
11	1.5664×10^{-3}	1.6561×10^{-3}	105
12	1.6244×10^{-3}	1.6110×10^{-3}	99.2
13	1.6534×10^{-3}	1.6373×10^{-3}	100
14	1.5229×10^{-3}	1.4640×10^{-3}	96.1
15	1.5084×10^{-3}	1.5717×10^{-3}	104
16	1.4069×10^{-3}	1.4631×10^{-3}	104
17	1.3198×10^{-3}	1.3117×10^{-3}	99.4
18	1.3634×10^{-3}	1.3264×10^{-3}	97.3
19	1.2763×10^{-3}	1.2190×10^{-3}	95.5
20	1.2473×10^{-3}	1.1940×10^{-3}	95.7
21	1.2183×10^{-3}	1.1754×10^{-3}	96.0
22	1.1603×10^{-3}	1.1638×10^{-3}	100
23	1.0878×10^{-3}	1.0703×10^{-3}	98.4
24	1.0298×10^{-3}	1.0102×10^{-3}	98.1
25	1.0080×10^{-3}	9.8599×10^{-4}	97.8
26	8.9923×10^{-4}	9.8957×10^{-4}	104
27	8.1221×10^{-4}	7.9712×10^{-4}	98.0
28	1.0878×10^{-3}	1.0686×10^{-3}	98.2
29	9.4274×10^{-4}	9.7144×10^{-4}	103
30	1.1117×10^{-3}	1.1094×10^{-3}	99.3
31	1.7405×10^{-3}	1.8258×10^{-3}	105
32	8.7023×10^{-4}	9.2018×10^{-4}	105
33	1.4504×10^{-3}	1.4963×10^{-3}	103
34	1.1603×10^{-3}	1.1099×10^{-3}	95.7

For Passing-Bablok plot shown in Fig. 3 the 95 % confidence interval of the slope is 0.9532 to 1.051 (slope equal to 1.002). The 95% confidence interval for the y-axis intercept is -6.509×10^{-5} to 5.815×10^{-5} (y-axis intercept equal to -3.468×10^{-6}). The confidence interval for the y-axis intercept includes zero and the confidence interval for the slope includes 1. Therefore, there are no constant differences between the actual molarity and the HPLC measured molarity, and that these values can be used interchangeably. Therefore, the actual molar values are representative of the HPLC measured molar values [22].

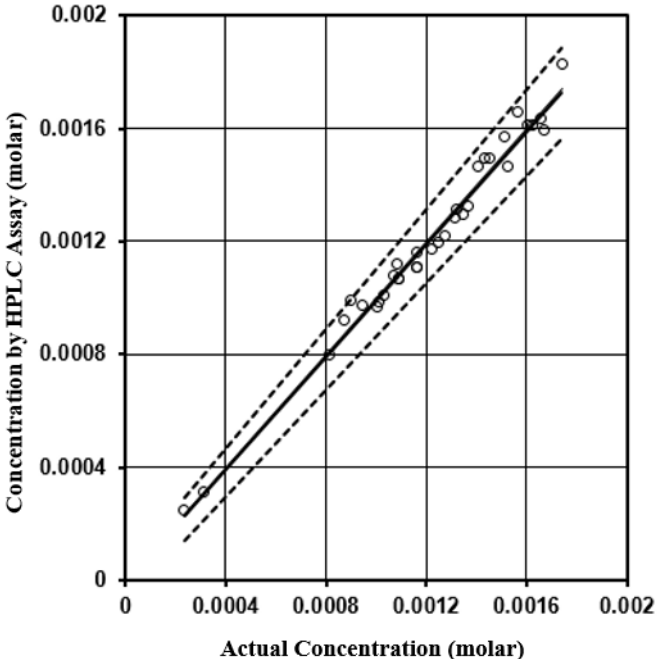

Fig. 3 Passing-Bablok plot of actual molar concentration values compared to molar concentration values obtained from assay by HPLC with standard curve. Value of 1 is contained within the 95% confidence interval of the slope (0.9532 to 1.051). The value of 0 is contained within the 95% confidence interval of the y-axis intercept (-6.509 x 10^{-5} to 5.815 x 10^{-5}). Pearson r correlation is 0.9909, with R^2 equal to 0.9819 (98.19 % of variance in peak area that is predictable from molarity of quinine sulfate).

Another means to determine interchangeability of the actual and measure concentrations is by using the Bland-Altman plot (or difference plot). The Bland-Altman plot is a graphical method to compare two measurements techniques[23]. In this graphical method the differences between the two techniques are plotted against the averages of the two techniques. Horizontal lines are drawn at the mean difference, and at the limits of agreement, which are defined as the mean difference plus and minus 1.96 times the standard deviation of the differences[23]. Presented in Figure 4 is the result of Bland-Altman plot of the actual and measured values of quinine sulfate concentration. Note that the mean difference is essentially zero, with limits of agreement above and below the mean difference. Note that all points are within the limits of agreement and scattered, indicating that the two sets of concentrations values are interchangeable and without bias [23].

The limit of detection (LOD) for any analytical procedure can be considered to be the point at which analysis is just feasible, whereas, the limit of quantitation (LOQ) is the concentration at which quantitative results can be reported with a high degree of confidence [24, 25]. The LOD and LOQ for an analytical method can be determined from the concentration of analyte that occurs at observed signal (S) compared to noise (N) observed in chromatogram results [24, 25]. Therefore, the LOD accepted to be at S/N = 3 and LOQ at S/N = 10, respectively[24]. For this study the LOD at the S/N of 3, is 2.2628 x 10^{-5} molar. For this study the LOQ at the S/N of 10, is 2.2628 x 10^{-5} molar.

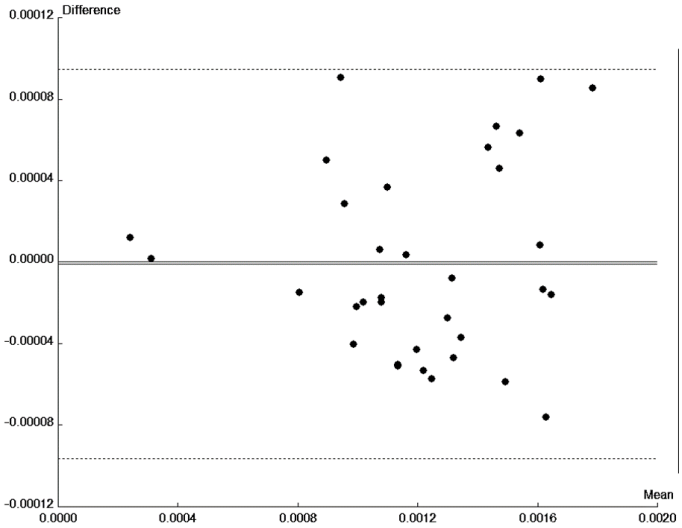

Fig 4. Bland–Altman plot with lines of agreement. The mean of the differences of the two sets is essentially zero. Dotted lines above and below the mean are limits of agreement mean +1.96 SD and mean −1.96 SD. The points are scattered above and below zero, so there is no consistent bias of one approach versus the other (the two methods are considered to agree and may be used interchangeably).

The column dead time is often designated as t_0 and represents the time it takes for an injected species to go through the column in a manner that does not interact with the column [16]. In addition, the dead volume (or hold-up volume), often designated as VM, is the volume of mobile phase inside the column. The dead volume for the column utilized in this study is 1.5 mL, calculated from VM = 0.01(length of column)[26]. The dead time for the column utilized in this study is 1.5 minute, calculated from t_0 = VM ÷ flow rate [26].

Drugs are most frequently administered in solid dosage formulations to be administered by the oral route [27]. The drug physicochemical characteristics and excipients added to the formulations all contribute to ensure a desired therapeutic activity. There are advantages in utilizing tablet forms for patient administration, including: 1) ease of handling, 2) variety of manufacturing methods, 3) mass production at low cost, 4) consistent quality, 5) dosing precision, and 6) can be self-administered[27]. Cellulose is a filler and diluent that has advantages for solid tablet application, which include improvement of tablet disintegration and good flow ability[27]. Quinine sulfate for antimalarial application is prepared in cellulose for tablet regimen, water, 0.9 % sodium chloride, and 5 % glucose matrixes[28].

HPLC assay of quinine sulfate samples stored with cellulose were accomplished following solubilization and filtering upon necessity (see Materials and Methods). Table 2 shows that determination of quinine sulfate following processing of solid matrix with cellulose was consistent. Cellulose matrix was separated from the quinine sulfate after dissolution in distilled water, followed by assay using HPLC. The expected molarity was 1.5254×10^{-3} molar, which all sample runs were centered, giving a standard deviation of only 2.5×10^{-5} molar. Grubb's test also indicated that there are no outliers among the determinedconcentrations of quinine sulfate (P=.05, two-sided). This determination of quinine sulfate was accomplished with various amounts of cellulose present (see column 2 in Table 2), with HPLC assay results of molarity being consistent.

Table 2. Assay of Quinine Sulfate from Samples Stored in Tablet Cellulose

Run	Amount of Cellulose (grams)	Concentration of Quinine Sulfate by HPLC (molar)
1	**0.0020**	**1.5795×10^{-3}**
2	**0.0060**	**1.5188×10^{-3}**
3	**0.0040**	**1.5187×10^{-3}**
4	**0.0210**	**1.5188×10^{-3}**
5	**0.0120**	**1.5176×10^{-3}**
6	**0.0290**	**1.5188×10^{-3}**
7	**0.0220**	**1.5523×10^{-3}**
8	**0.0360**	**1.4935×10^{-3}**
9	**0.0460**	**1.5105×10^{-3}**

This malarial drug is often prepared in sterile water as ampoules for injection in cases of severe or complicated conditions of malaria[28]. Advantages of this dosage form can be administered as intramuscular injection or intravenous infusion[28]. Table 3 shows that determination of quinine sulfate from water matrix to be consistent. The expected molarity was 1.4946×10^{-4} molar, which all sample runs were centered, giving a standard deviation of only 7.6×10^{-6} molar. Grubb's test also indicated that there are no outliers among the determined concentrations of quinine sulfate (P=.05, two-sided). This determination of quinine sulfate with HPLC, gives results of molarity that are consistent.

Table 3. Assay of Quinine Sulfate Stored in Water Solvent

Run	Concentration of Quinine Sulfate by HPLC (molar)
1	1.5079×10^{-4}
2	$1.4640 \text{ X } 10^{-4}$
3	1.4644×10^{-4}
4	1.4562×10^{-4}
5	1.4031×10^{-4}
6	1.6438×10^{-4}
7	1.5226×10^{-4}

This malarial drug is often prepared in 0.9 % saline (NaCl) for intravenous injection in severe cases of falciparum malaria[29]. Advantages of this dosage form are that the drug can be administered although the patient is severely ill and oral administration is not feasible[29]. Table 4 shows that determination of quinine sulfate from 0.9 % saline matrix to be consistent. The expected molarity was 1.1236×10^{-4} molar, which all sample runs were centered, giving a standard deviation of only 7.6×10^{-6} molar. Grubb's test also indicated that there are no outliers among the determined concentrations of quinine sulfate (P=.05, two-sided). Results of quinine sulfate assay are consistent.

Table 4. Assay of Quinine Sulfate Stored in 0.9% Saline Solvent

Run	Concentration of Quinine Sulfate by HPLC (molar)
1	1.0781×10^{-4}
2	1.0509×10^{-4}
3	1.0254×10^{-4}
4	1.2181×10^{-4}
5	1.0992×10^{-4}
6	1.2048×10^{-4}
7	1.2047×10^{-4}
8	1.1077×10^{-4}

This malarial drug is often prepared in 5 % glucose, for slow infusion as an intravenous injection in cases of severe malaria[18]. Advantages of this dosage form are that the drug can be administered although the patient is severely ill and oral administration is not feasible [29]. Table 5 shows that determination of quinine sulfate from 05 % glucose matrix to be consistent. The expected molarity was 1.4475×10^{-4} molar, which all sample runs were centered, giving a standard deviation of only 6.1×10^{-6} molar. Grubb's test also indicated that there are no outliers among the determined concentrations of quinine sulfate (P=.05, two-sided). This determination of quinine sulfate with HPLC, gives results of molarity that are consistent.

Table 5. Assay Quinine Sulfate Stored in 5% Glucose Solvent

Run	Concentration of Quinine Sulfate by HPLC (molar)
1	1.4697×10^{-4}
2	1.3557×10^{-4}
3	1.4660×10^{-4}
4	1.4034×10^{-4}
5	1.3604×10^{-4}
6	1.5196×10^{-4}
7	1.4648×10^{-4}
8	1.5234×10^{-4}

9	**1.4647×10^{-4}**

Therefore, quinine hemi-sulfate salt dihydratecan be assayed by HPLC from different matrixes including cellulose, water, 0.9 % saline, and 5 % glucose. The method presented in this study for determination of this drug will be useful in quality control within commercial drug manufacturing and verification of content for prepared pharmaceuticals. Using isocratic conditions in HPLC is advantageous for pharmaceutical assay

IV. CONCLUSION

Isocratic conditions with HPLC are shown to be effective for the assay of quinine sulfate from matrixes that include water, 5% glucose solution, 0.9% NaCl solution, mixtures of water with ethyl alcohol, and dissolved solid formulation having cellulose. The limit of detection (LOD) is 2.2628×10^{-5} molar and the limit of quantitation (LOQ) is 7.542×10^{-5} molar. With ultraviolet detection, monitor set at 222 nm, a visible peak for quinine sulfate is observed. This drug was consistently assayed from matrixes of cellulose (for tablet formulation), water (for intramuscular and intravenous), 0.9 % saline (intravenous), and 5 % glucose (intravenous). This method for determination of this drug can be used for quality control in manufacturing and toxicology determinations where appropriate.

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