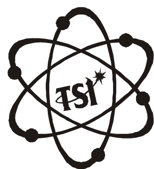

Trade Science Inc.

December 2009 *Volume 8 Issue 4*

Analytical CHEMISTRY
An Indian Journal

Full Paper

ACAIJ, 8(4) 2009 [406-410]

Direct determination of quinine from human excretory product by background reduction and single wavelength detection with reversed-phase H.P.L.C.

Ronald Bartzatt

University of Nebraska, College of Arts & Sciences, Durham Science Center, 6001 Dodge Street, Chemistry Department, Omaha, 68182, (NEBRASKA)

E-mail : rbartzatt@mail.unomaha.edu

Received: 20th July, 2009 ; Accepted: 30th July, 2009

ABSTRACT

The pupose of this work is to assay quinine which is used to treat Plasmodium falciparum, which is the most common cause of severe and life-threatening malaria. Prompt administration of parenteral or intravenous anti-malarial agents is crucial for patient recovery. The result of this work is a tractable process to monitor compliance of drug administration and levels of quinine drug in vivo will enhance clinical response. Quinine is assayed directly from human urine that is filter sterilized and injected itself onto a reversed-phase C-18 column with no column related problems. The major concluding outcomes include the detection at a single wavelength of 254 nm showed to be effective and efficaciously marginalized all chromatogram peaks associated with human urine. In this work the detection level of quinine was taken as low as 2.25E-05 molar to upper level 4.61E-04 molar. The standard curve produced a high Pearson r correlation of 0.9922 (very high correlation), with a coefficient of determination (R^2) at 0.9845. Percent recovery of quinine was 93 % to 115 % with standard deviation of 7.8 %. This highly sensitive methodology for quinine analysis directly from urine will be useful in determining patient compliance and regimen maintenance.
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KEYWORDS

Quinine;
Malaria;
Liquid chromatography;
HPLC;
Urine.

INTRODUCTION

Malaria is a dangerous infection caused by a protozoan parasite and transmitted by the female Anopheles mosquitoes. The most dangerous of these infections is by Plasmodium falciparum (or malignant malaria) because of the high rate of complications and mortality[1]. P. falciparum accounts for 80 % of all human malarial infections as well as 90 % of malaria related deaths. The variant P. falciparum is of the genus Plasmodium and family Plasmodiidae. The chemotherapy of malaria is divided into six categories: 1) Causal prophylaxis; 2) Suppresive treatment; 3) Clinical cure; 4) Radical cure; 5) Suppressive cure; and 6) Gametocytocidal therapy[1]. Use of quinine falls under clinical cure and is of great importance for treating P. falciparum.

P. falciparum causes the great majority of malaria related deaths and affects all age groups[1,2]. Prompt administration of the appropriate anti-malarial drugs is

crucial to effective clinical response, which in the case of deadly P. falciparum requires the administration of quinine whether by intravenous, intramuscular, or parenteral routes (whereas the parenteral path is recommended for severe cases of malaria)[2]. Quinine is used preferentially as a first line chemotherapeutic in most parts of the world partly due to a high bioavailability (85 % by intramuscular)[2] and tolerance of high loading dose in locations of less drug sensitivity[2].

Quinine, a cinchona alkaloid, is very effective and consistent in the treatment of chloroquinine resistant P. falciparum at all grades of severity[3]. Strains of P. falciparum resistant to quinine are suppressed by using quinine in combination therapy with other drugs[3]. Drug resistant malaria is treatable utilizing quinine which is also effective intravenously when patients are so ill as to not allow oral administration[4]. Even in locations showing decreased sensitivity to quinine it retains effectiveness when used in combination with tetracycline or doxycycline[5,6]. Should P. falciparum show multidrug resistance, than quinine combined with clindamycin or tetracycline retains clinical efficacy[7]. Quinine is the drug of choice in incidents of P. falciparum infection during pregnancy[8].

Side effects of quinine include cardiotoxcity, ototoxicity, and ocular toxicity[9,10]. For that reason and the efficacious application of quinine in clinical usage it is widely advocated that quinine levels be monitored[9] at a qualitative manner at minimum. Severe adverse side-effects due to quinine have been reported even in instances of adequate clinical administration[10]. Therefore various analysis for quinine utilizing high performance liquid chromatography (HPLC) approaches have been investigated. These include ion-pair reversed-phase[11], reversed-phase with fluorescent detection[12], and isocratic reversed-phase HPLC. However all the methods presented above[11,12], and[13] require solvent or instrumental extraction of quinine from biological fluids. The method presented in this work allows the direct injection of human urine for the determination of quinine levels for the purposes of monitoring patient compliance and/or clinical dosage levels in severely ill patients. The obvious advantages of direct injection includes convenience as well as effectiveness. The detection level of quinine possible with the presented method provides a beneficial device for the clinician.

EXPERIMENTAL

Reagents

Chemicals and reagents were obtained from Aldrich Co. (P.O. Box 2060, Milwaukee, WI 53201). The quinine sulfate utilized in this work provided by Matheson Coleman a Bell (East Rutherford, New Jersey).

HPLC instrumentation and settings

An Alltech 426 HPLC pump with a C-18 reversed-phase column was utilized and running at 1900 psig and 1.0 mL/minute. An Alltech 150 mm reversed-phase C-18 (octadecylsilyl) bonded phase packing column having a non-polar moiety extended into mobile solvent phase implemented analytical separation of quinine from urine. The detector was a Linear UV/Vis 200 detector set at 254 nm non-modulating wavelength, rise time 0.1 sec, and range at 1.00. The detector included data output of 1 AU/V and $\pm$ 1.0 nm wavelength precision. The SS Flow cell diameter was 6 millimeters. The solvent consisted of 20% ethanol, 4% acetic acid, and 76% water that optimized determination of water soluble components within the test samples and standards.

Preparation of sample and standards

A normal mid-stream human urine catch was obtained which was filtered sterilized through a 0.45 μm Millipore filter and sterilizing system (Millipore Corparation, Bedford Massachusetts, USA) . The filtered urine is them stored at 4° C until analysis. For test samples and standards quinine sulfate was utilized to make working concentrations of quinine at 0.000599 grams/milliliter (using solvent 10% ethanol and 90% distilled water). All test samples and standards were made from a mixture that included the previously filtered sterilized human urine that was diluted 1:20 for working applications. In this manner the test samples and standards contained the same component of human urine.

Statistical analysis and software

Test for normality and effectiveness of pairing by Wilcoxon matched pair test was performed by using GraphPad InStat version 3.00 for Windows 95, GraphPad Software, San Diego California USA, www.graphpad.com". Correlation and linear regression

analysis was accomplished by PAST v. 1.80 (copyright Hammer and Harper 1999-2008). Other statistical parameters were determined by Microsoft EXCEL 2003 (copyright Microsoft Corporation). Molecular properties of quinine were determined by Molinspiration (copyright Molinspiration Cheminformatics 2009).

RESULTS AND DISCUSSION

Reversed-phase chromatography accounts for the vast proportion of analysis performed in liquid chromatography wherein the more polar compounds elute faster and more non-polar compounds retained (hence reversed-phase)[14]. The most popular columns for this purpose are the C-18 bonded silica columns[14], which is utilized in this work. The mobile phase composition does not change during the separation period so this method is also isocratic. Faster elution and greater resolution are also traits of reversed-phase liquid chromatography. Quinine itself can generate significant side-effects and for this reason it is widely advocated that quinine levels be monitored at the clinical level[9].

The molecular structure of quinine is presented in Figure 1, showing the proximities of the ether, hydroxyl, and tertiary amine. Non-specific hydrophobic interactions affect the elution of quinine particularly due to the non-polar carbon chain of the stationary phase utilized in reversed-phase chromatography. In this respect then the quinuclidine ring and quinoline moiety will play a role in retaining the quinine in the column, however the alcoholic hydroxyl group (-OH) is expected to enhance aqueous solubility due to hydrogen bonding. The two nitrogen atoms and oxygen atom will function as hydrogen bond acceptors. The n-octanol/H_2O partition coefficient Log P for quinine is 3.037 with a polar surface area of 45.592 Angstroms^2.

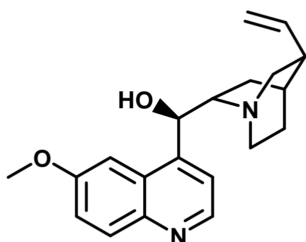
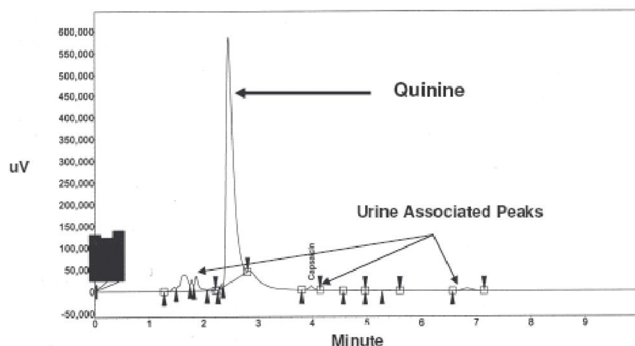


Figure 1 : The molecular structure of quinine is presented here showing relative proximities of an ether group, tertiary amine, hydroxyl group, and aromatic ring.

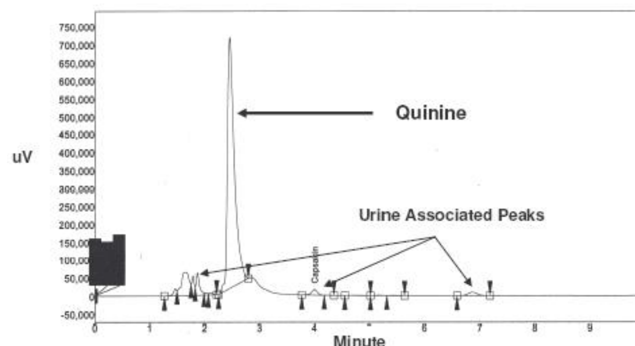
Human urine is first filter sterilized by Millipore 0.45 µm filtration system which removes bacteria and all particles larger. The specimen can be stored for later analysis at 4° C. The ability to utilize fresh patient urine by this approach increases clinical response efficiency so that real time compliance monitoring is possible. In addition, a rapid assay response is feasible to enable rapid treatment steps when patients appear with advanced malignant P. falciparum and/or advanced disabled conditions. This facet of injection of urine catch specimens is a strong benefit of this approach. The data presented here is accomplished for human urine dilution factor of 1:20, however it is noted that analogous results were observed with urine dilution factor of 1:10. No adverse results occurred with the C-18 column (ie. Clogging or diminished flow rate).

The molar extinction coefficient (molar absorptivity) is an intrinsic property of the analyte and is a measurement of how strongly an analyte absorbs light at a desired wavelength. The detector monitored wavelength at 254 nm and recorded strong absorption due to the quinine molecule. Using the typical equation of $A = \epsilon cl$, the molar absorptivity of quinine (ϵ) is 5441.9 Liter/cm•mole and utilizing a detector cell diameter of 0.60 cm

Typical chromatograms for both the test samples and standards having quinine at a predetermined concentration and in mixture of 1:20 (v/v) of patient urine are shown in Figure 2. Note that the absorbance peak for quinine at 254 nm is strong, stark, and well defined from any peaks associated with the sterile urine. The test sample the amount of quinine present is 3.74E-05 grams/milliliter. For the standard, quinine is present at a concentration of 5.99E-05 grams/milliliter. The background peaks associated with a media having human urine diluted 1:20 are not significant and pose no deficit to quinine determination. Quinine peak is indicated by inset arrow for comparison to urine associated peaks also indicated by inset arrow in chromatograms. The quinine is profoundly distinct within the chromatograms at all concentrations of quinine used for this study. The average percent recovery of quinine in test samples is 100.3 %, with a median and mode values of 96.1 %. Observed values for percent recovery in human urine diluted 1:20 ranged from 93% to 115 % and having a standard error of only 2.76 %.



Example of urine test sample with quinine



Standard prepared in urine for quinine analysis

Figure 2 : Representative chromatograms are presented for each of a test sample in filtered sterilized human urine (urine diluted 1:20 in distilled water) having quinine and a standard of quinine prepared in filtered urine. For the test sample the amount of quinine present is 3.74E-05 grams/milliliter. For the standard, quinine is present at a concentration of 5.99E-05 grams/milliliter. Note that the background peaks associated with the human urine media are not significant and pose no deficit to quinine determination. Quinine peak is indicated by inset arrow. The quinine is profoundly distinct within the chromatograms at all concentrations of quinine used for this study.

All results for test samples and standards were determined to pass the normality test (by GraphPad). In addition the Wilcoxon matched pair test showed that the test sample elution results were highly consistent with those of the standards and test samples were well matched with standards. The standard curve plot is presented in Figure 3 (plot of uv/min versus grams/milliliter for quinine). The standard curve produced a high Pearson r correlation of 0.9922 (very high correlation), with a coefficient of determination (R^2) at 0.9845. In addition the nonparametric Spearman correlation coefficient for the curve is a very high 1.000. The equation of line for this plot is y = 9.1709E+08 + 42978. All test samples analyzed were observed to fall within the 95% confidence interval of the 2-way plot for the standards.

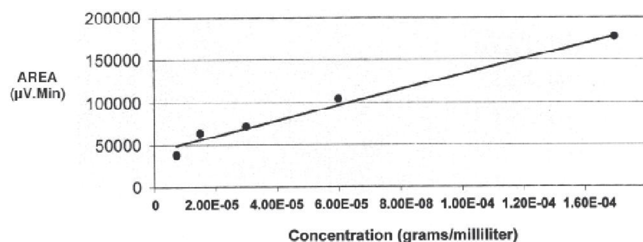


Figure 3 : A typical standard curve for quinine determination obtained at concentrations going to 7.00E-06 grams/milliliter of quinine and prepared in sterile filtered human urine diluted to 1:20 (distilled water) in preparation of the standard. Thus both test samples and standards are prepared similarly in human diluted urine. This insures similarity of column interaction and reproducibility in detection. The Pearson r correlation is 0.9922 (very high correlation), coefficient of determination (R^2) is 0.9845 (indicating 98.45 % of the total variance is shared by the two variables).

This approach which allow for the expedient analysis of captured patient human urine in the clinical environment will be of great efficacy for enforcement of patient compliance to anti-malarial regimen. Of course an additional benefit is the rapid and effective monitoring of administered quinine utilized for vital treatment of malignant P. falciparum. This approach will enhance patient survival in the clinical treatment of drug resistant forms of malaria as well.-

CONCLUSIONS

The analytical approach presented is a means to assay quinine directly from a clinical patient urine that is filter sterilized prior to aqueous dilution to 1:20 (or 1:10) by volume. The dilution gradation reduces background peaks of urine yet preserves the high sensitivity detection of excreted quinine in the urine itself. Test samples and standards together produced highly linear plots having Pearson correlation r greater than 0.9700. Percent recovery of analyte ranged from 94.3 % to 115 % in a range of 7.30E-06 g/mL to 1.50E-04 g/mL quinine. All test samples fell within the 95 % confidence interval of the standard curve. This methodology allows clinicians to directly monitor patient compliance of parenteral administered anti-malarial quinine which is a vital treatment for survival of drug resistant and/or malignant P. falciparum infections.

ACKNOWLEDGEMENTS

This work was supported by the College of Arts & Sciences, University of Nebraska, Omaha, Nebraska 68182 USA.

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