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Determination of quinine in drugs and beverages by fluorimetric method

A highly sensitive and simple fluorimetric method for the determination of quinine in pharmaceuticals and non-alcoholic beverages is proposed. The optimal conditions for quinine fluorimetric determination in drugs and beverages were found: solvent — 0.01 M sulfuric acid, excitation wavelength 353 nm, luminescence wavelength 452 nm, strobe parameters — signal delay 0.85 μs, signal duration 21.25 μs. To increase the sensitivity of the developed method, quinine luminescence was studied in sulfuric acid of various concentrations — from 0.005 to 1.000 M, and the quantum yield of quinine luminescence was calculated in all studied concentrations of sulfuric acid. It has been established that the highest luminescence intensity, the highest quinine quantum yield and the smallest background signal of the solvent was observed in 0.01 M H_2SO_4. The calibration curve exhibited the linear range from 0.10 to 1.00 mg/l. The limit of detection (LOD) was found to be 0.0029 mg/l for quinine in 0.01 M H_2SO_4. The suggested approach was successfully applied to determine the amount of quinine in tablets «Analgin-quinine» and in the non-alcoholic beverage «Schweppes Bitter Lemon». The proposed method can be used to control the quality of pharmaceuticals and food products.

Keywords: quinine, pharmaceuticals, beverages, fluorimetry, quantum yield, strobe parameters.

Introduction

Quinine is the alkaloid derived from Cinchona bark. Since 1633, quinine has been used as an antimalarial drug [1]. It also has antipyretic and analgesic properties [2]. In addition, due to the bitter taste, quinine is actively used in tonic water with the taste of «bitter lemon» or «bitter lime» [3]. In medicine, quinine is used to increase labor activity [4], but overdosing can lead to abortion [2]. Recent studies in rats have shown that quinine completely blocks ovulation and causes oxidative stress in the ovary of rats [5]. Quinine overdose is dangerous to human health and might be fatal. Therefore, the use of quinine as a food additive is limited up to 83–85 mg/l [6].

Different chromatographic techniques are applied for quinine determination in pharmaceuticals [7–9]. Despite the high prevalence of chromatography, these methods are expensive and toxic solvents are contained in mobile phases in most cases. Electrochemical [10–11], spectrophotometric [12] and fluorimetric [13] methods of analysis are used for quinine determination in beverages. Electrochemical and spectrophotometric methods have lower cost instrumentation, but suffer from less sensitivity. Fluorimetric methods have the highest sensitivity, and often are used as detectors in chromatography for the quinine determination in beverages [14–15].

It is known that quinine has the highest luminescence intensity in sulfuric acid solution. But different concentrations of sulfuric acid are used by researches. Thus, the authors [13] used 0.05 M sulfuric acid to determine quinine in tonic water. In work [12] 0.0005 M H_2SO_4 is used for quinine determination in beverages by capillary electrophoresis. For the determination of quinine in soft drinks by sequential injection analysis (SIA) 0.1 M H_2SO_4 was used [16]. Unfortunately, the authors of these works do not justify the choice of a particular acid concentration.

Qualitative and quantitative determination of quinine in drugs and beverages is a pressing issue in pharmaceutical and food industries, thus the development of highly sensitive methods for quality control is encouraged. The aim of the work is to develop a highly sensitive fluorimetric method for the determination of quinine in drugs and beverages.

Experimental

Reagents. The working solutions of quinine were prepared using the standard quinine substance (95 %; manufactured by Vekton, St. Petersburg, Russia). Quinine working solutions were prepared in 0.01 M H_2SO_4.

The following research objects were selected: 1. Tablets «Analgin-quinine», manufacturer Sopharma (Bulgaria). Ingredients: active ingredients — metamizole sodium 200 mg and quinine hydrochloride 50 mg;

excipients — microcrystalline cellulose, sodium carboxymelitic starch, Coplidon-25, talc and magnesium stearate. 2. Non-alcoholic beverage «Schwepps Bitter Lemon». Ingredients: water, sugar, citric acid, natural flavors, stabilizers, antioxidant ascorbic acid, quinine, carotene dye.

Equipment. All measurements were performed on a Fluorat-02-Panorama spectrofluorimeter fluid analyzer (manufactured by Lumex-Marketing LLC, St. Petersburg, Russia). Spectrophotometric measurements were carried out using Agilent Technology Cary 60 UV-Vis spectrophotometer.

Results and Discussion

As previously noted quinine has good luminescence in sulfuric acid solution [16]. Therefore a synchronous scan of a standard quinine solution (C = 10 mg/l) in sulfuric acid was performed at various monochromator displacements, to reveal the optimal excitation wavelength at which the maximum quinine luminescence occurs (Fig. 1).

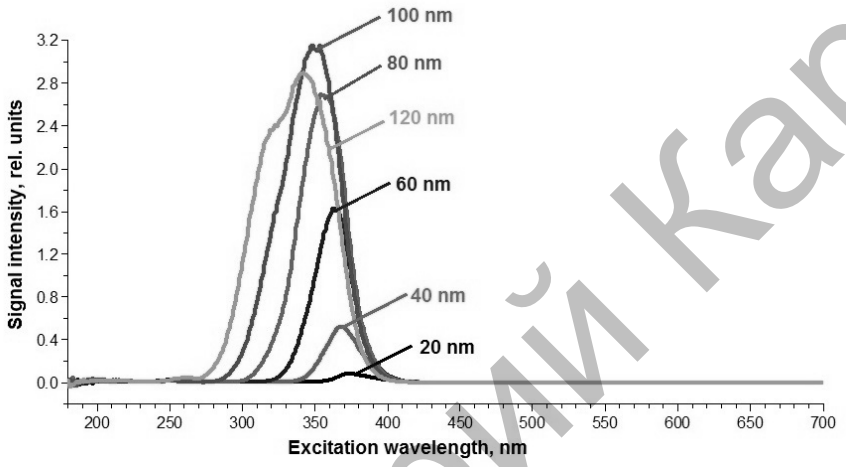


Figure 1. Synchronous scan mode of quinine standard solution (C = 10 mg/l) in sulfuric acid at different shifts of the monochromator

From the synchronous scanning mode an excitation wavelength of 353 nm was established and the maximum quinine luminescence in sulfuric acid at 452 nm was observed (Fig. 2).

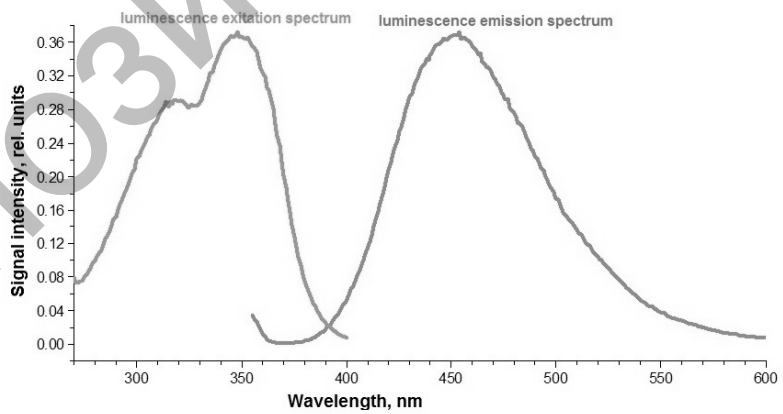


Figure 2. Excitation spectrum and luminescence spectrum of quinine in sulfuric acid

To increase the accuracy and sensitivity of the developed method different concentrations of sulfuric acid were studied, as well as the background and quinine intensity signals and the quantum yield in the studied acid solutions. The results are presented in Table 1.

It is known that quantum yield is one of the most important characteristics of a substance [17]. The quinine quantum yield in sulfuric acid solutions of different concentrations was calculated by the standard method. As a standard we have chosen a solution of fluorescein in 0.1 M NaOH with well-known quantum

yield 0.64 and the luminescence emission of fluorescein at a wavelength of 520 nm. For calculations accuracy the concentrations of the standard (fluorescein) and the test substance (quinine) were selected so that their optical density was lower than 0.1. The areas under the emission spectrum of both the investigated and standard substances (S_i), the optical densities of these substances at the excitation wavelength (D_i) and the refractive indices of solvents (n_i) were measured under the same conditions. The calculation of the quantum yield was carried out according to the formula

$$\varphi_{test} = \frac{\left(1-10^{-D_{st}}\right) * S_{test} * n_{test}^2}{\left(1-10^{-Dtest}\right) * S_{st} * n_{st}^2} * \varphi_{st},$$

where S_{test} is the area under the emission spectrum of the investigated substance; S_{st} is area under the emission spectrum of the standard substance; n_{test} is refractive index of the investigated solvent H_2SO_4; n_{st} is refractive index of the standard solvent NaOH; D_{test} is optical density of the investigated substance quinine at the excitation wavelength; D_{st} is optical density of the standard substance fluorescein at the excitation wavelength; φ_{test} is quantum yield of investigated substance quinine; φ_{test} is quantum yield of standard substance fluorescein.

According to the literature, the quinine quantum yield in 0.1 M H_2SO_4 is 0.58 [18].

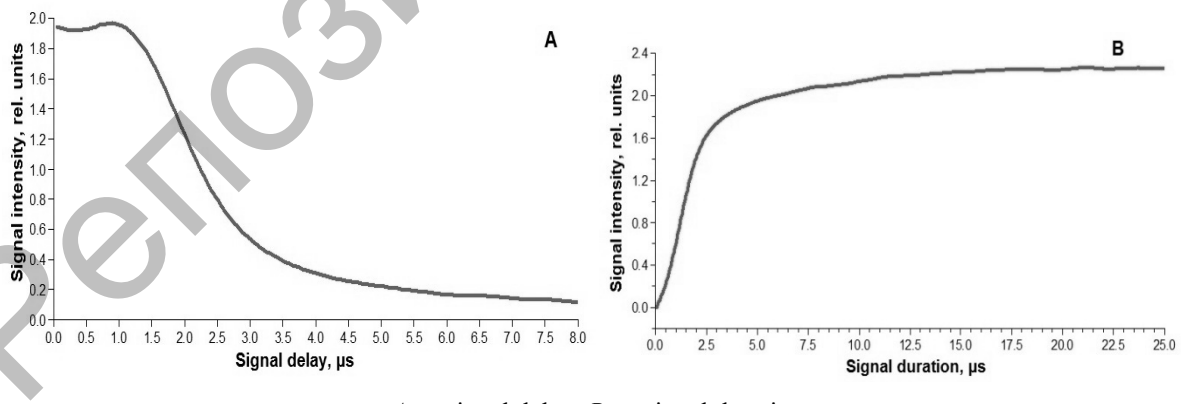
Table 1

Investigation of the luminescent properties of quinine in solutions of sulfuric acid of various concentrations

Investigated parameter	Concentration of sulfuric acid, M					
	0.005	0.01	0.05	0.1	0.5	1.0
Background signal intensity (sulfuric acid), rel. units	0.00199	0.00025	0.00327	0.00357	0.00542	0.01626
Quinine signal intensity (C = 100 mg/l) in sulfuric acid, rel. units	16.724	18.928	19.043	19.186	19.438	19.267
Quinine quantum yield, rel. units	0.555	0.609	0.420	0.590	0.608	0.610

As can be seen from the table the highest quantum yield of quinine is observed in 0.01 M and 0.1 M sulfuric acid solutions. With increase sulfuric acid concentration acid, the intensity of the quinine luminescence signal also increases, but rather slightly. Therefore 0.01 M sulfuric acid was chosen for quinine quantitative determination in the studied pharmaceuticals and food products. Furthermore the lowest background signal intensity was observed at a given acid concentration, which will further increase the detection limit.

For sensitivity enhancement of quinine determination, the strobe parameters were selected — delay time (signal intensity versus time) and signal duration (recording time at one wavelength) (Fig. 3).



A — signal delay; *B* — signal duration

Figure 3. Dependence of quinine luminescence intensity on strobe parameters

When studying the dependence of the luminescence intensity on the signal delay in the range from 0.05 to 8.00 μs, the optimum value of the signal delay for quinine was set to 0.85 μs. From the signal duration range from 1.00 to 25.00 μs the duration is set to 21.25 μs. The highest quinine luminescence intensity was observed under selected strobe parameters.

Depending on the nature of the excited electronic state the luminescence is divided into two types – fluorescence and phosphorescence. In practice, the processes of fluorescence and phosphorescence differ in temporal characteristics. Instantaneous attenuation of the emission after excitation cessation from 10^{-7} to 10^{-10} s is typical for fluorescence, the continuation of a certain glow time after excitation cessation from 10^{-6} to 10^{-1} s — for phosphorescence. To establish the type of quinine luminescence process the dependence of the luminescence signal intensity on the signal time was plotted (Fig. 4).

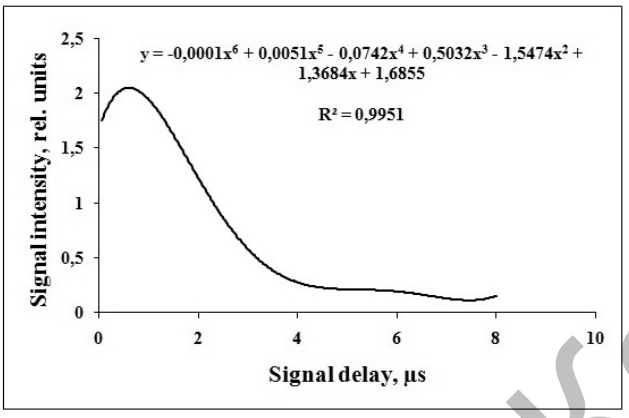


Figure 4. The decay kinetics curve for quinine in 0.01 M H_2SO_4

Using the quinine attenuation curve obtained, the average luminescence lifetime was calculated through the area under the attenuation curve (a definite integral from 0 to 8 μs for the function):

$$\int_0^8 (-0.0001x^6 + 0.0051x^5 - 0.0742x^4 + 0.5032x^3 - 1.5474x^2 + 1.3684x + 1.6855)dx.$$

For quinine the lifetime was $1.505 \cdot 10^{-5}$ s. From the calculations it can be concluded that phosphorescence is characteristic for quinine in 0.01 M H_2SO_4.

Thus, the following working conditions were selected: solvent — 0.01 M H_2SO_4, excitation wavelength 353 nm, luminescence wavelength 452 nm, signal delay 0.85 μs, signal duration 21.25 μs. Under optimized conditions a linear calibration curve of the luminescence intensity on quinine concentration was plotted in the range of 0.10–1.00 mg/l (Fig. 5*A*).

Spectrophotometry was used as a comparison method. Quinine was determined by its own absorption. The dependence of the optical density from the quinine concentration in 0.1 M sulfuric acid at an absorption wavelength 347 nm showed linear response in concentration range of 1.00 to 10 mg/l (Fig. 5*B*).

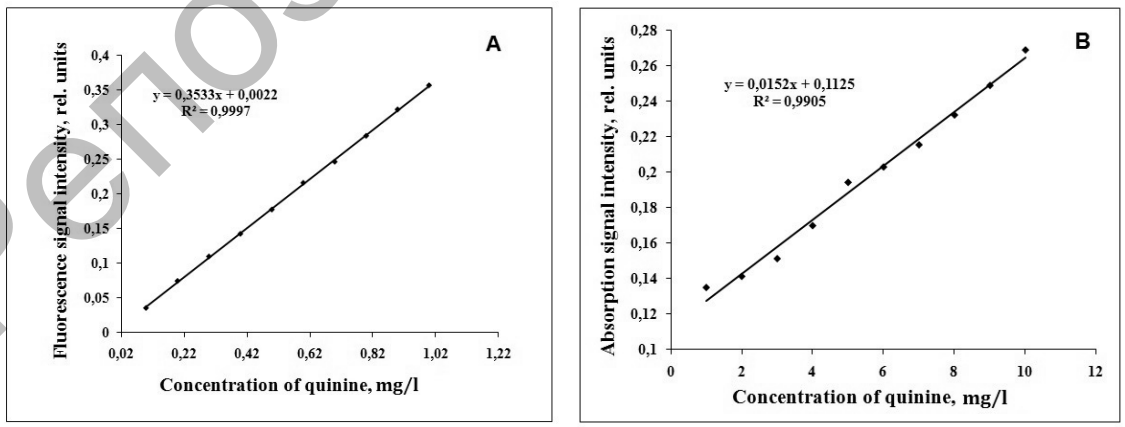


Figure 5. Calibration curves of the intensity of the luminescence (*A*) and absorption (*B*) on the concentration of quinine in 0.01 M sulfuric acid

Sample preparation of the objects was as follows. The tablet was previously dissolved in 20 ml of 0.01 M sulfuric acid. The resulting solution was diluted 10 000 times. The intensity of the diluted solution was

measured and quinine concentration was recalculated into 20 ml of the initial solution. The original sample of the beverage was degassed and diluted 10 times with distilled water. The results of quantitative determination of quinine by two methods are presented in Table 2.

Table 2

The results of the determination of quinine in tablets and soft drinks by fluorimetric and spectrophotometric methods, n = 3, p = 0.95, t_{table} = 2.78

Sample	Fluorimetric method, mg/l	S_r	Spectrophotometric method, mg/l	S_r	t_{exp}
Tablet «analgin-quinine»	51.41±0.43	0.003	52.33±2.74	0.021	0.074
Beverage «Schweppes Bitter Lemon»	53.74±1.86	0.014	54.42±5.25	0.039	0.373

As can be seen from Table 2 there is a good agreement between results obtained by the developed fluorimetric method and spectrophotometric method. The quinine content does not exceed the maximum allowable value of 85 mg/l in a beverage. But it is content in the tablet is slightly higher than the stated amount of 50 mg per tablet, which might have a bearing on human health on scheduled administration.

Additionally the limit of detection of quinine in 0.01 M sulfuric acid was calculated as 0.0029 mg/l. The value of the limit of detection is much lower in comparison with the majority of works [7–8, 11–16].

Conclusions

An effective, highly sensitive, simple and low-cost fluorimetric method for the determination of quinine in pharmaceuticals and soft drinks has been developed. The quantum yield of quinine in sulfuric acid of various concentrations was calculated. Optimal conditions for quinine determination in 0.01 M sulfuric acid were selected. The quinine luminescence process was studied and the quinine phosphorescence process was observed in 0.01 M H_2SO_4. Due to the selected assay conditions the high detection sensitivity and the limit of detection for quinine determination in pharmaceuticals and beverages has been achieved.

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Дәрілік препараттар мен сусындардағы хининді флуориметрия әдісімен анықтау

Фармацевтикалық препараттар мен алкогольсіз сусындарда хининді анықтаудың жоғары сезімтал және қарапайым флуориметрлік әдісі ұсынылған. Дәрілік заттар мен сусындарда хининді флуориметрлік анықтаудың оңтайлы шарттары табылды: еріткіш — 0,01 М күкірт қышқылы, қозу толқынының ұзындығы 353 нм, люминесценция толқынының ұзындығы 452 нм, строб параметрлері — сигналдың кідірісі 0,85 мкс, дабылдың ұзақтығы 21,25 мкс. Әзірленетін әдістеменің сезімталдығын арттыру үшін 0,005-нан 1,000 М-ге дейін күкірт қышқылының әртүрлі концентрацияларында хининнің люминесценциясына зерттеу жүргізілді, сондай-ақ күкірт қышқылының барлық зерттелетін концентрацияларында хининнің кванттық шығыны есептелді. Хининнің люминесценциясының ең үлкен қарқындылығы, хининнің ең үлкен кванттық шығымы және еріткіштің ең аз фонының сигналы 0,01 М H_2SO_4-те байқалатыны анықталды. Хининді анықтау 0,10-ден 1,00 мг/л дейінгі концентрация аралығында жүргізілді. Табылған жағдайда «Анальгин-хинин» дәрілерінде және «Schweppes Bitter Lemon» алкогольсіз сусынында хининнің мөлшері зерттелді. Салыстыру әдісі ретінде талдаудың спектрофотометриялық әдісі қолданылды. Хининді салыстыру әдісімен анықтау толқын ұзындығы 347 нм кезінде хининді жұтқан кезде 1,00-ден 10,00 мг/л-ге дейінгі концентрация диапазонында жүргізілді. Ұсынылған әдістеме фармацевтикалық препараттар мен тамақ өнімдерінің сапасын бақылау үшін пайдаланылуы мүмкін.

Кілт сөздер: хинин, фармацевтикалық дәрілік заттар, сусындар, квантты шығым, строб параметрлері.

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Определение хинина в лекарственных препаратах и напитках методом флуориметрии

Предложен высокочувствительный и простой флуориметрический метод определения хинина в фармацевтических препаратах и безалкогольных напитках. Найдены оптимальные условия флуориметрического определения хинина в лекарствах и напитках: растворитель — 0.01 М серная кислота, длина волны возбуждения 353 нм, длина волны люминесценции 452 нм, параметры строба — задержка сигнала 0.85 мкс, длительность сигнала 21.25 мкс. Для увеличения чувствительности разрабатываемой методики проведены исследования люминесценции хинина при различных концентрациях серной кислоты от 0.005 до 1.000 М, а также подсчитан квантовый выход хинина при всех исследуемых концентрациях серной кислоты. Установлено, что наибольшая интенсивность люминесценции хинина, наибольший квантовый выход хинина и наименьший сигнал фона растворителя наблюдается в 0.01 М H_2SO_4. Определение хинина проводилось в диапазоне концентраций от 0.10 до 1.00 мг/л. Рассчитан предел обнаружения хинина в 0.01 М H_2SO_4 при заданных условиях, который составил 0.0029 мг/л. При найденных условиях исследовано содержание хинина в таблетках «Анальгин-хинин» и в безалкогольном напитке «Schweppes Bitter Lemon». В качестве метода сравнения использован спектрофотометрический метод анализа. Определение хинина методом сравнения проводили в диапазоне концентраций от 1.00 до 10.00 мг/л при поглощении хинина при длине волны 347 нм. Предложенная методика может быть использована для контроля качества фармацевтических препаратов и пищевых продуктов.

Ключевые слова: хинин, фармацевтические препараты, напитки, квантовый выход, параметры строба.