Current Topics in Toxicology
Vol. 11, 2015

Original Communication

Comparison of constant-wavelength synchronous spectrofluorimetry method and HPLC method for the rapid analysis of quinine

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ABSTRACT

Quinine is included in the positive list of the European Union as a food flavouring additive and as it is a potentially toxic drug, it is used with a limit of 100 mg/l in different non-alcoholic beverages. Two rapid and sensitive methods were utilized. The first method is based on measuring the synchronous fluorescence spectra of quinine at $\Delta\lambda$ = 100 nm in 0.05 M H_2SO_4. The second method, high-performance liquid chromatography (HPLC) separation was performed in less than 10 min; UV detection was performed at 250 and 275 nm and fluorescence detection at 375 nm after excitation at 325 nm; standard solutions of quinine were prepared in Milli-Q water. Both methods are suitable for the analysis of tonic waters. Constant-wavelength synchronous spectrofluorimetry method is considered as a good method, which is selective and more sensitive, quick and inexpensive for determining quinine content.

KEYWORDS: quinine, HPLC, constant wavelength synchronous spectrofluorimetry, tonic waters

INTRODUCTION

The cinchona alkaloids are an important subgroup of the polycyclic β-carbonile alkaloids. Quinine (6´-methoxycinchonan-9-ol), a naturally occurring alkaloid is a bitter tasting powder extracted from the bark of the cinchona tree native to South America. Quinine is an old antimalarial drug that has been in use for over 350 years [1-3]. Additionally, it is often used as a flavouring in soft drinks such as tonic water because of its bitter taste [3-7]. However, quinine is a potentially toxic drug. The typical syndrome caused by very high therapeutic doses of quinine is called cinchonism. The side effects of quinine include ringing in the ears, nausea, abdominal pain, headache and fever, renal failure, asthma, [6, 8] and allergies [9-10]. Quinine in tonic water may also cause hypersensitivity and anaphylactic shock [9-12]. For these reasons it should not be prescribed during pregnancy, as it can cause birth defects and miscarriages. Therefore, some countries such as the United States and Germany have instructed that quinine concentration should be declared on food labels [13-14] (with an upper limit between 83 and 85 mg/Kg) while other countries like Japan [10] does not legally permit quinine to be added to drinks. In Spain and other European countries the maximum permitted limit of quinine is set at 100 mg/L for different beverages, and the quinine content must be included in the list of ingredients [6-7, 15-16].

Various techniques have been reported for the determination of quinine. These methods include luminescence spectrometry and high performance liquid chromatography, methods that have been used by us. Fluorescence spectroscopy is an extremely sensitive technique and quinine is one of the most active fluorescent agents known [17-19]. However, the HPLC is the most used method for the determination of quinine. Most of the reported

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methods refer to the analysis of biological fluids [20-25] and only a few are focused on the analysis of alcoholic and non-alcoholic soft drinks, such as tonic water and bitter lemon [13-14, 26].

The aim of this study was to compare two analytical methods for the identification and quantification of quinine. The first method, constant-wavelength synchronous spectrofluorometry, is a technique that is very simple, sensitive and cheap, and is usually not used. It is based on measuring the synchronous fluorescence spectra of quinine at $\Delta\lambda$ = 100 nm. The second method, HPLC with PDA and fluorescence detection is the most used in the literature. These techniques were applied in the present study for the determination of quinine in tonic waters.

MATERIALS AND METHODS

Chemicals and standard solution

All reagents used in this work were of analytical grade. Acetonitrile, methanol, ammonium acetate ($NH_4CH_3CO_2$) and sulfuric acid (H_2SO_4) were supplied by Merck (Darmstadt, Germany). Quinine was supplied by Alfa-Aesar (Germany). Water used for all the solutions was obtained from a Milli-Q water purification system (Millipore) (Belford, MA, USA). Stock standard solutions of quinine were prepared in Milli-Q water. The working solutions were prepared in 0.05 M H_2SO_4. Quinine solutions were prepared daily and should be protected from light.

Spectrofluorimetry analysis

All spectrofluorimetric measurements were performed with a Perkin-Elmer LS-50 luminescence spectrometer (Beakons Weld, UK), equipped with a xenon discharge lamp, Monk-Gillieson monochromators and 1 cm quartz cuvettes. Spectral data acquisition and processing were carried out by means of the program Fluorescence Data Manager (v. 2.5 and 3.5), on a personal computer serially interfaced (RS232C) to the spectrometer. The spectra were obtained with the following instrumental parameters: excitation and emission slits of 2.5 nm and 5 nm, respectively and scan speed of 480 nm/min. Thirty synchronous scans of the quinine in 0.05 M H_2SO_4 were recorded between 200 and 500 nm, with an initial interval of 10 nm between the monochromators of excitation and emission in the fluorescence range of samples. In successive scans, the wavelength interval between the monochromators was increased by 5 nm ($\Delta\lambda$ range of 10 to 155 nm). Optimum excitation-emission wavelength interval for synchronous spectrofluorimetric analysis was 100 nm.

Quantification was performed using the fluorescence intensity at 350 nm of spectra obtained with the wavelength interval of 100 nm between the monochromators of excitation and emission. Calibration lines were constructed based on five concentration levels of standard solutions within 0.100-0.800 mg/L range. Limits of detection and quantification were calculated in accordance with American Chemical Society [27]. Sulfuric acid, 0.05 M, used as solvent of standard working solutions did not interfere with the results.

The precision and recovery were determined by applying this procedure to six replicate samples of tonic water spiked with 0.240 mg/L of quinine.

HPLC analysis

The HPLC consisted of a quaternary pump (Jasco PU-2089 Plus), a manual injector setup (50 µL loop) a degasser, a photodiode array detector (Spectra System UV 8000) and a fluorescence detector (Spectra-Physic, FL 2000). The HPLC system was controlled by a Software ChromQuest 5.0. Chromatographic separation was carried out with a Kromasil C18 column (15 x 0.4 cm; 5 µm particle size) and a flow rate of 0.5 mL/min. Isocratic mobile phase consisted of CH_3OH:CH_3CN:0.1 M CH_3COONH_4 (45:15:40 v/v/v) [22]. UV detection was set at 250 and 275 nm and fluorescence detection was set at 325 nm (excitation) and 375 nm (emission). The column oven temperature was set at 23 ºC (Croco-Cil column heater). The total run time was 10 min. Quantification was carried out by the external standard method. Parameters of linearity, limit of detection (LOD), limit of quantitation (LOQ), precision and recovery (spiked with 4 mg/L) were calculated in the same way as for the spectrofluorometric method.

Samples

The method was applied to six samples of tonic water of six different brands, acquired from different local shops of Santiago de Compostela (Northwest Spain).

Spectrofluorimetry: Sufficient quantity (5-10 mL) of tonic water was added to a small beaker.

The sample was degassed (Ultrasons Selecta, Spain) for 10-15 min. 0.125 mL of the tonic water sample was poured into a 10 mL volumetric flask and 0.05 M volume of H_2SO_4 was added. Synchronous spectra were recorded between 200 and 500 nm, with wavelength interval of 100 nm. The fluorescence intensities were measured in duplicate.

HPLC: 5 mL of the tonic water sample was poured into a 100 mL volumetric flask and was diluted to 100 mL with Milli-Q water.

Statistical analysis

The Statgraphics-Plus 5.1 software was used for simple regression analysis and variance (ANOVA) of data obtained by HPLC and spectrofluorimetry to identify the differences between both methods. The level of significance was $p < 0.05$.

RESULTS AND DISCUSSION

The quinine dissolved in 0.05 M H_2SO_4 has two analytically useful excitation wavelengths, 250 and 350 nm. Regardless of which is used, the maximum wavelength fluorescence is 450 nm. Normally, 350 nm as excitation wavelength and 450 nm as emission for the scan spectra is used [17, 19]. In constant wavelength synchronous spectrofluorimetry, the optimum $\Delta\lambda$ is very important with regard to resolution and sensitivity. It can directly influence the spectral shape, bandwidth and signal value. The wavelength interval between the emission and excitation monochromators that afforded the best signal was 100 nm (Fig. 1). Fig. 2 shows the fluorescence spectra ($\Delta\lambda$ = 100 nm) of quinine solution and all tonic waters analyzed. All the peaks are at the wavelength of maximum fluorescence (350 nm).

In HPLC, the retention time of quinine was about 6 minutes. In table 1, the parameters of linearity, precision (% RSD) and recovery (% R) obtained in both methods are shown. It can be observed that the limit of detection of spectrofluorimetric method is much lower than that obtained in HPLC, while the precision and recovery are similar. The results obtained in HPLC with Dyode Array Detector

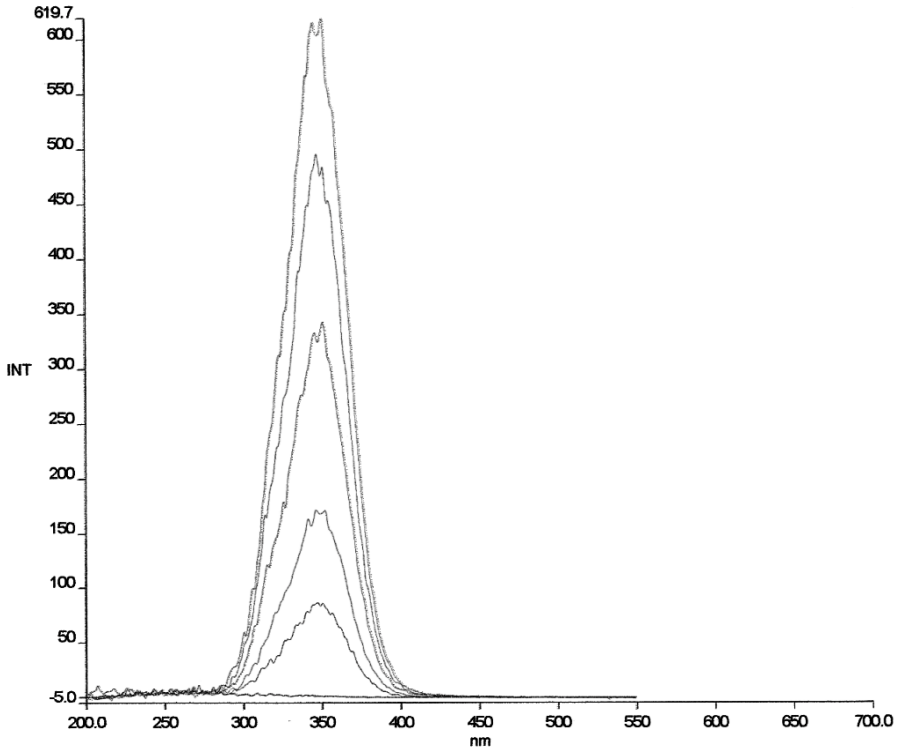

Fig. 1. Constant-wavelength synchronous fluorescence spectra ($\Delta\lambda$ = 100 nm) of quinine concentrations (0.1-0.8 mg/L) in 0.05 M H_2SO_4

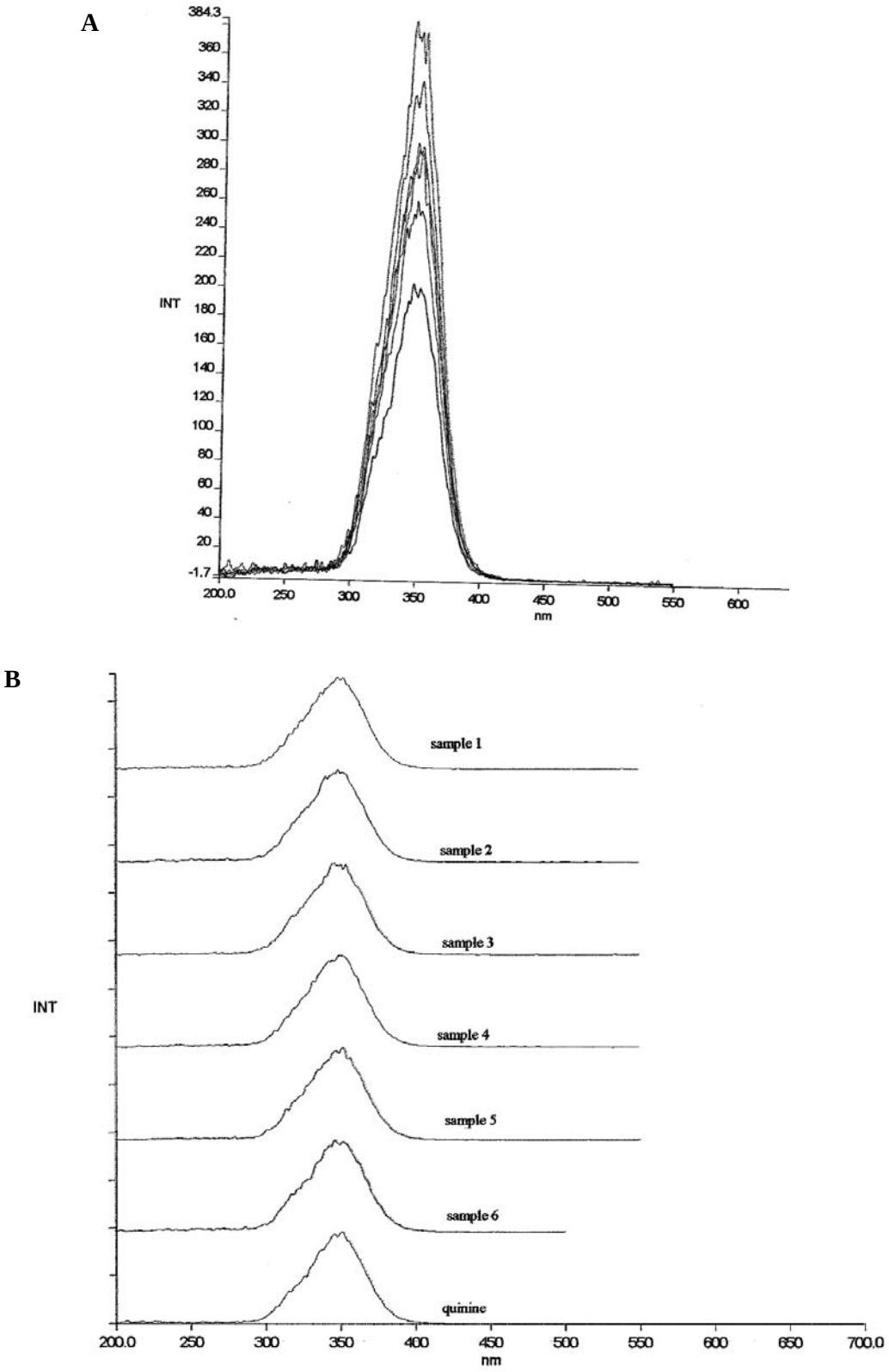

Fig. 2. Constant-wavelength synchronous fluorescence spectra ($\Delta\lambda$ = 100 nm) of quinine solution and all tonic waters analyzed together (A) and separately (B).

Table 1. Parameters of linearity, limits of detection, limits of quantification, precision and recovery.

	Intercept	Slope	r^2	Range mg/L	LOD mg/L	LOQ mg/L	Precision (RSD %)	Recovery (%)
Spectrofluorimetry $\Delta\lambda$ = 100 nm	13.16	1.050	0.9969	0.100 - 0.800	0.0077	0.014	3.84	94
HPLC								
UV 250 nm	19331	383977	0.9985	1.00 - 8.00	0.026	0.045	2.54	97
UV 275 nm	24573	364373	0.9984	1.00 - 8.00	0.033	0.058	2.20	101
FL	94975	481544	0.9993	1.00 - 8.00	0.046	0.085	2.51	105

Table 2. Quinine concentration in six commercial tonic water samples, expressed in mg/L.

Samples	HPLC 250 nm	HPLC 275 nm	HPLC Fluorescence	Spectrofluorimetry $\Delta\lambda$ = 100 nm
1	68.4	68.1	68.0	66.7
2	48.6	49.1	51.1	51.9
3	75.2	75.4	81.6	75.5
4	63.1	65.9	65.9	58.1
5	56.8	55.7	59.9	55.2
6	44.6	43.7	47.7	44.2

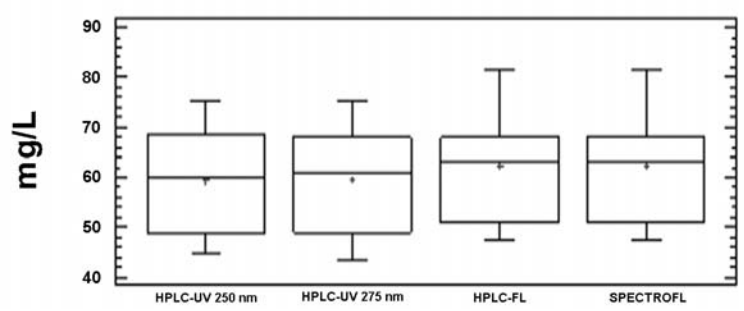

Fig. 3. Box and whisker diagram of quinine content in the samples (n = 6) analyzed by HPLC and spectrofluorimetry methods.

(DAD) (λ = 250 nm and λ = 275 nm) and FL (λ_{ex} = 325 nm and λ_{em} = 375 nm) are similar.

The quinine concentrations obtained from the tonic waters analysed are listed in table 2. The quinine concentration found is between 44.6 and 81.6 mg/L, always below 100 mg/L. In fig. 3 the box and whisker diagrams of quinine content is presented.

Statgraphics Plus 5.1 software was used in paired sample test. In the study of the data obtained by fluorescence spectroscopy with constant wavelength interval and HPLC (HPLC UV 250 nm-HPLC UV 275 nm, HPLC UV 250 nm-HPLC FL, HPLC UV 275 nm-HPLC FL, HPLC UV 250 nm-Spectrofluorimetry, HPLC UV 275 nm-Spectrofluorimetry and HPLC FL-Spectrofluorimetry), the p-value in the ANOVA table is less than 0.01, indicating that there is a statistically significant relationship for a confidence level of 99% in all cases. The correlation coefficient range is 0.9564-0.9937 indicating a relatively strong relationship between the variables. This shows that synchronous spectrofluorimetry with constant wavelength interval is a good alternative method.

The results are similar to those reported elsewhere [13] in soft drinks using HPLC-FL; however, the

performance parameters obtained were worse. Other authors have obtained similar contents by direct analysis of quinine in beverages and soft-drinks [14], with recoveries about 100%. If the results obtained in this work are compared with those that have been reported by other authors [8], our values are slightly lower (between 54 and 95 mg quinine/L), but with almost the same range of variation. Other authors [20-23] have studied quinine and other compounds in plasma and urine samples using HPLC and have found high coefficients of variation.

Recoveries of quinine in real samples of biological fluid [17, 23] were above 100% or lower [22, 25, 28]. Other authors [26] have performed direct potentiometric determinations of quinine in soft drinks and the results obtained showed low standard deviations and high recoveries.

In the Regulation (European Union (EU) [7] the use of three quinine salts, quinine hydrochloride, quinine sulfate and quinine monohydrochloride dehydrate in non-alcoholic beverages, at concentration not exceeding 100 mg/Kg is included. All the samples analysed meet the requirements of Regulation EU [7].

HPLC method is compatible with aqueous samples; the samples can be injected directly, with a simple dilution with Milli-Q water. Besides, a good separation is possible if other compounds are present. Constant-wavelength synchronous spectrofluorimetry is a technique rarely used, but cheap; samples are simply diluted with 0.05 M H_2SO_4. It is faster than HPLC method, because the result is immediate; however, its precision is slightly lower. Constant-wavelength synchronous spectrofluorimetry is a very sensitive method, with more acute and selective peaks than those obtained with conventional spectrofluorimetry and allows a high throughput of samples; the solvent consumption is limited to that required for the preparation of samples. The selectivity of the technique is very high, because eliminates interferences from Rayleigh scattering and the virtual absence of non-fluorescent compounds in the sample.

CONCLUSION

In this work we have studied two analytical methods, constant-wavelength synchronous spectrofluorimetry and HPLC with PDA and Fluorescence detection, for identification and quantification of quinine. Both techniques have been applied to the analysis of tonic waters containing quinine, and no significant differences have been found. Constant-wavelength synchronous spectrofluorimetry is a selective and sensitive technique and has also shown good reproducibility. Moreover it is a reliable analytical technique, inexpensive, quick, free of organic solvents and easy to use for determining quinine content in tonic waters samples. Therefore it can be recommended for routine and quality control analysis of non-alcoholic beverages or drugs.

CONFLICT OF INTEREST STATEMENT

None of the authors has any conflicts of interest to declare.

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