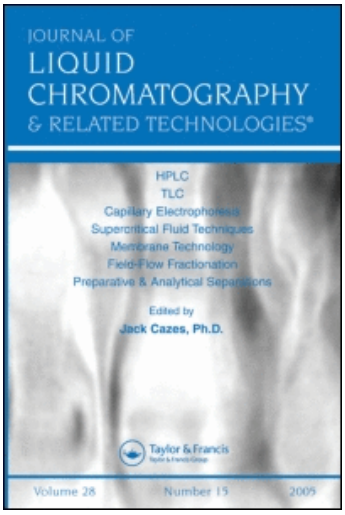

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Simple and Rapid HPLC Method for the Determination of Quinine in Soft Drinks Using Fluorescence Detection

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ABSTRACT

A simple and rapid reversed-phase high performance liquid chromatographic (RP-HPLC) method was developed for the routine determination of quinine in soft drinks, which is added when a bitter taste is required. The analytical column, an MZ Kromasil, C_{18}, 5 μm, 250 × 4 mm^2, was operated at ambient temperature with backpressure of 230 kg/cm^2. The mobile phase consisted of $CH_3OH-CH_3CN-CH_3COONH_4$ 0.1 M, (45 : 15 : 40 %v/v/v) and was delivered at a flow rate of 1.0 mL/min. Fluorescence detection was performed at 325 nm (excitation) and 375 nm (emission). For the quantitative determination of quinine, salicylic acid was used as internal standard at a concentration of 0.5 ng/μL, resulting

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in a detection limit (signal-to-noise ratio 3 : 1) of 0.3 ng, while the upper limit of linear range was 0.7 ng/μL. Analysis time was less than 5 min. The statistical evaluation of the method was examined performing intra-day ($n = 8$) and inter-day calibration ($n = 8$) and was found to be satisfactory, with high accuracy and precision results. The method was applied to the analysis of soft drinks containing quinine, such as tonic water and bitter lemon. No interferences from other food additives were observed.

Key Words: Quinine; Beverages; Soft drinks; Tonic water; Bitter lemon; HPLC; Fluorescence detection.

INTRODUCTION

Quinine (6′-methoxycinchonan-9-ol), a natural occurring alkaloid, is a bitter tasting powder extracted from the bark of the cinchona tree of South America. It is well known for its activity against malaria and, for this reason, it has been used in medicine as anti-malarial agent.[1,2] Moreover, it has been prescribed for the treatment of muscle cramps.[3,4] Due to its bitter taste, it is also used as an additive in soft drinks, such as tonic water and bitter lemon.[5,6]

However, quinine is a potentially toxic drug. The typical syndrome of quinine side effects is called cinchonism, and it can be mild in usual therapeutic dosage or severe in larger doses. Quinine side effects include ringing in the ears, vertigo, disturbed vision, nausea, diarrhoea, abdominal pain, headache and fever, renal failure, chest pain, and asthma. For these reasons it should not be prescribed during pregnancy, as it can cause birth defects and miscarriages. As a consequence, some countries, such as the United States and Germany, order that quinine concentration must be declared on food labels (with an upper limit of 83 and 85 mg/kg, respectively). In other countries, like China, quinine is not legally permitted to be added to drinks. Greek legislation decrees that the upper limit of quinine content in alcohol free drinks must be 100 mg/L.[7,8]

Various techniques have been reported for the determination of quinine. These techniques include UV-Vis spectrometry, fluorimetry, polarography, atomic absorption spectroscopy, membrane electrodes, and chromatography.[7–15] In the latter, both normal and reversed-phase methods have been reported, with either UV[12] or fluorescence detection.[11,13] The second is better because of its greater sensitivity. Ion pairing techniques are also reported in literature, though retention times are higher in this case.[13,14] Most of the reported methods refer to the analysis of biological fluids and only a few are focused on the analysis of alcoholic beverages and non-alcoholic soft drinks, such as tonic water and bitter lemon.[14,15]

A simple and rapid reversed-phase high performance liquid chromatographic (RP-HPLC) method was developed and described herein for the routine determination of quinine in soft drinks.

EXPERIMENTAL

Instrumentation

An SSI 222D pump (SSI, State College, PA) was used to deliver the mobile phase to the analytical column, Kromasil, C_{18}, 5 μm, 250 × 4 mm^2, MZ analytical (Mainz, Germany).

Sample injection was performed via a Rheodyne 9125 injection valve (Rheodyne, Cotati, CA) with a 50-μL loop. Detection was achieved by a fluorescence detector, RF-551 Shimadzu (Kyoto, Japan). A Hewlett-Packard (Avondale, PA) HP3396A integrator was used for quantitative determination of eluted peaks. A glass vacuum-filtration apparatus obtained from Alltech Associates was employed for the filtration of the buffer solution, using 0.2-μm-membrane filters obtained from Whatman (Maidstone, England). Degassing of solvents was achieved by helium sparging before use. Dissolution of compounds was enhanced by sonication in a Transonic 460/H Ultrasonic bath (Elma, Germany).

Reagents and Materials

Quinine was supplied by Sigma (St. Louis, MO). Methanol and acetonitrile, ammonium acetate, and salicylic acid were supplied by Merck (Darmstadt, Germany). Water used for all dilutions was bis deionised.

Quinine containing soft drinks were purchased from the local market. These include: Ivi tonic water (Pepsico-Ivi, Athens, Greece), Britvic Indian tonic and Britvic bitter lemon drink (Britvic Soft Drinks LtD, Chelmsford, England), Tuborg tonic water (Tuborg, Denmark), Schweppes Indian tonic water, Schweppes bitter lemon (By 3E, Athens, Greece, under the license of Schweppes Holdings Limited), tonic water (DIA S.A. Madrid, Spain).

Preparation of Standard Solutions

Aqueous stock standard solutions were prepared at a concentration of 100 ng/μL. Working standards were prepared from these stocks in the range of 0.01–0.7 ng/μL, by appropriate dilution and contained the internal

standard salicylic acid at a concentration of 0.5 ng/μL; these were found to be stable for at least three months, when stored refrigerated.

Chromatographic Conditions

The analytical column was a Kromasil, C_{18}, 5 μm, 250 x 4 mm^2. Mobile phase consisted of $CH_3OH–CH_3CN–CH_3COONH_4$ 0.1 M, (45 : 15 : 40% v/v/v). This was delivered at a flow rate of 1.0 mL/min. Backpressure observed was 230 kg/cm^2. Fluorescence detection was performed at 325 nm (excitation) and 375 nm (emission). Under these chromatographic conditions, retention time of quinine was 4.853 min. A typical chromatogram is shown in Fig. 1.

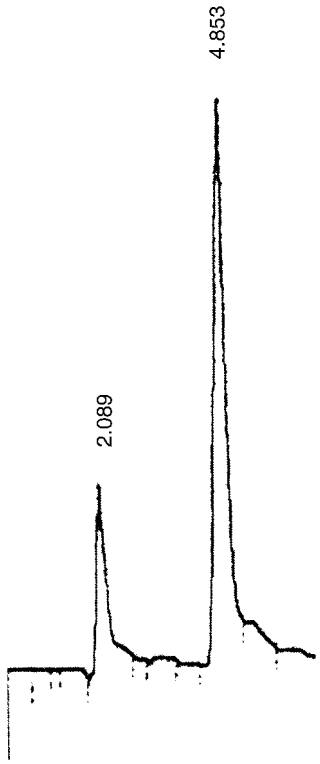

Figure 1. Chromatogram of quinine (4.853 min) in the presence of salicylic acid as internal standard (2.089 min).

Method Validation

Method validation was performed in terms of linearity, sensitivity, stability, accuracy, and precision. The intra-day repeatability was determined by analyzing three standards at eight replicates on the same day. The inter-day precision (defined as the RSD of triplicate analyses) was obtained by analyzing the three standards on eight consecutive days.

Sample Preparation

An indicative concentration of up to 60 ppm of quinine was stated on the label of one product (Tuborg tonic water). All other products stated that they contain quinine, with no further information concerning its concentration. After opening the soft drinks can, an aliquot was transferred into a glass beaker, and was sonicated for 10 min in an ultrasonic water bath, to remove carbon dioxide. Sample dilution was subsequently performed to reach the working range. Diluted samples were filtered through a 0.2 μm filter prior to injection to the chromatographic system. Three concentration levels were prepared and six measurements were performed at each level.

RESULTS AND DISCUSSION

Standardization, Determination of Sensitivity, Validation, and Stability

Standardization was carried out by injecting a series of standards ranging in concentration from 0.01 to 0.7 ng/μL in order to determine sensitivity. Analysis was carried out at ambient temperature. The response factors for each concentration were calculated from the ratio of peak area of analyte to that of IS (Salicylic acid 0.5 ng/μL). Regression analysis revealed the parameters of the equation shown in Table 1.

Correlation coefficient, intercept, and slope were calculated. Each point of the calibration curve represents an average of five determinations. Detection limit was calculated using the criterion of signal-to-noise ratio 3 : 1. Validation of the procedure was determined by the consistency of relationship between each concentration and the corresponding factor. The intra-day repeatability was determined by analyzing three standards with eight replicates, on the same day. The inter-day precision (defined as the RSD of triplicate analyses) was obtained by analyzing the three standards on eight consecutive days.

Table 1. Performance parameters for the quantitation of quinine in the presence of salicylic acid as IS.

Parameter	Value
Slope $(ng/\mu L)^{-1}$	3.8677 ± 0.1110
Intercept	0.1713 ± 0.0330
Correlation coefficient	$R = 0.9975$
LOD (ng)	0.3
LOQ (ng)	0.5
Upper limit $(ng/\mu L)$	0.7

Note: x, analyte's concentration.

Accuracy was estimated by the following equation: the results obtained are reported in Table 2.

$$\text{Accuracy}\,(\%) = \frac{[\text{Mean determined value} - \text{Theory (added amount)}]}{\text{Theory}} \times 100$$

Stability of the detector response was checked daily at three concentrations.

Real Sample Analysis

Analysis of seven commercial soft drinks containing quinine was performed. Typical chromatograms of quinine in tonic water and bitter

Table 2. Intra-day and inter-day accuracy and precision data for quinine determination.

Added (ng)	Found $\pm$ SD (ng)	RSD	Relative error (%)
		Intra day $n = 8$	
0.5	0.56 ± 0.05	8.0	12.0
3.5	3.74 ± 0.30	8.0	6.8
25	27.82 ± 3.01	10.8	11.3
		Inter day $n = 8$	
0.5	0.52 ± 0.04	8.2	4.0
3.5	3.49 ± 0.22	6.3	-0.3
25	25.12 ± 2.32	9.3	3.5

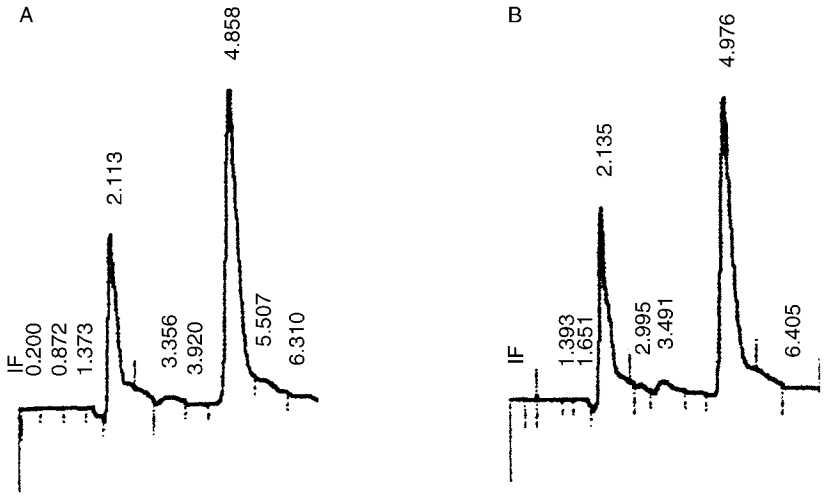

Figure 2. Chromatogram of quinine determination in (A) Britvic Indian tonic (*Peaks*: 2.113 min = IS and 4.858 min = quinine). (B) Schweppes bitter lemon (*Peaks*: 2.135 min = IS and 4.976 min = quinine).

lemon are illustrated in Fig. 2. Results from real samples' analysis are summarized in Table 3. The concentration levels of quinine measured in the soft drink samples that were analysed were in good agreement with the declared amount of 60 mg/L in tuborg tonic water labels. Quinine levels in all soft drinks were significantly lower than the maximum allowed concentration by Greek legislation that is 100 mg/L. No interference was noticed from other food additives such as citric acid, sugar, glucose, artificial sweeteners mainly saccharin, preservatives mainly sodium benzoate, etc.

CONCLUSIONS

A simple, rapid, accurate, and sensitive method for the routine determination of quinine in soft drinks was developed. Analysis was completed within 5 min. Salicylic acid was proven to be a suitable internal standard for quantitation of quinine eluting earlier. Detection limit was 0.3 ng for 50 μL injection volume. Method validation performed in terms of intra-day ($n = 8$) repeatability and inter-day precision ($n = 8$) was found to be satisfactory, with high accuracy and precision results. The method was

Table 3. Analysis of seven commercial soft drinks containing quinine. Results are mean values of six measurements.

Theoretical[a] (ng)	Measured ± SD (ng)	Quinine content (mg/L)
Ivi tonic water		
2.5	2.63 ± 0.15	65.03 ± 2.06
5	5.60 ± 0.43	
15	16.19 ± 0.24	
Britvic Indian tonic		
2.5	2.57 ± 0.05	64.83 ± 3.69
5	5.74 ± 0.35	
15	15.97 ± 0.74	
Britvic bitter lemon drink		
2.5	2.61 ± 0.07	65.3 ± 4.68
5	5.89 ± 0.70	
15	15.64 ± 0.38	
Tuborg tonic water		
2.5	2.55 ± 0.05	61.60 ± 0.61
5	5.19 ± 0.05	
15	15.31 ± 0.25	
Schweppes Indian tonic water		
2.5	2.62 ± 0.03	65.80 ± 2.85
5	5.72 ± 0.31	
15	16.46 ± 0.62	
Schweppes bitter lemon		
2.5	2.58 ± 0.06	63.01 ± 2.11
5	5.45 ± 0.18	
15	15.39 ± 0.22	
DIA tonic water		
2.5	2.61 ± 0.01	66.80 ± 4.15
5	5.91 ± 0.54	
15	16.74 ± 0.40	

[a]Only Tuborg tonic water stated the concentration of quinine 60 mg/L. All other estimations were based on the supposition of similar concentration level.

successfully applied to the analysis of soft drinks containing quinine, such as tonic water and bitter lemon. No interference from other food additives was observed.

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