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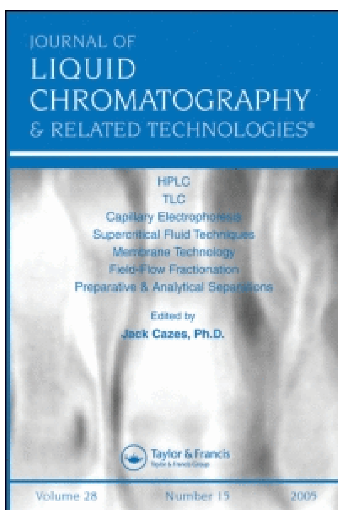

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DETERMINATION OF QUININE IN DRINKS BY REVERSED-PHASE ION-PAIR CHROMATOGRAPHY

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ABSTRACT

A reversed-phase ion-pair chromatographic method was developed for the determination of quinine in drinks. The separation was performed by an isocratic elution with 0.4% glacial acetic acid-methanol (45:55, v/v), which contained 7.5 mmol/L sodium 1-heptanesulfonate as mobile phase (1 mL/min), and detection by ultraviolet spectrometry at 236 nm and fluorimetry at 375 nm (with excitation at 334 nm). Good linearities between the concentrations of quinine and relevant peak area responses were achieved in the range of 0.1 - 20 mg/mL by both detection modes.

The detection limits (signal-to-noise ratio 3:1) for quinine were 0.02 μg/mL and 0.004 μg/mL for spectrometric and fluorimetric detections, respectively. The method has been successfully applied to the analysis of commercial drinks, and the average recoveries for various samples ranged from 91% to 106%.

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INTRODUCTION

Quinine is a naturally occurring quinoline alkaloid obtained from the bark of mature trees of the tropical species *Cinchona* (Rubiaceae), where it is found at a concentration of 1 - 2% in the presence of related alkaloids. The anti-malarial properties of quinine have been recognized for many centuries. It has been widely used as an anti-malarial drug, as well as in analgesic preparations. In addition, quinine, as its sulfate or hydrochloride salt, is usually administered as a flavoring agent to carbonated drinks, in particular tonic water. This compound produces a distinctly bitter taste in the drinks, and the bitterness blends well with the other tastes and provides a refreshing gustatory stimulation.

In the United States, quinine can be added in carbonated drinks with an upper limit of 83 mg/Kg.[1] However, the excessive consumption of this compound may cause a series of adverse effects collectively called cinchonism: arrhythmia, hypotension, vomiting, certain neurological complications, and so on.[2-4] All these side effects suggested that the use of drinks containing quinine should be avoided by children or by women during pregnancy and lactation. As a result, a few countries, including the United States, decree that soft drinks containing quinine must be declared on food label.[1,3,4]

At present, quinine is not legally permitted to be added to drinks in China.[5]

In recent years, although several methods were proposed for the determination of quinine in drinks including spectrometry,[6,7] flow injection analysis,[8] cyclic voltammetry,[9] isotachophoresis,[4] micellar electrokinetic capillary chromatography,[10] and immunoassay,[11] most of which suffer from various disadvantages such as poor sensitivity, poor selectivity, time-consuming procedure, or specific requirement for an instrument, high performance liquid chromatography (HPLC) is still the most popular choice because of its facilitation of separation and detection.[12-15] However, the peak shapes of quinine were not satisfactory in most HPLC methods because of the interaction between residual silanol groups of stationary phase and quinine, which results in tailing peak and, thus, relatively poor sensitivity.

In this study, a reversed-phase ion-pair chromatographic method was proposed for the rapid determination of quinine in soft drinks including tonic water, with satisfactory results. The separation procedure can be completed within 10 min. In addition, the effects of some experimental variables on separation and detection were studied in detail.

EXPERIMENTAL

Apparatus

A Waters liquid chromatograph (Milford, MA, USA) equipped with a 600 gradient pump was employed, along with a Millennium 2010 chromatography

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manager workstation (version 2.10) for instrument control as well as data acquisition and processing. The separation was performed by a Shimadzu Shim-pack CLC-C_8(M) column (5 μm, 4.6 × 150 mm, Kyoto, Japan) with 0.4% glacial acetic acid-methanol (45:55, v/v), which contained 7.5 mmol/L sodium 1-heptanesulfonate as mobile phase (1.00 mL/min). The detection was simultaneously conducted by a Waters 486 tunable absorbance detector and a Waters 474 scanning fluorescence detector, which were connected in series.

The ultraviolet detection wavelength of spectrometry was set at 236 nm, and the excitation wavelength of fluorimetry was set at 334 nm (excitation bandwidth 18 nm), and the emission wavelength at 375 nm (emission bandwidth 18 nm). The column temperature was maintained at 40°C by using a Waters temperature control module, and the injection volume of standard and sample solutions at 20 μL by a Waters 717plus autosampler. In this study, the peak area measurements for all calculations were adopted.

The preliminary spectrometric experiments were carried out by using a Hitachi U-3000 spectrophotometer (Tokyo, Japan) with 1 cm quartz cells at room temperature.

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Reagents

The quinine stock solution (0.5 mg/mL) was prepared by dissolving an appropriate amount of quinine sulfate (purity: 99.0 – 101.0%, Beijing Chemical Factory, Beijing, China) in water. Sodium 1-heptanesulfonate (purity: ≥98.0%) was obtained from Tokyo Kasei Kogyo Co. (Tokyo, Japan), and HPLC grade of methanol from Fisher Scientific (Fair Lawn, NJ, USA). The water for the preparation of all solutions was made by a Millipore Milli-Q RG ultra-pure water system (Bedford, MA, USA). All other chemicals were of analytical grade.

RESULTS AND DISCUSSION

Selection of Separation Mode

In principle, quinine as a diprotic weak base, which pKa_1 and pKa_2 values were 4.13 and 8.52, respectively,[16] existed mainly as divalent or/and monovalent cations in the mobile phase for the classical reversed-phase silica-based bonded stationary phase, which pH value was in the range of 2 - 8. As a result, it could chemically interact with residual silanols of the stationary phase, which results in tailing peak, long retention time, and so on.

In order to reduce analysis time and minimize residual silanols of stationary phase as much as possible, a short length of end capped Shim-pack CLC-

C_8(M) column, which is generally less retentive than a C_{18} column,[17] was employed. Even so, this interaction was so strong that the retention time of quinine was more than 90 min when water-methanol (45:55, v/v) was used as mobile phase; this made it impossible for use in routine analysis. This is partly because of the incomplete end capping of stationary phase.[18]

Considering that low pH could suppress the ionization of silanols, 0.4% glacial acetic acid-methanol (45:55, v/v), whose pH value was approximately 3.0, was selected for a trial. However, the retention factors (k) of quinine, which were obtained by spectrometric and fluorimetric detections, were still as high as 7.78 and 6.96, respectively. Furthermore, the peak shapes were severely tailing, which made it very difficult for accurate quantitation. Under this condition, the addition of an ion-pair reagent in mobile phase, i.e., adoption of an ion-pair chromatographic assay for the determination of this alkaloid, was a rational choice.

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Selection of Mobile Phase

Because the identical separations can be achieved with different ion-pair reagents, the choice of ion-pair reagent is not terribly important in ion-pair chromatography.[19, 20] Because column equilibration is generally slower when the ion-pair reagent is more hydrophobic,[19] a higher concentration of a short-chain ion-pair reagent is necessary in order to accomplish the same results. Since a lower concentration of a long-chain ion-pair reagent is used,[20] in this study heptanesulfonate with a medium length of alkyl chain was chosen as a compromise.

Since the pH value of mobile phase controlled the ionization of silanols and the charge of quinine, the influences of the acidity of mobile phase, which was adjusted by glacial acetic acid, on the retention behaviors of 20 μg/mL of quinine were first studied, and the results illustrated in Figure 1. At that time, the mobile phase was chosen as aqueous phase-methanol (50:50, v/v), the concentration of ion-pair reagent in the mobile phase as 5 mmol/L, and the column temperature as 40°C. From Figure 1, it can be found that the retention factors of quinine decreased by increasing the glacial acetic acid concentrations in aqueous phase, which was likely a result of suppression of residual silanols of stationary phase.

In addition, the increase of mobile phase acidity also caused the significant increase of fluorimetric detection sensitivity and the slight decrease of ultraviolet detection sensitivity for quinine. The former was probably related to the fluorimetric characteristics of quinine in various acidities, and the latter was probably related to the increase of ultraviolet background absorbance of mobile phase caused by the increase of acetic acid concentrations. Because too low pH value of mobile phase was not beneficial for short-chain silica-based bonded stationary

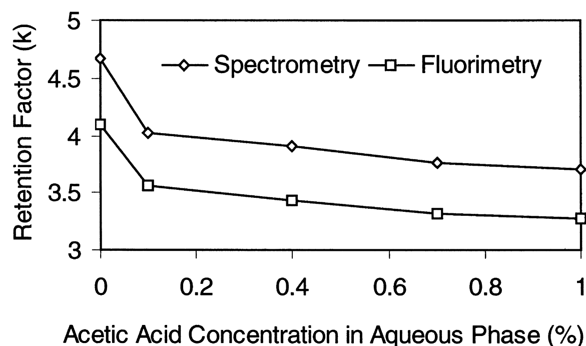

Figure 1. Effect of acetic acid concentration in aqueous phase on the retention factor (k) of quinine.

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phase,[21] the concentration of acetic acid in aqueous phase was selected at 0.4%, and the pH value of mobile phase was near 3.0.

The effects of heptanesulfonate concentrations on retention of quinine are demonstrated in Figure 2. It can be found, that the retention factors of quinine increased as the concentrations of the ion-pair reagent increased. However, it can also be noticed, that when the ion-pair reagent concentration was as low as 5 mmol/L, the peaks of quinine were slightly tailing, which would make it difficult to accurately quantify; when the ion-pair reagent concentration was 2.5 mmol/L, the distortion of quinine peaks could even be observed. Considering that too high a concentration of ion-pair reagent was unnecessary for separation while

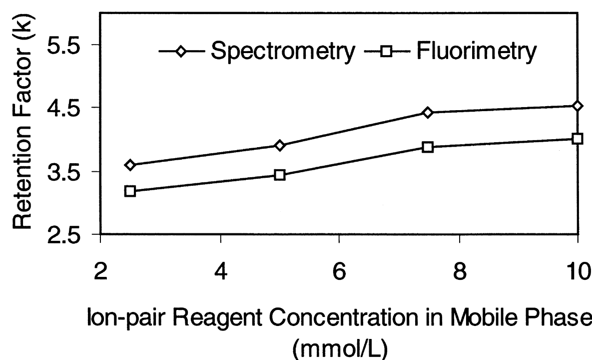

Figure 2. Effect of ion-pair reagent concentration in mobile phase on the retention factor (k) of quinine.

increasing the analysis time, the concentration of heptanesulfonate was finally chosen as 7.5 mmol/L.

Due to the better solubility for salts, methanol was utilized as organic phase rather than acetonitrile in this study. Table 1 illustrates the effects of the methanol concentrations in mobile phase on the retention behaviors of quinine. The retention factors of quinine decreased by increasing the methanol concentrations, which can be easily understood. Additionally, the increase of the organic phase concentrations resulted in the gradual increase of the spectrometric detection sensitivity and the gradual decrease of the fluorimetric detection sensitivity for quinine. The reasons for this were not very clear. When the methanol concentration was too low, the retention time of quinine would be prolonged unnecessarily and the peak would be tailing. Finally, the volume ratio between aqueous phase and methanol was set at 45:55 (v/v).

In general, triethylamine is frequently added in the mobile phase as a modifier in ion-pair chromatography to reduce peak tailing and, consequently, improve peak shape of the analyte.[20] Therefore, in this study, 0.1% of triethylamine was added into the mobile phase for a trial. Compared with the results obtained by the mobile phase without triethylamine, the ultraviolet sensitivity of quinine increased by 32.8%, for which the cause was not yet clear, while the fluorimetric sensitivity of quinine decreased by 87.2%, which may be caused by fluorescence quench.

Besides, after addition of triethylamine in mobile phase, the retention time of quinine was reduced and the peak shapes were somewhat improved, while the ultraviolet and fluorimetric backgrounds remained approximately constant. Since the addition of amine modifier will make the column equilibration slow and contribute to the complexity of the method,[22] taking all the above factors into consideration, finally, triethylamine was not included in the mobile phase.

Table 1. Effect of the Volume Ratios Between Aqueous Phase and Methanol in Mobile Phase on the Retention of Quinine

Aqueous Phase : Methanol (v/v)	Retention Factor	
	Spectrometry	Fluorimetry
40:60	1.14	1.01
45:55	2.28	2.02
50:50	4.43	3.89
55:45	8.22	7.35
60:40	17.19	15.31

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Effect of Column Temperature

Because the fluctuation of column temperature can directly produce some impacts on several equilibrium processes in ion-pair chromatographic assay,[19, 20] it is important to control the column temperature for reproducible separations. The results obtained showed that by increasing the column temperature in the range of 30 – 50°C, the retention factors of quinine and the column pressure reduced gradually, the ultraviolet sensitivity of quinine had no obvious changes, while the fluorimetric sensitivity decreased slightly. In comparison with the results at 30°C, the fluorimetric intensity of quinine at 50°C decreased by 9.31%. Finally, the column temperature was maintained at 40°C.

Selection of Detection Conditions

It is relatively easy to detect quinine because it has ultraviolet and fluorimetric characteristics, simultaneously. In this study, these two detection modes were compared. From Figure 3, it can be seen that the optimal ultraviolet detection wavelength was 236 nm at which the absorbance of quinine was a maximum, while the background of mobile phase was very low. The excitation wavelength

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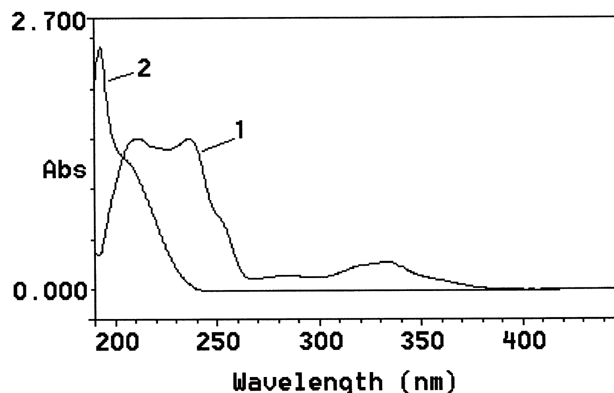

Figure 3. The ultraviolet absorbance spectra of 20 μg/mL quinine against mobile phase blank (curve 1) and of mobile phase against water (curve 2). Mobile phase: 0.4 % glacial acetic acid-methanol (45:55, v/v) which containing 7.5 mmol/L heptanesulfonate. Spectrometric measurements were carried out using a Hitachi U-3000 spectrophotometer with 1 cm quartz cells. Scanning wavelength range: 190-450 nm; Scanning speed: 300 nm/min; Slit: 2.0 nm.

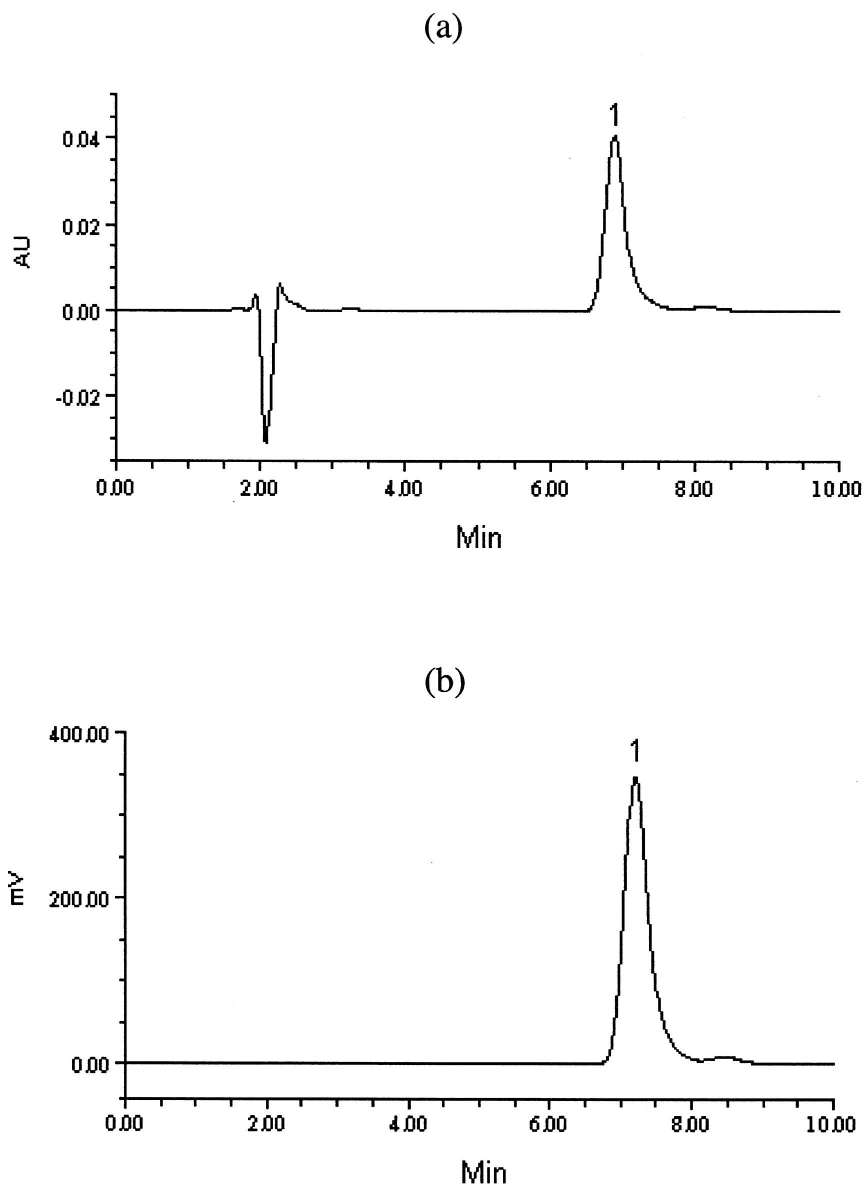

Figure 4. The chromatograms of 10 μg/mL quinine standard solution (a and b) and of Sample A final solution (c and d). Detection mode: spectrometry (a and c), fluorimetry (b and d). Peaks: 1 = quinine.

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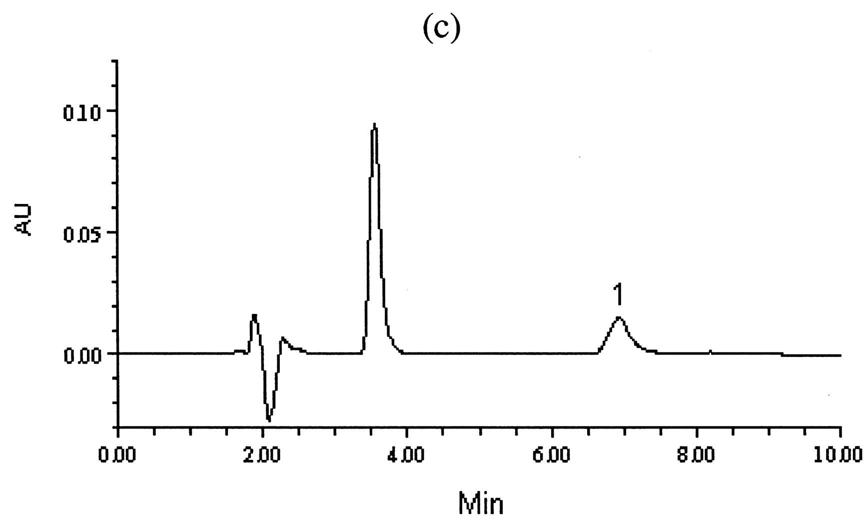

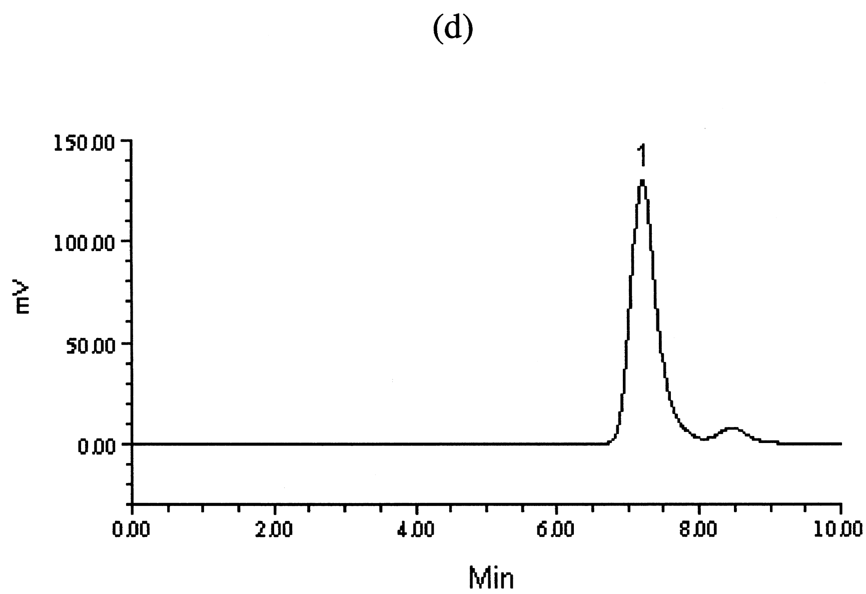

Figure 4. Continued.

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of fluorimetry was adopted as 334 nm rather than 236 nm because at the latter wavelength the fluorimetric background of mobile phase and its noise would increase significantly, although, the fluorimetric intensity of quinine was much higher. When the emission wavelength of fluorimetry varied from 350 to 460 nm, the fluorimetric intensity of quinine reached a maximum at 375 nm, which was finally chosen. Under the optimal experimental conditions, the detection limits (signal-to-noise ratio 3:1) of the two detection modes were 0.02 μg/mL and 0.004 μg/mL for ultraviolet spectrometry and fluorimetry, respectively.

Because the taste threshold of quinine was at μg/mL level,[23] as a food-flavoring agent intentionally added into foods, the concentration of quinine in commercial drinks should generally not be less than that in order to realize its functions. As a result, both of the detection modes can completely meet the requirements for the analysis of real samples, and the sensitivity and selectivity of fluorimetric detection were much better. Due to the simplicity and ease of availability of a spectrometric detector in HPLC, in practical application, the ultraviolet absorbance detection can be used as an alternative to fluorimetric detection for the determination of quinine. Finally, both of the detection modes were adopted in this study.

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Study on Interferences

Under the specified experimental conditions, the commonly used food additives in soft drinks such as artificial sweeteners (saccharin, aspartame, cyclamate, acesulfame-K), preservatives (benzoic acid, sorbic acid), organic acids (citric acid, malic acid, tartaric acid), ascorbic acid, caffeine, theobromine, and theophylline did not interfere with the determination of quinine.

Linearity and Precision

Under the optimized experimental conditions, quinine showed good linearity between the concentrations and peak area responses by both detection modes. In the range of 0.1 - 20 μg/mL, the correlation coefficients were 0.9996 and 0.9997 for spectrometry and fluorimetry, respectively. Besides, the precisions were evaluated by conducting seven replicate analyses of two concentration levels of standard solutions. The relative standard deviation values of 1 μg/mL of quinine were 1.27% and 0.65% for spectrometric and fluorimetric detections, respectively, and the relative standard deviation values of 4 μg/mL of quinine were 0.50% and 0.73% for spectrometric and fluorimetric detections, respectively.

Table 2. Analysis of Real Samples

Sample	Content[a] (mg/Kg) Spectrometry	Fluorimetry	Added (mg/Kg)	Recovery[a] (%) Spectrometry	Fluorimetry
A	37.24±0.74	36.50±0.42	40	102.1±1.1	97.8±0.6
			80	103.2±0.8	97.2±0.5
B	ND[b]	ND	40	91.8±1.7	99.0±0.7
			80	98.6±1.1	102.8±0.5
C	ND	ND	40	99.7±0.6	105.2±0.5
			80	101.2±0.5	105.5±0.5

[a] Average of five determinations ± standard deviation.
[b] Not detected.

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Analysis of Real Samples

All the samples including one tonic water (Sample A), one cola (Sample B), and one carbonated drink (Sample C), were purchased from the local market. Approximately 2.5 g of sample was weighed accurately into a 25 mL volumetric flask and deaerated in an ultrasonic water bath for 5 min prior to the dilution to volume with water. The final sample solution was injected into the chromatographic system after filtering through a 0.45 μm filter. The results are summarized in Table 2, and the chromatograms of the quinine standard solution and Sample A final solution shown in Figure 4. The results obtained by two detection modes were consistent. It can also be noticed that, in Sample A quinine was illegally added, which violated the present Chinese regulation.[5] In addition, the spike recovery studies were carried out by all samples, and the results also shown in Table 2 are satisfactory.

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REFERENCES

1. *Code of Federal Regulations, Title 21*; U.S. Government Printing Office: Washington DC, 1998, sec172.575.

2. *The Dictionary of Substances and their Effects*; Richardson, M.L.; Gangolli, S., Eds.; The Royal Society of Chemistry: Cambridge, 1994; Vol. 6, 949-950.
3. Gennaro, M.C.; Giacosa, D.; Abrigo, C.; Biglino, P.; Campi, E. Organic Bases. In *Handbook of Food Analysis;* Nollet, L.M.L., Ed.; Marcel Dekker: New York, 1996; Vol. 1, 791.
4. Reijenga, J.C.; Aben, G.V.A.; Lemmens, A.A.G.; Verheggen, T.P.E.M.; De Bruijn, C.H.M.M.; Everaerts, F.M. J. Chromatogr. **1985**, *320*, 245-252.
5. *Hygienic Standards for Uses of Food Additives*; National Standard of the People's Republic of China: 1996;GB 2760-1996.
6. Rao, M.V.; Krishnamacharyulu, A.G.; Sattigeri, V.D.; Manjunath, M.N.; Nagaraja, K.V.; Kapur, O,P. J. Food Sci. Technol. **1984**, *21*, 266-268.
7. Castro, J.C.G.; Sánchez, M.J.; Delgado, M.A.R.; Romero, C.D. Mikrochim. Acta **1993**, *110*, 263-268.
8. Gong, Z.L.; Zhang, Z.J.; Yang, X.F. Analyst **1997**, *122*, 283-285.
9. Krause, J.; Umland, F. Fresenius' Z. Anal. Chem. **1989**, *335*, 791-795.
10. Trenerry, V.C.; Ward, C.M. J. Capill. Electrophor. **1996**, *3*, 271-274.
11. Ward, C.M.; Morgan, M.R.A. Food Addit. Contam. **1988**, *5*, 555-561.
12. Kral, K.; Sontag, G. Z. Lebensm. -Unters. -Forsch. **1982**, *175*, 22-24.
13. Valenti, L.P. J. Assoc. Off. Anal. Chem. **1985**, *68*, 782-784.
14. Wörner, M.; Gensler, M.; Bahn, B.; Schreier, P. Z. Lebensm. -Unters. -Forsch. **1989**, *189*, 422-425.
15. Lander, V.; Wörner, M.; Kirchenmayer, C.; Wintoch, H.; Schreier, P. Z. Lebensm. -Unters. -Forsch. **1990**, *190*, 410-413.
16. *CRC Handbook of Chemistry and Physic*, 79th Ed.; Lide, D.R., Ed.; CRC Press: Boca Raton, 1998; 8-56.
17. Dolan, J.W. LC-GC Int. **1997**, *10*, 570-572.
18. Dolan, J.W. LC-GC Int. **1999**, *12*, 156-163.
19. Snyder, L.R.; Kirkland, J.J.; Glajch, J.L. *Practical HPLC Method Development*, 2nd Ed.; John Wiley & Sons: New York, 1997; 317-341.
20. Dolan, J.W. LC-GC Int. **1996**, *9*, 624-628.
21. Dolan, J.W. LC-GC Int. **1998**, *11*, 292-297.
22. McCalley, D.V. LC-GC Asia Pacific **1999**, *2* (3), 37-44.
23. Lindsay, R.C. *"Flavors," in Food Chemistry*, 3rd Ed.; Fennema, O.R., Ed.; Marcel Dekker: New York, 1996; 729.

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