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Original Article

Simultaneous determination of ascorbic acid and caffeine in commercial soft drinks using reversed-phase ultraperformance liquid chromatography

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ABSTRACT

A new reversed-phase ultraperformance liquid chromatography method with a photodiode array detector was developed for the quantification of ascorbic acid (AA) and caffeine (CAF) in 11 different commercial drinks consisting of one energy drink and 10 ice tea drinks. Separation of the analyzed AA and CAF with an internal standard, caffeic acid, was performed on a Waters BEH C_{18} column (100 mm × 2.1 mm, 1.7 μm i.d.), using a mobile phase consisting of acetonitrile and 0.2M H_3PO_4 (11:89, v/v) with a flow rate of 0.25 mL/min and an injection volume of 1.0 μL. Calibration graphs for AA and CAF were computed from the peak area ratio of AA/internal standard and CAF/internal standard detected at 244.0 nm and 273.6 nm, respectively. The developed reversed-phase ultraperformance liquid chromatography method was validated by analyzing standard addition samples. The proposed reversed-phase ultraperformance liquid chromatography method gave us successful results for the quantitative analysis of commercial drinks containing AA and CAF substances.

1. Introduction

Caffeine (CAF; 1,3,7-trimethylxanthine), which is a xanthine alkaloid, has widely been used in tea (black, white, and green), coffee, guarana, chocolate, cocoa, soft and energy drinks, and pharmaceutical products. In recent years, the use of CAF in energy drinks has increased significantly due to its excitation and analgesic properties. However, the use of a high dosage of CAF gives rise to some symptoms such as headache, slowness, fatigue, and depression. Ascorbic acid {AA; (5R)-[(1S)-1,2-dihydroxyethyl]-3,4-dihydroxyfuran-2(5H)-one} is one of the most important vitamins, which plays an important role for hydroxylation reactions and antioxidants. Symptoms of lack of AA are physical and mental infirmity, fatigue, weight loss, bruising, dry hair and skin, and increased sensibility of infections. Nowadays, the production of commercial drinks as a function of the developments in the food industry has

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increased tremendously. Taking such situations into account, quality control and routine analysis of commercial drinks have very vital importance for the human health and life quality. In this context, quantitative analysis and quality control of commercial drinks require new powerful analytical methods giving reliable, precise, and accurate results with short runtime and low cost of analysis.

Several analytical methods including spectrophotometry for CAF [1,2] and AA [3], high-performance liquid chromatography for CAF [4–16] and AA [17–22], liquid chromatography–mass spectrometry for CAF [23] and AA [24], voltammetry for CAF [25–27] and AA [28–32], Fourier transform infrared spectrophotometry for CAF [33–35] and AA [36], chemiluminescence for CAF [37], gas chromatography–mass spectrometry for CAF [38], ion chromatography for CAF [39], capillary electrophoresis for CAF [40], and ultra-high-performance liquid chromatography for CAF [41] and AA [42] have been reported for the analysis of the related compounds in drinks and pharmaceuticals. A literature survey revealed that there was no report about the simultaneous estimation of AA and CAF in the mentioned samples. Hence, the authors have attempted to develop a rapid, precise, and accurate method for the simultaneous determination of these active compounds in commercial drink samples. Some typical studies related to AA [43,44] and CAF [45] were reported.

Today, the ultraperformance liquid chromatography (UPLC) method is preferable to high-performance liquid chromatography for the analysis of raw samples, food products, drug preparations, and compounds in biological fluids due to short runtime and less solvent consumption. Moreover, the UPLC technique offers new possibilities in liquid chromatography, giving short analysis time and better chromatographic elution for the simultaneous determination of compounds in samples with adequate precision and accuracy.

In this study, a new reversed-phase UPLC (RP-UPLC) method was developed for the simultaneous quantitative analysis of AA and CAF in 11 different commercial drinks. The validation of the proposed UPLC method was carried out analyzing standard addition samples to evaluate its precision, accuracy, and selectivity. It was concluded that the UPLC method provided successful results for the quantitative estimation and quality control of the analyzed commercial drink samples containing CAF and AA. The analysis results provided by the developed and validated RP-UPLC method were compared with those obtained by the literature methods.

2. Experimental

2.1. Instrument and software

Chromatographic separation was carried out using the Waters ACQUITY UPLC H-Class system, including a quaternary solvent manager photodiode array detector, a cooling autosampler, and an oven enabling the control of column temperature. Chromatographic data collection and evaluation were made by Waters Empower2 chromatography software. Chromatographic elution of AA and CAF was performed via a Waters BEH C_{18} column (100 mm × 2.1 mm, 1.7 μm i.d.).

2.2. Chromatographic conditions

In the chromatographic analysis, the analytical column was the Waters BEH C_{18} column (100 mm × 2.1 mm, 1.7 μm i.d.). The mobile phase for the elution of AA and CAF in samples in the presence of an internal standard (IS) was a mixture of acetonitrile and 0.2M H_3PO_4 (11:89 v/v). The mobile phase was filtered through a 0.20 μm microfilter. The total runtime of AA and CAF with IS was 14 minutes, with a flow rate of 0.25 mL/min and column temperature of 50°C. AA and CAF were detected at 244.0 nm and 273.6 nm, respectively.

2.3. Reagents

Acetonitrile was of high-performance liquid chromatography grade (Sigma-Aldrich, Germany), and H_3PO_4 (Merck, Germany), CAF (Sigma-Aldrich, USA), and AA (Merck, Germany) were of guaranteed reagent grade. Water purified with Milli-Q Gradient A10 Milipore System (Merck Milipore, ABD, USA) was used during chromatographic analysis. All solutions were filtered through a 0.20 μm hydrophilic PTFE syringe filter (Minisart, Germany).

2.4. Commercial drink products

A commercial energy drink (Burn energy drink) and 10 commercial ice tea drinks, consisting of Didi bargamot tea, Didi lemon tea, Fuse melon tea, Fuse lemon tea, Fuse pine-mango tea, Fuse peach tea, Lipton apple tea, Lipton green tea, Lipton lemon tea, and Lipton peach tea, were analyzed by the proposed RP-UPLC method. All the commercial drink products were purchased from local supermarkets.

2.5. Standard solutions

Standard stock solutions of AA, CAF, and caffeic acid were separately prepared by dissolving 10 mg of each compound in 100 mL of 0.1M HCl. A standard calibration set of five mixtures containing 2.5–40 μg/mL of AA and 4.0–44.0 μg/mL of CAF in the presence of 12 μg/mL of caffeic acid as an IS was

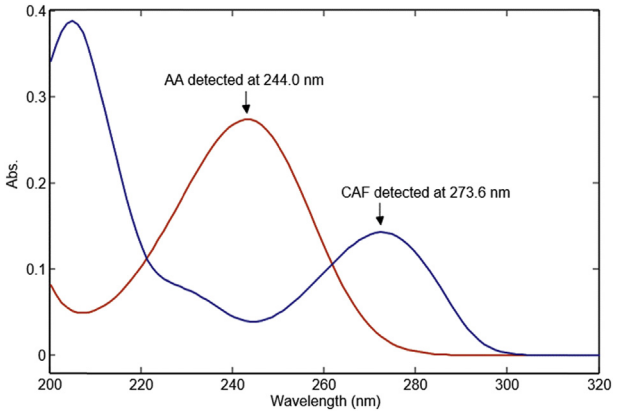

Figure 1 – UV spectra showing the detection wavelengths of the AA and CAF compounds. AA = ascorbic acid; CAF = caffeine.