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Simple and environmental friendly procedure for the gas chromatographic–mass spectrometric determination of caffeine in beverages

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Abstract

A simple, quick and organic solvent saving procedure has been developed for the GC/MS determination of caffeine in beverages. The procedure involves the mixing of 25 μL sample with 1 mL ethyl acetate, and a following simple desiccation procedure in a 1.5 mL autosampler vial. A linear calibration curve was generated with caffeine concentration ranging from 0.005 mg/L to 30.0 mg/L. The procedure developed provides a 0.001 mg/L detection limit of caffeine in the final solution by injection of 1 μL solution and the relative standard deviation (RSD) was less than 2% for independent measurement. The total amount of organic solvent used for individual detection is 1 mL of nontoxic ethyl acetate. The developed method was repeatable and could be applied to determine trace amounts of caffeine in popular commercial beverages.

Keywords: Beverage analysis; GC/MS; Caffeine; Environmental friendly method

1. Introduction

Caffeine, a naturally occurring alkaloid found in tea leaves, coffee beans, kola nuts, cocoa beans and other plants, is used as a flavoring agent in a variety of beverages, including some soft drinks and energy drinks. The amount of caffeine in soft drinks varies among brands and it is closely regulated by the US Food and Drugs Administration (FDA) to no more than 6 mg/oz fluid or 200 mg/L [1]. Therefore, caffeine analysis is requested to ensure proper caffeine levels in beverages and to meet regulatory standards. Several methods for caffeine determination in soft drinks are available in the literatures. These are UV spectrometry [2,3], HPTLC [4], capillary electrophoresis (CE) [5], continuous flow autoanalyzer of solid-phase extraction (SPE) coupled with UV, evaporative light scattering (ELSD) [1] or IR detectors [6], HPLC [7,8], GC [9], TLC–SPE–MS [10] and solid-phase microextraction (SPME) combined with GC/MS [11,12], etc.

However, the methods previously reported usually involve large quantities of organic solvents in liquid solvent extraction, in mobile phases or in the elution procedure of SPE. Among these methods reported, the UV methods were found to yield results with positive errors in some cases [2,3]. Moreover most of them are somewhat expensive and time-consuming. Although organic solvents are not necessary in the SPME/GC/MS techniques, it usually requires the isotopically labeled caffeine as an internal standard.

Method which is simple, rapid and can greatly reduce the amount of organic solvent used in the analysis of caffeine in beverages is therefore desirable, particularly in the quality control applications. GC/MS is a powerful separation and identification instrument and has been applied for its high sensitivity for many years. Here, we report a new method for the determination of caffeine in beverages. It involves the mixing of beverage sample (25 μL) with 1 mL ethyl acetate, drying over $MgSO_4$ and the following GC/MS analysis. The procedure is simple, inexpensive, and the total amount of organic solvent consumed is 1 mL. All these advantages enable this procedure to be a preferable method for the content control of caffeine in beverage industry and regulatory analysis.

2. Experimental

2.1. Materials and reagents

Caffeine containing beverages with different brand names such as Coca Cola, Pepsi, Future, Diet Coca Cola, and Diet Pepsi

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as well as the caffeine-free soft drink Wahaha were purchased from the local market. All the reagents used in this method were the analytical grade. Ethyl acetate was purchased from Zhengxin Chemical Co. (Shanghai, China). Anhydrous sodium sulfate, calcium chloride, and magnesium sulfate were obtained from Hewei Chemical Co. (Shanghai, China). Standard caffeine was purchased from Fluka (Shanghai, China). Standard ethyl acetate solutions of caffeine with a varied concentration ranging from 0.005 mg/L to 30.0 mg/L were prepared from the dilution of a stock solution. The caffeine-free soft drink Wahaha was used to prepare the stock solution with a caffeine concentration of 100 mg/L.

2.2. Sample preparation

Soft drink sample (25 μL) was taken in a 1.5 mL autosampler vial (Agilent, USA) and 1 mL ethyl acetate was added. The mixture was shaken by ultrasonic for 2 min. Anhydrous magnesium sulfate (0.005 g) was then added to the vial and the resulting mixture was maintained for 3 min. The dried ethyl acetate solution was directly used for GC/MS analysis.

2.3. Instrumentation

The GC/MS analysis was carried out on a HP6890 gas chromatograph with a HP5973 mass spectrometric detector equipped with an electron ionization source and a single-stage quadrupole. Analytes were separated on a HP-5MS capillary column, 30 m × 0.25 mm I.D. and 0.25 μm phase thickness. The helium carrier gas was set at a constant flow-rate of 1 mL/min. Sample injection was done with the HP 7683 autosampler. The 10 μL syringe was washed three times with ethyl acetate before and after each run and rinsed with 8 μL of sample solution before 1 μL of sample solution was injected in the splitless mode. The temperatures of the GC/MS instrument were set at 250 °C at the injector, 280 °C at the transfer line, 230 °C at the ion source and 150 °C at the quadrupole. The GC oven was maintained at 120 °C for 2 min, ramped at a rate of 40 °C/min to 240 °C and held at this temperature for 2 min. The mass spectrometer was operated in the electron impact mode (EI, 70 eV) and *m/z* 194 and 109 ions are selected for monitoring. Caffeine was identified by direct comparison with caffeine standard on the basis of the retention time and mass spectral ion ratios.

3. Results and discussion

3.1. Calibration curve and detection limit

The strong molecular ion peak of caffeine at *m/z* 194 and the less strong ion at *m/z* 109 were selected as the quantitative signal. The calibration curve was constructed by plotting the measured peak areas versus concentration. Excellent linearity was obtained within the concentration range of 0.005–30.0 mg/L, giving the correlation coefficient as 0.9999.

The detection limit was determined when the concentration of caffeine had a signal-to-noise ratio of 3:1. In this way, the detection limit of caffeine was found to be 0.001 mg/L in the final solution.

3.2. Optimization of the sample preparation procedure

It is known that the solubility of water in ethyl acetate is 2.94% (w/w) at room temperature [13]. Thus, it was assumed that water could be completely dissolved in ethyl acetate when small quantity of beverage sample was added to a great deal of ethyl acetate because the volume of ethyl acetate is 40 times of the beverage volume. No specific phase separation could be observed when less than 30 μL beverage was added to 1 mL ethyl acetate. In order to get the appropriate volume ratio of sample to solvent, 10 μL, 15 μL, 20 μL, and 25 μL samples were tested. It was reported that the regular caffeine content in soft drinks is 3 mg caffeine/oz fluid (around 100 mg/L) [14], so the caffeine concentrations in the final solutions should be around 1 mg/L, 1.5 mg/L, 2 mg/L, and 2.5 mg/L when 10 μL, 15 μL, 20 μL, and 25 μL beverages were dissolved in 1 mL ethyl acetate, respectively. In order to obtain accurate and repeatable results, a concentration with 25 μL/1 mL was selected for the following tests.

Two different shaking modes, mechanical and ultrasonic, were used for comparison. Shaking time from 1 min to 6 min for both modes was evaluated. It was found that the ultrasonic mode was better than the mechanical shaking mode. The mean peak area (*n* = 3) of caffeine (sample with the caffeine concentration of 100 mg/L) versus the time of ultrasonic shaking is shown in Fig. 1. As it could be clearly seen from Fig. 1, the peak area is going to be stable after 2 min shaking. Therefore, a 2 min ultrasonic shaking mode was chosen for the following tests.

To select the appropriate desiccant, sodium sulfate, calcium chloride and magnesium sulfate were assayed. Obviously agglomeration was observed for sodium sulfate and calcium chloride, respectively, which would inevitably effect the deter-

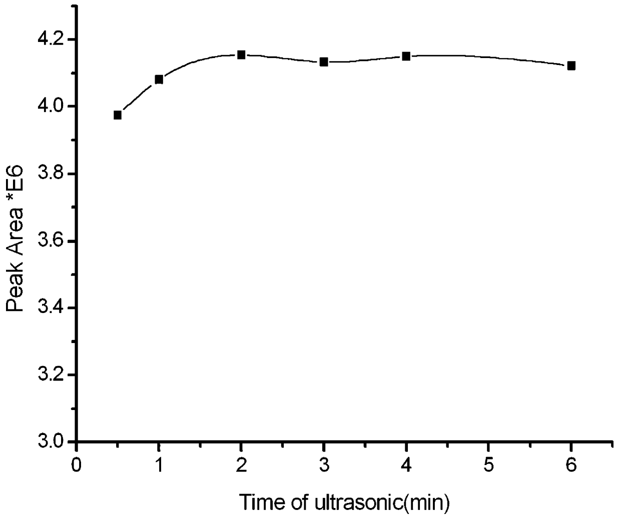

Fig. 1. The mean peak area (*n* = 3) of caffeine (sample with the caffeine concentration of 100 mg/L) vs. time of ultrasonic shaking.