Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.jfda-online.com

Review Article

Certification of caffeine reference material purity by ultraviolet/visible spectrophotometry and high-performance liquid chromatography with diode-array detection as two independent analytical methods

A.B. Shehata [a,*], M.S. Rizk [b], E.A. Rend [a]

[a] Reference Materials Department, National Institute of Standards, El-Matbah, Haram, Giza, Egypt
[b] Department of Chemistry, Faculty of Science, Cairo University, Giza, Egypt

ARTICLE INFO

Article history:
Received 3 January 2016
Received in revised form
26 May 2016
Accepted 14 June 2016
Available online 30 July 2016

Keywords:
caffeine
certification
coffee
extraction
purity analysis

ABSTRACT

Caffeine reference material certified for purity is produced worldwide, but no research work on the details of the certification process has been published in the literature. In this paper, we report the scientific details of the preparation and certification of pure caffeine reference materials. Caffeine was prepared by extraction from roasted and ground coffee by dichloromethane after heating in deionized water mixed with magnesium oxide. The extract was purified, dried, and bottled in dark glass vials. Stratified random selection was applied to select a number of vials for homogeneity and stability studies, which revealed that the prepared reference material is homogeneous and sufficiently stable. Quantification of caffeine purity % was carried out using a calibrated UV/visible spectrophotometer and a calibrated high-performance liquid chromatography with diode-array detection method. The results obtained from both methods were combined to drive the certified value and its associated uncertainty. The certified value of the reference material purity was found to be 99.86% and its associated uncertainty was ±0.65%, which makes the candidate reference material a very useful calibrant in food and drug chemical analysis.

1. Introduction

Caffeine (1,3,7 trimethyl xanthine) is a natural component of tea, coffee, guarana, and cocoa. It is also present in chocolate, cola beverage, and soft drinks [1]. The caffeine content of raw Arabica coffee is 0.9–1.4%, while in Robusta coffee it varies from 1.5% to 2.6%. Caffeine obtained by the decaffeination process and synthetic caffeine are used by the pharmaceutical and soft drink industries [2]. Caffeine has numerous

* *Corresponding author*. National Institute of Standards, Tersa Street, El-Matbah, Haram, P.O. Box 136, Giza Code No 12211, Egypt.
E-mail address: adelshehata63@yahoo.com (A.B. Shehata).

http://dx.doi.org/10.1016/j.jfda.2016.06.009

physiological effects, such as stimulation of the central nervous system, and enhancement of blood circulation and respiration [2]. Analytical measurement of the caffeine content is, therefore, of fundamental importance for nutritional and pharmaceutical applications. The accuracy and credibility of the data produced by measurements depend largely on the traceability of the measurement results to the international system of units. In chemical analysis, certified reference materials (CRMs) are the measurement standards by which metrological traceability can be achieved. It is reported that reference materials (RMs) are generally desired for determining compliance with the existing regulations and for determining the systematic errors when developing a new analytical method. RMs are widely used for calibration of equipment, and for quality control and quality assurance programs in many fields. Caffeine CRM is produced by some national metrology institutes such as the National Metrology Institute of Australia and by some companies such as Sigma, Alfa Aesar, and others. However, no published research work on the certification process of pure caffeine RM is available in the literature, and only CRM certificates issued by the producers can be obtained. There are very few reports in the literature on the certification of caffeine in some food matrixes. Sander et al [3] certified three green tea RMs characterized for catechins, xanthine alkaloids, theanine, and toxic elements using five analytical methods. Thomas et al [4] developed a rapid and selective isocratic reversed-phase liquid chromatographic method to measure caffeine, theobromine, and theophylline simultaneously in baking chocolate. In addition, Thomas et al [5] determined the concentration of caffeine and caffeine-related compounds in two ephedra-containing RMs by three independent analytical methods. Sharpless et al [6] collaborated to produce a series of CRMs for dietary supplements. In this series, values were assigned for ephedrine alkaloids and toxic elements in all certified materials and for other analytes (e.g., caffeine, nutrient elements, proximates, etc.) in some of the RMs. In this study, we report for the first time, a full scientific process of the extraction, purification, and certification of caffeine RM. In this work, high-performance liquid chromatography with diode-array detection (HPLC-DAD) and UV/visible (Vis) spectrophotometry was used as two independent analytical methods; data from these methods was combined to produce the certified value and uncertainty.

2. Experimental

2.1. Materials and reagents

Roasted and ground coffee was purchased from the local market in Cairo, Egypt. Magnesium oxide (reagent grade), hexane, dichloromethane, and acetonitrile (HPLC grade) were purchased from Merck, Darmstadt, Germany. Caffeine calibrant (99.7%) was obtained from Alfa Aesar, Karlsruhe, Germany.

2.2. Extraction of caffeine from roasted and ground coffee

Fats were removed from the coffee sample via three successive extractions by hexane for 24 hours. After that, 10 g of defatted coffee was added to 50 g of magnesium oxide in a 1 L measuring flask, and 800 mL deionized water was added [7]. The flask was heated at 90°C under stirring for 20 minutes and then left to cool to room temperature, and the volume was made up to 1 L. After settling of the solids, the solution was filtered and the filtrate was extracted with dichloromethane [8]. The solvent was evaporated and caffeine powder was obtained. An amount of 600 g of coffee was extracted by this method, and a total yield of 6 g was obtained. The extracted caffeine was then purified on a chromatographic column (1 cm i.d. × 24 cm) packed with 4.2 g silica gel. Acetonitrile (10 mL) was pooled and drained into the column to ensure column conditioning; 6 mg of the extracted caffeine in 10 mL of acetonitrile/water mixture (95:5%) was poured into the column. Thus, the whole amount of extracted caffeine was purified.

2.3. Equipment

The purity measurement was carried out using the UV/Vis spectrophotometer Analytikjenaspecord 250 Plus equipped with a 15-sample tray. Measurements were made using a quartz cell at 273 nm. A reversed-phase liquid chromatography , Agilent 1100 series system, equipped with a G1379A vacuum degasser, a G3111A quaternary pump, a G1313A autosampler, a G1315B diode-array detector, and a G1364C fraction collector, was used. Chromatographic separation of caffeine and other compounds was achieved by a Zorbax-Eclipse-XDB-C18 column (4.6 mm × 250 mm, 5 μm).

2.4. Calibration

For calibration of the HPLC-DAD method, a stock solution was prepared by weighing 0.10011 g of caffeine and dissolving it in 0.1 L ultrapure water. From the prepared stock solution, 10 calibration solutions of concentrations 10 mg/L, 20 mg/L, 30 mg/L, 40 mg/L, 50 mg/L, 60 mg/L, 70 mg/L, 80 mg/L, 90 mg/L, and 100 mg/L were prepared and injected into the HPLC system. Meanwhile, a stock solution for calibration of the UV/Vis spectrophotometer was prepared by weighing 0.10037 g of the same caffeine and dissolving it in 0.1N HCl [9]. Six calibration solutions of concentrations 10 mg/L, 20 mg/L, 30 mg/L, 40 mg/L, 50 mg/L, and 60 mg/L were prepared from the stock solution and were measured by the UV/Vis spectrophotometer. Detection was done by a photodiode array detector at 273 nm wavelength.

2.5. Homogeneity study

Five sealed vials, including the first and the last ones, were randomly selected for the homogeneity study. The between- and the within-vial variability were studied by dividing each of the selected vials into three subsamples. Measurements were performed by Method 1 (M1).

2.6. Assay of caffeine purity

The purity of caffeine was measured by two methods. In M1, reversed-phase liquid chromatography with a Zorbax-Eclipse-XDB-C18 column (4.6 mm × 150 mm, 5 μm) was used. Solvent