Simultaneous Determination of Caffeine in Cola Drinks and Other Beverages by Reversed-Phase HPTLC and Reversed-Phase HPLC

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Abstract: A simple reversed-phase high-performance thin-layer chromatographic (HPTLC) method for the identification and quantitative determination of caffeine in Coca-Cola is described. The chromatographic conditions of the HPTLC method are further adapted by developing the HPLC method for identification and determination of caffeine in cola drinks and other caffeine-containing beverages. The results of the quantitative determinations of caffeine in Coca-Cola obtained by using both HPTLC and HPLC methods were 96.1 ± 1.1 mg L⁻¹ and 95.4 ± 0.2 mg L⁻¹, respectively. The experimental objective for the students is to determine the unknown amount of caffeine in a complex matrix by two different chromatographic techniques. The pedagogical objective is to make students familiar with these well-established techniques. Furthermore, they learn about the usefulness, difficulties, and limitations of comparative analytical studies. The two-part experiment has been developed for second-year chemistry and biology undergraduate students and is performed in the instrumental analysis course in two three-hour laboratory sessions.

Introduction

Recently, the development of chemical and instrumental methods for the separation, identification, and quantitative analysis of individual components in food and beverages has become extremely important. The food industry as well as academic and governmental institutions needs to assess the nutritional value of food and beverages for human health [1]. High-performance thin-layer chromatography (HPTLC, planar chromatography) and high-performance liquid chromatography (HPLC), among other chromatographic methods, offer many advantages and can be applied very widely in the analysis of food and beverage components [2–4]. Both separation techniques are of considerable chemical interest, and it is necessary to introduce chemistry students to these techniques [5].

HPTLC is a very simple, economical analytical method that exhibits high performance in the qualitative characterization and even quantitative determination of mixtures of substances carried out on efficient fine-particle layers [6]. The TLC layers on which planar chromatography is carried out can be prepared by each user from loose-powdered sorbent materials or can be purchased as layers on glass, aluminum, or plastic-foil bases. The great majority of TLC separations today are performed on silica-gel layers. Universal application for nonpolar as well as very polar substances is found for the chemically modified layers, such as reversed-phase layers (RP) [6]. These are gels incorporating chemically bound n-alkyl groups such as n-octyl (RP-8) or n-octadecyl (RP-18). For sample application various techniques are used, including spotting with the help of disposable-glass capillaries, micropipettes, syringes, capillary dispensers, and autosamplers [7]. Planar chromatography is easy to perform. In its classic development, the dry TLC plate is immersed in the mobile phase at the bottom of the horizontal glass chamber. The development is based on capillary forces. The visualization of the sample distribution on the HPTLC plate can be performed using several methods [8–12]. The most popular is UV–vis detection using stationary phases containing a fluorescent indicator with excitation at 254 nm. When quantitative evaluation is performed, the reflection mode is generally used. The vast majority of quantitative evaluations are based on the method of external standards (calibration curves) using a densitometer (TLC scanner) for direct evaluation of the chromatograms.

Advantages of reversed-phase HPLC are shorter column-equilibration times, insensitivity to humidity, and the use of polar organic/aqueous phase systems where most substances are soluble [6]. HPLC is used increasingly in the analysis of food samples to separate and detect additives and contaminants [3–4]. Because separation and detection occur at or slightly above ambient temperature, this method is ideally suited for compounds of limited thermal stability, which often occur in food and beverages. Each modern HPLC apparatus consists of the mobile-phase container, the injection system (manual injector or autosampler) with the sample loop, the pumping system (isocratic or gradient), the analytical column with the thermostatted column compartment, the detector and a data handling system.

The commonly used HPLC detectors are: UV–vis detectors, diode-array detectors (DADs), fluorescence detectors (FLDs), refractive index detectors (RIDs), electrochemical detectors (ELDs), and mass selective detectors (MSDs).