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Standard addition strip for quantitative electrostatic spray ionization mass spectrometry analysis: Determination of caffeine in drinks.

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ABSTRACT

Standard addition strips were prepared for the quantitative determination of caffeine in different beverages by electrostatic spray ionization mass spectrometry (ESTASI-MS). The gist of this approach is to dry spots of caffeine solutions with different concentrations on a polymer strip, then to deposit a drop of sample mixed with an internal standard, here theobromine on each spot and to measure the mass spectrometry signals of caffeine and theobromine by ESTASI-MS. This strip approach is very convenient and provides quantitative analyses as accurate as the classical standard addition method by MS or liquid chromatography.

1. Introduction

Many food and drinks with caffeine content are now commercially available. Consequently, there is a great interest for development of fast analytical tools to measure caffeine and other alkaloids, such as theobromine, theophylline and paraxanthine. These alkaloids have been found in more than 100 different plants and are widely used in the preparation of food, beverages and medicines with analgesic effect [1]. The most well known drinks containing caffeine are coffee and tea. Nowadays, these two beverages are the most consumed drinks all over the world [2]. Caffeine works as a natural drug to stimulate the central nervous and metabolic system [3]. It has positive influence, such as to prevent lung diseases and metabolic disorders [4]. However, its overconsumption can lead to caffeine overdose, in extreme cases to death [5]. The daily intake of caffeine is suggested to be less than 400 mg [6]. Therefore, it is important to quantify caffeine in various food and drink in order to control its consumption and prevent caffeine intoxication.

Caffeine quantitative analyses have been performed with various analytical techniques, including high-performance liquid chromatography (HPLC) with ultraviolet (UV) detection [7–9], capillary electrophoresis [10], gas chromatography (GC) coupled to mass spectrometry (MS) [11], HPLC-MS [12,13], Fourier transform infra-red (FTIR) spectroscopy [14], high-performance thin-layer chromatography (HPTLC) [15] and etc. In addition to these widely used analytical methods, several ambient ionization MS techniques have been introduced for caffeine analysis, such as direct analysis in real time (DART) MS [16], probe electrospray ionization MS and low temperature plasma MS [17,18]. Using ambient ionization MS can avoid time-consuming sample preparation procedures, therefore highly suitable for high throughput analyses.

Recently, we have developed a new contactless ambient ionization technique called electrostatic spray ionization (ESTASI), where a droplet of a liquid containing analytes on a plastic substrate is ionized by application of a pulsating high voltage (HV) for MS detection [19]. The main advantage of ESTASI over other electrospray techniques is that the samples require minimal or no preparation, meaning that ESTASI can provide fast and real-time measurements whilst generating accurate data. To date, ESTASI has been successfully used to analyze protein/peptide dried on a plastic plate [19] or inside a porous matrix [20], to couple MS with capillary electrophoresis [19] and gel electrophoresis [20], to quickly characterize perfume [21] and perform MS imaging of biomolecules on a substrate[22].

In this work, ESTASI-MS is applied for a rapid quantitative analysis of caffeine in beverages with only a few sample pretreatment procedures. The quantitative analysis method is based on plastic strips containing spots of standards at different concentrations, and is named as standard addition strip-ESTASI-MS. The quantification of caffeine in various samples has been performed both with the standard addition strip-ESTASI-MS method and compared with classical standard addition methods by MS or LC. Proof of concept results have been obtained, indicating that this strip strategy can be applied for fast and accurate food analysis and quality control.

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2. Material and methods

2.1. Chemicals and materials

Caffeine (≥ 99%) and theobromine (≥ 99%) were obtained from Sigma-Aldrich (Steinheim, Germany). Methanol (99.9%, HPLC grade) was purchased from Applichem GmbH (Darmstadt, Germany). Acetic acid (100%) was obtained from Merck (Zug, Switzerland). All these reagents and materials were used as received without further purification. Black coffee, tea, and soft drinks (Coca-Cola™, Coca-Cola Zero™, Nestea™) were purchased from the local store. Deionized (DI) water (18.2 MΩ cm) was purified by an alpha Q Millipore system (Zug, Switzerland) and used for all experiments. Gelbond PAG film, 0.2 mm thickness, from Amersham Pharmacia Biotech AG (Uppsala, Sweden) was used as the insulating plate during ESTASI.

2.2. Sample preparation

Soluble coffee (2 g) was diluted in 100 ml of boiled water. Black tea leaves (2 g) were poured with 100 ml of boiling water and filtered. Soft drinks (Coca-Cola™, Coca-Cola Zero™, Nestea Lemon™) were decarbonized using the sonication bath. All liquid samples were diluted 50 times with an acidic solution (50% methanol, 49% water and 1% acetic acid). In standard addition methods, pure caffeine of different concentrations was added into the diluted samples till a final concentration between 5 and 50 μg/ml. In all MS experiments, a fixed amount (25 μl) of an aqueous solution containing 200 μg/ml of theobromine was added into 975 μl of each diluted sample as an internal standard, giving a final concentration of 5 μg/ml.

2.3. High performance liquid chromatography quantitation of caffeine

HPLC analyses of caffeine were performed on a Waters 1525 (Milford, MA, USA) apparatus equipped with a binary pump, a Rheodyne injector with a 5 μL injection loop and a dual wave length UV detector 2487. The compounds were separated on a 4.6 mm i.d. × 250 mm, 5 μm particle reversed-phase Nucleodur C_{18} gravity column (Macherey–Nagel, AG, Switzerland). Methanol/water 50/50 (v/v) was used as a mobile phase. Isocratic elutions were performed at a flow rate of 0.8 ml/min. The column was operated at room temperature and the adsorption wavelength was set at 280 nm. The analysis time was 7 min. 20 μl of black tea or black coffee infusion previously diluted 50 times in the acidic solution, with or without pure caffeine additions (from 5 to 50 μg/ml) were injected. For each sample, experiments were repeated three times. Caffeine in the sample was identified by comparing its retention time with that of pure caffeine standard.

2.4. ESTASI-MS

A linear ion trap mass spectrometer of Thermo LTQ Velos (Reinach, Switzerland) with an ion transfer capillary modified into an "L" shape [21] was used for ESTASI-MS. Electrospray voltage of the LTQ Velos was set as 0 V. An enhanced ion trap scanning rate (10,000*m/z* units/s) was used for the MS analysis. During the experiments, the commercial ion source of the instrument was replaced by an ESTASI ion source. 5 μl of sample in the acidic solution (50% methanol, 49% water and 1% acetic acid) was loaded on top of a polymer insulating plate by Eppendorf pipette. An electrode was placed behind the droplet and under the insulating plate. The distance between the MS inlet and the droplet was around 2 mm. By applying HV (9 kV) to the electrode, the droplet became polarized and as soon as the charge was large enough at the tip of the droplet, a spray of charged microdroplets occurred. When grounding the electrode, a spray of counter charges took place to re-establish the electroneutrality of the droplet. By applying a pulsating square wave HV (0–9 kV) at a given frequency, alternating spray of cations and anions took place. The square wave HV was amplified from a function generator by an amplifier (10HVA24-P1, HVP High Voltage Products GmbH, Martinsried/Planegg, Germany). A digital oscilloscope was used to monitor current and HV pulse. All mass spectra were recorded in positive ion mode. Data analysis was performed by Xcalibur Qual Browser (ThermoFisher Scientific, Reinach, Switzerland). More detailed information on ESTASI can be found elsewhere [19].

2.5. Standardization on ESTASI-MS

To determine the concentration of caffeine in unknown sample, theobromine (5 μg/ml) was mixed with pure caffeine of various concentrations (5–50 μg/ml) in an acidic solution containing 50% methanol, 49% water and 1% acetic acid. The mixtures were analyzed by ESTASI-MS, and the ratios of the peak intensities of caffeine and theobromine were plotted as a function of the caffeine concentration to demonstrate the quantitative analysis performance of ESTASI-MS.

2.6. Standard addition calibration by ESTASI-MS

The method of standard addition by ESTASI-MS was performed by adding small amounts of pure caffeine into the diluted samples in the acidic solution that contained also the internal standard of theobromine for ESTASI-MS analyses. Peak intensities of caffeine and theobromine from mass spectra were used for calculation. The experiments for each sample were repeated several times for accurate calibration and to obtain the standard deviation (SD). The calibration curves and figures were plotted using IGOR Pro (Version 6.00 for Macintosh, WaveMetrics, Lake Oswego, OR, USA).

2.7. Standard addition strip-ESTASI-MS

Arrays of wells (1 mm diameter, 2 mm depth) were drilled on a plastic strip. 5 μl of pure caffeine of known concentration (5–50 μg/ml) was deposited in the wells and left to dry. The strip containing the dried spots of pure caffeine was placed under the MS inlet. 5 μl of a diluted sample mixed with theobromine in the acidic solution was deposited carefully to fully cover the drilled well in order to extract all the dry caffeine just before ESTASI-MS analysis. The electrode and ion transfer capillary were set in such a way to be in line with the center of the drilled hole to achieve good reproducibility. The obtained signal intensities of caffeine and theobromine were used to calculate the caffeine amount in the sample.

3. Results and discussion

3.1. Quantitative analysis from droplets of standard solution by ESTASI-MS.

ESTASI-MS is very sensitive in the detection of trace amount of caffeine. The limit of detection (LOD) was found as 51 nM (10 ng/ml) (see Supporting information (SI)-1). To demonstrate the quantitative analysis performance, a series of standard solution was prepared in 50% methanol, 49% water and 1% acetic acid containing pure caffeine at different concentrations (5–50 μg/ml) and a constant amount of internal standard (5 μg/ml) of theobromine. Theobromine was selected as the internal standard since it has a structure similar to that of caffeine. 5 μl of each solution was