Forensic Science International 220 (2012) 279–283

Contents lists available at SciVerse ScienceDirect

Forensic Science International

journal homepage: www.elsevier.com/locate/forsciint

# Rapid analysis of caffeine in ‘‘smart drugs’’ and ‘‘energy drinks’’ by microemulsion electrokinetic chromatography (MEEKC)

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ARTICLE INFO

*Article history:*
Received 1 June 2011
Received in revised form 13 March 2012
Accepted 16 March 2012
Available online 11 April 2012

*Keywords:*
Caffeine
Energy drink
Smart drug
Microemulsion electrokinetic chromatography

ABSTRACT

A novel method based on microemulsion electrokinetic chromatography (MEEKC) with diode array detection (DAD) for rapid determination of caffeine in commercial and clandestine stimulants, known as ‘‘energy drinks’’ and ‘‘smart drugs’’, is described. Separations were carried out in 50 cm × 50 μm (ID) uncoated fused silica capillaries. The optimized buffer electrolyte was composed of 8.85 mM sodium tetraborate pH 9.5, SDS 3.3% (w/v), *n*-hexane 1.5% (v/v) and 1-butanol 6.6% (v/v). Separations were performed at a voltage of 20 kV. Sample injection conditions were 0.5 psi, 3 s. Diprofilline was used as internal standard. The determination of the analytes was based on the UV signal recorded at 275 nm, corresponding to the maximum wavelength of absorbance of caffeine, whereas peak identification and purity check was performed on the basis of the acquisition of UV radiation between 200 and 400 nm wavelengths. Under the described conditions, the separation of the compounds was achieved in 6 min without any interference from the matrix. Linearity was assessed within a caffeine concentration range from 5 to 100 μg/mL. The intra-day and inter-day precision values were below 0.37% for migration times and below 9.86% for peak areas. The present MEEKC method was successfully applied to the direct determination of caffeine in smart drugs and energy drinks.

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## 1. Introduction

Caffeine is the most ancient and widely consumed psychoactive drug, being naturally present in coffee and cacao beans, kola nuts, guarana berries, tea leaves etc. which are used worldwide in many cultures. The main effects of caffeine include physical endurance, reduction of fatigue and enhancement of mental alertness [1]. Because of its positive activity on the cardio-respiratory system and on the brain function, from year 1984 to year 2004 caffeine was included in the list of doping drugs, when detected in urine above 12 μg/mL. The physical and mental stimulation exerted by caffeine meets the modern trends of the young generations towards the use of ‘‘legal’’ stimulants, instead of the traditional but illegal cocaine and amphetamines. Also, it is noteworthy that caffeine availability has expanded since this compound is present as an additive in ‘‘energy drinks’’ and dietary supplements, often perceived as ‘‘safe’’, but not free from relevant adverse effects. Quite recently, different preparations containing caffeine (capsules, strips, powders) have become available through the Internet and in the so called ‘‘smart shops’’ as ‘‘legal’’, easily available stimulant drugs (smart drugs).

In recent years, the use of alcohol in combination with caffeine-containing drinks or drugs has become fairly popular, for the ability of caffeine to offset the sedating effects of alcohol and to enhance alertness [2–4].

On the other hand, evidence of clinical syndromes of caffeine dependence and overdosing have been reported [5] as well as numerous caffeine-related intoxications and even deaths [6–9].

Current methods for caffeine analysis are based on: gas chromatography–mass spectrometry (GC–MS) [10,11] and HPLC–MS [12,13]. Unfortunately, these techniques are available only in specialized laboratories, but rarely in the laboratories of clinical chemistry and clinical toxicology, causing a clear underestimation of the phenomenon of caffeine abuse in the population.

Because a higher versatility and easiness to switch between different analytical conditions, capillary electrophoresis (CE) may look preferable to the above mentioned techniques for the analysis

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doi:10.1016/j.forsciint.2012.03.015

of caffeine, which at present is only rarely requested in laboratories of clinical and forensic toxicology.

In recent years, indeed, CE methods for the determination of caffeine have been reported [14,15]. For the neutral characteristics of the molecule of caffeine precluding any charge-to-mass ratio based separations, usually micellar electrokinetic chromatography (MEKC) methods were proposed. In MEKC analytes are separated on the basis of their partitioning between an aqueous separation buffer and the hydrophobic core of charged micelles, which acts as a pseudo stationary hydrophobic phase [16,17].

More recently, microemulsion electrokinetic chromatography (MEEKC) has been introduced as an attracting alternative to MEKC for the separation of neutral as well as charged molecules. The MEEKC separation buffer is a microemulsion in which an organic water immiscible solvent forms the core of the microdroplets, which are stabilized by a charged surfactant located at their surface, which confers to them a net electric charge and consequently an electrophoretic mobility. According to this scheme, the separation of neutral compounds in MEEKC is based on the analyte partitioning between the moving charged "oil" droplets and the aqueous buffer phase. Particularly, the oil-in-water (o/w) microemulsions are similar to micelles for their ability of solubilizing hydrophobic compounds, but display a much larger capacity due to a larger droplet size [18]. Moreover, in comparison to MEKC, MEEKC, because of a higher complexity of the buffer, is more flexible and can be more finely tuned to optimize separations.

To date, the most common applications of MEEKC are in the pharmaceutical field [19,20], but, to the best of our knowledge, only two method were reported applying this separation mode for the determination of caffeine (and catechins) in green tea [21] or for the detection of caffeine as adulterant in illicit preparations of heroin and amphetamine [22].

The present work was aimed at the development and validation of a rapid and simple MEEKC method for the quantitative analysis of caffeine in commercial beverages and in "smart drugs" preparations.

## 2. Materials and methods

### 2.1. Chemicals and reagents

Ultrapure deoxycholic acid, sodium tetraborate and pure caffeine (Sigma Reference Standard) were obtained from Sigma–Aldrich (St. Louis, MO, USA). Diprofilline [used as the internal standard (IS)] was obtained from a pharmaceutical product named Katasma$^{TM}$ (Bruschettini s.r.l., Genova, Italy). Stock solutions of caffeine and diprofilline were prepared in 50/50 methanol/water at individual concentrations of 1 mg/mL and stored at +4 °C until use.

Sodium dodecyl sulphate (SDS), *n*-hexane and 1-butanol were obtained from Merck (Darmstadt, Germany). Buffer electrolytes were prepared by proper dilution of the stock solutions of sodium tetraborate 100 mM and SDS 100 mg/mL, each one obtained by dissolution of the respective powders in deionised water.

The deionised water used throughout the study was obtained from an Aqua MAX-Ultra 370 Series water purification system (Young Lin Instrument, Anyang, Korea).

### 2.2. Sample preparation

Six different energy drink cans, namely Imola® (Getranke GmbH Traisental, Austria), Semtex® (Pinelli spol s.r.o., Czech Republic), Burn® (Coca-Cola Company, USA), Red Bull® (Red Bull GmbH, Austria), Shock® (Al.Namura spol s.r.o., Czech Republic), Mixxed Up® (LIDL Stiftung &Co. KG, Germany) were collected from Italian supermarkets. Samples of "smart drugs", namely Minikikke®, Koru®, Finalkat®, Happy caps XXX®, Happy caps 4U®, were purchased in different Italian Smart Shops, in the frame of a research project (Smart Search) in collaboration with the National Early Warning System.

All the samples were stored in their original cans or packages at room temperature until analysis.

### 2.3. Capillary electrophoresis

The present study was performed by using a P/ACE MDQ automated capillary electropherograph (Beckman Coulter, Fullerton, CA, USA) equipped with a diode array detector. The software "32 Karat" Version 5.0 (Beckman Coulter) controlled hardware operation, data acquisition and data reporting.

The electrophoretic analysis was performed in an uncoated fused-silica capillary (50 μm i.d., 50 cm total length) from Composite Metal Services (The Chase, Hallow, UK), with an effective length of 40 cm. Separations were carried out by applying a constant voltage of 20 kV at a capillary temperature of 25 °C. The optimized buffer electrolyte was composed of 8.85 mM sodium tetraborate pH 9.5, SDS 3.3% (w/v), *n*-hexane 1.5% (v/v) and 1-butanol 6.6% (v/v). Under these conditions, the generated current was about 60 μA. In order to obtain reproducible separations, the fresh buffer was prepared at the beginning of each day and degassed by sonication for 15 min before use.

Before each run, the capillary was rinsed sequentially with NaOH 1 M, water and buffer electrolyte, for 5 min each.

Hydrodynamic injections were carried out by applying 0.5 psi for 3 s at the inlet of the capillary. Detection was performed by monitoring the wavelengths corresponding to the maxima of absorbance of caffeine: 200 nm and 275 nm. However, for peak identification and peak purity check the UV spectrum in the range of 200–400 nm was also recorded.

Before CE analysis, all beverages were centrifuged at 10,000 rpm in a benchtop centrifuge for 5 min to remove particulate material and then diluted 1:2 with buffer containing 100 μg/mL diprofilline (IS). An aliquot of each smart drug in powder form was weighed and diluted in methanol to a final concentration of 20 mg/mL. The obtained solutions were then sonicated for 15 min and centrifuged at 3500 rpm for 10 min. The surnatants were diluted in buffer solution containing the IS (to a final concentration of 50 μg/mL). Each solution was sonicated for 10 min before injection to avoid outgassing.

Quantification was carried out on the basis of peak areas detected at 275 nm by using the internal standard method (IS: diprofilline). Standard curves were prepared by spiking buffer solutions with caffeine to obtain concentrations of 5, 10, 20, 40, 75 and 100 g/mL. which were diluted with the IS solution and injected.

## 3. Results and discussion

On the basis of existing literature [15], for the separation of caffeine, plain capillary zone electrophoresis (CZE) with a basic background electrolyte (15 mM sodium tetraborate at pH 9.5–11.0) was initially tested. However it was soon clear that at these pH values, caffeine poorly ionized and consequently migrated close to the EOF, not resolved from the neutral compounds present in the samples.

Thus, in order to solve this problem, the introduction of a new separation mechanism, in addition to electrophoresis, looked necessary. To this aim, MEEKC looked attractive, because of its ability to deal with charged and neutral compounds as it is reported by a number of recent papers [19]. In MEEKC the background electrolyte is composed of a dispersion of two immiscible liquids, consisting either in "oil" finely dispersed in an aqueous buffer (o/w microemulsion) or water dispersed in "oil" (w/o microemulsion). In this system, the resulting droplets are formed in the presence of an ionic surfactant coating their surface which reduces the surface tension, thus allowing the formation of a stable emulsion. The MEEKC buffer is further stabilized by the addition of a short-chain alcohol, such as butanol or octanol. By application of voltage across the capillary, the oil droplets bearing on the surface the charged surfactant molecules migrate toward the electrode with opposite polarity. In the present case, the droplets covered with SDS and hence negatively charged move towards the anode, i.e. in the direction opposite to the electroosmotic flow (EOF). However, at high EOF values, such as in the present case (because of the basic pH of the buffer electrolyte), the negative droplets are swept to the cathode (i.e. towards the detector) by the prevailing velocity of the EOF, which exceed their own electrophoretic velocity directed backwards in the capillary.

In o/w MEEKC, as in the present system, neutral solutes are separated on the basis of their solubility in the "oil" phase (log P) with the more water-insoluble solutes migrating last.

In this fairly complex separation system, specific conditions to be optimized include the choice and concentration of the "oil" phase, of surfactant and co-surfactant. Moreover, it should be stressed that the buffer pH plays an important role in any

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