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Fully-automated in-syringe dispersive liquid-liquid microextraction for the determination of caffeine in coffee beverages

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ABSTRACT

A novel fully-automated magnetic stirring-assisted lab-in-syringe analytical procedure has been developed for the fast and efficient dispersive liquid-liquid microextraction (DLLME) of caffeine in coffee beverages. The procedure is based on the microextraction of caffeine with a minute amount of dichloromethane, isolating caffeine from the sample matrix with no further sample pretreatment. Selection of the relevant extraction parameters such as the dispersive solvent, proportion of aqueous/organic phase, pH and flow rates have been carefully evaluated.

Caffeine quantification was linear from 2 to 75 mg L^{-1}, with detection and quantification limits of 0.46 mg L^{-1} and 1.54 mg L^{-1}, respectively. A coefficient of variation (n = 8; 5 mg L^{-1}) of a 2.1% and a sampling rate of 16 h^{-1}, were obtained. The procedure was satisfactorily applied to the determination of caffeine in brewed, instant and decaf coffee samples, being the results for the sample analysis validated using high-performance liquid chromatography.

1. Introduction

Caffeine is an alkaloid present in plants as a natural compound against certain insects (Xia, Ni, & Kokot, 2013). It is a derivative from the group of xanthines, and its consumption has an effect over the nervous central system. Caffeine is consumed on a large scale as a stimulant and is typically added to many products and beverages. Studies have reported the beneficial effects of caffeine consumption as an ergogenic aid in aerobic and anaerobic exercises that influence the athlete's mood swings (Carneiro et al., 2013). There are reports of its use among athletes to promote an increase in muscle strength and power, increased mental alertness, decreased fatigue and improvement in mood due to direct action on the central nervous system. However, excessive amounts of caffeine might cause the "caffeinism" syndrome, such as ringing in the ears, mood swings, diarrhea, delirium, muscle tension and tremors (Andrade et al., 1995). In addition, other adverse physiological effects are observed like gastric acid secretion, diuresis (Belay, Ture, Redi, & Asfaw, 2008), increased of blood pressure, or excessive stimulation of the central nervous system. In view of the physiological action produced by caffeine consumption, it is essential the quantification in drinks, foods and pharmaceutical formulations containing it, in order to ensure safe intake levels.

Procedures for the determination of caffeine require the use of expensive instrumentation such as HPLC (Belguidoum, Amira-Guebailia, Boulmokh, & Houache, 2014; Camargo & Toledo, 1998; Rostagno et al., 2011; Wang et al., 2011), capillary electrophoresis (Marra et al., 2014; Meinhart et al., 2010), infrared spectroscopy (Huck, Guggenbichler, & Bonn, 2005), Raman spectroscopy (Najafi, Hamid, & Afshin, 2003), or hyphenated techniques (Huang et al., 2012; Marra et al., 2014; Shrivas & Wu, 2007). The official method for quantification of caffeine, recommended by the Association of Official Analytical Chemists "AOAC" (Association of Official Agriculture Chemists (AOAC), 1945) is laborious, time consuming, and is being subject to errors in view of the multiple steps involved in the extraction process. In this regard, the development of simple, reproducible and sensitive procedures is essential, with a concomitant low cost and small waste generation, in order to quantify caffeine in beverages.

To remove matrix effects and/or concentrate the analyte avoiding the use of large volumes of solvents, liquid-liquid microextraction is a widely used procedure for sample preparation in food analysis (Asensio-Ramos, Ravelo-Perez, Gonzalez-Curbelo, & Hernandez-Borges, 2011). However, these approaches are time consuming and difficult to automate. Automated flow injection techniques are a practical solution to overcome this bottleneck (Silvestre, Santos, Lima, & Zagatto, 2009). Using these techniques sample treatments such as chemical derivatization, separation or preconcentration steps are easily carried out using small volumes

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of sample and/or reagents. Since these procedures do not require reaching an equilibrium state, this aspect can improve the precision, thus increasing the sampling rate. Furthermore, since the extraction steps are carried out in a closed system, the possibility of sample contamination is reduced, as well as minimizing the exposure of the analyst to toxic and volatile chemicals. The lab-in-syringe system (Maya, Horstkotte, Estela, & Cerdà, 2014) is a recently proposed strategy, which enables the miniaturized on-line sample preparation showing highly reproducible sample-processing conditions. These systems combine the strategies of sequential injection systems (Ruzicka & Marshall, 1990) with flow-batch approaches (Diniz, Almeida, Harding, & Araújo, 2012), and the entire step of sample preparation (*i.e.* extraction and/or preconcentration) and detection is performed in an integrated system. In this sense, the lab-in-syringe stirring-assisted liquid–liquid microextraction (Horstkotte, Suárez, Solich, & Cerdà, 2013; Henriquez, Horstkotte, Solich, & Cerdà, 2013), dispersive liquid–liquid microextraction (DLLME) (González, Avivar, & Cerdà, 2015; Maya, Horstkotte, Estela, & Cerdà, 2012), gas–liquid separation (Giakisikli, Miró, & Anthemidis, 2013) and on-line cloud point extraction (Frizzarin, Portugal, Estela, Rocha, & Cerdà, 2015) procedures have been proposed recently.

The aim of this work is to develop for the first time a fast, simple and fully-automated lab-in-syringe methodology for the stirring-assisted DLLME of caffeine due to current interest in food, agronomic and environmental applications. The DLLME using the lab-in-syringe procedure is exploited to automatically extract caffeine from the matrix of coffee beverage with no further sample treatment. Simple on-line UV–vis spectrophotometry was feasible since the separation/extraction procedure showed high selectivity and sensitivity.

2. Experimental

2.1. Apparatus

The flow-based system (Fig. 1) comprised an eight-way selection valve (Sciware Systems S.L., Bunyola, Spain) coupled to an automatic burette with a 5 mL glass syringe (BU 4S, Crison) and a three-way solenoid valve in the head section. The stirring system was composed by a lab made device and a miniature magnetic stirrer (10 mm length, 3-mm diameter) placed inside the syringe, as detailed in previous work (Henriquez et al., 2013). All tubing of the flow system was made from polytetrafluoroethylene (PTFE) tubing (0.8 mm id).

The control of active devices for method execution, acquisition and data processing was developed by using the AutoAnalysis 5.0 software (Sciware). A CCD spectrophotometer (Ocean Optics, Dunedin, FL, USA; model USB4000) was directly coupled to a 10 mm optical-path flow cell. Radiation from a D_2 source (Ocean Optics; model DH-2000-BAL) was conducted to the flow cell by using an optical fiber.

For method validation (Andrade et al., 1995), a HPLC instrument equipped with a dual solvent pump (PV-4180 Jasco) with gradient controller and mixing interface, a C_{18} column (Kinetex 5 μm EVO C18 100A, 150 × 4.6 mm), a 20 μL injection loop and a diode array UV–vis detector (MD-4017 Jasco) was used.

2.2. Reagents and solutions

All solutions were prepared using analytical grade chemicals and deionized water (18 MΩ cm). A stock solution of 1000 mg L^{-1} was prepared by appropriate dissolution of caffeine (Sigma-Aldrich) in water and maintained under refrigeration avoiding light radiation. Reference solutions were daily prepared by stepwise dilution of the caffeine stock solution. Water was used as washing stream.

Dichloromethane, cyclohexane, n-hexane, chloroform, xylene, toluene, ethanol, acetonitrile, acetone, 2-propanol and methanol HPLC grade solvents were used in appropriate proportion for all experiments.

Brewed coffee, instant coffee and decaf coffee samples were used for caffeine extraction and determination. The brewed coffee samples were obtained from different coffee shops from the city of Palma de Mallorca (Balearic Islands, Spain). Instant coffee (2.0 g) obtained from a local grocery store was dissolved in 50 mL of hot

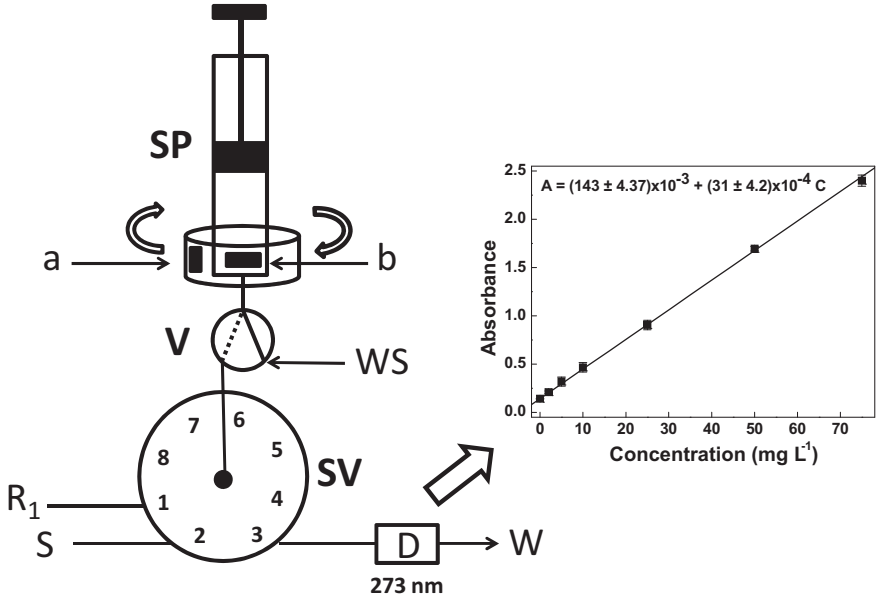

Fig. 1. Lab-in-syringe system for the DLLME of caffeine. SP: syringe pump; a: agitation system; b: magnetic bar; SV: solenoid selection valve; V: three-way solenoid valve; D: detection system (flow-cell coupled to the spectrophotometric detector); S: sample; R_1: mixture of dichloromethane and methanol (15:85) v/v; WS: washing stream; W: waste. The inset represents the obtained calibration curve.