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Stepwise injection potentiometric determination of caffeine in saliva using single-drop microextraction combined with solvent exchange

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ABSTRACT

A flow potentiometric method for determination of caffeine in saliva is suggested. This task is important for non-invasive assessment of drug metabolizing system activity in hepatocytes. In the current study, stepwise injection analysis (SWIA) was successfully combined with single-drop liquid microextraction (SDLME) and solvent exchange procedure. The method is based on the caffeine SDLME with subsequent solvent evaporation and dissolution of analyte in sulfuric acid followed by potentiometric detection using poly(vinyl chloride) membrane electrode containing potassium tetrakis[3,5-bis(trifluoromethyl)phenyl] borate as electroactive component. SDLME was employed for elimination of interfering matrix effects of saliva and caffeine metabolites such as theophylline, theobromine and paraxanthine. A linear range of 10^{-5}–10^{-2} M was established for caffeine with detection limit at 6×10^{-6} M. The sample throughput was 6 samples h^{-1}. The proposed method was successfully applied to the determination of caffeine in saliva and the analytical results agreed well with the results obtained with reference HPLC method.

1. Introduction

A wide variety of foreign or toxic substances (drugs, pesticides, food preservatives, etc.) are being metabolized by microsomal oxidase (MO) enzyme system in hepatocytes [1]. This system serves as a detoxification route and, in contrast, also as a route of metabolic activation to yield reactive metabolites which initiate toxic and carcinogenic events [2–4].

There are invasive and noninvasive methods for determination of MO activity. Presently, noninvasive methods are more attractive, since they do not require liver biopsy. The activity of MO system of liver is normally examined by noninvasive measurement of the rate of model drug biotransformation which is only metabolized by the hepatic P-450 enzymes [5]. The procedure is based on per os ingestion of model drug followed by the estimation of pharmacokinetic parameters of drug (or its metabolites) elimination in biological fluids (saliva, urine or blood) after its oral ingestion. Caffeine (3,7-dihydro-1,3,7-trimethyl-1H-purine-2,6-dione or 1,3,7-trimethylxanthine) has been proposed as a model drug in clinical practice [6,7]. Caffeine is one of the readily accessible drugs, and its rate of metabolism by the liver may reveal the presence of many acute and chronic diseases, and allows estimating their degree [8–10]. Saliva is employed for therapeutic monitoring of a variety of drugs. Moreover, the easy noninvasive and stress-free nature of saliva collection makes it one of the most accessible body fluids.

Various analytical methods have been developed (Table 1) for determination of caffeine in biological fluids based on micellar LC [11], HPLC [12,13], HPLC–MS [14], electrochemistry [15–18], capillary electrophoresis (CE) [19,20] and CE–MS [21]. These methods are characterized by high sensitivity, good reproducibility and recovery, but they usually involve long stages of sample preparation consuming large number of chemicals and respectively large volume of waste generation.

An important trend in modern analytical chemistry is the automation of analytical procedures and one of the successful directions in this field is devoted to the development of flow analysis methods. A large number of methods based on flow analysis have been proposed for caffeine determination in different objects [22–25]. To the best of our knowledge, the procedures for determination of caffeine in saliva in a flow system have not been reported so far. Also, the flow potentiometric determination of caffeine has not been developed.

The aim of this work was to develop an automated procedure for determination of caffeine in saliva in the flow conditions. In our work plasticized poly(vinyl chloride) (PVC) membrane electrode containing potassium tetrakis[3,5-bis(trifluoromethyl)phenyl]borate (KTFPB) as an electroactive component [26] was applied for caffeine determination in saliva samples. In order to eliminate interfering matrix effects of saliva and main products of caffeine

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Table 1
Comparison of the suggested method with previously reported for the determination of caffeine in biological fluids.

Technique	Sample pretreatment	Sample	Linear concentration range, mg^{-1} L	LOD, mg^{-1} L	Reference
Micelar LC	Filtration	Urine	–	1.2	[11]
HPLC	Filtration	Urine	3.21–71.2	–	[12]
HPLC	Solid-phase extraction	Meconium	0.01–0.25	10^{-2}	[13]
HPLC-MS	Liquid–liquid extraction	Breast milk	–	1.6	[14]
VM	No	Urine	0.004–19.419	1.6×10^{-3}	[15]
CV	No	Serum	4.85–38.84	9.7×10^{-2}	[16]
CV	Filtration	Urine, blood serum	0.06–12.18	1.7×10^{-2}	[17]
DPV	Filtration	Urine	0.02–19.42	3.3×10^{-3}	[18]
SWV	Filtration	Urine	0.02–9.71	3.9×10^{-3}	[18]
MEKC	Filtration	Urine, serum	–	0.4	[19]
MECC	Filtration	Rat serum	1.5–194	1.5	[20]
CE–MS	Solid-phase extraction	Urine	–	20	[21]
SWIA-PM	SDLME	Saliva	2–2000	1.2	This work

HPLC – high performance liquid chromatography, HPLC–MS – high performance liquid chromatography mass spectrometry, VM – voltammetry, CV – cyclic voltammetry, DPV – differential pulse voltammetry, SWV – square-wave voltammetry, MEKC – micellar electrokinetic capillary chromatography, MECC – micellar capillary electrophoresis, CE–MS – capillary electrophoresis mass spectrometry, SWIA-PM – stepwise injection analysis with potentiometric detection.

metabolism such as theophylline, theobromine and paraxanthine, we investigated single-drop liquid microextraction (SDLME) of caffeine with subsequent solvent evaporation and dissolution of analyte in aqueous phase for potentiometric detection. The whole method was realized in a stepwise injection system (SWIA), which is a universal solution for the automation of different analytical procedures [27–30]. The SDLME was chosen due to its simplicity, reliability and efficiency [31–33]. However, the extraction solvents used in the SDLME are not always suitable for the subsequent detection. The solvent exchange after extraction is widely used in different techniques. In this research, the SDLME combined with solvent exchange based on flow system was realized for the first time and used for separation of caffeine and its main metabolites.

2. Experimental

2.1. Reagents and solutions

All chemicals were of analytical reagent grade. Ultra pure water from Millipore Milli-Q RG (Millipore, USA) was used for solution preparation and dilution.

Stock solutions of caffeine, theophylline, theobromine and paraxanthine (10^{-2} M) were prepared by dissolving the corresponding amount of substances (Sigma-Aldrich, USA) in water. The solutions were stored at room temperature and used within 60 days. Working solutions of caffeine and its metabolites were prepared immediately before the experiments by dilution of the stock solutions.

2.2. Manifold and apparatus

2.2.1. SWIA system

The SWIA manifold (Fig. 1) includes: six-way solenoid valve (Cole-Parmer, USA), syringe pump (SP) (Cavro XLP 6000, Tecan, USA), mixing chamber (a polymer syringe, 50 mm in height and 10 mm in i.d.), holding coil (HC), flow-through potentiometric detector equipped with the saturated Ag/AgCl reference electrode (Warminster, PA, USA), Hamilton® syringe (700 series, fixed needle, 10 μL) and laboratory-made caffeine-sensitive electrode and communication tubes (PTFE, 1 mm in i.d.). The system was controlled automatically by the PC.

2.2.2. Caffeine-sensitive electrode

The caffeine-sensitive electrode employed in this study was based on polymeric sensor membrane. The membrane was

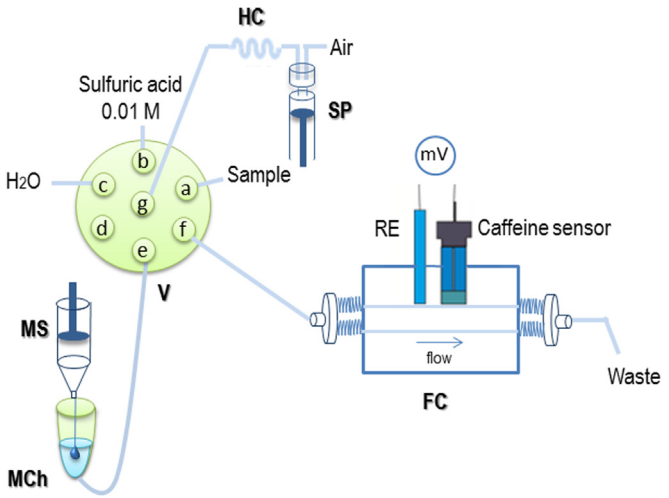

Fig. 1. The SWIA manifold for the potentiometric determination of caffeine in saliva: FC – flow-cell, MCh – mixing chamber, MS – microsyringe, V – valve, HC – holding coil, SP – syringe pump, RE – reference electrode, ICE – ion selective electrode.

prepared using 33 wt% PVC as a polymer, 65 wt% 2-fluorophenyl 2-nitrophenyl ether as a solvent-plasticizer and 2 wt% KTFPB as a cation-exchanger using the approach similar to the one reported in [26]. All membrane components were purchased from Sigma-Aldrich (Germany) in Selectophore® grade. Sensor membranes were prepared following a standard procedure by dissolving the weighed amounts of membrane components in freshly distilled tetrahydrofurane under stirring with subsequent casting in a flat bottom Teflon beaker. After tetrahydrofurane evaporation the disks 4 mm in diameter were cut from the parent membrane, equipped with solid inner contact made of graphite suspension in PVC-cyclohexanone mixture (coated wire type design) and glued upon PVC tubes (sensor bodies). Potentiometric measurements were performed in the following galvanic cell:

Cu|Ag |AgCl, KCl_{sat}| sample solution| sensor membrane |solid contact |Cu.

All measurements were performed against Ag/AgCl reference electrode using digital mV-meter I-510 (Aquilon, Russia).

2.2.3. HPLC system

HPLC analysis was performed with a liquid chromatograph LC-20 (Shimadzu, Japan) with UV detection at 205 nm (diode array). Chromatographic separation was performed on a column