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Repetitive injection field-amplified sample stacking for cationic compounds determination

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ABSTRACT

The development of a field-amplified sample stacking technique is presented. Sensitivity enhancement in this technique was obtained by repetitive injections of a sample followed by steps of sample matrix removal through the application of counter-pressure. Under optimized conditions the background electrolyte (BGE) was composed of 80 mM H_3PO_4 while the sample matrix contained 0.5 mM H_3PO_4 and 30% (v/v) methanol. The elaborated method enabled a 4-fold effective injection of the sample (53 s, 0.5 psi). Each injection was followed by a focusing step during which the application of a voltage (2 kV) and counter-pressure (−1 psi) was performed for 0.65 min. The method was developed for the determination of six psychiatric drugs (opipramol, hydroxyzine, promazine, amitriptyline, fluoxetine, and thioridazine). The elaborated method was applied for analysis of human urine samples after a simple liquid–liquid extraction procedure. The detection limits obtained were in the range of 2.23–6.21 ng/mL.

1. Introduction

The separation of chemical compounds is one of the most intensively developed fields in analytical chemistry. Among applied techniques chromatography is the most popular and most widely used. In the last 20 years capillary electrophoresis (CE) has gained in popularity and is more often considered as a supplementary or even alternative to liquid chromatography (LC). Despite many advantages, like a shorter analysis time and high separation efficiency, CE usually provides higher detection limits in comparison to LC. This limitation can be overcome by the application of on-line preconcentration techniques.

Field-amplified sample stacking (FASS) is the oldest and the simplest of techniques whose theoretical foundations were created by Mikkers et al. [1]. The principle of this technique is based on the rapid decrease in migration velocity of the analyte on the boundary of a high electric field sample zone and a low electric field background electrolyte (BGE) zone. An interesting review on this topic was provided by Quirino and Terabe [2].

The FASS technique can be easily implemented for the elaborated separation method by a simple lowering of the sample conductivity. However, it has some limitations. The most crucial is a greatly limited volume of the sample that can be injected. The application of a sample injection longer than a few percents of the capillary length can cause current failures and analysis breakdowns. A longer injection plug can be obtained using large volume sample stacking (LVSS) techniques [3,4]. In these techniques greater sensitivity improvement can be achieved, in comparison to FASS, and it is limited by the capillary volume [5,6]. Electrokinetic injection (EKI) usually provides better signal strength enhancement. This is caused by the additional electrophoretic mechanism of the injection of the ions. EKI is used in the most powerful on-line preconcentration techniques developed so far [7,8]. However, the application of these techniques requires the optimization of many parameters. It has been also proved that hydrodynamic injection can be more repeatable than EKI [9]. Pressure-based injection modes also enable the simultaneous introduction (into the capillary) of analytes with different charge states (cations, anions, ampholytes and non-charged molecules) [10]. Thus, the development of preconcentration strategies coupled with hydrodynamic injection mode is still an important topic despite the fact that a greater sensitivity improvement can be achieved with the use of EKI [9].

The limitation of the capillary volume has been overcome by Urban et al. [11]. The application of charged β-cyclodextrines (β-CD) as a pseudostationary phase enabled the separation and double stacking of anabolic steroids using full-capillary injection. The authors have shown that the third injection, although it was possible, resulted in low separation efficiency and poor repeatability [11]. Wang et al. reported up to a fivefold whole-capillary

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injection [12]. The developed method was based on the LVSS technique followed by a sweeping of analytes coupled with the removal of the sample matrix after each injection. The elaborated method provided three orders of magnitude sensitivity enhancement.

This work presents a simple and repeatable way of sensitivity enhancement in the FASS technique. The low conductivity of samples used in the FASS technique does not enable the performance of injections longer than a few % of the whole capillary volume due to the inefficiency of stacking effect. This can result in the broadening of bands and loss of repeatability. Moreover, longer sample injection can lead to current destabilization during analysis and run collapse. The proposed repetitive injection field-amplified sample stacking (RI-FASS) technique was elaborated as a solution to this problem. The applicability of this method was shown for determination of selected psychiatric drugs in human urine samples after a simple liquid–liquid extraction clean-up step.

2. Experimental

2.1. Instrumentation

All experiments were performed on a PA 800 plus CE system (Beckman Instruments, Fullerton, CA, USA) equipped with a diode-array detector. Analytical wavelength was set at 200 nm. Analyses were carried out using uncoated fused-silica capillaries (50 μm × 60 cm, 50 cm to detection window; Beckman) thermostated at 25 °C. The pH values were measured using a Crison GLP-21pH meter (Barcelona, Spain). The pKa values of the analytes were calculated using ACD/ChemSketch (version 12.01) software (Advanced Chemistry Development, Inc., Toronto, ON, Canada). Injection plug length was calculated using CE Expert software (Beckman). pH values in Section 3.2 were calculated using PeakMaster 5.3 Complex software [13].

2.2. Reagents and solutions

All solvents and reagents were of analytical grade. Solvents used in experiments were sodium hydroxide 0.1 M (Beckman Coulter, Brea, USA), sodium hydroxide (Sigma-Aldrich, St. Louis, MO, USA) phosphoric acid 85% (Sigma-Aldrich), methanol (Baker, Analyzed LC–MS reagent, NJ, USA), dichloromethane (Sigma-Aldrich) and redistilled water (Millipore, Mili Q Direct 16). The analyzed drugs (opipramol, hydroxyzine, promazine, amitriptyline, fluoxetine, thioridazine and oxazepam) were purchased from Sigma-Aldrich. Drug stock solutions were prepared in pure methanol to a concentration of 1 mg/ml. The calculated pKa values were as follows: 3.45 and 7.45 for opipramol, 2.10 and 6.62 for hydroxyzine, 9.43 for promazine, 9.15 for amitriptyline, 10.05 for fluoxetine, and 9.84 for thioridazine.

2.3. General electrophoresis procedure

Optimal composition of BGE for both separation and preconcentration was found to be 80 mM H_3PO_4. The sample matrix was composed of 30% methanol (v/v) and 1 mM H_3PO_4.

Before each analysis, capillaries were rinsed with 0.1 M NaOH (0.5 min), water (1 min) and BGE (1.5 min) at 20 psi. Samples were injected for 53 s at 0.5 psi followed by a short BGE plug (12 s, 0.5 psi). Next, the stacking step with simultaneous sample matrix removal was performed for 0.65 min (2 kV, −1.0 psi). Under optimized conditions, injections and stacking were repeated four times in total. In the end, high voltage (30 kV) was applied for separation of the compounds.

2.4. Sample preparation

The extraction procedure applied in the presented assay was a modified method reported by Rabanes et al. [14]. Urine samples were collected from healthy volunteers and stored frozen (−20 °C). Before CE analysis, the samples were defrosted and centrifuged at room temperature (7378g for 10 min) to remove visible sediments. Each of the urine supernatants (3 ml) was spiked with 15 μL of a drug mixture that contained 6.0–200.0 μg/mL of each analyte and 10 μL of I.S. (200 μg/mL). This was equivalent to 0.03–1.0 μg/mL final concentration in an enriched sample. 2 M NaOH was added to the urine sample until the pH was 12 to render the analytes electrically neutral. The solution was extracted 3 times with 1 mL of dichloromethane. The organic extract was recovered by centrifugation for 15 min at 3000 rpm. Next, 1.2 mL of the pooled extract was collected in a clean tube and evaporated at 25 °C to dryness in an argon stream. Finally, the residue was dissolved in a matrix solution buffer, centrifuged and analyzed.

3. Results and discussion

3.1. Mechanism of preconcentration in a repetitive injection field-amplified sample stacking technique

The scheme of the preconcentration mechanism is presented in Fig. 1. At the beginning a low conductivity sample was introduced into the capillary, preliminarily filled with the BGE (Fig. 1A). The sample injection was followed by a buffer plug (Fig. 1A). Next, a high voltage with counter-pressure was applied (Fig. 1B). Due to the applied voltage the analytes migrated towards the capillary outlet, stacking on the sample/BGE boundary. Simultaneously, under the presence of counter-pressure, the low conductivity sample matrix was removed from the capillary. This stacking/removing step was conducted until the current value reached 95% of the current obtained when the capillary is filled only with the BGE (Fig. 1C). The sample and BGE injection followed by stacking/removing steps was repeated (Fig. 1D, F). In the end, a high voltage was applied and separation was performed (Fig. 1G).

There are a few crucial factors influencing the sensitivity enhancement in the presented technique, such as the sample

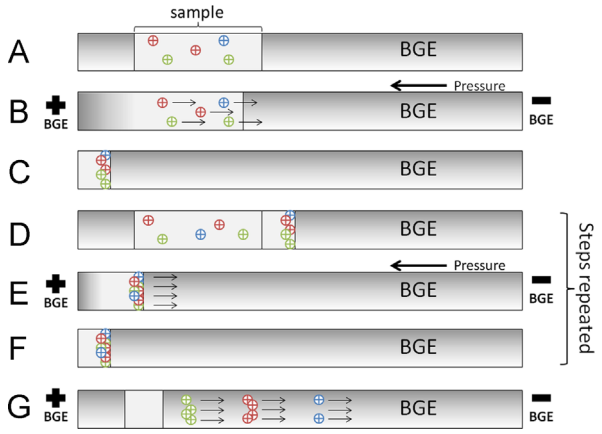

Fig. 1. Scheme presenting mechanism of the repetitive injection field-amplified sample stacking technique. (A) cationic analytes in low conductivity matrix were introduced into the capillary followed by short BGE plug. (B) Application of voltage induced cations to migrate toward the cathode and stacking on the sample/BGE boundary. Simultaneous counter-pressure removed the sample matrix from the capillary. (C) pressure and voltage were stopped when current value reached ~95% of current value when capillary was filled only with BGE. (D, E, F) Injection of sample and short BGE plug and stacking/matrix removal steps were repeated. (G) In the end high voltage was applied and the separation occurred.