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Development of a chromatographic low pressure flow injection system: Application to the analysis of methylxanthines in coffee

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ABSTRACT

In this work, the coupling of a commercial monolithic column to a traditional low pressure FIA system is proposed for the analysis of theobromine, theophylline and caffeine in coffee brewed samples using UV detection.

The parameters mobile phase composition, flow rate and loop volume were evaluated and discussed considering the various chromatographic parameters in order to enable resolution of the methylxanthines studied within the coffee brewed sample matrix. The analyses of methylxanthines in coffee brewed samples by the proposed methodology were in good agreement with those obtained by the reference procedure based on HPLC. Relative errors were below 6% for all samples analyzed. Detection limits in the selected experimental conditions were within 10^{-6} M range for theobromine and theophylline, and 10^{-5} M for caffeine. The determination rate of the three methylxanthines for coffee brewed samples was ca of 10 h^{-1}.

The main advantage of the proposed flow system was the possibility to perform chromatographic separations in low pressure flow systems. This substantial improvement was achieved due to the compatibility of monolithic columns within the flow injection system surpassing in this way one of the main handicaps of traditional flow analysis systems. Additional features of the strategy presented were low cost, efficiency, high versatility and low reagent consumption comparing to HPLC methodologies usually followed in the case study herein presented.

1. Introduction

Recently, the utilization of monolithic columns in the development of chromatographic methodologies imparted to this technique lower time of analysis without decreasing peak resolution. These features result from the bimodal pore structure of monolithic columns based on mesopores, which provide high surface area required for sample equilibrium establishment, and macropores which enable high flow rates with low back pressure [1]. The performance of monolithic columns not only enabled the development of analytical methodologies with columns of reduced length [2,3] but also minimized the usual apparatus of chromatographic systems, allowing the replacement of the traditional piston pumps by alternative propelling devices that were used exclusively in low pressure flow systems. In this context, sequential injection chromatography can be viewed as a milestone of low pressure chromatography [4], where a syringe driven propulsion device is used for eluent propulsion. Still, since the appearance of sequential injection chromatography, scarce low pressure chromatographic methodologies have been developed namely analytical systems based on syringe driven pistons [5,6], milliGAT pumps [7] and peristaltic pumps [8,9]. Syringe driven pistons and milliGAT pumps take advantage of withstand higher back-pressures, comparing to peristaltic pumps. The main handicap of using syringe driven pistons is the non-continuous flow mode inherent to the defined barrel volume. The analytical systems based on the use of a peristaltic pump, propelling device usually employed in flow analysis systems, encompass columns length of 0.5 mm [8] and 0.3 mm [9]. Although columns of reduced length develop less backpressure, facilitating their coupling with peristaltic pumps, it can also be limitative when higher resolution is required. Furthermore, low pressure chromatographic systems based on gradient elution have also been proposed [7–9], although requiring more complex manifolds.

Flow systems experienced progressive developments, aiming to easily perform sample analysis with high selectivity and automatically. Some developments were achieved through several strategies, such as the exploitation of the performance of different carrier propelling devices, tube and apparatus different sizes, in-line sample treatment strategies that enabled to perform p.e. derivatizations, dilutions, extraction steps, and-so-on, but still no strategy has yet been described capable to separate in-line the different constituents of a sample, with efficiency similar to

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chromatographic systems. In this scenario, coupling of monolithic columns to low pressure flow systems can be a viable way to overcome this original handicap assigned to flow – based techniques, since its beginning. This strategy enables to carry out a chromatographic approach over a sample matrix using a traditional low pressure flow system, and therefore reducing alternative in-line or off-line sample treatment, many times source of additional uncertainty and labor.

In this work, a chromatographic low pressure flow manifold for the determination of methylxanthines, namely caffeine, theobromine and theophylline in coffee brewed samples, is presented. Methylxanthines have been thoroughly studied regarding their effect in human health, and, besides the well-recognized stimulant effect over the central nervous system, these compounds have recently been studied over cancer diseases [10] as bowel [11], esophageal [12] or breast cancer [13]. Since coffee is one of the main beverages that account for the human uptake of methylxanthines, particularly of caffeine, it is of great relevance to develop fast, low cost and accurate methodologies for the determination of these compounds. Additionally, from an organoleptic point of view, caffeine, with a threshold of only 75–155 mg L^{-1} [14], is partially responsible for bitterness and astringency of brewed coffee. Therefore, it is of interest to the coffee industry to quantify and to control the concentration of these compounds in the final product in order to assure a good feedback from the consumer.

The methodologies described for the determination of methylxanthines in coffee samples include ion-chromatography with UV detection [15], near-infrared spectroscopy [16], and electrochemical methods, namely using glassy carbon electrodes [17], and conductive diamond electrodes [18]. Still, due to the complexity of coffee matrix, the determination of methylxanthines is most frequently, carried out using reversed phase high performance liquid chromatography with UV detection [19,20] justifying, in this way, the development of simple alternative analytical methodologies for routine determination of these compounds.

For the case study herein presented, an alternative methodology exploiting the major benefits of flow injection low pressure systems combined with the separation capacity of monolithic columns was evaluated. The major contributions expected from such an approach in this context were to present a procedure capable to perform chromatographic separations, as simple as possible, of low cost and with high throughput, especially if compared to HPLC methodologies. The low pressure flow manifold used was based on a monolithic guard column of 10 mm length coupled to a peristaltic pump as the propelling device and to a low pressure injection valve. General operational conditions of the flow system proposed are discussed and parameters like the mobile phase composition, flow rate and loop volume, were evaluated considering the analytical application presented. Furthermore, the adopted strategy aimed to highlight the potentialities of such a general purpose low pressure flow system capable of performing chromatographic separations with the aforementioned potentialities, comparing to the most frequently used high performance liquid chromatography.

2. Experimental

2.1. Reagents and solutions

All solutions were prepared with deionized water with a specific conductance of less than 0.1 μS cm^{-1} provided by a Millipore deionization water system. All chemicals were analytical-reagent grade. Acetonitrile (Merck, 1.00030.2500) was used for eluent preparation. Standard solutions of caffeine (Merck, 2584) theophylline (Sigma, T1633) and theobromine (Sigma, T4500) were prepared through rigorous dilution of stock solutions previously prepared by rigorous weighing. All solutions were kept at room temperature.

Different brands of coffee powder were purchased from local stores.

2.2. Apparatus

The low pressure flow manifold consisted of a Minipuls 3 peristaltic pump (Gilson, Villiers-le-Bel, France) equipped with Tygon tubing (Gilson, Villiers-le-Bel, France), and a four-way valve (Rheodhyne 5020, Wertheim-Mondfeld, Germany) to inject defined volumes of samples and standard solutions. The detection system consisted of a CCD detector (Ocean Optics HR4000, Winter Park, USA), two 200 μm fiber optic cables (Ocean Optics P200-2-UV–Vis, Winter Park, USA) and a light source (Ocean Optics Mikropack DH-2000, Winter Park, USA). A 1 cm cuvette holder (Ocean Optics, Winter Park, USA) and a flow-through cell with an internal volume 8 μL (Hellma 178.713QS) was used throughout. The chromatographic column was a guard column Chromolith RP-18e 10 mm length × 4.6 mm id coupled into the flow system using a column holder (Merck, 1.51471.0001).

The connection of the different components of the set up was carried out with end-fittings and connectors from Gilson (Villiers-le-Bel, France) and PTFE tubing 0.8 mm id (Omnifit, Cambridge, UK) in order to best match the id of flow channels of the injection valve (0.8 mm id) and the spectrophotometric flow-through cell (1.0 mm id). In this way the establishment of mixing chambers throughout the flow system is minimized.

In the assembling of the flow manifold, attention was taken to reduce as much as possible the distance between the injection valve and the column, as well as between the column and the detector, minimizing, as much as possible, signal broadening. The flow system is schematically depicted in Fig. 1.

A personal computer with SpectraSuite software from OceanOptics was used for data acquisition. Peak area was calculated using trapezoid's rule [21] through a home-made worksheet in Excel2007.

2.3. Comparison method

In order to assess the quality of the results obtained by the proposed methodology, concentration of methylxanthines in brewed coffee samples was also determined by a comparison method, based on HPLC with UV detection. The comparison method was adapted from the reference methodology aimed for caffeine determination in coffee [22,23] in order to enable quantification of theobromine and theophylline as well. Following the experimental conditions referred by the comparison method, a chromatographic system comprised a piston pump JASCO PU-2080 and a diode array detector JASCO MD 205, controlled through ChromNav software was used. The column was an X-Terra MS C18 5 μm 3.9 mm × 150 mm with a guard column 3.9 mm × 10 mm. The remaining experimental conditions were as follows: mobile phase composition, acetonitrile, water, 5:95, v/v, 1% acetic acid, flow rate of 1.0 mL min^{-1}, loop volume of 20 μL and wavelength detection set at 273 nm.

2.4. Sample analysis procedure

Methylxanthines quantification was performed, for both the proposed methodology and reference methodology, after extraction of the analytes from coffee samples according to the following experimental procedure: 0.5 g of ground coffee rigorously weighed and 1.0 g of MgO were added into a 200 mL Erlenmeyer flask fitted with a stopper. The weight of the flask and reagents was measured.