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Automated sample preparation based on the sequential injection principle Solid-phase extraction on a molecularly imprinted polymer coupled on-line to high-performance liquid chromatography

Georgios Theodoridis*, Constantinos K. Zacharis, Paraskevas D. Tzanavaras, Demetrius G. Themelis, Anastasios Economou

Laboratory of Analytical Chemistry, Department of Chemistry, Aristotle University of Thessaloniki, GR-541 24 Thessaloniki, Greece

Abstract

A molecularly imprinted polymer (MIP) prepared using caffeine, as a template, was validated as a selective sorbent for solid-phase extraction (SPE), within an automated on-line sample preparation method. The polymer produced was packed in a polypropylene cartridge, which was incorporated in a flow system prior to the HPLC analytical instrumentation. The principle of sequential injection was utilised for a rapid automated and efficient SPE procedure on the MIP. Samples, buffers, washing and elution solvents were introduced to the extraction cartridge via a peristaltic pump and a multi-position valve, both controlled by appropriate software developed in-house. The method was optimised in terms of flow rates, extraction time and volume. After extraction, the final eluent from the extraction cartridge was directed to the injection loop and was subsequently analysed on HPLC. The overall set-up facilitated unattended operation, operation and improved both mixing fluidics and method development flexibility. This system may be readily built in the laboratory and can be further used as an automated platform for on-line sample preparation.

Keywords: Molecular imprinting; Solid-phase extraction; Sequential injection; Automation; Instrumentation; Injection methods; Caffeine; Xanthine; Theophylline; Theobromine

1. Introduction

Sample preparation is one of the most important and crucial steps in chemical analysis, and usually the most labour-intensive [1–3]. It is often the most time-consuming and costly part of the analytical process, especially if multi-step procedures are used. Many of the conventional procedures are not amenable to automation, a fact which increases the amount of labour needed. Therefore, it is not surprising that the development of novel methods for sample preparation is an important field in contemporary research. Over the last decade, researchers have invested a considerable effort in the development of novel sample preparation techniques to address various pragmatic issues: increased sample loads, increasing regulatory constraints, decreased labour forces, need for enhanced productivity, better quality of data, need for greater information management and worker safety. An ideal sample preparation technique should be simple, inexpensive, efficient, selective and compatible with various analytical methods. It should also reduce the consumption of organic solvents and be able to simultaneously separate and concentrate the analytes [1–3].

Solid-phase extraction (SPE) is recognised as a principal sample pre-treatment technique with a broad application area. SPE offers significant advantages such as ease in operation, high load abilities, high recoveries, wide spectrum of stationary phases available, automation capabilities, enhanced reproducibility and so forth [4]. However, despite its many attractive features, SPE bears an important drawback (common in many chromatographic methods) namely the generic selectivity. Non-specific interactions often control the retention mechanism and may deteriorate the purification potential of the method.

An elegant way to overcome such problems is the implementation of molecular recognition mechanisms in separation. Molecular imprinting is an established method for the production of media of predetermined selectivity to be applied in separations and chemical analysis. Molecularly

* Corresponding author. Tel.: +30-2310-997718; fax: +30-2310-997719.
E-mail address: gtheodor@chem.auth.gr (G. Theodoridis).

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imprinted polymers (MIPs) have been widely used as selective sorbents for a wide range of analytes of biological, pharmaceutical and environmental interest [4–11]. The combination of MIPs and SPE (MIP–SPE) has been developed for both off-line and on-line approaches. For the on-line approach, the MIP is packed typically in a stainless steel cartridge to be used in a column switching approach prior to HPLC analysis [7,12–15].

The present communication reports the development of an on-line sample preparation method exploiting the principle of sequential injection (SI) analysis. In SI analysis, a desired chemical reaction occurs as overlapping sample and reagents zones pass through a reaction coil [16]. The 'heart' of SI is the multi-port selection valve; each port of the valve may perform a different operation. Although SI is based on the same principles as flow injection (FI), it offers significant advantages in terms of manifold simplicity, robustness and low consumption of reagents and sample. In SI, there is no need to reconfigure the manifold in order to apply it to different chemical systems, while all major parameters such as reagents and sample volumes, direction of the flow rate, order of mixing and reaction time can be computer-controlled. These attractive features make SI a very elegant method for automated sample preparation prior to HPLC.

Exploiting the above-mentioned advantages of SI, a method was developed employing a multi-step extraction protocol. Sample preparation was performed automatically using conventional low pressure SI instrumentation (pump, multi-position valve, tubing). At the end of the extraction, the eluted fraction (containing the analytes) is transferred to the injection loop and is subsequently injected on the HPLC. Method optimisation included investigation of flow rate, time and solvent volume in the various extraction steps. Instrument control via a software developed in-house enhanced method flexibility, operation control and improved mixing fluidics. Such a system may be built in-house with a significantly reduced cost and be further used as an automated platform for on-line sample preparation. In the present investigation, a MIP produced towards caffeine has been used as a selective sorbent for the on-line SPE of biological samples and beverages. The method facilitated unattended operation and provided selective and enhanced purification of the analyte prior to HPLC analysis.

To the best of our knowledge, this is the first paper reporting on the combination of SI with HPLC. Due to its special characteristics, SI may ideally be placed in front of the HPLC to provide a valuable tool for on-line sample preparation.

2. Materials and methods

2.1. Materials

Caffeine (a), theophylline (b) and theobromine (c) (Fig. 1) were from Sigma (St. Louis, MO, USA). Ethylene glycol dimethacrylate, methacrylic acid and azo-*N*,*N*′-disisobutylnitrile were from Fluka (Buchs, Switzerland). All solvents used (acetonitrile, methanol, ethanol) were of HPLC quality and were obtained from Merck (Darmstadt, Germany). All other chemicals were of analytical-reagent grade and were provided from Merck. Water was double de-ionised and filtered through a 20 μm filter (Schleicher & Schuell, Dassel, Germany).

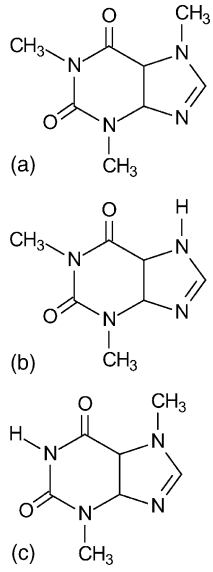

Fig. 1. Structures of caffeine (a), theophylline (b) and theobromine (c).

2.2. Polymer preparation

The method used for the preparation of imprinted polymer was adapted from a method previously developed by our group [17]. In brief, azobis-isobutylnitrile (AIBN, initiator, 15 mg) and the template caffeine (0.50 mmol) were dissolved in 2 ml of acetonitrile in a 20 ml glass tube. Methacrylic acid (MAA, 2 mmol) and ethylene glycol dimethacrylate (EDMA, 6 mmol) were added to the solution and the tube was sealed and heated in a water bath at 60 °C for 16 h. At the end of the polymerisation, the tube was smashed and the polymer monolith was ground in a laboratory mortar and pestle. Remains of the monomers and the template were removed by Soxhlet extraction using a mixture of methanol–acetic acid (9:1, v/v). The polymer was extracted for 16 h, resulting to a sum of 30–35 solvent cycles. To estimate the effectiveness of the Soxhlet extraction, the concentration of caffeine in the extraction solvent was determined by HPLC. Next, the polymer was ground in a laboratory mortar and pestle under wet conditions (water). The particles were sieved through 71 and 20 μm sieves with water. Particles that passed through the 71 μm sieve were collected; the retained particles were reground, until all the material could pass through the sieve. Fine particles were removed from the polymer by repeated sedimentations in methanol–water (1:1, v/v). Finally, the particles were dried

under vacuum and stored at ambient temperature until use. A conventional polypropylene SPE tube was adapted to be used as the housing for the SPE cartridge. Two hundred milligrams of the polymer were packed dry in the cartridge between two 0.2 μm frits.

2.3. Instrumentation

HPLC analysis were performed using an LKB 2150 pump (Broma, Sweden), a Jasco (Tokyo, Japan) 875 UV variable wavelength detector operating at 272 nm, a Shimadzu CR5 A Integrator (Kyoto Japan), and an automated injector from ABI Spectroflow bearing a Rheodyne (Cotati, CA, USA) 7010 six-port injection valve. The injection volume was 250 μl throughout the study. Separations were carried out on a 250 mm × 4.6 mm, Taxsil 2 analytical column from Metachem Technologies (Torrance, CA, USA). The mobile phase was a mixture of methanol–acetonitrile–0.05 mol l^{-1} aqueous CH_3COONH_4–CH_3COOH (17:12:70.4:0.6, v/v/v/v) and the flow rate was maintained at 0.8 ml min^{-1}.

A schematic diagram of the SI manifold and the connection to the HPLC is shown in Fig. 2. The SI manifold was comprised of the following parts: a micro-electrically actuated 10-port valve (Valco, Switzerland), and a peristaltic pump (Gilson Minipuls3, France). The flow system used 0.7 mm i.d. PTFE tubing throughout. Tygon pump tubes of 0.5 mm i.d. were used for aspirating/delivering the solutions. The hardware was interfaced to the controlling PC through a multi-function I/O card (6025 E, National Instrument, Austin, TX). The control of the system and the data acquisition from the detector were performed through a software program developed in the laboratory using the LabVIEW 5.1.1 instrumentation software package (National Instrument, Austin, TX, USA).

2.4. Operation of the system

SI is based on the sequential aspiration of zones of reagents and samples via a selection valve in a holding coil (HC). By reversing the pump flow the aspirated zones are propelled towards the detector (or, in this case, towards the HPLC separation system). A multi-position valve is used for the selection of the flow channel. Samples, washing and eluting solvents and buffers are transferred by means of a peristaltic pump. The low-pressure system is connected to a two-position selection valve bearing the injection loop where the eluted fraction (containing the analytes) is stationed before injection to HPLC. Appropriate volumes of the conditioning solvents, the loading buffers, the sample, the washing solvents and the elution solvent were aspirated in the holding coil, through the corresponding ports of the selection valve and subsequently delivered to the MIP cartridge. Each zone was propelled to the cartridge individually, with the exception of the loading buffer and the sample zone which were mixed in the holding coil. The experimental set-up is illustrated in Fig. 2.

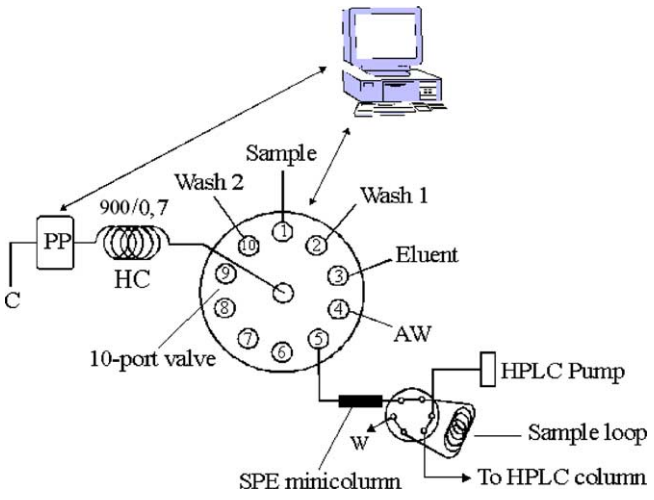

Fig. 2. SI–MIP–SPE–HPLC analysis instrumentation set-up.

The sequence for the performance of the automated extraction by the SI approach is shown in Table 1. As a rule, aspiration occurred at higher speed than delivery to the MIP cartridge (2 and 1 ml min^{-1}, respectively). For the MIP conditioning sequence, the system started with the delivery of 2 ml of water to the MIP cartridge (selection valve position 5). Subsequently, the system first aspirated and then delivered to the MIP cartridge 1 ml of methanol. In the loading step, the system aspirated in the holding coil 0.5 ml of loading buffer, 1 ml of sample and another 0.5 ml of loading buffer. The 2 ml of the mixture were delivered to MIP cartridge where analytes were trapped.

For the washing sequence, the pump aspirated the washing buffer (same as the loading buffer) and next delivered it to the MIP. Next, the MIP was washed with acetonitrile or other washing solvents/mixtures and finally the analytes of interest were recovered with 1 ml MeOH. Extraction fractions to be analysed were stationed in the HPLC injection loop by stopping the peristaltic pump at an optimised propulsion time when the fraction/zone had reached the loop. Next, the injection valve was switched to the Inject position and the plug containing the analytes was transferred to the HPLC column. Three replicates of the whole procedure were made per sample in all instances. Extraction experiments consisted of loading the MIP–SPE column with 5 μg ml^{-1} of the corresponding xanthine ((a), (b), (c) or their mixtures). A calibration curve was constructed loading the MIP with different concentration xanthines standard solutions (0.45–15 μg ml^{-1}), and subsequent analysis of the final elution fraction by the HPLC.

2.5. Pre-concentration/single standard calibration

In order to investigate the pre-concentration potential of the developed method, three different volumes of samples were loaded on the MIP and next extracted with the developed protocol, keeping a constant analyte mass loading (extraction protocol in Table 2).

Table 1
The selected sequence for the performance of the automated extraction by the SI approach

Time (s)	Pump action	Flow rate (ml min^{-1})	Valve position	Action description
120	Deliver	1	5	Propulsion/delivery of water to MIP
30	Aspirate	2	3	Aspiration of MeOH in holding coil
60	Deliver	1	5	Propulsion/delivery of MeOH to MIP (conditioning)
15	Aspirate	2	10	Aspiration of loading buffer solution
30	Aspirate	2	1	Aspiration of sample
15	Aspirate	2	10	Aspiration of loading buffer solution
120	Deliver	1	5	Propulsion/delivery of sample to MIP (MIP loading)
30	Aspirate	2	10	Aspiration of buffer solution in holding coil
60	Deliver	1	5	Propulsion/delivery of buffer solution to MIP (wash)
30	Aspirate	2	3	Aspiration of CH_3OH (eluent) in holding coil
51	Deliver	1	5	Propulsion/delivery of CH_3OH to MIP (elution step)
0	Off	–	5	End of measuring cycle

An interval of 1 s is also calculated between each step as it required for changing the valve position. Total time = 572 s

Table 2
Pre-concentration potential of the MIP–SPE–HPLC method

Sample volume (ml)	Sample concentration (μg ml^{-1})	Total analyte mass loaded (μg)	Average peak area count[a]	s_r (%)
1.00	2.25	2.25	664340.6	11.1
0.50	4.50	2.25	711555.3	1.66
0.25	9.00	2.25	671422.7	10.22

Constant analyte loading (2.25 μg) on the MIP and subsequent elution to the HPLC system.
[a] Average value of three measurements.

Additionally, loading experiments of increasing volumes of one standard solution were performed. By altering the aspiration time, increasing volumes (Table 3) of a solution of 4.5 μg ml^{-1} of caffeine was introduced in the MIP cartridge. With this experimental set-up, the feasibility of constructing a calibration curve using one standard solution was also examined.

2.6. Breakthrough

To estimate the breakthrough volume and the sorbent capacity, loading of the MIP cartridge was performed in four different concentrations (10.13, 20.27, 40.45 and 81.10 μg ml^{-1}) and three different flow rates (0.25, 0.5 and 1 ml min^{-1}). The peristaltic pump was used to continuously deliver the caffeine solution to the MIP. After a certain time, the MIP binding sites were saturated and caffeine was not further retained. Breakthrough volume was calculated as the product of the time in which non-retained caffeine solution reached the detector and the used flow rate.

Table 3
Single standard calibration

Aspiration volume (μl)	Mass loaded (μg)	Area[a]	Calibration curve	r^2
100	0.450	185000	$y = 281063x + 84267$	0.9991
250	1.125	386247		
500	3.250	714500		
750	3.375	1067626		
1000	4.500	1375000		
1500	6.750	1996666		
2000	9.000	2580000		

Loading of increasing volumes of a reference solution (4.5 μg ml^{-1}) on the MIP and subsequent elution to the HPLC system.
[a] Average value of three measurements.

2.7. Sample extraction

2.7.1. Spiked human urine

Urine was collected from a male subject abstaining from caffeine ingestion for more than a week. Twenty millilitres of urine were filtered through a 0.2 μm filter and portions of 2.5 ml of the filtered urine were diluted 10-fold and spiked to end up with 25 ml of solutions of the following concentrations: 0.18, 0.36, 0.72 and 1.8 μg ml^{-1}. The selected on-line SPE protocol was applied and the resulting elution zone was introduced in the injection loop and analysed on HPLC.

2.7.2. Beverages and coffee

An amount of 5.0 g of instant coffee from the Greek market were dissolved in 40 ml of water. Ten millilitres of the resulting solution were filtered through a 0.2 μm syringe filter (Whatman) and next 2.5 ml of the coffee solution was diluted with water to a volume of 100 ml and this solution was next assayed with the developed MIP–SPE–HPLC protocol.

For beverages analysis, 10 ml of beverages containing caffeine (Cola) and non-caffeine (Sprite) were degassed in an ultrasonicator and were next filtered through a 0.2 μm filter. An aliquot of 2.5 ml was diluted with water to a volume of 50 ml and was next assayed by the selected on-line SPE method.

3. Results and discussion

3.1. MIP–SPE

Analyte sorption and trapping on the MIP may be accomplished by either selective or non-selective interactions.

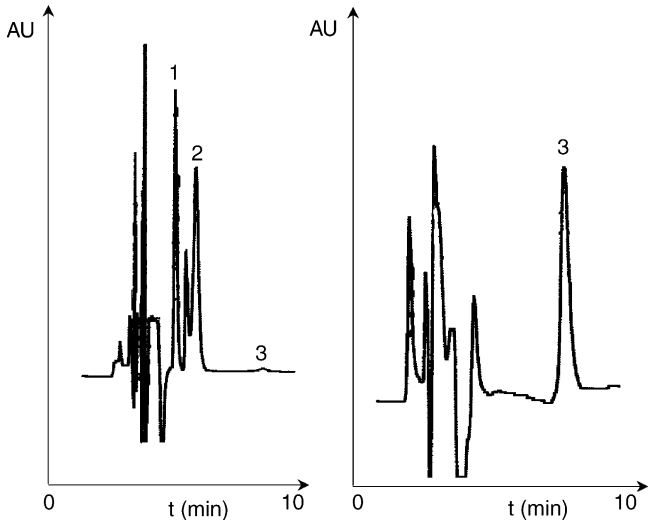

Fig. 3. Monitoring the on-line MIP–SPE system. Chromatograms of loading fraction of a reference standard solution of theophylline (1), theobromine (2) and caffeine (3) 2 μg ml^{-1} of each on the MIP–SPE (left). Elution fraction following the extraction protocol (right).

The two different modes may be paralleled to normal-phase and reversed-phase mode chromatography. In the first case, matrix interferences are not retained, thus the sample is purified and pre-concentrated within a single step [7]. Selective adsorption occurs in normal phase as the sample is introduced in an organic solvent (often the porogen used in polymer preparation). In the latter case, the retention mechanism is governed mainly by hydrophobic non-specific interactions. In order to facilitate selective analyte isolation and purification, a "molecular recognition step" is included in the extraction procedure. At this step, only the non-specific interactions should be disrupted in order to facilitate removal of unwanted matrix interferences from the sorbent. Such protocols have been described by the authors [17,18] and other groups [7,19] for the selective pre-treatment of caffeine, scopolamine, clenbuterol, and other analytes. In our previous report utilising off-line SPE for the isolation of caffeine [17], an alkaline buffer (0.05 mol l^{-1} CH_3COONH_4–$NH_{3(aq)}$, pH 9) was chosen for conditioning, loading and washing the extraction cartridge. The role of the buffer was to suppress non-specific interactions, by masking reactive acidic moieties on the surface of the polymer. In such an environment, it was expected that specific binding would be enhanced. Indeed, when loading the MIP–SPE cartridge in the dynamic mode noteworthy differences were observed: direct loading of theophylline and theobromine resulted in considerable breakthrough, whereas caffeine was almost quantitatively retained (see Fig. 3 and Section 3.4). The MIP was next washed with 1 ml of acetonitrile containing 1% triethylamine. As expected, in this step non-specifically bound analytes were eluted off the MIP cartridge. However, it was also observed that washing the cartridge in dynamic mode with the acetonitrile fraction eluted a considerable portion of caffeine. This fact was attributed to the heterogeneity of the binding sites. Therefore, in the finally selected protocol this step was omitted.

Analytes of interest were finally eluted with a portion of methanol (see Table 1). Methanol being a protic solvent shows capacity to disrupt hydrogen bonds, which are expected to play a dominant role in selective analyte binding on the polymer. This scheme resulted to the satisfactory extraction recovery and also to the highest recovery differences between the MIP and the control polymer. In general, analytes were eluted from the control polymer either in the loading step (theophylline, and theobromine) or in the washing steps (all three xanthines). In the final elution step, from the control polymer no trace of the xanthines was found [17].

3.2. Sequential injection extraction

A sequential injection extraction system for sample preparation has been proposed by J. Ruzicka, one of the inventors of the technique [20]. In that report, the authors injected sequentially an aqueous sample and an organic solvent into a reaction coil where the phases where mixed and subsequently separated by differential flow velocities. In the present communication, a sequential injection low pressure instrument configuration is coupled on-line HPLC functioning as an automated SPE system. The experimental scheme for the coupling of SI with HPLC is illustrated in Fig. 2. The advantages of this coupling are: full instrument control by the computer, automation of the extraction process, reduced solvent consumption, method flexibility allowing easy introduction and (if required) mixing of various solvents/buffers to the SPE cartridge.

3.3. Pre-concentration/single standard calibration

The SI–MIP–SPE system exhibited satisfactory potential for the pre-concentration of dilute samples. A scheme was applied keeping a constant analyte mass loading (2.25 μg) by loading three varying volumes of three different caffeine concentrations. Table 2 depicts the results, which indicate an overall similar behaviour of the sorbent when loading samples of varying concentration. This was further verified by loading a series of increasing volumes of one standard solution (Table 3). No analyte loss was observed allowing construction of a calibration curve by using only one reference standard solution. The calibration curve was constructed plotting the areas of the caffeine peaks versus the mass loaded. The method exhibited very satisfactory linearity ($y = 281063x + 84267$, $r^2 = 0.9991$), where y is the area and x the analyte mass.

3.4. Breakthrough

The breakthrough volume is a key parameter in SPE as it indicates the sample volume and thus the analyte amount to be enriched-purified [21,22]. In frontal chromatography, the breakthrough volume can be related to the affinity constant

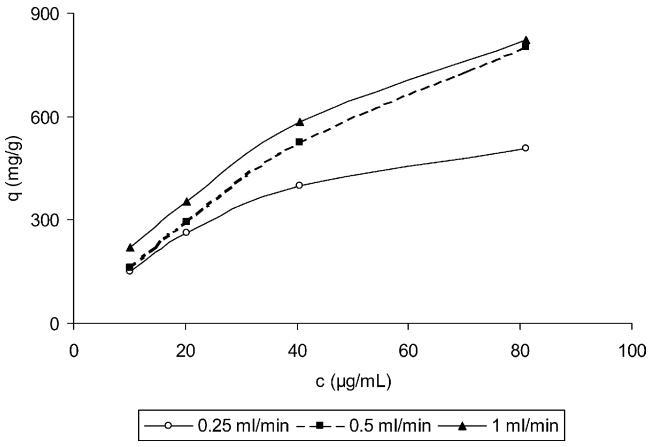

Fig. 4. Adsorption isotherms of caffeine obtained from loading the MIP in three different flow rates.

of the equilibrium between the analyte and the stationary phase [23]. Guiochon and co-workers [24–26] have investigated the thermodynamics and the mass transfer kinetics of methacrylate molecularly imprinted polymers using Langmuir and bi-Langmuir isotherm equations. Our experiments indicated that kinetics do not seriously affect the retention on the MIP. The study of the sorbent capacity revealed a typical non-linear adsorption profile. The results are illustrated in Fig. 4, where q is the ratio of the adsorbed analyte mass (mg) versus the bed mass (g) and c the analyte concentration in the mobile phase. In the experimental conditions considered in this work, the adsorption behaviour of caffeine on the MIP followed a Langmuir isotherm model in all the three flow rates. Fit curves exhibited values of r^2 ranging from 0.9964 to 0.9999.

3.5. Optimisation of the SI extraction procedure

The fine tuning of the several different extraction parameters is of utmost importance in the development of on-line sample preparation schemes. Flow assays are depended on flow rates and thus differences in elution velocity may alter extraction efficiency. An error in stationing a desired plug (buffer, wash solvent, etc.) may prove more detrimental, as this may result in total analyte loss. By varying the capture time of the eluted analyte plug, a concentration profile can be obtained. The highest concentration was acquired at a time of 51 s with a flow rate of 1 ml min^{-1}. An aliquot of 250 μl was stationed in the loop and was subsequently injected to the HPLC. Typical chromatograms of the loading fraction and the elution fraction are depicted in Fig. 3.

To calculate extraction efficiency, Bjarnason et al. have connected the MIP column directly to the detector, bypassing the HPLC column [12]. In this way, the extraction efficiency gives the amount that could have been obtained using off-line protocols. In the present work such a coupling was not feasible as the injection of the methanol plug directly to the detector resulted in high background signal hindering the quantitation of the caffeine peak. Instead, the overall extraction efficiency was determined by comparing the signal intensities obtained by the whole SI–SPE–HPLC procedure, with the signal intensities when loading manually the same analyte mass directly on the HPLC injection loop. The ratios of the peak areas obtained for caffeine were significantly low ranging from 45 to 52%. This was attributed to zone dispersion and analyte migration during the on-line extraction procedure, but also to losses in the extraction process itself. Off-line MIP–SPE extraction of caffeine on the same MIP has given extraction yields of ∼70% [12]. Additionally, in the developed experimental set-up only a fraction of the eluted plug is actually injected in the HPLC. Therefore, losses were actually expected. In a flow injection analysis (FIA) based extraction system, Tolokan et al. reported low extraction efficiencies due to losses in FIA and in a manual deproteinisation step [27].

Carry-over is a common problem in automated SPE assays. To minimise such phenomena following the elution of the bound caffeine on the MIP, the low-pressure system operated parallel to the HPLC analysis washing and regenerating the MIP cartridge and the tubing. Preparation for the next sample loading was accomplished automatically by serial washing with 2 ml fractions of water, methanol and water. Analysis of blanks between the assay of samples or standards revealed minimal carry-over effects when large concentrations have been previously processed.

3.6. Analysis of real samples

To demonstrate the suitability of the method and its potential for sample pre-treatment, a number of real samples (human urine, coffee and beverages) were processed. For urine analysis, a calibration curve was constructed by analysing a series of urine samples containing caffeine at five concentrations (0.18–1.8 μg ml^{-1}). On-line extraction on the MIP provided good linearity $r^2 = 0.9975$ with a calibration

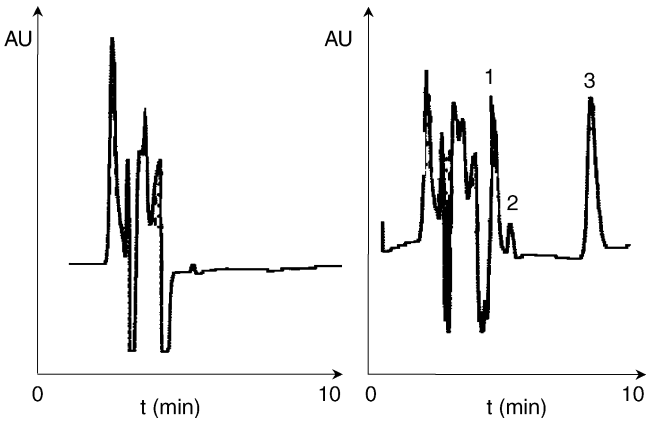

Fig. 5. Chromatograms obtained by on-line MIP–SPE on the SI–SPE–HPLC system. Analysis of human urine diluted 1/10 with water. Blank urine (left), urine spiked with theophylline, theobromine and caffeine (0.36 μg ml^{-1}) (right).

Table 4
Quantitation of caffeine in coffee and beverages

Sample	Caffeine content	Reference method	Literature values
Coffee[a]	43.65 ± 0.163 mg	45.21 ± 0.087 mg	59 mg
Cola	81.2 ± 2.76 μg ml^{-1}	86.1 ± 1.02 μg ml^{-1}	81 ± 2.0 μg ml^{-1} [29]
Sprite	0 μg ml^{-1}	0 μg ml^{-1}	–

[a] Caffeine amount in 2 g of coffee.

equation $y = 490371x - 18495$, where y is the peak area and x the concentration of caffeine (in μg ml^{-1}). Additionally, satisfactory purification was obtained as can be seen in Fig. 5, indicating the potential of the method in bioanalysis.

For the determination of caffeine in coffee and beverages, a calibration curve was constructed analysing a series of aqueous caffeine samples. The curve showed satisfactory linearity in a wide mass concentration range (0–20 μg ml^{-1}, linearity data: $y = 448964x+142372$, $r^2 = 0.9953$). Quantitative results from the analysis of beverages are summarised in Table 4. The results were in agreement with experimental results obtained from an HPLC reference method and with data from the literature [28,29].

4. Conclusions

The automated sample preparation scheme described here allows the on-line extraction of a sample prior to HPLC analysis utilising a low-pressure system. The instrumentation set-up facilitated method flexibility and full instrument control via a software developed in the lab. Additionally, setting up the device required only for a peristaltic pump and a multi-position micro-electrically actuated 10-port valve. There was no need for high pressure fittings or accessories and the system worked satisfactorily for the extraction of a significant number of samples, prior to repacking the MIP cartridge. Full automation of the extraction process and direct coupling to HPLC is documented to enhance robustness, ease in sample handling, precision and reproducibility [30,31]. The developed scheme offers a cost effective and versatile alternative to existing automated SPE instrumental set-ups. Cost effectiveness is realised in instrumentation but also in running costs as a result of the reduction of solvent consumption. In typical column switching approaches, a non-stop operation of at least two high-performance pumps is required. In the developed system, only the necessary volume of as many as seven solvents/buffers may be loaded on the SPE cartridge via a single low pressure peristaltic pump.

However, a major objective of such a set-up is its suitability for on-line pre-treatment such as derivatisation or other analyte modifications/reactions. Improved fluidics and mixing dynamics (achieved in sequential injection by reagent stacking) hold promise for the development of an attractive alternative to conventional derivatisation techniques. The combination of SI with HPLC may provide a useful tool for the development of integrated sample preparation methodologies. Research towards this direction is already on its way in our laboratory.

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