Synthesis and application of molecularly imprinted polymers for the extraction of caffeine from food and beverage samples

Síntese e aplicação de polímeros com impressão molecular para a extracção de cafeína de amostras de alimentos e bebidas

DOI:10.34117/bjdv7n4-151

Recebimento dos originais: 08/03/2021
Aceitação para publicação: 06/04/2021

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ABSTRACT

In this present study molecularly imprinted polymers were synthesized and characterized in order to extract caffeine from different food and beverage samples. The characteristics of molecular recognition of these polymers were used to develop a MIP-SPE methodology, a pre treatment able to eliminate interferences. The accuracy of the proposed method reached 96,4 ± 4,7% and the intermediate precision 2.30%. MIP-SPE cartridges here produced can be reused for at least 3 cycles, being much more affordable than the traditional commercial SPE cartridges. Caffeine was analyzed in fifteen food and beverage samples by the here developed method. It is particularly interesting to emphasize that the analyses of soft drinks and coffee powder samples made it possible to attest that, for this sort of samples, the proposed method is able to completely eliminate all the interferences present in these matrices, being unnecessary to apply chromatographic separation previously to analyte quantification. This result assures the method's selectivity and turns its application to these samples much faster,

environmentally friendly and economically affordable. There are other works in literature that propose the use of MIP-SPE to perform caffeine determination in some food and beverage samples, but none of them reaches good accuracy and precision. Also, none of them presented selectivity enough not to need the use of chromatographic separation.

Keywords: molecularly imprinted polymers, caffeine, MIP-SPE.

RESUMO
Neste estudo foram sintetizados e caracterizados polímeros com impressão molecular, a fim de extrair cafeína de diferentes amostras de alimentos e bebidas. As características de reconhecimento molecular destes polímeros foram utilizadas para desenvolver uma metodologia MIP-SPE, um pré-tratamento capaz de eliminar interferências. A precisão do método proposto atingiu 96,4 ± 4,7% e a precisão intermédia 2,30%. Os cartuchos de MIP-SPE aqui produzidos podem ser reutilizados durante pelo menos 3 ciclos, sendo muito mais acessíveis do que os cartuchos comerciais tradicionais de SPE. A cafeína foi analisada em quinze amostras de alimentos e bebidas através do método aqui desenvolvido. É particularmente interessante salientar que as análises de amostras de refrigerantes e café em pó permitiram atestar que, para este tipo de amostras, o método proposto é capaz de eliminar completamente todas as interferências presentes nestas matrizes, sendo desnecessário aplicar previamente a separação cromatográfica para analisar a quantificação. Este resultado assegura a selectividade do método e torna a sua aplicação a estas amostras muito mais rápida, amiga do ambiente e economicamente acessível. Existem outros trabalhos na literatura que propõem a utilização do MIP-SPE para efectuar a determinação da cafeína em algumas amostras de alimentos e bebidas, mas nenhum deles atinge uma boa precisão e precisão. Além disso, nenhuma delas apresentava selectividade suficiente para não necessitar do uso da separação cromatográfica.

Palavras-chave: polímeros de impressão molecular, cafeína, MIP-SPE.

1 INTRODUCTION

Caffeine is a well-known analyte, as it is an alkaloid found in more than 60 species of plants. It is a diuretic substance and stimulant of nervous and cardiovascular systems, so that its ingestion in elevated doses may be a potential risk to neurological and cardiovascular sicknesses. More frequently, its high consumption may lead to gastritis, bad functioning of the liver, chemical dependence and anxiety (1–4). For that reason it is considered a drug of abuse by the International Olympics Committee (IOC) and many agencies such as WHO (World Health Organization), EFSA (European Food Security Authority) and ANVISA (*Agência Nacional de Vigilância Sanitária* - Brazilian national agency for sanitary surveillance) suggest that its consumption should not exceed 400 mg per day by an adult of 70 kg and 200 mg per day by pregnant and lactating women. These agencies affirm that there are not studies enough to attest the safe consumption of caffeine

by children and teenagers (5–7). Thus, the monitoring of caffeine content in food and beverage samples is essential.

Since Polyakov's pioneering work in the 1930's using silica matrices, the continuous development of design, preparation, characterization and application of molecularly imprinted polymers (MIP) has attracted the scientific community's attention. The number of published works in this field is continuously increasing as a reflect of the rapid development of new trends in the area (8–14) .

These polymers are most commonly prepared by a process in which monomers form complexes with template molecules through covalent or non-covalent interactions. The complexes are joined by a cross-linking reagent and, after removing the template by chemical reaction or extraction, binding sites are exposed. These cavities are complementary to the analyte in size, shape, and reactivity of functional groups, consequently allowing its selective extraction (15,16).

In that context, molecularly imprinted polymers have attracted much attention due to their outstanding advantages, such as recognition ability, stability, ease and low cost of preparation, and potential application to a wide range of target molecules (17,18). Compared to traditional sorbents, MIP can not only pre concentrate but also selectively separate the analytes from complex matrices. As a consequence, the imprinted polymers have been successfully applied to the pretreatment of analytes in food, drugs, biological and environmental samples in the past twenty years (15,19).

The use of molecularly imprinted polymers (MIP) for the selective extraction of caffeine from complex samples by solid phase extraction (MIP-SPE) started with Theodoridis and Manesiotis in 2003 (20), who reached recovery percentages about 82%. Other research groups tried to improve the polymer synthesis and analyze other types of samples, but none of them reached acceptable recovery percentages, varying from 45 to 65% (21–23).

This present work proposes a MIP-SPE alternative methodology for caffeine extraction. The SPE protocol here developed is different from the previous others. By doing so, much better caffeine recovery percentages were obtained for fifteen different matrices. In addition, the analyses of soft drinks and coffee powder samples revealed that the here developed method is able to completely eliminate all the present interferences, being unnecessary to apply chromatographic separation previously to analyte determination. This result attests the selectivity and innovation of the proposed method

and turns its application to this kind of samples much faster, environmentally friendly and affordable.

2 EXPERIMENTAL PROCEDURES

2.1 REAGENTS AND SOLUTIONS

All reagents were of analytical grade and employed without further purification. Caffeine, Theobromine, Metacrylic Acid (MAA), Benzoyl Peroxide (BPO), and Ethylene Glycol Dimethacrylate (EGDMA) were purchased from Sigma-Aldrich (USA). Acetonitrile (ACN), Methanol, Ethanol and Acetic Acid were acquired from J.T.Baker (USA).

A 50 mmol L^{-1} phosphate buffer (VETEC, Brazil) at pH 10.6 was prepared by dissolving 2.050 g sodium phosphate in 200 mL of deionized water under magnetic stirring. The mixture's pH was adjusted by adding a small volume of NaOH (VETEC, Brazil) 6.0 mol L^{-1} and then transferred to a volumetric flask. The final volume was made up to the mark of 250.0 mL with deionized water. This solution was prepared weekly and stored in a refrigerator.

200 mg L^{-1} caffeine solutions in ACN were prepared daily as stock solutions by dissolving 5.0 mg of the analyte in 10.0 mL of ACN. The mixture was then transferred to a 25.00 mL volumetric flask and the volume was completed to the mark with the same solvent. Working standard solutions were prepared daily by the dilution of this stock solution, in order to built analytical curves for quantifications.

2.2 INSTRUMENTATION

The aqueous solutions employed in this work were prepared by using the Sartorius Arium Pro (Germany) purification system, which produces deionized water with resistivity of 18.2 MΩ cm. For pH buffers adjustments the pHmeter Digimed DM-22 (Brazil) was used. The MIP-SPE procedure was developed using an Agilent Technologies (USA) manifold coupled to a Thomas Scientific OOA-P104E-AA (USA) vacuum pump.

IR characterization spectra of the polymers were obtained with a FT-IR Spectrum Two Spectrometer using KBr pastille. Morphologic characterization in terms of grain size and mean rugosity was achieved with the aid of a Nanosurf Atomic Force Microscope (Switzerland), using a TAP190AI-G needle in contact mode and 1 second per line sweeping speed.

Spectrophotometric measurements were performed with the aid of the equipment UV-Vis Evolution 600 Thermo Scientific (USA) operating from 200 to 330 nm. Chromatographic analyses were accomplished with the equipment named Shimadzu Prominence (Japan), operating in isocratic mode with a C-18 Shim-Pack VP-ODS column (250 x 4.6 mm; 4.60 µm particle size) and UV-Vis detection at 273 nm. The injection volume was 10 µL to all chromatographic experiments and the mobile phase consisted of a mixture of ACN and water 1:1 in 0.600 mL min^{-1} flow. The mobile phase was daily degassed in a Unique USC-800A (Brazil) ultrasonic bath and filtered with 0.45 µm Unifil PTFE membranes.

2.3 POLYMER PREPARATION

The polymers were synthesized as an adaptation of the in bulk methodology proposed by Theodoridis and Manesiotis (20). Metacrylic acid (MAA) was used as monomer, Benzoyl Peroxide (BPO) as radical initiator and Ethylene Glycol Dimethacrylate (EGDMA) as cross linking reagent, in Acetonitrile (ACN) as solvent.

97 mg of caffeine (0.50 mmol) and 30 mg of BPO (0.10 mmol) were solubilized in 172 mg of MAA (2.0 mmol) and 1.98 g of EGDMA (10 mmol) in polypropylene tubes. In sequence 4.00 mL ACN were added and the tubes were degassed under nitrogen flow for 5 minutes. These flasks were sealed and heated in a water bath for 16 hours at 65 °C. Around 30 minutes after starting the heating process the first nuclei of polymerization could be noticed. After finishing the process, a rigid white polymeric matrix was obtained.

The flasks were then broken and the product ground with mortar and pestle in order to reduce particle size and increase superficial area. After sieved, the solid material presented particles smaller than 63 µm.

In order to remove monomer and radical initiator residues, and also the template molecules, the polymers were washed in soxhlet for 6 hours with a mixture of methanol and acetic acid 9:1. Finally, the material was dried in an oven at 60 °C and stored in a desiccator.

With the aim to verify the effectiveness of caffeine retention by molecular recognition, control polymers not containing caffeine (non imprinted, NIP) were prepared under the same procedure, including the soxhlet washing step.

2.4 MIP-SPE

Previously used commercial SPE cartridges were emptied, cleaned and dried, the same way as their seals. 90 mg of the synthesized polymers were added inside each cartridge and sealed.

The extraction experiments were realized by conditioning the cartridges with 4.00 mL ACN, adding 1.00 mL of sample or 25.0 mg L^{-1} caffeine aqueous solution, washing with 1.00 mL of 50 mmol L^{-1} phosphate buffer at pH 10.6 and eluting the analyte with 1.00 mL ACN.

In order to spectrophotometrically analyze the collected fractions of each SPE step, it was necessary to dilute them. That way, the 1.00 mL extracts were diluted to 4.00 mL to make spectrophotometric analyses. To chromatographic analyses the 1.00 mL extracts were diluted to 2.00 mL, with the aim to minimize errors related to solvent retention inside the cartridge or solvent evaporation.

2.5 SAMPLE PREPARATION

2.5.1 Soft drinks

An aliquot of 10.0 mL of each sample of soft drink was degassed in ultrasonic bath for 10 minutes. In sequence 1.00 mL of each sample was diluted 25 times with deionized water in volumetric flasks. The resulting solutions were subjected to the MIP-SPE method described in item 2.4 and the analyte was quantified by spectrophotometric and chromatographic techniques in triplicate.

2.5.2 Coffee Powder

An amount of 1.00 g of each sample of coffee powder was added to a polypropylene tube containing 30.0 mL of deionized water. The mixtures were heated in a water bath at 65 °C for 1 hour. Aliquots of 5.00 mL of the extract were filtered with 0.22 μm PVDF membranes. 1.00 mL of the filtered solutions was diluted 100 times with water in volumetric flasks. The resulting solutions were subjected to the MIP-SPE method described in item 2.4 and the analyte was quantified by spectrophotometric and chromatographic techniques in triplicate.

2.5.3 Black Tea

An amount of 1.00 g of each sample of black tea was added to a polypropylene tube containing 20.0 mL of deionized water. The mixtures were heated in a water bath at

65 °C for 1 hour. Aliquots of 5.00 mL of the extract were filtered with 0.22 µm PVDF membranes. 1.00 mL of the filtered solutions was diluted 100 times with water in volumetric flasks. The resulting solutions were subjected to the MIP-SPE method described in item 2.4 and analyzed by liquid chromatography in triplicate.

2.5.4 Chocolate

An amount of 2.00 g of each sample of chocolate was added to a polypropylene tube containing 20.0 mL of deionized water. The mixtures were heated in a water bath at 65 °C for 1 hour. Aliquots of 5.00 mL of the extract were filtered with 0.22 µm PVDF membranes. 1.00 mL of the filtered solutions was diluted 25 times with water in volumetric flasks. The resulting solutions were subjected to the MIP-SPE method described in item 2.4 and analyzed by liquid chromatography in triplicate.

2.5.5 Energy Drinks

An aliquot of 10.0 mL of each sample of soft drink was degassed in ultrasonic bath for 10 minutes. In sequence 1.00 mL of each sample was diluted 100 times with deionized water in volumetric flasks. The resulting solutions were subjected to the MIP-SPE method described in item 2.4 and analyzed by liquid chromatography in triplicate.

3 RESULTS AND DISCUSSION

This present work intends to evaluate molecularly imprinted polymers as sorbent phases to perform solid phase extraction (SPE) of caffeine. The synthesized polymers are supposed to selectively recognize the analyte and thus to make feasible its extraction from a complex matrix.

To do so, the SPE procedure was studied and optimized, in order to guarantee maximum sorption and recovery. Also, intending to distinguish the imprinted and non imprinted polymers, they were characterized in terms of morphology, chemical composition and sorption capacity.

3.1 PHYSICAL CHARACTERIZATION

Figure 1 shows the IR spectra for the non imprinted and imprinted polymers. In this last case, before and after removing the template by soxhlet wash. The imprinted polymer presents bands in 1661 cm^{-1} and 1552 cm^{-1} that characterize, respectively, amide's C=O angular deformation in caffeine and N-H angular deformation (24) because

of the caffeine/MAA bond. It can be noticed that both non imprinted and washed polymers do not present these bands, assuring the efficiency of the processes of template's impression and removal.

Figure 1. IR spectra for the non impressed and impressed polymers.

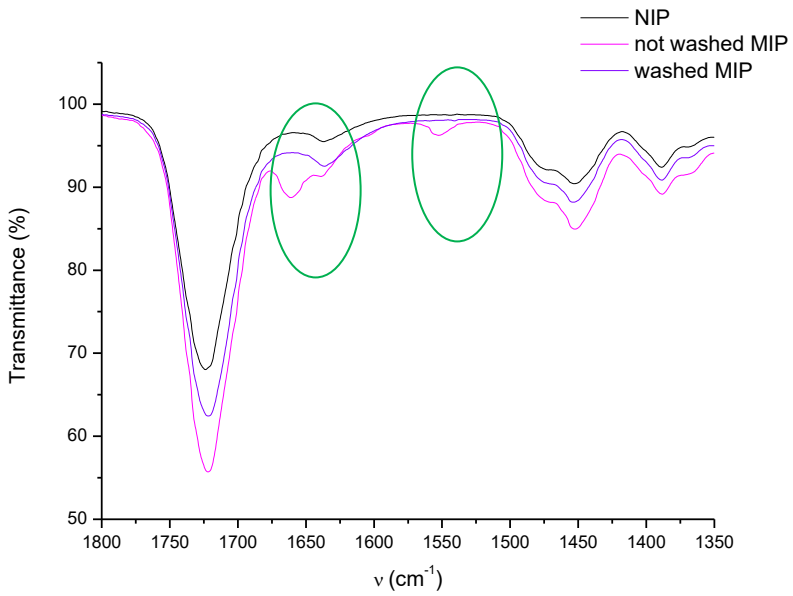


Figure 2 shows the images of Atomic Force Microscopy for the non imprinted and imprinted polymers, before and after removing the template by soxhlet wash. A simple visual evaluation leads to the conclusions that the polymers' morphology is different, and the washed imprinted polymer turns similar to the non imprinted one. This visual observation is confirmed by the grain size and mean rugosity values (25–29) presented in Table 1.

Figure 2. Tridimensional images by AFM for (a) NIP; (b) not washed MIP; (c) washed MIP.

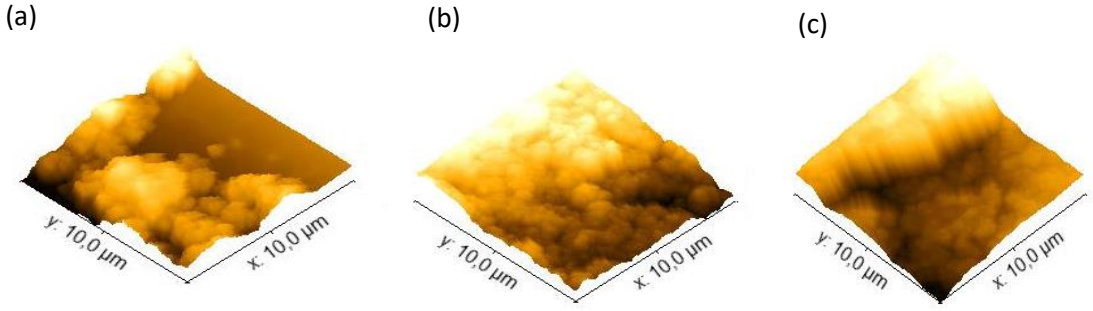


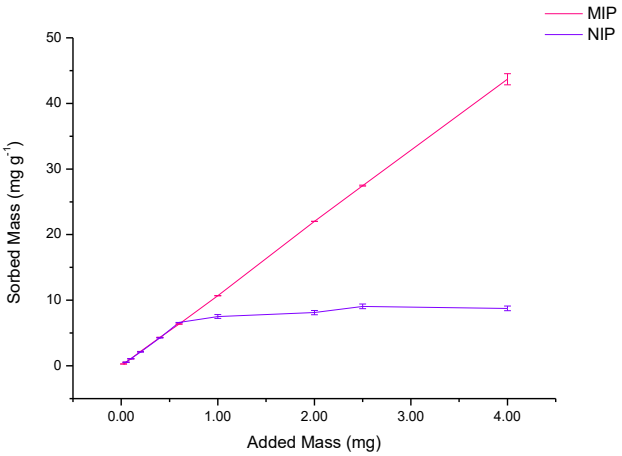
Table 1. Grain size and mean rugosity values for the studied polymers.

Polymer	Grain size (μm)	Mean Rugosity (μm)
NIP	0.966	0.209
not washed MIP	5.02	0.624
washed MIP	1.46	0.276

3.2 SORPTION CAPACITY

Figure *3* shows the breakthrough curve built for the imprinted and non imprinted polymers. This curve was constructed by inserting in the MIP-SPE cartridges fixed volumes (1.00 mL) of caffeine aqueous solutions progressively more concentrated. By means of spectrophotometric analyses it was possible to determine the percentage of analyte retained and the retained mass in each experiment.

Figure 3. Breakthrough curves for MIP and NIP



By this study it was possible to evaluate the sorption capacity of the polymers, which equals to 8.01 mg g^{-1} to NIP and is higher than 43.7 mg g^{-1} to MIP. In view of the difference between the imprinted and non imprinted polymers in terms of morphology, chemical composition and sorption capacities, their physical and chemical distinctions are evident. For that reason, further MIP-SPE experiments were performed exclusively with MIP.

3.3 MIP-SPE OPTIMIZATION

The first study related to the process of MIP-SPE optimization consisted in evaluating the amount of solvent used in the conditioning step. This step was performed with the aim to eliminate residues of the template, the monomer or the radical initiator from the synthesized polymers.

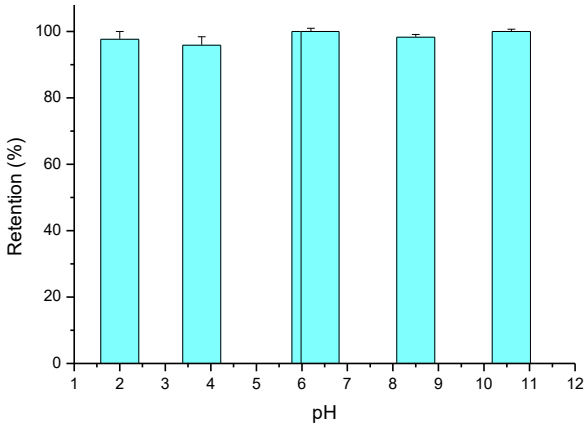
To do so, each MIP cartridge was treated with increasing volumes of ACN. Successive aliquots of 1.00 mL ACN were passed through each cartridge and analyzed separately. It was noticed that the addition of 4.00 mL of this solvent was enough to eliminate the residual analyte from the solid phase in the conditioning step.

The addition step was performed in aqueous media, in view of research previously proposed in literature (20,21). Though it is known that MIPs generally perform better in terms of molecular recognition and rebinding when they are in the media they were produced, in the present case acetonitrile, it was noticed that the analyte was not retained in this solvent, probably due to its strong eluting characteristics. In fact, the addition in aqueous media facilitates sample preparation and reduces costs during the method application.

In this study 1.00 mL of 25 mg L^{-1} caffeine was added in different aqueous media: in deionized water (pH 6.4) and in 50 mmol L^{-1} phosphate buffer in different pH values (Figure 4. Caffeine retention after the addition in different pH intervals (conditioning with 4.00 mL ACN, addition of 1.00 mL 25 mg L-1 caffeine).). The use of this same buffer was possible due to the fact that it presents pKa,1 = 2.1, pKa,2 = 7.2 and pKa,3 = 12.3 (30).

Caffeine presents pKa equal to 8.3 (31), so that it forms different species in aqueous media depending on the pH. That might suggest greater possibilities of retention in a media on which it was protonated or not, but the results exposed in Figure 4 show that this analyte is highly retained in all the evaluated conditions, including the absence of buffering. Thus, the following experiments were performed in unbuffered aqueous solutions, to facilitate samples' preparation.

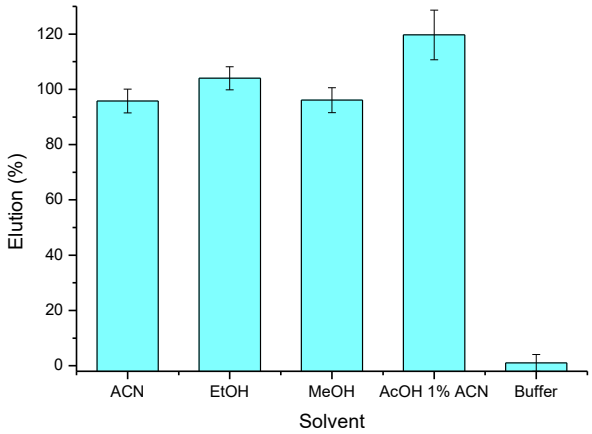
Figure 4. Caffeine retention after the addition in different pH intervals (conditioning with 4.00 mL ACN, addition of 1.00 mL 25 mg L^{-1} caffeine).



Considering that caffeine was highly retained in all the tested conditions, including the absence of buffering, the following experiments were performed by adding the analyte in non buffered deionized water media, to facilitate sample preparation.

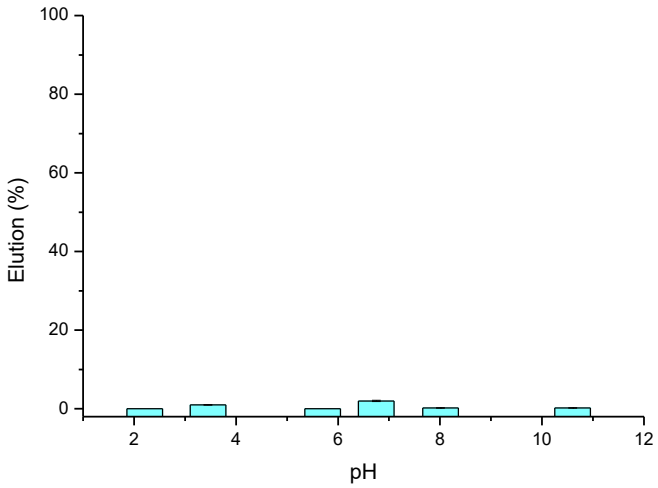
The elution step was studied by testing 1.00 mL of different solvents: acetonitrile, ethanol, methanol, 1% acetic acid in acetonitrile and 50 mmol L^{-1} phosphate buffer at pH 10.6. The results from this study (Figure 5. Caffeine elution with 1.00 mL of different solvents (conditioning with 4.00 mL ACN, addition of 1.00 mL 25 mg L-1 caffeine aqueous solution)show that all the tested solvents are adequate to quantitatively remove caffeine from the polymers, but the aqueous buffer. For that reason the 50 mmol L-1 phosphate buffer was in sequence studied as an adequate solvent for the washing step.

Figure 5. Caffeine elution with 1.00 mL of different solvents (conditioning with 4.00 mL ACN, addition of 1.00 mL 25 mg L^{-1} caffeine aqueous solution)



The results of the washing step optimization are shown in Figure 6. As it can be observed, the removal of the analyte in the washing step was minimum using 50 mmol L^{-1} phosphate buffer at different pH intervals. Theodoridis and Manesiotis (20) and Farrington and co-workers (21) affirm that a basic buffer can suppress non-specific interactions by masking the reactive acidic moieties on the surface of the polymers, so that the 50 mmol L^{-1} phosphate buffer was employed at pH 10.6 for the washing step in following experiments.

Figure 6. Caffeine removal during the washing step with 1.00 mL 50 mmol L^{-1} phosphate buffer at different pH intervals (conditioning with 4.00 mL ACN, addition of 1.00 mL 25 mg L^{-1} caffeine aqueous solution)



The washing step proposed in this present method differs significantly from some already present in literature (20,21), in which the authors perform a second basic wash with 1% triethylamine in acetonitrile to achieve reasonable differentiation between the imprinted and non imprinted polymers (about 108% recovery for MIP and 82% for NIP (20)). Surprisingly, the use of this second basic wash of the polymers here synthesized resulted in complete retention of the analyte in the solid phase, occasioning in recoveries close to zero.

Table 2 shows a summary of the optimized MIP-SPE conditions employed for the here developed method. Under these conditions, recovery of 104 ± 8% was achieved with the molecularly imprinted polymers.

Table 2. Summary of the optimized MIP-SPE protocol

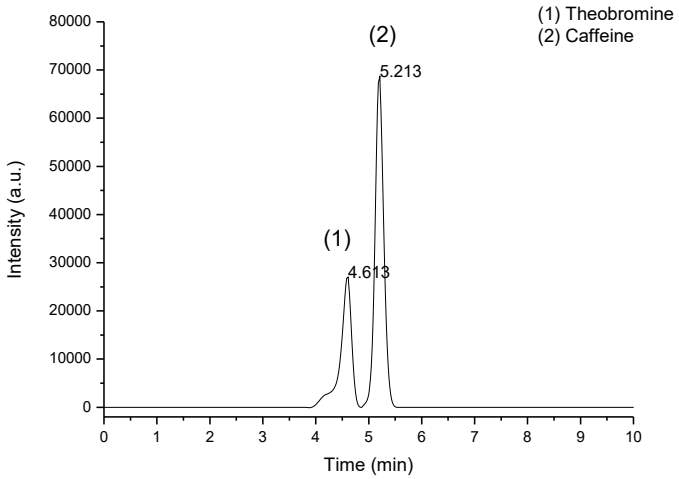
Step	Optimized condition
Conditioning	4.00 mL ACN
Addition	aqueous media
Wash	1.00 mL 50 mmol L^{-1} phosphate buffer pH 10.6
Elution	1.00 mL ACN

3.4 STUDY OF POSSIBLE INTERFERENCES

It was expected that theobromine, another xanthine commonly present in food and beverage samples (32–34), interfered in the analyses considering that caffeine and theobromine are very close in structure, differing only by a methyl group. In fact, it was observed that these substances were not separated simply by employing the polymeric solid phase and spectrophotometric UV Vis detection. Thus, the separation method using the synthesized MIP and UV-Vis detection is able to quantify total xanthines, but not each of them individually.

To overcome this drawback liquid chromatography separation was used together with MIP-SPE, previously to UV Vis detection, in order to distinguish caffeine, the analyte, from theobromine. Figure 7. Caffeine and theobromine separation by HPLC UV-Vis (ACN/H2O 1:1, 0.600 mL min-1, 10 µL). shows that the interference caused by theobromine was resolved by using this strategy (chromatographic conditions specified in item 2.3).

Figure 7. Caffeine and theobromine separation by HPLC UV-Vis (ACN/H_2O 1:1, 0.600 mL min^{-1}, 10 µL).



3.5 FIGURES OF MERIT

Table 3 shows the figures of merit for the proposed methodology. Most of the parameters are subdivided in UV Vis and HPLC items, meaning that for samples containing theobromine chromatographic separation was also necessary. For samples not containing this substance, only MIP-SPE separation was enough to remove all other interferents present in the matrices.The limits of detection and quantification of the method were calculated by the criteria of $3\delta/S$ and $10\delta/S$, respectively, in which δ corresponds to the standard deviation to the analytical signals obtained to ten 0.250 mg L-1 caffeine solutions and S to the slope of the analytical curve. The method's accuracy was determined by subjecting three aliquots of 1.00 mL of 25 mg L-1 caffeine solution to the MIP-SPE process. The method's precision was estimated by the standard deviation of three aliquots analyzed the same way.

It should be emphasized that the accuracy here obtained is much better than the one previously presented in literature (20–23), due to the synthesis characteristics and SPE optimization procedure. Also, cartridges can be reused for three cycles, an interesting and funds saving innovation when compared to SPE methods using commercial cartridges. Finally, it should be noticed that this is not a pre concentration, but a clean up procedure, in view of the great caffeine concentration in the analyzed food and beverage samples.

Table 3. Figures of merit for the proposed method

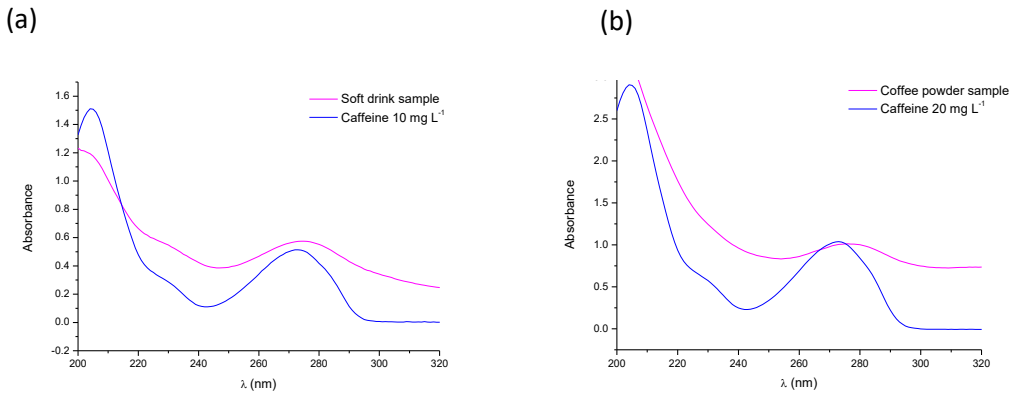
Parameter		
Typical Analytical Curve	UV Vis	Abs = 1.267 [caf, mg L^{-1}] – 0.1327
	HPLC	Abs = 95810 [caf, mg L^{-1}] – 4120
r^2	UV Vis	0.9995
	HPLC	0.9986
Limit of Detection	UV Vis	0.0565 mg L^{-1}
	HPLC	0.0860 mg L^{-1}
Limit of Quantification	UV Vis	0.188 mg L^{-1}
	HPLC	0.287 mg L^{-1}
Accuracy	96,4 ± 4,7%	
Precision	2.30 %	
Cartridge reuse	3 cycles	

3.6 APPLICATION OF THE METHODOLOGY

Figure 8. UV Vis spectra of (a) soft drink and (b) coffer powder samples, compared to a caffeine aqueous standard solution.shows the UV Vis spectra for one of the soft drink samples and one of the coffee powder samples, compared to a caffeine aqueous standard solution. It can be clearly observed that the interferents present in the

matrix provoke shift and overlapping of the caffeine characteristic absorption band at 273 nm. These phenomena justify the use of MIP-SPE to eliminate interferences.

Figure 8. UV Vis spectra of (a) soft drink and (b) coffer powder samples, compared to a caffeine aqueous standard solution.



The method's accuracy was evaluated by two different approaches: first, in terms of recovery tests, spiking the samples' solutions with 10.0 mg L-1 caffeine (

Table 4), and after statistically comparing the results obtained by spectrophotometric UV Vis quantification to liquid chromatography (HPLC) employing UV Vis detection (Table 5). All samples were evaluated in triplicates. In both cases MIP-SPE previous separation was applied. It is highlighted that if the use of MIP-SPE wasn't enough to totally remove the interferents present in these matrices, their presence would be evidenced by the employment of chromatographic separation. After the application of paired t-test to the results in Table 5 at 95% confidence level, it was possible to conclude that the difference between the determined concentrations is due to random errors. In other words, there is no statistical difference between the results obtained by the two methods. These observations, together with the recovery tests prove that the method here proposed presents good accuracy (35).

Table 4. Recovery tests for soft drink and coffee powder samples, spiked with 10.0 mg L^{-1} caffeine.

Sample	Caffeine content	Recovery (%)
soft drink I	115 ± 21 mg L-1	95.4 ± 0.2%
soft drink II	151 ± 6 mg L-1	93.4 ± 3.3%
soft drink III	149 ± 6 mg L-1	91.4 ± 3.3%
coffee powder I	15.5 ± 1.7 mg g-1	97.6 ± 3.6%
coffee powder II	16.5 ± 1.0 mg g-1	88.1 ± 3.2%
coffee powder III	16.1 ± 0.2 mg g-1	92.0 ± 2.7%

Table 5. Quantifications of soft drinks and coffee powder samples employing UV Vis and HPLC UV Vis.

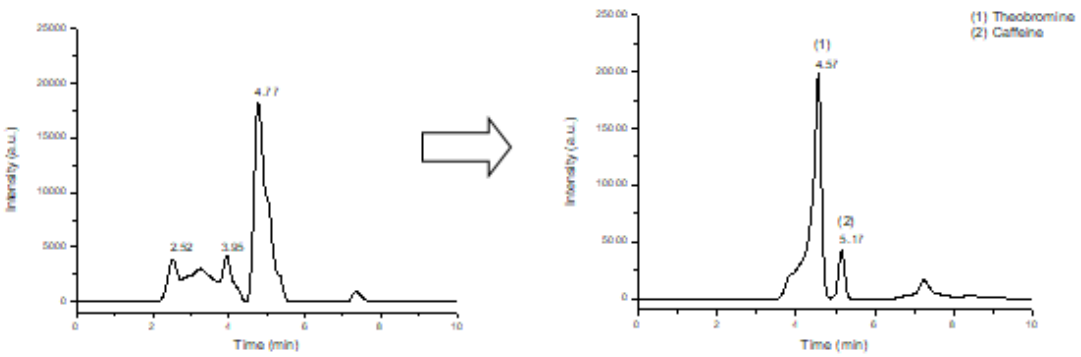
Sample	UV Vis	HPLC UV Vis
soft drink I	114 ± 18 mg L^{-1}	134 ± 12 mg L^{-1}
soft drink II	151 ± 3 mg L^{-1}	157 ± 11 mg L^{-1}
soft drink III	149 ± 6 mg L^{-1}	142 ± 9 mg L^{-1}
coffee powder I	16.2 ± 1.0 mg g^{-1}	17.0 ± 0.6 mg g^{-1}
coffee powder II	15.5 ± 1.7 mg g^{-1}	17.8 ± 1.3 mg g^{-1}
coffee powder III	16.1 ± 0.2 mg g^{-1}	15.4 ± 0.8 mg g^{-1}

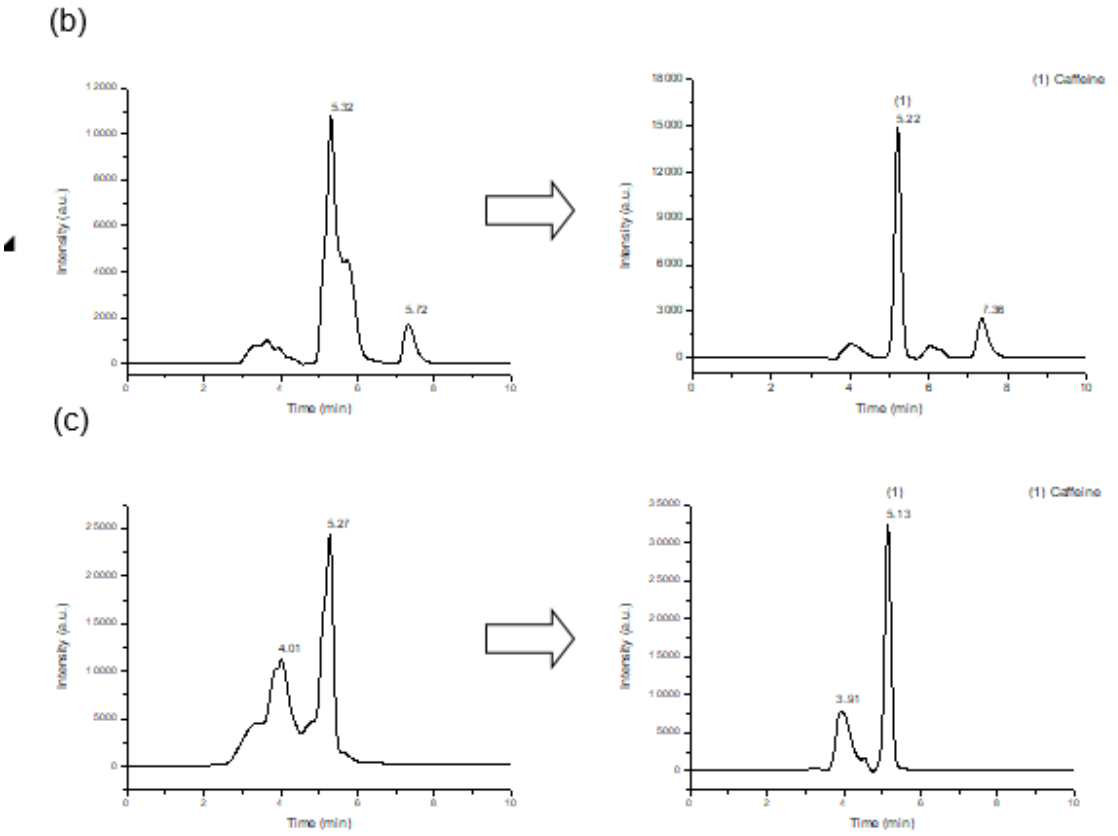
As mentioned before, the analyses of samples containing other xanthines required chromatographic separation associated to MIP separation.

Figure 9. shows the chromatograms for one of the chocolate samples, one of the energy drink samples and one of the black tea samples, before and after MIP-SPE. It can be noticed that the interferents present in the matrix provoke peaks overlapping, something that is resolved after employing the procedure here proposed.

Figure 9. Some samples' chromatograms, before and after MIP-SPE (a) chocolate sample (b) energy drink sample (c) black tea sample (ACN/H2O 1:1, 0.600 mL min-1, 10 µL).

(a)





For these samples the method's accuracy was evaluated in terms of recovery tests, spiking the samples' solutions with 10.0 mg L^{-1} caffeine (Table 6). All samples were evaluated in triplicates. The high values obtained in recovery tests prove that the MIP-SPE proposed method presents good accuracy (35).

Table 6. Quantifications of chocolates, energy drinks and black teas employing HPLC.

Sample	Caffeine content	Recovery (%)
chocolate I	0.268 ± 0.009 mg g^{-1}	101 ± 1
chocolate II	0.258 ± 0.010 mg g^{-1}	101 ± 3
chocolate III	0.276 ± 0.012 mg g^{-1}	102 ± 2
energy drink I	381.4 ± 7.5 mg L^{-1}	92.6 ± 2.6
energy drink II	376.1 ± 14.5 mg L^{-1}	95.5 ± 3.2
energy drink III	386.7 ± 9.3 mg L^{-1}	91.6 ± 3.7
black tea I	15.6 ± 0.8 mg g^{-1}	101 ± 2
black tea II	14.7 ± 0.7 mg g^{-1}	103 ± 3
black tea III	16.2 ± 0.8 mg g^{-1}	99.3± 2.9

4 CONCLUSIONS

The MIP herein synthetized exhibited strong molecular recognition characteristics even under extremely polar media in aqueous samples. Also, the addition of samples in

aqueous media made the method much simpler, more feasible and environmentally friendly.

The MIP-SPE conditions were optimized in order to achieve maximum sorption and desorption percentages. Under these conditions the prepared solid material demonstrated high selectivity towards xanthines, though being unable to separate each of them. The separation of xanthines in chocolates, energy drinks and black teas was accomplished by liquid chromatography and UV Vis detection.

Thus, the quantification of soft drinks and coffee powder samples, which do not contain xanthines other than caffeine, can be easily achieved by MIP-SPE separation followed by spectrophotometric detection. In other words, the synthesized MIP was able itself to eliminate interferences, being unnecessary the use of chromatographic separation. This fact assesses the here proposed method as simpler and more accessible than the ones previously proposed in literature, something specially interesting for research groups from developing countries, in which funding is scarce.

ACKNOWLEDGEMENTS

The authors assume that there are no conflicts of interest to declare and are grateful to the Brazilian institutions CAPES (*Coordenação de Aperfeiçoamento de Pessoal de Nível Superior*), CNPq (*Conselho Nacional de Desenvolvimento Científico e Tecnológico*) and FAPERJ (*Fundação Carlos Chagas de Amparo à Pesquisa do Estado do Rio de Janeiro*) for grants, scholarships and financial support.

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