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A Molecularly Imprinted Polymer-Disposable Pipette Tip Extraction-Capillary Electrophoresis (MISPE-DPX-CE) Method for the Preconcentration and Determination of Scopolamine in Synthetic Urine Samples

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Abstract: Alcoholic beverages contaminated with scopolamine (SCP) are often employed for criminal purposes due to their sedative effect. The determination of the residual levels of SCP in body fluids (e.g., urine) can help to track possible victims of induced ingestions. Biological sample analysis usually requires a preconcentration step to enhance their detectability and to provide sample clean-up. Molecularly imprinted polymers (MIPs) in lieu of conventional solid sorbents represent an enhancement of selectivity, due to their specific recognition sites. Additionally, the adaptation of the solid-phase extraction (SPE) cartridge into a disposable pipette tip extraction (DPX) contributes to the miniaturization of the sample preparation step. Herein, an analytical method for the determination of SCP in synthetic urine samples via the integration of molecularly imprinted solid-phase extraction (MISPE) with DPX as a preconcentration step prior to capillary electrophoresis analysis (also known as MISPE-DPX-CE) is presented. The extraction and elution steps were optimized using a factorial design. Using the optimized conditions, a preconcentration factor of 20 was obtained, leading to a working range of 0.5–6 μM with LOD of 0.04 μM and repeatability of 6.4% (n = 7) and adequate recovery values (84 and 101%) The proposed MISPE-DPX-CE approach was successfully applied to selective extraction, preconcentration, and determination of SCP in synthetic urine samples.

Keywords: sample preparation; molecularly imprinted polymer; solid-phase extraction; disposable pipette extraction; forensic chemistry

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1. Introduction

Hyoscine hydrobromide or scopolamine (SCP) is a tropane alkaloid naturally found in several plants of the Solanaceae family, such as *Datura stramonium* and *Atropa belladonna*. These plants are traditionally used for medicinal purposes with anticholinergic, antiemetic, antispasmodic, and parasympatholytic actions. Scopolamine butylbromide (SCP-BB) was synthesized to minimize toxic and unwanted effects as its polarity prevents the absorption of the gastrointestinal drug. These changes allow SCP-BB to be present in various formulations that occasionally make changes to the central nervous system [1]. Although SCP has medicinal properties, when used in excess it can temporarily impair the ability of judgment, and concentration, and can generate visual and auditory hallucinations, characterized by a state of intoxication, followed by a deep sleep accompanied by amnesia [2–4]. It is important to note that the negative effects mentioned above are related to the usage of SCP, however, the conversion from SCP-BB to SCP can be easily achieved by heating at 100 °C [3,5,6].

Such substances can be intentionally mixed with alcoholic beverages for criminal purposes, popularly known as "Good Night Cinderella", in which the ingestion of a relatively small quantity of the mixture facilitates assault or sexual abuse [7]. The felonious

takes advantage of a state of amnesia, drowsiness, and unconsciousness of the victim to perpetrate the crime. In addition, the ingestion of such mixtures with alcoholic beverages can be misunderstood as the victim being drunk and not doped, resulting in an impression of the victim's collaboration [7,8]. The number of cases of this crime is increasing worldwide, especially among women, and the LGBTQIA+ community [6,9,10]. There is still no official study that determines a lethal dose of SCP to an adult organism, but there are already some clinical cases where 2 mg of SCP is sufficient to cause poisoning with severe symptoms [11].

For these reasons, analytical methods devoted to the quantification of such drugs and their metabolic residues in beverages and body fluids (e.g., urine, hair, serum, saliva, and blood) are an essential demand to help to elucidate possible crimes [12]. The direct detection of SCP has been performed by spectrophotometry (e.g., by measuring its absorbance at 250 nm) [13], colorimetry (e.g., by its reaction with a specific reagent) [14], or using electrochemical methods (e.g., potentiometry or voltammetry) [15]. Although their optical and electrochemical behavior allows the quantification of low concentrations of SCP in simple matrices, the complexity of biological samples affects the method selectivity [16]. Scopolamine can be found at a concentration of 0.6 µM in urine [17]. Several analytical methods for the quantification of tropane alkaloids in a variety of matrices have been developed based on separation techniques, such as capillary electrophoresis (CE) [5], thin-layer chromatography (i.e., TLC) [18], and high-performance liquid chromatography (HPLC) [19] coupled to mass spectrometry (MS), electrochemiluminescence (ECL) [20], electrochemical and optical detection [21]. Such techniques can provide high separation performance, for instance, SCP and SCP-BB were separated by capillary electrophoresis with capacitively coupled contactless conductivity detection (CE-C^4D) in less than 2 min. Despite the selectivity enhancement resulting from the inherent separation capability of the instrumental technique, the complexity of the composition of biological samples can still produce analytical interference. Additionally, the sensitivity required for the analysis of scopolamine in urine samples using CE is usually not achieved without sample treatment steps. In this scenario, sample preparation that involves extraction and preconcentration steps is crucial, especially when the analytes are found at low concentration levels (i.e., 0.6 µM in urine). Based on this approach, several studies were developed for the quantification of SCP in biological samples by hyphenating conventional solid-phase extraction (SPE) with subsequent HPLC [22] or CE analyses [23,24].

In this context, highly selective molecularly imprinted polymers (i.e., MIP) in lieu of conventional unspecific solid adsorbents have been employed to improve the performance of the extraction and preconcentration steps [25,26]. Molecularly imprinted technology produces porous materials with specific recognition sites with the ability to preferably bind to the desired analyte, thereby improving the efficiency in the solid-phase extraction step [27,28]. Compared to other selective sorbents, MIPs demonstrate high mechanical strength, and high thermal and chemical stability, enabling their applicability in a variety of chemical environments [29–32]. Due to these advantages, MIPs represented a great advance in the field of sample preparation, acting as stationary phases in affinity chromatography [33] and as sorbent in solid-phase extraction [34]. The employment of MIPs as solid sorbents for SPE is commonly defined as a molecularly imprinted solid-phase extraction (MISPE) [35–37].

Miniaturization of analytical methods related to the sample preparation step has shown great advances toward the principles of green analytical chemistry [38]. For instance, the employment of disposable pipette extraction (i.e., DPX) technique rather than conventional solid-phase extraction cartridge significantly reduces the volumes of sample and solvents as well as the solid-phase amount used in each analysis. Therefore, less waste is generated as well as lower consumption of reagents and samples [26,39]. DPX is based on the accommodation of the solid phase—usually a few milligrams—into a pipette tip followed by sampling aspiration enabling the contact between the sample and the adsorbent for a suitable time. After sample disposal, either the analyte or the interferents are retained in the solid material. The application of DPX as a sample preparation technique has been demonstrated for a few analytes; however, the number of commercially avail-

able extraction phases and the high cost compared to traditional solid-phase cartridges represent a limitation to its applicability in routine analysis [27,40]. Therefore, developing solid phases for application in DPX suggests a promising field, mainly selective sorbents for tailored applications [41]. Previous studies have demonstrated the development of DPX-based methods using MIPs as solid sorbents and separation techniques [26,42,43], however, traditional reverse-phase sorbents are still the most used for preconcentration-based methodologies [39].

In this study, we described the application of a selective molecularly imprinted polymer solid-phase for the extraction and preconcentration of SCP in synthetic urine samples and further quantification by CE-C^4D. The MISPE was adapted into a pipette tip enabling its application as a DPX cartridge, therefore reducing the number of polymers and sample volume used in each analysis. The experimental conditions of the hyphenated MISPE-DPX-CE procedure were optimized using factorial experiments.

2. Experimental

2.1. Reagents and Materials

Scopolamine hydrobromide (SCP; 99% purity) was purchased from Sigma-Aldrich (Darmstadt, Germany), as well as acrylamide (ACR), 1,1′-azobis(cyclohexanecarbonitrile) (ABCN) 98%, ethyleneglycol dimethacrylate (EGDMA), and butyric acid. Sodium hydroxide was obtained from Vetec (Rio de Janeiro, Brazil). Methanol and glacial acetic acid were obtained from Dinâmica (São Paulo, Brazil). All chemicals, standards, and solvents used were of analytical grade. Solutions were prepared using deionized water from a Milli-Q system (Millipore, Bedford, MA, USA).

The reagents used in the preparation of synthetic urine, such as citric acid (99%), ascorbic acid (99%), and sodium sulfate (99%) were purchased from Vetec (Duque de Caixas, Brazil). Sodium bicarbonate (99%), calcium chloride dihydrate (78%), sodium chloride (99%), and ammonium chloride (99%) were obtained from Synth (Diadema, Brazil). Potassium hydrogen phosphate (99%) and potassium dihydrogen phosphate (99%) were obtained from Merck (Darmestádio, Germany). Finally, urea (99%), uric acid (99%), and magnesium sulfate heptahydrate (99%) were acquired from Dinâmica (São Paulo, Brazil), Sigma-Aldrich (Saint Louis, USA), and Neon (São Paulo Brazil), respectively.

2.2. Synthesis of Molecular Imprinted Polymers (MIPs) and Non-Imprinted Polymers (NIPs)

Imprinted polymers were synthesized as described by a previous study from our research group [44]. Briefly, a non-covalently imprinted polymer was prepared by dissolving 0.33 mmol of SPC as a template, 1.8 mmol of the acrylamide (functional monomer) followed by 6.0 mL of methanol (porogenic solvent) in a glass vial. The mixture was let to rest for 4 h and then 6.7 mmol of ethylenedimethacryl (EGDMA) and 0.2 mmol of cyclohexanecarbonitrile (ABCN) were added. The mixture was sonicated and purged with nitrogen for 10 min and the glass tube was sealed with a stopper. The polymerization was performed in a water bath at 80 °C for 5 h. The resulting solid was firstly rinsed with methanol, followed by washing in a Soxhlet system with 1 round of methanol/acetic acid (9:1, *v*/*v*) and 1 round of methanol for complete extraction of the template. Non-imprinted polymers (NIPs) were submitted to the same method, but with no addition of the imprinting template.

2.3. Procedure for Preparation, Conditioning, and Application of MISPE-DPX Cartridges for the Extraction and Preconcentration of Scopolamine

Each DPX cartridge was fabricated as follows: 30 mg of glass wool was inserted into a 5 mL pipette tip followed by 20 mg of a previously macerated MIP. The optimized conditioning procedure comprises 6 cycles of aspiration/disposal of 3 mL of methanol prior to each analysis. Next to it, 3 mL of the solution containing SCP (i.e., standard solution or synthetic urine) was aspired into the pipette tip, let to rest for 1 min, and dispensed back to the flask. This procedure was repeated 10 times. After the extraction step, SCP was desorbed from the DPX cartridge by aspiring 500 µL of methanol. An amount of 1 mL of

the background electrolyte (BGE) solution was added and the final mixture was injected into the capillary electrophoresis system. The procedure is represented in Figure 1.

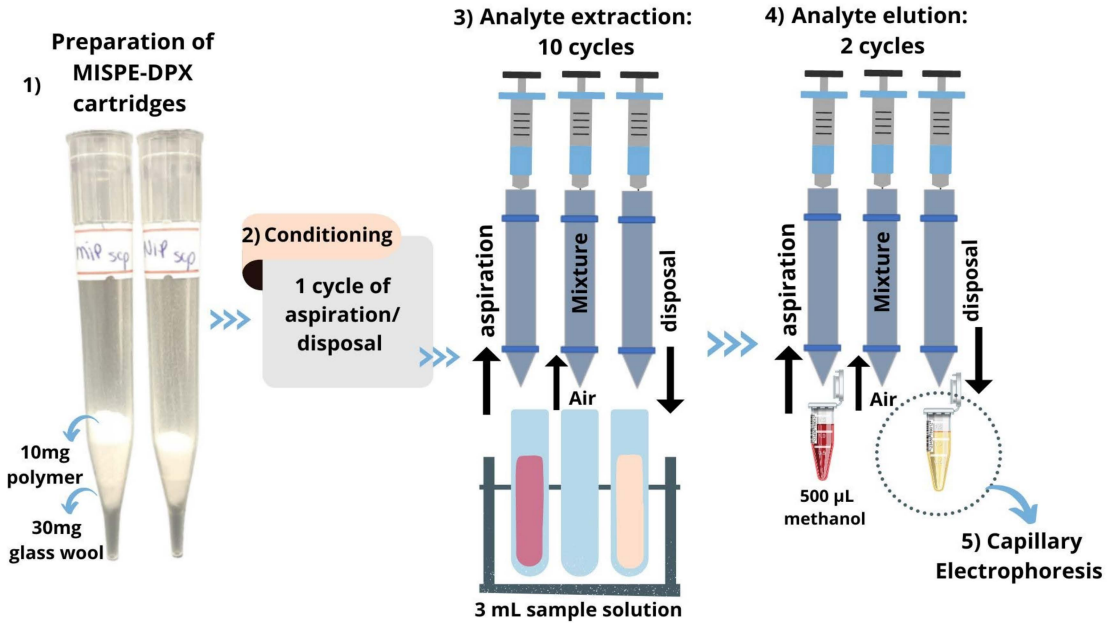

Figure 1. Representative diagram of the steps from the preparation of the cartridges to the elution of the analyte.

The CE equipment used for the analysis is equipped with two C^4D detectors that operate with a constant potential of 4 Vpp and a frequency of 1.1 MHz. The detectors were positioned at 10 cm (effective length–first detector) and 40 cm (effective length–second detector) along the fused-silica capillary column with a 50 cm total length. The capillary was kept thermostatic at 25 °C. A constant voltage of 20 kV, was applied during electrophoretic running. Sample hydrodynamic mode was selected to inject the sample into the capillary at 25 kPa for 2.0 s. For the electrophoretic separation, the BGE solution was comprised of 20 mmol of butyric acid and the pH was adjusted to 4.5 with NaOH. Daily, prior to the first analysis, the silica capillary was flushed with deionized water for 500 s, then with 1.0 mol L^{-1} NaOH for 500 s followed by deionized water for 500 s, and finally by the BGE solution for 500 s [5].

2.4. Synthetic Urine Samples

The synthetic urine samples were prepared based on the procedure described by Brooks et al. For this, the following substances were added to 250 mL of deionized water: 0.1 g of citric acid, 0.093 g of ascorbic acid, 0.525 g of sodium bicarbonate, 2.5 g of urea, 0.0175 g of uric acid, 0. 01 g of calcium chloride dihydrate, 1.3 g of sodium chloride, 0.353 g of sodium sulfate, 0.123 g of magnesium sulfate heptahydrate, 0.325 g of ammonium chloride, 0.238 g of potassium hydrogen phosphate, and 0.305 g of dihydrogen phosphate of potassium. The final solution was transferred to an amber bottle and stored at 4 °C. The pH of the sample was adjusted to 6.

3. Results and Discussion

3.1. Evaluation of the Solvent for the Preconditioning of the MISPE-DPX Device

The synthesized molecularly imprinted polymer employed in this study was characterized in terms of physical proprieties and selectivity performance in our previous work [44]. The adsorption capacity (Q) and the imprinted factors (IF) to different concentrations of SCP were obtained as well as a molecular dynamics (MD) study demonstrating that acrylamide was the most suitable monomer to enhance the selectivity of the MIP for the recognition of SCP. In addition, the relative selectivity coefficient for SCP was higher than

for similar molecules, such as atropine and hyoscine. Such experiments have demonstrated the improved selectivity caused by the molecularly imprinted polymer synthesis using scopolamine as a template [44].

In view of the successful results obtained in the computational and experimental studies dedicated to the synthesis of a highly selective MIP for the recognition of SCP, the polymer was employed for extraction and preconcentration purposes. For this, each MISPE-DPX device was prepared by transferring 20 mg of the polymer to a disposable pipette tip sealed with 30 mg of glass wool. In the DPX technique, the sample solution is aspirated and mixed with the sorbent in a dispersive way enabling the equilibrium of the analyte with the sorbent. After a suitable time, the sample solution is dispensed in the sample flask. Each aspiration/dispensing procedure is defined as a cycle.

Initially, the effect of the solvent for preconditioning the MISPE-DPX cartridge before the extraction of SCP was evaluated using water (100%), methanol (100%), and water–methanol mixture (1:1 v/v). The device was initially flushed using 1 cycle of 3 mL of the selected solvent followed by six cycles with a solution of SCP of 10 mg L^{-1}. The resulting solution was injected into the CE system and the peak area of SCP was used to calculate its concentration using a previously established calibration curve. The adsorptive capacity (Q) and the imprinting factor (IF) for each preconditioning were calculated according to Equations (1) and (2). Table 1 demonstrates the Q and IF values for each solvent used for preconditioning.

$$Q = \frac{[Co] - [Ce]}{m} * V, \quad (1)$$

where Co = concentration of SCP before the extraction procedure (10 mg L^{-1}); Ce = concentration of SCP after the extraction with the MISPE-DPX procedure (mg L^{-1}); m= polymer mass (g); V = volume of solution (L).

$$IF = \frac{Q_{MIP}}{Q_{NIP}}, \quad (2)$$

where Q_{MIP} and Q_{NIP} are the adsorptive capacity of MIP and NIP in mg g^{-1}, respectively.

Table 1. Evaluation of the preconditioning using different solvents (n = 3).

Solvent	Polymer	Q (mg/g)	IF
Water	MIP	1.60 ± 0.02	1.42
	NIP	1.13 ± 0.01	
Methanol	MIP	1.33 ± 0.01	1.17
	NIP	1.14 ± 0.01	
Water:Methanol (50:50)	MIP	1.50 ± 0.02	1.25
	NIP	1.20 ± 0.02	
No conditioning	MIP	1.42 ± 0.03	1.27
	NIP	1.12 ± 0.01	

Higher values of Q_{MIP} and IF indicate a superior capability of the extraction procedure to retain the analyte in the solid phase. As can be seen in Table 1, the preconditioning with water resulted in the highest Q_{MIP} and IF. The SCP solution was prepared in water and it is expected that the preconditioning of the solid phase to be more efficient using the same solvent of the sample. It is important to note that for the other solvents, the higher affinity of SCP towards the MIP is higher than the NIPs, indicating a superior recognition ability of the MIP.

3.2. Optimization of the Extraction Conditions

After the definition of the preconditioning solvent, the influence of other relevant variables for improving the efficiency of the extraction via the MISPE-DPX approach was

evaluated. Polymer mass, number of extraction cycles, and contact time were evaluated by applying a 2^3 factorial design set of experiments. This analytical strategy is useful in screening the most relevant variables of a given procedure where several parameters can influence the result. The combination of variables generates a reasonable number of experiments, and different situations can be evaluated through the response obtained in each experiment performed. The percentage of absorption (%), adsorptive capacity, and the imprinting factor were calculated, and the IF was used as the extraction efficiency evaluation parameter (i.e., analytical response). Table 2 shows the maximum, minimum, and central point values for each variable as well as the analytical response. All experiments were performed in triplicate.

Table 2. The 2^3 factorial design parameters and analytical response for the MISPE-DPX extraction procedure.

Experiment	Coded Values			Real Values			Q (mg/g)		Response
	Time (s)	Mass (mg)	Cycles of Extraction	Time (s)	Mass (mg)	Cycles of Extraction	Q_{MIP}	Q_{NIP}	IF
1	+	+	+	60	10.0	10	2.24	1.72	1.30
2	−	+	+	20	10.0	10	1.51	1.01	1.50
3	+	−	+	60	5.0	10	1.84	1.80	1.02
4	−	−	+	20	5.0	10	2.39	2.34	1.02
5	+	+	−	60	10.0	2	1.69	1.67	1.01
6	−	+	−	20	10.0	2	1.35	1.21	1.12
7	+	−	−	60	5.0	2	0.46	1.69	0.27
8	−	−	−	20	5.0	2	0.19	1.23	0.15
cp1	0	0	0	40	7.5	6	1.97	0.99	1.99
cp2	0	0	0	40	7.5	6	2.06	1.99	1.04
cp3	0	0	0	40	7.5	6	2.00	2.89	0.69

The effect of each variable was calculated using the software Octave (version 4.2.1) (https://octave.org; accessed on 20 July 2022) and can be seen in Table S1 and Figure S1, where it is possible to determine the effect of each variable and the related effect in their secondary (i.e., 12, 13, and 23) and tertiary (i.e., 123) combinations. Higher effects in the extraction procedure were related to the adsorbent mass (2) and the number of cycles (3). As DPX implies a rapid contact between the solid phase and the solution, followed by air mixing, the variation in contact time did not show a considerable effect. Therefore, 20 s of contact time, 10 mg of polymer, and 10 cycles of extraction were the optimized conditions chosen for further experiments.

3.3. Optimization of the Elution Conditions

Once the extraction conditions were optimized, a new factorial design was developed to obtain the best conditions for analyte elution after the extraction procedure. An efficient elution enables the application of the MISPE-DPX device as an SCP preconcentration procedure. Herein, the concentration and volume of the elution solvent (i.e., water/methanol mixture), and the number of elution cycles were the selected variables. The SCP concentration evaluated was 10 mg L^{-1}. The concentration (C) for the MIP and NIP were calculated using a calibration curve and the ratio (MIP/NIP) for each experiment was used as the analytical response. Table 3 shows the maximum, minimum, and central point values for each variable as well as the analytical response.

Table 3. The 2^3 factorial design parameters and analytical response for the MISPE-DPX elution procedure.

Experiment	Coded Values			Real Values			Concentration (mg L^{-1})		Response
	Volume (mL)	[Solvent] %	Cycles Elution	Volume (mL)	[Solvent] %	Cycles Elution	C_{MIP}	C_{NIP}	MIP/ NIP
1	+	+	+	2.0	100	10	9.52	5.06	1.88
2	–	+	+	0.50	100	10	15.26	16.85	0.91
3	+	–	+	2.0	50	10	6.60	5.55	1.19
4	–	–	+	0.50	50	10	14.57	14.35	1.02
5	+	+	–	2.0	100	2	5.14	4.27	1.20
6	–	+	–	0.50	100	2	21.76	15.25	1.43
7	+	-	–	2.0	50	2	7.48	6.37	1.18
8	–	–	–	0.50	50	2	10.79	10.90	0.99
cp1	0	0	0	1.25	75	6	10.30	6.19	1.66
cp2	0	0	0	1.25	75	6	8.00	7.92	1.01
cp3	0	0	0	1.25	75	6	10.26	7.38	1.39

The individual and combined effects from each variable are shown in Table S2 and Figure S2. The volume of solvent (1) is responsible for 66.2% of the influence on the analytical response, which is expected since the volume of solvent can improve the desorption efficiency as well as dilute the final concentration of the analyte. The optimized conditions were achieved using 500 µL of a solution of pure methanol as elution solvent, and two elution cycles.

3.4. Analytical Validation

The evaluation of the analytical parameters of the proposed preconcentration method for the determination of SCP was performed with the optimized extraction/elution conditions. Initially, the intraday repeatability was obtained as the relative standard deviation (RSD) of seven replicates of a solution of SCP with a concentration of 2 µM submitted to the MISPE-DPX-CE procedure. A concentration value of 1.77 ± 0.11 µM was obtained, which corresponds to an RSD of 6.43%. A linear relation between the averaged values of peak area (n = 3) registered by the electrophoretic separation at a migration time of 60 s versus SCP concentrations was established in the range of 0.5 to 6 µM. The limit of detection expressed as three times the standard deviation of the blank was calculated at 0.04 µM. The preconcentration factor was determined by the ratio between the limit of quantification—calculated as ten times the standard deviation of the blank—obtained with and without the preconcentration procedure.

The method accuracy was obtained through the determination of SCP in spiked synthetic urine samples with two concentration levels. The recovery was calculated by the relationship between the spiked concentration and the concentration found after the analysis. The obtained results were within the tolerance range of ±20% for the evaluated concentration levels, according to the validation guidelines, indicating a suitable accuracy of the proposed method. All the analytical parameters are summarized in Table 4. In addition, the analytical performance of the developed MISPE-DPX-CE analytical method was compared with similar studies for the preconcentration of scopolamine in a variety of samples (Table 5). As described in Table 5, the sensitivity of the proposed method was superior when compared to other CE methods, indicating that it is suitable for the determination of scopolamine in urine samples.

Table 4. Summary of the analytical parameters of the proposed MISPE-DPX-CE method for the determination of SCP.

Parameters	Values
Linear range	0.50–6.00 μM
r	0.9988
R^2	0.9972
Intraday repeatability (DPR%, n = 7)	6.43%
LD (μM)	0.04
LQ (μM)	0.12
Recovery 1 (2 μM)	84%
Recovery 2 (6 μM)	101%
Preconcentration factor	20
Migration time	59.4 ± 1.1 s

Table 5. Comparison of the analytical performance of the MISPE-DPX-CE method with previously published studies for the preconcentration of scopolamine.

Method	Solid-Phase	Linear Range	Sample	LD	Ref.
MISPE-DPX-CE	MIP	0.5–6 μM	Synthetic urine	0.04 μM	This work
MISPE–LC–MS/MS	MIP	0.011–2.28 μM	Extracts of *Przewalskia tangutica* Maxim. fruits	0.005 μM	[45]
MISPE–HPLC–UV	MIP	91–82 μM	Plant samples	0.005 μM	[23]
Column–switching HPLC–UV	MIP	0.0068–0.205 μM	Pharmaceutical preparations	0.002 μM	[46]
Reversed-phase HPLC–UV	MIP	2.28–228 μM	Human urine	–	[47]
CE–ELC	–	10–1000 μM	Chinese herb	0.05 μM	[48]
CE–C^4D	–	100–350 μM	Pharmaceutical samples	2.5 μM	[49]
CE–C^4D	–	10–1000 μM	Beverages and pharmaceutical formulations	2.4 μM	[5]

3.5. Application in Synthetic Urine Samples

The linear range obtained by the MISPE-DPX-CE method is suitable to be employed in the determination of SCP in urine samples. However, due to the difficulty to obtain permission to use human urine samples, we have applied the MISPE-DPX-CE method for the determination of SCP in synthetic urine samples, prepared as described in the literature. The samples were previously spiked with 2 and 6 μM of SCP and submitted to the analytical procedure. Figure 2 shows representative electropherograms for the blank and spiked synthetic urine samples. Additionally, the obtained concentration levels used in the spiked samples revealed that the method has suitable sensitivity and accuracy to be employed in the determination of SCP in urine samples for forensic purposes.

As can be seen, the potential of the proposed MISPE-DPX-CE method was clearly shown in Figure 2 The urine sample without pretreatment (Figure 2C) shows the presence of a high concentration of cations in the migration time between 0.5 and 0.9 min and the absence of the peak of SCP (without detectability). However, the sample pretreatment by MISPE-DPX (Figure 2B) shows a minimum signal for cations (cleanup process) and an easily visible peak for SCP at around 1.1 min (increase in detectability).

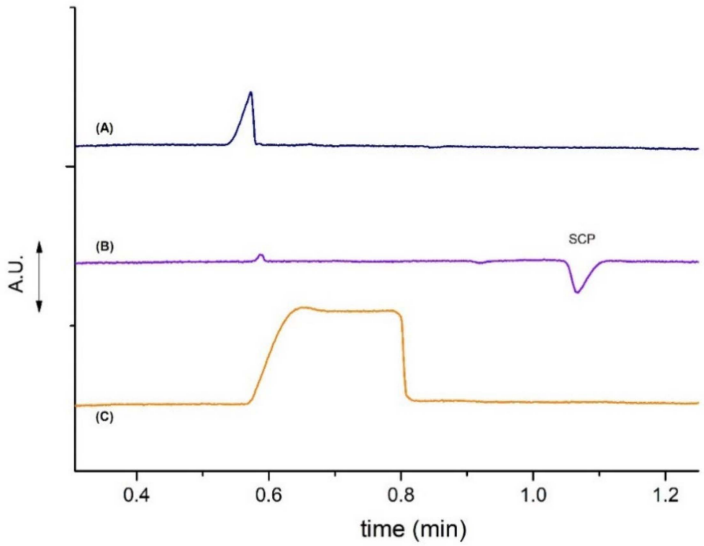

Figure 2. Representative electropherograms of a synthetic urine sample submitted to the MISPE-DPX-CE procedure before (**A**) and after (**B**) spiked with SCP (6 μM) and, (**C**) synthetic urine sample without submission to the MISPE-DPX-CE procedure and also spiked with SCP (6 μM). BGE: 20 mmol L^{-1} of butyric acid with 7 mmol L^{-1} NaOH (pH = 4.5). C4D detector positioned at 10 cm along the fused-silica capillary column. A.U.: arbitrary units.

4. Conclusions

The determination of SCP in body fluids—such as urine—can be helpful to identify possible victims of drug abuse. The application of separation techniques might enhance the selectivity of the analytical method; however, the low concentration of the analyte and the sample complexity are relevant analytical issues that impair their application in biological samples. In this scenario, preconcentration techniques are an excellent alternative to improve the detectability and clean up potential interferences.

In this study, we have proposed a preconcentration method based on the usage of a selective molecularly imprinted polymer (MIP) as a solid-phase (MISPE) into a disposable pipette tip (DPX) device. This fact contributes to the miniaturization of the sample preparation step, resulting in a less amount of polymer and a lower volume of sample (i.e., 3 mL) and solvents used, therefore reducing the generation of waste. Additionally, the employment of a selective MIP in lieu of a conventional solid-phase adsorbent (i.e., NIP) enhanced the method's selectivity and efficiency. The sample preparation step was followed by the quantification of SCP using capillary electrophoresis, which is an analytical technique that employs water-based electrolytes and low sample volumes. The analytical performance of the method was evaluated and the working range is suitable for the determination of SCP in urine samples in the usually found concentration levels.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/chemosensors10100387/s1, Figure S1—Percentage of effects found for individual variables (1, 2, 3), secondary effects (12, 13, 23), and tertiary effects (123); Figure S2—Percentage of effects found for individual variables (1, 2, 3), secondary effects (12, 13, 23), and tertiary effects (123); Table S1—Evaluation of between the variables: 1—time, 2—mass, 3—extraction cycles; Table S2—Evaluation of the between the variables: 1—solvent volume, 2—methanol concentration, 3—elution cycles.

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