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Mixed-mode adhesive tape integrated in a syringe plunger: an affordable microextraction device for isolating basic drugs from saliva samples

Yuri Arrates [a,b], Carlos Calero-Cañuelo [a], Ángela I. López-Lorente [a], Andréa Rodrigues Chaves [b], Rafael Lucena [a,*], Soledad Cárdenas [a,**]

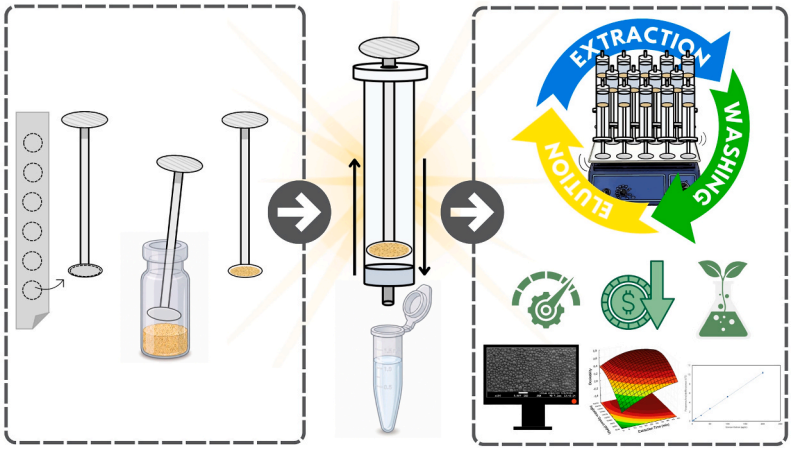
[a] Affordable and Sustainable Sample Preparation (AS2P) Research Group, Departamento de Química Analítica, Instituto Químico para La Energía y El Medioambiente IQUEMA, Universidad de Córdoba, Campus de Rabanales, Edificio Marie Curie, Córdoba, E-14071, Spain

[b] Centro de Excelência Em Estudos Moleculares, Energia e Petróleo (CEMEP), Instituto de Química, Parque Tecnológico, Universidade Federal de Goiás-UFG, Rodovia R2 C/ Av. Esperança Rodovia R2, Campus Samambaia, Goiânia, Goiás, 74690-631, Brazil

HIGHLIGHTS

- A plastic syringe with a modified plunger is proposed as a microextraction device.
- Mixed-mode particles are immobilized in the plunger using adhesive tape as a binder.
- The resulting SP-ISME is evaluated for the isolation of seven basic drugs from saliva.
- Simultaneous extraction of samples provides a high sample throughput.
- Analytes are stable in the sorptive phase, thus enabling on-site extraction.

GRAPHICAL ABSTRACT



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ABSTRACT

Background: Current bioanalytical laboratories must cope with increasing pressure on workflow due to the high number of samples to process and the speed required to generate analytical information. The development of cost-effective extraction procedures capable of processing multiple samples simultaneously can address, or at least mitigate, this enormous challenge. These devices must be affordable and cost-effective, taking into account that disposable units are preferred when biosamples are processed to avoid contamination of operators and carryover effects.

Results: In this work, sorptive plunger-based in-syringe microextraction (SP-ISME) is presented and evaluated for the isolation of seven basic drugs from saliva samples. The extraction device consists of a conventional plastic syringe whose plunger has been coated with mixed-mode cationic exchange (MCX) microparticles using a

* Corresponding author.
** Corresponding author.
E-mail addresses: q62luror@uco.es (R. Lucena), qa1caarm@uco.es (S. Cárdenas).

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double-sided adhesive tape as binder. In this case, the sample is aspirated into the syringe, where it is incubated for a defined time under continuous agitation, minimizing handling and allowing multiple samples to be extracted simultaneously. The main variables involved in the SP-ISME (i.e., sample dilution, ionic strength, stirring rate, extraction time, and elution time) have been evaluated in detail. After extraction, the analytes were analyzed by high-performance liquid chromatography coupled with tandem mass spectrometry. The analytical performance showed competitive results, yielding limits of quantification of 0.5 μg L^{-1} for all analytes, with intra- and inter-day precision lower than 6.1% and 13.2%, respectively, and relative recoveries between 81% and 110%.

Significance and novelty: The microextraction device is highly versatile, as it can be modified with various types of microparticles, whether commercial or synthesized in the laboratory. Also, its design avoids backpressure, a common issue in some in-syringe devices, because the sample is not flowed through the sorptive phase. The stability of the analytes retained in the sorptive phase after extraction opens the door to on-site applications of this technique.

1. Introduction

The analysis of drugs in biofluids remains challenging due to the low concentrations at which these compounds are typically present and the complexity of the sample matrices. In this context, sample preparation plays a crucial role in achieving sufficient sensitivity and selectivity while ensuring compatibility with the detection technique. Conventional extraction methods, such as liquid-liquid extraction and solid-phase extraction, are effective but often involve extensive solvent consumption, multiple handling steps, and limited adaptability to simplified or portable analytical workflows. These drawbacks have driven the development of microextraction techniques that reduce solvent use and simplify operations, in line with current trends in analytical chemistry [1] and green sample preparation [2].

Among the existing microextraction techniques, thin-film microextraction (TFME) uses a planar sorptive phase, which increases the surface-area-to-volume ratio, leading to faster extraction kinetics and efficient analyte sorption while reducing the amount of sorbent [3,4]. The incorporation of particulate material into planar phases has proven advantageous, as the particles increase the number of active sites accessible at the planar surface [5]. Different strategies have been described for immobilizing particulate materials on flat supports, including polymeric binders [6] and adhesive interfaces [7], with the latter offering a particularly simple route.

Adhesive tapes (ATs) are versatile, cost-effective materials that have been used to design planar phases that can serve as both sampling substrates and sorptive media. These approaches take advantage of the pressure-sensitive polymeric adhesive layer of the ATs, either for the direct extraction of target analytes [8], or for the immobilization of a wide variety of functional materials, such as commercial sorbents, e.g., hydrophilic-lipophilic balance microparticles [9,10] and mixed-mode anionic exchange particles [11], among others. They have been employed as sampling probes in combination with direct analysis by ambient ionization mass spectrometry (AIMS), including thermal desorption [12], and paper-spray [13,14], as well as in matrix-assisted laser desorption/ionization MS [15]. Also, they have been used in spectroscopic techniques such as surface-enhanced Raman spectroscopy [16], for example by surface decoration with plasmonic nanomaterials like gold nanoparticles [17].

Mixed-mode cation-exchange (MCX) microparticles are especially attractive particulate sorbents for drug analysis, as they provide complementary ionic and hydrophobic interactions that enhance selectivity toward basic compounds in complex matrices [18] [19], enabling isolation of analytes at physiological pH. The integration of MCX-based sorbents into thin-film formats has demonstrated clear analytical potential for the determination of opioids [20]. Nevertheless, the performance of such systems is strongly influenced not only by sorbent chemistry but also by the configuration of the extraction phase in contact with the sample and the mass-transfer regime governing analyte-sorbent interaction.

Syringe-based extraction systems are attractive platforms since they enable sample handling and extraction within a closed and well-controlled environment [21,22]. In syringe configurations, the sorptive phase is typically dispersed or immobilized inside the syringe body, as a plug or packed bed located within the barrel [23–26]. In this latter case, they can be supported on paper [27] or implemented in microextraction by packed sorbents (MEPS) formats [28]. Alternatively, they can be positioned in the needle assembly [29–31], while the plunger mainly fulfils a passive mechanical function. Exceptions include plunger-in-needle solid phase microextraction devices, where sorbent coatings applied to the plunger act as fiber-like extraction phases exposed in external sample vials, outside the syringe environment [32, 33]. Although planar sorptive phases attached to the plunger have also been reported for ultrasound-assisted extraction [34], their integration is based on a metallic gauze support, thus leaving unexplored the use of adhesive-based particle immobilization.

In this work, sorptive plunger-based in-syringe microextraction is presented and evaluated for the determination of seven drugs in saliva. The microextraction device consisted of a commercial plastic syringe whose plunger is coated with MCX microparticles using ATs as binder. The design avoids backpressure, a common issue in some in-syringe devices, because the sample is not flowed through the sorptive phase. In this case, the sample is incubated for a defined time under continuous agitation, minimizing handling and allowing multiple samples to be extracted simultaneously.

2. Materials and methods

2.1. Reagents and samples

Unless indicated, the reagents used for the present study were acquired from Sigma-Aldrich (Madrid, Spain). All the analytes (cocaine, codeine, fentanyl, methadone, methamphetamine, oxycodone and tramadol) were prepared at 1 g L^{-1} in methanol and stored in the freezer at – 20°C. An intermediate standard containing all analytes at 20 mg L^{-1} was prepared and used to prepare the working solutions. Cocaine-d_3, codeine-d_3, fentanyl-d_5, methadone-d_3, methamphetamine-d_5 and oxycodone-d_6 served as internal standards (ISs), and a mixture of all of them was prepared at 4 mg L^{-1}.

Double-sided adhesive tape (Milan, Mont-Ras, Spain) was used to attach the polymeric microparticles to the syringe plunger. Oasis MCX microbeads (30 μm in particle size and 80 Å in pore size) purchased from Waters Corporation (Milford, Massachusetts, USA) were employed as the actual sorptive phase.

A pool of blank saliva was prepared by mixing samples from different volunteers (see ethical statement) to account for inter-personal variability and was used throughout optimization and validation. Before analysis, saliva samples were centrifuged at 10,000 rpm (11,069 g) for 3 min to remove debris.

2.2. In-syringe microextraction

2.2.1. Preparation of the in-syringe microextraction device

A 2 mL commercial plastic syringe was used as starting microextraction device. The syringe plunger was detached, and an 8-mm-diameter circular segment of double-sided adhesive tape was adhered to the plunger seal. After removing the second protective layer of the tape, the plunger was introduced into a vial containing the MCX microparticles. The microparticles were adhered to the tape, and their excess was removed by manual shaking. A total of 0.52 ± 0.08 mg of particles (measured using 32 replicates) were effectively adhered. Fig. 1 presents a scheme of the procedure. The plunger is finally reattached to the syringe barrel to build the microextraction device.

The MCX-coated plunger was characterized by scanning electron microscopy (SEM) using a JEOL JMS-7800 F microscope (Akishima, Tokyo, Japan), available at the Central Service for Research Support (SCAI) of the University of Córdoba. Before micrograph acquisition, a gold coating was applied to improve surface conductivity.

2.2.2. Sorptive plunger-based in-syringe microextraction

1 mL of saliva at physiological pH was aspirated into the syringe, which was immediately turned upside down to bring the MCX-coated plunger into close contact with the sample. The syringe was vortexed (1750 rpm) for 40 min to favor the diffusion of the analytes from the matrix into the MCX coating. Following extraction, the matrix was discarded, and the syringe was cleaned by aspirating 1 mL of ultrapure water and agitating for 5 min at 1000 rpm. Elution was subsequently carried out by aspirating 200 μL of a methanol: ammonia 99:1 (v/v) solution, which was maintained in contact with the sorbent for 2 min under agitation at 1000 rpm. The overall procedure is illustrated in Fig. 2. 5 μL of the final eluate was analyzed by liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) as indicated in the supplementary information (section 1, Table S1).

3. Results and discussion

Current bioanalytical laboratories have to cope with increasing pressure on workflow due to the high number of samples to process and the speed required to generate analytical information. The frequency of analysis can be increased using two main strategies, which can be combined synergistically: accelerating sample preparation or increasing the speed of instrumental measurements. The first strategy, considered in this research, can be achieved by minimizing sample preparation or by designing approximations that can be applied simultaneously to a large number of samples. In the latter case, extraction units, which are typically disposable because they work with biofluids, must be as affordable as possible. In this work, we have evaluated the possibility of modifying conventional plastic syringes to incorporate a small amount of sorbent particles that allow the isolation of target analytes. For this purpose, double-sided adhesive tape has been used to immobilize MCX particles in the syringe plunger as described in the experimental section. The efficient and homogeneous immobilization of these particles on the adhesive tape is a key factor for a robust extraction unit. Initially, the amount of particles retained in the plunger was evaluated, yielding 0.52 ± 0.08 mg (n = 32). SEM analysis revealed a homogeneous distribution of the MCX microparticles across the plunger surface, with the particles remaining well dispersed throughout the support material. The micrographs also indicated the formation of a predominantly single-layer coating of microparticles adhered to the adhesive tape, as shown in Fig. S1A–B.

The microextraction device consists of three elements: a syringe, adhesive tape, and MCX particles. A preliminary study was developed to fully understand the role of each element in the isolation of the target compounds. For this purpose, 1 mL of saliva containing the analytes were extracted (see initial conditions in the supplementary information) using an unmodified plunger syringe, a plunger modified with just double-sided AT, and the proposed microextraction device that integrates the MCX particles into the plunger. The results, expressed as absolute extraction recoveries (AERs), are shown in Fig. 3. As can be seen, the syringe does not contribute to analyte extraction because, at physiological pH (selected to enhance mixed-mode interactions), the analytes are charged and do not interact with the smooth surface of the plastic. Fentanyl and methadone, the most hydrophobic analytes, are partially retained in the acrylic glue due to the potential combination of hydrophobic and H-bonding interactions [19,35]. Nevertheless, the presence of microparticles improved the overall recovery and precision, enhancing the sensitivity of the extraction method compared to the other configurations demonstrating the active role of the MCX particles.

According to the results, the MCX-coated plunger enabled the extraction of a broad range of compounds with distinct polarity profiles, covering logD values from −1.02 to 2.44 at pH 6.5 (see Table S2). This broad extraction range reflects the versatility of the device, which can be adapted to other organic cationic molecules through a combination of reversed-phase and cation-exchange interactions [36,37].

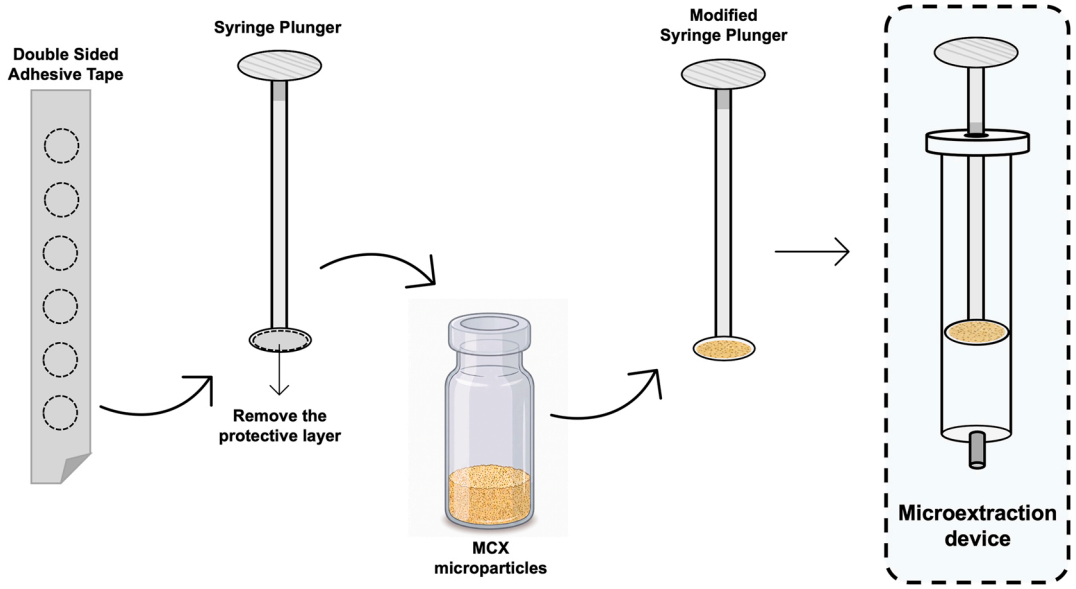

Fig. 1. Scheme of the preparation of the in-syringe microextraction device.

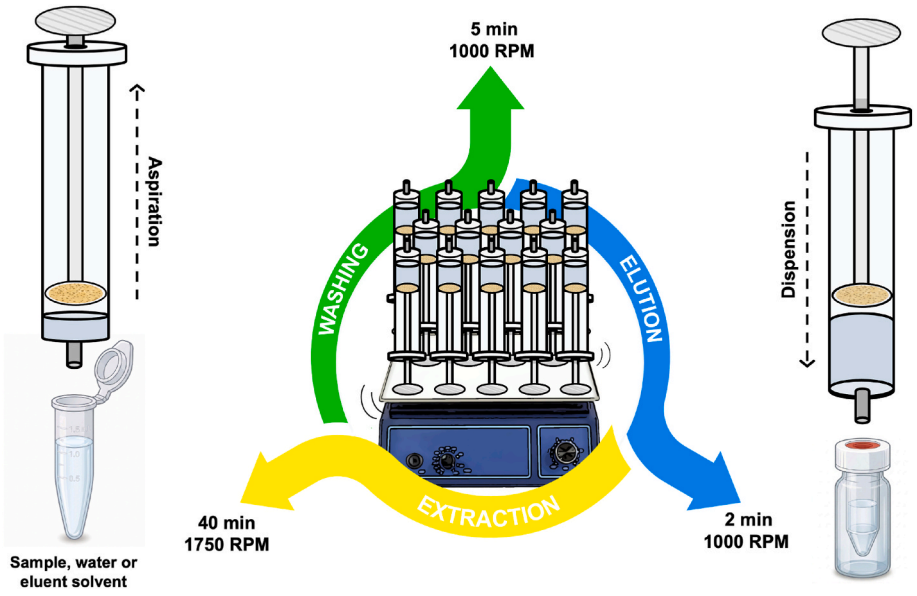

Fig. 2. Sorptive plunger-based in-syringe microextraction.

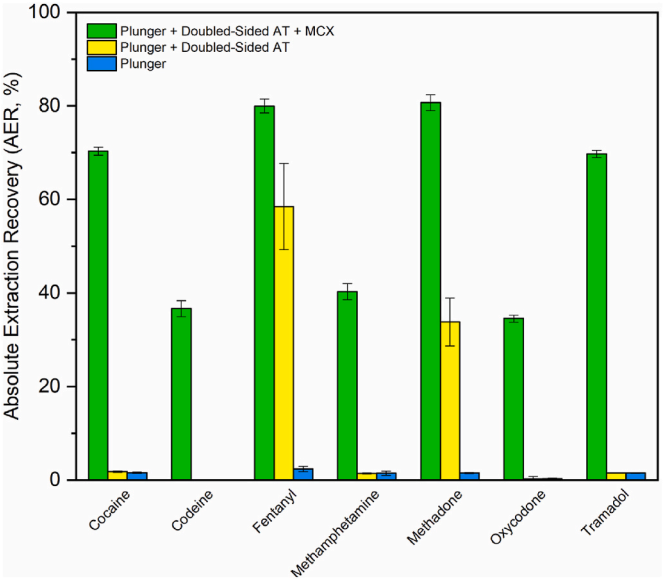

Fig. 3. Comparison of the extraction efficiency of the abuse drugs in saliva between different microextraction configurations.

3.1. Optimization of the microextraction procedure

Once the good performance of the new microextraction device was preliminarily demonstrated, the main variables involved in the extraction were studied. Saliva samples exhibit high viscosity, which typically restricts the diffusion of analytes from the matrix into the sorptive phase, slowing extraction kinetics. This shortcoming can be overcome by diluting the sample before extraction. This strategy typically improves the AER but at the expense of the analyte dilution. The effect of sample dilution on the peak area of the analytes was evaluated by extracting a spiked blank saliva sample diluted at different ratios (1:2 and 1:4) with Milli-Q water. As shown in Fig. S2, a consistent decrease in the analytical signal was observed, with some analytes approaching low response levels, suggesting a potential detrimental effect on method sensitivity. Thus, subsequent steps were performed without sample dilution, since this condition better reflects the native sample composition, preserves analytical sensitivity, and avoids the introduction of additional preparation steps.

Ionic strength is also a relevant variable when mixed-mode ion-exchange mechanisms are involved in analyte isolation. As indicated in Fig. S3A, the addition of sodium chloride (selected as the model electrolyte) negatively affects the AER. Analysis of variance (ANOVA) indicated that NaCl addition at different concentrations had a statistically significant effect ($p < 0.05$; $p = 0.034$). Although the magnitude of this effect was modest, the condition without salt addition (0%) provided superior extraction recoveries. Thus, no adjustment of ionic strength was implemented, thereby preserving the simplicity of the sample preparation procedure and higher recoveries values. Nevertheless, any potential variations in the analytical signal arising from differences in ionic strength among saliva samples are normalized by adding an internal standard before extraction (Fig. S3B), eliminating the need to adjust for this variable and reducing the number of processing steps.

The extraction time and agitation speed are closely related variables, so a full factorial design with central composite and axial points (2^2) was used to identify the optimum conditions for extraction efficiency. The extraction time was investigated from 5 to 45 min, while agitation speed was studied over a range from 500 to 2000 rpm. The studied factor levels are presented in Table S3, while the complete experimental matrix, including replicates in standard order, is provided in Table S4. All statistical analyses for the multivariate design were performed using STATISTICA® version 7 (StatSoft, USA). The inclusion of axial points ($\pm\alpha$) enabled the estimation of quadratic terms, allowing the model to capture curvature in the response surface that would not be described by a simple factorial design. According to the ANOVA results, the linear effects of both factors were statistically significant for all analytes. In addition, the quadratic term associated with agitation speed was consistently significant, whereas the quadratic effect of extraction time was not significant for all analytes. This result suggests that, for some compounds, the response with respect to agitation speed deviates from linearity and may exhibit a maximum or minimum within the studied domain. The interaction between factors was not statistically significant ($p > 0.05$), indicating that extraction time and agitation speed act independently on analyte recovery. These results are detailed in Table S5 and Fig. S4 (ANOVA and Pareto charts).

Given the multiresidue nature of the method and the need to simultaneously optimize all analytes, a desirability function was applied. This approach transforms individual responses into a

dimensionless scale ranging from 0 (undesirable) to 1 (fully desirable) and converges the results into an equation that maximizes the average recovery of the system without compromising individual responses. The resulting response surface and contour plots (Fig. 4A–B) indicate an overall optimal region at the +1 level, approximately 40 min of extraction time and 1750 rpm for the global extraction. It is important to note that beyond this region, further increases in either factor do not improve recovery and may even reduce it, likely due to analyte re-equilibration.

Elution time was finally investigated over the range of 2 to 15 min, maintaining a constant agitation rate of 1000 rpm. The optimization of the elution time demonstrated that this factor influences analyte recovery (Fig. S5). An increase in contact time led to a gradual decline in extraction efficiency, with the average's recoveries decreasing from 66.7% at 2 min to 62.4% at 15 min. This phenomenon can be attributed to the co-elution of adhesive residues at longer times, which may induce ion suppression of the analytes. Therefore, an elution time of 2 min was selected, as it provides the highest recovery while supporting high-throughput analytical performance. Based on our previous experience using MCX sorbent to isolate basic drugs [19,20], methanol containing 1% ammonia was selected as the elution solvent. This combination ensures a good elution by simultaneously disrupting hydrophobic and ionic interactions.

3.2. Analytical performance of the proposed method

The analytical method was validated under the optimum extraction conditions using matrix-match calibration. The analytical performance was evaluated in accordance with criteria established by recognized regulatory frameworks for bioanalysis procedures. Specifically, the guideline "ICH guideline M10 on bioanalytical method validation and study sample analysis" was adopted as reference [38].

A 1/x-weighted calibration curve was built for all analytes, evaluating linearity over 0.5-200 μg L^{-1} (Fig. S6). The analytical features of the method are presented in Table 1.

According to the validation guidelines, the relative standard deviation (RSD) should not exceed 15%, except at LOQ, where it should not exceed 20%. For accuracy, each concentration level should be within ± 15% of the nominal concentration, except LOQ, where it should be within ± 20%. To meet the requirements for sensitivity (signal-to-noise ratio), precision, and accuracy outlined in the validation guideline, the limits of quantification (LOQs) and limits of detection (LODs) were set at 0.5 μg L^{-1} and 0.15 μg L^{-1}, respectively, for all analytes. Linear ranges spanned from LOQ to 200 μg L^{-1} with a R^2 value of 0.999 in all cases.

Method precision and accuracy were assessed at four quality control (QC) levels: 0.5 μg L^{-1} (LOQ), 1 μg L^{-1} (low QC), 100 μg L^{-1} (medium QC), and 200 μg L^{-1} (high QC). The precision of the method was evaluated within day (intra-day) and between days (inter-day), and both were expressed as relative standard deviation (RSD, %). Intra-day precision was studied in quintuplicate and was better than 6.1% for all analytes, while inter-day precision was assessed using 11 replicates over 3 different days, with RSD lower than 13.2% for all analytes, demonstrating the method's repeatability and reproducibility. Further, accuracy was expressed as relative recovery (%), calculated as the ratio of the measured concentration to the true concentration across all QCs. The accuracy values ranged from 81 to 110%, in accordance with the validation guideline specifications.

To further assess the analytical robustness of the method, the stability of analytes retained in the sorptive phase after extraction was investigated at two concentration levels (i.e., 10 μg L^{-1} and 100 μg L^{-1}) over a period of 4 weeks under two storage conditions: room temperature (25°C) and 4°C, aiming to determine the preservation of analyte integrity during storage. Fig. S7 shows the evolution of the relative area (analyte/IS) response ratios throughout the storage period under each evaluated condition. For comparative purposes, the results at the beginning of the experiments (week 0) were used to normalize the data collected over 4 weeks. Assuming ±15% as an acceptable value, the analyte/IS ratios remained essentially unchanged over the four-week study for almost all the analytes, indicating that no significant degradation or loss of the target compounds occurred during storage at the evaluated temperatures. Only methamphetamine showed unexpected behavior in the second week, with an increase in the signal above the 15% limit (10 μg L^{-1}: 25.1 and 25.6% for room and 4°C temperatures, respectively; 100 μg L^{-1}: 26.1 and 27.4% for room and 4°C temperatures, respectively). These results open the door to using this microextraction technique for in situ sample preparation. This characteristic is particularly advantageous in forensic and environmental investigations, where immediate instrumental analysis is often impractical, and sample transport may be required. Consequently, the ability to preserve analyte integrity during storage enhances the operational flexibility and practical applicability of the method in routine laboratory workflows and field-sampling scenarios. Importantly, although the method was

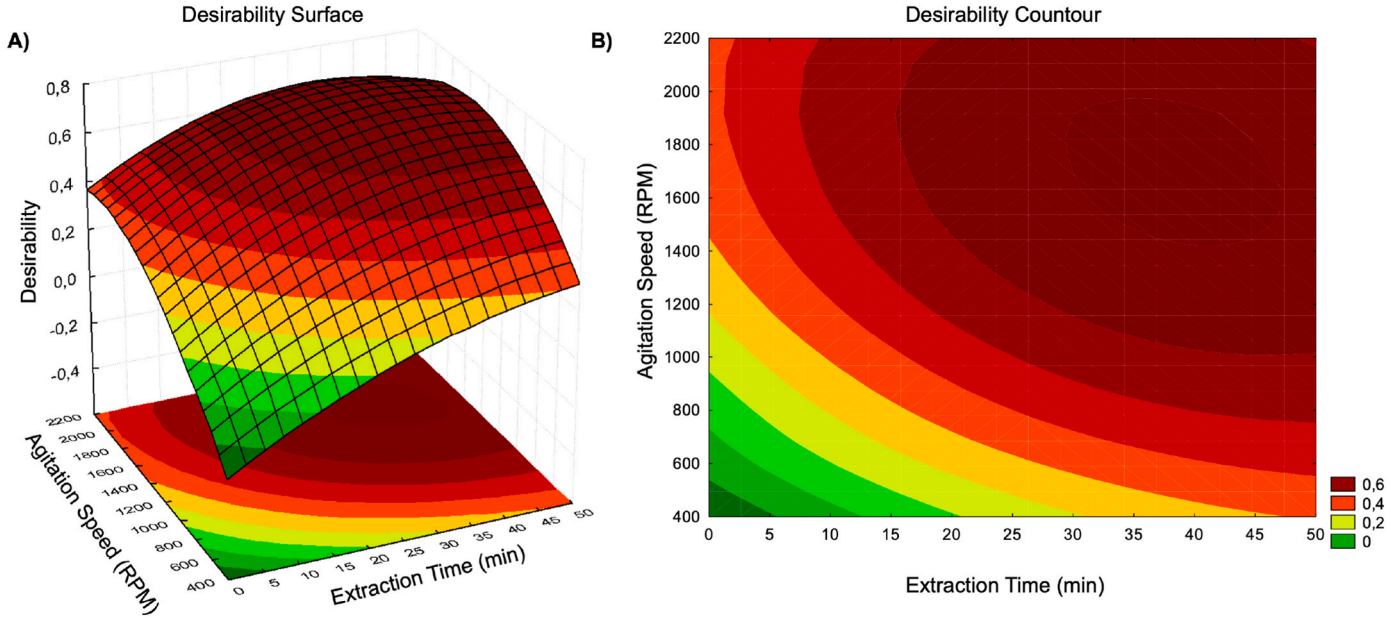

Fig. 4. Responses of desirability of the 2^2 full factorial design for extraction of the analytes, A) Surface and B) Contour.

Table 1
Analytical features of the proposed method.

Analyte	LOQ (μg·L^{-1})	Precision							Accuracy (% Relative recovery, n = 5)			
		Intra-day precision (%, n = 5)				Inter-day precision (%, n = 11)						
		0.5 μg L^{-1}	1 μg L^{-1}	100 μg L^{-1}	200 μg L^{-1}	0.5 μg L^{-1}	1 μg L^{-1}	100 μg L^{-1}	200 μg L^{-1}	0.5 μg L^{-1}	1 μg L^{-1}	100 μg L^{-1}	200 μg L^{-1}
Cocaine	0.5	4.2	0.9	0.5	1.2	8.8	4.2	3.4	3.3	84 ± 5	100 ± 1	96 ± 1	98 ± 1
Codeine	0.5	5.6	6.1	3.0	0.4	13.2	8.9	3.6	4.6	98 ± 8	95 ± 7	96 ± 3	94 ± 1
Fentanyl	0.5	1.7	1.3	1.0	0.5	5.6	9.7	4.8	1.6	110 ± 2	110 ± 1	102 ± 1	104 ± 1
Methadone	0.5	1.8	0.7	0.4	0.3	6.2	4.5	3.2	3.8	86 ± 2	99 ± 1	95 ± 1	95 ± 1
Methamphetamine	0.5	2.2	1.6	1.8	1.5	8.6	1.7	11.5	5.7	82 ± 2	97 ± 2	84 ± 1	91 ± 1
Oxycodone	0.5	2.5	1.4	0.7	1.3	11.1	5.7	2.9	2.4	81 ± 3	97 ± 2	94 ± 1	95 ± 1
Tramadol	0.5	2.5	1.8	0.8	1.5	8.8	2.2	7.7	7.6	86 ± 2	96 ± 2	89 ± 1	90 ± 1

successfully validated using spiked saliva, its application to authentic clinical and forensic samples remains an important step for future studies.

3.3. Critical evaluation of the method in terms of performance and sustainability

The proposed method was compared with other methods reported in the literature (Table 2) for the analysis of saliva and oral fluid samples in terms of sensitivity, precision, and accuracy [19,27,39–45]. The figures of merit of the new method were comparable to those of previously reported methods, including more traditional and well-established alternatives such as solid-phase extraction (SPE) and solid-phase microextraction (SPME). Moreover, our procedure has been compared with direct analysis of the eluate or sorbent by MS, which are alternatives designed to reduce analysis time and the associated costs. However, these methodologies may be ascribed to ion suppression, which can reduce sensitivity, a problem exacerbated for less polar compounds or those that are more difficult to extract.

It should be noted that the sorptive phase preparation is very simple and does not require complex synthesis, reducing the high costs associated with this process. In this case, a single SPE cartridge containing 150 mg of MCX microparticles enables the preparation of up to 288 MCX-plunger units, without the need for time-consuming synthesis and providing a solvent- and reagent-free preparation approach. Also, its design allows all sample handling in commercial syringes and conventional laboratory stirring plates, without requiring additional equipment throughout the extraction process. Importantly, the number of parallel extractions is ultimately determined by the dimensions and capacity of the stirring platform. The proposed device itself does not impose any intrinsic limitation on sample throughput. In the present study, up to 30 samples were processed simultaneously without compromising extraction performance. The current cost per extraction unit, calculated based on the overall materials used and quantities employed, was estimated at 0.17 €/unit. Reusing the extraction devices may reduce this cost even further. However, when processing biofluids, we prefer to use the devices only once to avoid carry-over and reduce washing-solvent consumption. In addition, the use of large volumes of these solvents can affect the adhesive and, therefore, the mechanical stability of the particles.

Finally, the proposed method was quantitatively evaluated using AGREEprep [46,47], BAGI [48], and RAPI [49] metrics. These evaluation tools provide standardized frameworks for assessing analytical methods based on principles of green analytical chemistry, enabling objective comparison with alternative techniques through quantitative sustainability scores. By considering factors such as solvent consumption, energy requirements, waste generation, operational complexity, and overall environmental burden, these metrics offer a comprehensive perspective on method greenness and practical sustainability. The resulting assessments, presented in Fig. 5A–C, provide an integrated evaluation of the environmental performance of the proposed method. As discussed in detail in the supplementary material, the scores demonstrate the sustainable character of the new technique as well as its practicality and applicability.

4. Conclusions

In this work, sorptive plunger-based in-syringe microextraction is presented as a simple, disposable, and low-cost device for bioanalytical sample preparation. The proposed device uses a commercial plastic syringe whose plunger is modified with MCX microparticles using double-sided adhesive tape as binder. 0.52 ± 0.08 mg of MCX microparticles are retained in each plunger, so up to 288 extraction devices can be prepared with a single MCX SPE cartridge. In addition, the device allows the operator to conveniently collect and dispense samples and solvents directly through the syringe system, thereby improving analytical

Table 2
Comparison of the proposed method with other methods reported in the literature in terms of analytical performance.

Analytes	Sample preparation	Instrumental technique	Sample volume (mL)	Extraction procedure time (min)	LOD ($\mu g \cdot L^{-1}$)	RSD intra-day (%)	RSD inter-day (%)	Recovery (%)	Reference
Fentanyl	SPE	GC-MS	2	N/S	0.5	<2.5	<13.8	80-100	[45]
Fentanyl	Protein precipitation	LC-MS/MS	0.1	20 + evaporation and reconstitution	0.1	<3.49	<13.70	102.63-113.87	[39]
Oxycodone					0.3	<2.58	<13.19	97.85-114.66	
Tramadol					0.6	<4.51	<12.35	110.32-116.95	
Codeine	In-syringe-μ-SPE	DI-MS/MS	1.25	<5*	1.8	<12.3	-	99.7-105.9	[27]
Methadone					0.0064	<4.6		89.9-101.4	
Tramadol					0.92	<14.9		90.9-108.6	
Tramadol	TFME	DIC	10	40.08	1.7	2.2	2.4	93.1-102.5	[40]
Cocaine	SPME	MOI-MS	1.5	20	0.05	1	5	91-107	[41]
Codeine					1	6	6	99-110	
Fentanyl					0.01	2	2	89-100	
Methamphetamine					0.1	2	1	93-110	
Codeine	TFME	DI-MS/MS	1.5	19	3	<9.2	<13.7	81-120	[42]
Methadone					1.5	<2.4	<10.6	86-109	
Oxycodone					1.5	<5.4	<6.3	89-118	
Tramadol					1.5	<9.5	<11.8	89-104	
Cocaine	SPE	UHPLC-MS/MS	1	>10*	0.04	5	-	80-99	[43]
Cocaine	c-WT-SPME	LC-MS/MS	1	730 + evaporation and reconstitution	0.3	9	-	84-102	[44]
Codeine					1.6	9		71-88	
Fentanyl					0.3	4		81-111	
Methadone					0.3	5		72-119	
Methamphetamine					1.6	8		68-90	
Tramadol					0.3	4		99-113	
Fentanyl	PT-TFME	LC-MS/MS	2	N/S	0.15	< 10.8	< 15.5	99-113	[19]
Methadone					0.45	< 5.2	< 8.3	89-98	
Oxycodone					0.45	< 6.3	< 14	89-108	
Tramadol					0.45	< 13.8	< 24.7	87-111	
Cocaine	SP-ISME	LC-MS/MS	1	47	0.15	<4.2	<8.8	84-100	This work
Codeine					0.15	<6.1	<13.2	94-98	
Fentanyl					0.15	<1.7	<9.7	102-110	
Methadone					0.15	<1.8	<6.2	86-99	
Methamphetamine					0.15	<2.2	<11.5	82-97	
Oxycodone					0.15	<2.5	<11.1	81-97	
Tramadol					0.15	<2.5	<8.8	86-96	

Sample preparation: SPE: solid phase extraction; TFME: thin-film microextraction; SPME: solid phase microextraction; c-WT-SPME: carboxylmethylated wooden toothpicks solid phase microextraction; PT-TFME: pipette-tip thin-film microextraction; SP-ISME: sorptive plunger-based in-syringe microextraction. **Instrumental technique**: GC-MS: gas chromatography mass spectrometry; LC-MS/MS: liquid chromatography tandem mass spectrometry; DI-MS/MS: direct infusion tandem mass spectrometry; DIC: digital image colorimetry; MOI-MS: microfluidic open interface mass spectrometry; UHPLC-MS/MS: ultra-high performance liquid chromatography tandem mass spectrometry.
N/S: not specified.
*: the exact time of the extraction procedure was not clearly specified. Either the authors provided an approximate value, or it was estimated based on the intermediate times indicated in the manuscript.

throughput and operational efficiency. This design also minimizes direct contact with biological materials and chemical reagents, enhancing user safety and reducing the risk of contamination during sample handling. Moreover, the device avoids backpressure because the sample is not flowed through the sorptive bed.

The evaluation of the SP-ISME goes beyond a proof-of-concept since it has been applied to determine 7 basic drugs in saliva samples by LC-MS/MS. The analytical performance demonstrated that the proposed method is competitive with alternative approaches reported in the literature, while offering the additional advantages of low cost and reduced sample preparation complexity. Furthermore, the extraction procedure was performed through controlled agitation, minimizing excessive manual handling and significantly improving sample throughput. Although in this first approach, a relatively high volume of saliva sample is processed, the extraction device could be scaled down to process lower volumes. This adaptation can be done using smaller syringes (e.g., 0.5 or 1 mL) or by partially filling them with samples.

The results suggest that the platform may be adapted to other biological matrices (such as urine, blood, and plasma), although dedicated optimization would be required to evaluate the matrix effect in any case. Moreover, stability studies confirmed its suitability for in situ sample preparation, an attribute of considerable value for routine laboratory applications, as it can simplify logistics and expand operational flexibility.

CRediT authorship contribution statement

Yuri Arrates: Writing – original draft, Validation, Investigation, Conceptualization. **Carlos Calero-Cañuelo:** Writing – original draft, Supervision, Conceptualization. **Ángela I. López-Lorente:** Writing – review & editing, Supervision, Methodology. **Andréa Rodrigues Chaves:** Writing – review & editing, Funding acquisition. **Rafael Lucena:** Writing – review & editing, Supervision. **Soledad Cárdenas:** Writing – review & editing, Resources, Project administration, Funding acquisition.

Ethical statement

Experiments were performed in accordance with the Helsinki Declaration and were approved by the Research Ethics Committee of the Province of Córdoba (SICEIA-2023-000030) accredited body that depends on the Andalusian Health Service (Regional Government of Andalucia). Each volunteer provided written informed consent to participate in this study, in accordance with the guidelines established

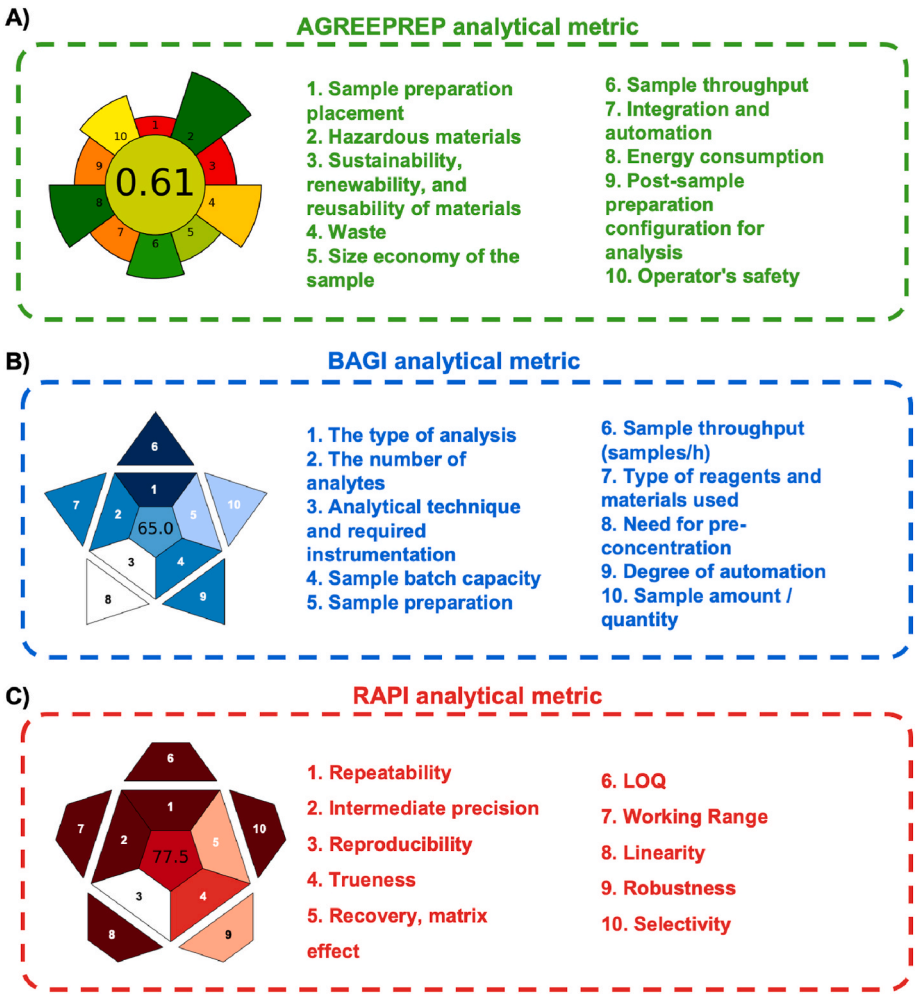

Fig. 5. Analytical metrics evaluating the sorptive plunger-based in-syringe microextraction technique by: A) AGREEprep, B) BAGI and C) RAPI metrics.

by the regulators (Real Decreto 1716/2011).

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Declaration of competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.aca.2026.346173.

Data availability

Data will be made available on request.

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