Journal of Pharmaceutical Analysis 13 (2023) 1346–1352

Contents lists available at ScienceDirect

Journal of Pharmaceutical Analysis

journal homepage: www.elsevier.com/locate/jpa

Original article

Nylon 6-cellulose composite hosted in a hypodermic needle: Biofluid extraction and analysis by ambient mass spectrometry in a single device

Jaime Millán-Santiago, Rafael Lucena*, Soledad Cárdenas

Affordable and Sustainable Sample Preparation (AS_2P) Research Group, Analytical Chemistry Department, Chemical Institute for Energy and Environment (IQUEMA), University of Cordoba, E-14071, Cordoba, Spain

ARTICLE INFO

Article history:
Received 27 January 2023
Received in revised form
28 May 2023
Accepted 27 June 2023
Available online 29 June 2023

Keywords:
ESI emitter
Mass spectrometry
Hypodermic needle
Composite
Methadone

ABSTRACT

This study proposes a hypodermic needle (HN) as a sorbent holder and an electrospray (ESI) emitter, thus combining extraction and analysis in a single device. A novel nylon 6-cellulose (N6-Cel) composite sorbent is proposed to extract methadone from oral fluid samples. The cellulosic substrate provides the composite with high porosity, permitting the flow-through of the sample, while the polyamide contributes to the extraction of the analyte. The low price of the devices (considering the holder and the sorbent) contributes to the affordability of the method, and their small size allows easy transportation, opening the door to on-site extractions. Under the optimum conditions, the analyte can be determined by high-resolution ambient ionization mass spectrometry at a limit of detection (LOD) as low as 0.3 μg/L and precision (expressed as relative standard deviation, RSD) better than 9.3%. The trueness, expressed as relative recovery (RR), ranged from 90% to 109%. As high-resolution mass spectrometers are not available in many laboratories, the method was also adapted to low-resolution spectrometers. In this sense, the direct infusion of the eluates in a triple quadrupole-mass spectrometry provided an LOD of 2.2 μg/L. The RSD was better than 5.3%, and the RR ranged from 96% to 121%.

1. Introduction

Opioids are a family of drugs used as anesthetics, painkillers, sedatives, or palliative care. However, their extended ingestion can lead to dependence, addiction, overdose, and even death. Rapid treatment response to patients suspected of drug overdose or intoxication is crucial to apply therapeutic protocols [1,2]. Mass spectrometry (MS) is a powerful technique in bioanalysis due to its high selectivity/sensitivity levels. Its combination with chromatographic techniques is considered as one of the most reliable tools in the toxicological field [3], and it has been widely used for determining drugs in biosamples [4,5]. However, chromatographic separation, essential to reduce some matrix effects, slows down information acquisition. Direct analysis of samples by ambient ionization mass spectrometry (AIMS) is a faster analytical approach but is more prone to ion suppression effects [6]. Combining sample preparation and MS (bypassing chromatography) is interesting since it unites a lower ion suppression effect with a faster analysis [7].

Although different ionization techniques can be applied, electrospray (ESI) has been extensively used in these approaches for drug analysis. A particular substrate, sometimes called ESI-emitter, is used to create an electrospray containing the ionized analytes, allowing their transference to the MS inlet. Different substrates have been used in these developments, including paper [8,9], wooden tips [10,11], coated blades [12,13], threads [14–16], probes [17,18], solid phase microextraction (SPME) fibers [19], Teflon [20], graphite rods [21], volumetric absorptive microsampling [22], and needles [23,24].

Cotton is a natural, inexpensive, and versatile biopolymer that can be used as a sorbent. It is composed of cellulose fibers that confer it a hydrophilic character due to the superficial hydroxyl groups. In the microextraction context, cotton has been used in different formats, including cotton fibers [25], threads [15], swabs [26], and gauzes [27]. Different authors have reported using unmodified cotton fibers for microextraction purposes [28,29]. However, cellulose fibers are usually chemically modified to promote interaction with the target analytes.

Peer review under responsibility of Xi'an Jiaotong University.
* Corresponding author.
E-mail address: q62luror@uco.es (R. Lucena).

https://doi.org/10.1016/j.jpha.2023.06.015

The presence of superficial hydroxyl groups in raw cotton also allows its modification with different groups, including sulfhydryl moieties [30,31], graphene oxide composites [32,33], ionic liquids [34], β-cyclodextrins [35], polydopamine [25], and metal-organic frameworks [36]. Likewise, cotton can be used as a substrate for the physical deposition of sorptive polymers [37].

Cotton-based materials have been used in microextraction techniques under different configurations [27,31,33,38,39]. Although cotton threads have been successfully coupled to AIMS [14–16], the potential of cotton wool has been scarcely considered. The complex fabrication of a stable and reproducible ESI substrate made of cotton wool somewhat restricts this combination. This article addresses this problem by placing the cotton wool sorbent in the hub of a hypodermic needle (HN), which acts as a holder and a reproducible ESI emitter. The determination of methadone in saliva samples has been studied as proof of principle to evaluate the potential of this new approach. Methadone is a synthetic opioid suitable for opioid substitution therapies: it presents a pharmacokinetic half-life of approximately 24 h, and plasmatic concentrations of methadone accumulate for at least 5 days [40–42]. Polyamide-coated cotton composite has been selected as sorbent in such a way that the normal-phase interactions of raw cotton are complemented by hydrogen bonds and hydrophobic interactions provided by the polyamide backbone. The composite is easily packed into the HN, allowing the sample flow-through and improving the extraction kinetics.

2. Materials and methods

2.1. Reagents and samples

All reagents were of analytical grade or better. Unless otherwise indicated, they were purchased from Sigma Aldrich (Madrid, Spain). A stock standard solution of methadone was prepared at a concentration of 10 mg/L in methanol and stored at −20 °C. Methadone-d_3 was used as the internal standard (IS) in direct infusion tandem mass spectrometry (DI-MS/MS) and AIMS. IS stock standard solution was prepared at 20 mg/L in methanol and stored at −20 °C. Working solutions were prepared by diluting the stock solutions in Milli-Q water (Millipore Corp., Darmstadt, Germany) or saliva as needed. Cotton wool was purchased in a local supermarket. N6 pellets were dissolved in formic acid (≥98%), and this solution was used for coating the cotton fibers. Stainless-steel HNs (Becton Dickinson, Huesca, Spain) were used to host the sorptive phase and as ESI emitters. Methanol, Milli-Q water, and formic acid were used as components of the carrier phase in DI-MS/MS analyses, while methanol and formic acid were used as the eluent and ionizing agent in AIMS. 25% (*V*/*V*) ammonium hydroxide solution was used to adjust the pH of the aqueous and saliva samples. NaCl was used to study the effect of ionic strength on the extraction of the analyte.

2.2. Oral fluid collection

Blank saliva samples were obtained using Salivette® collection devices (Sarstedt, Nümbrecht, Germany) for the study of the variables and the validation of the proposed methods. The consumption of any food or drink was prohibited 30 min before sampling. The Salivette® cotton roll was introduced in the mouth until complete permeation of the device. Then, the cotton roll was centrifuged at 3,000 rpm for 2 min. The obtained blank saliva was stored at 4 °C until analysis.

2.3. Preparation and characterization of the sorptive phase

A 0.1% (*m*/*V*) N6 solution was prepared by dissolving N6 pellets in formic acid. Two milligrams of cotton was weighed and placed with a tweezer in the Luer hub of each HN. Afterward, 20 μL of the N6 solution was deposited on the cotton bead. Finally, the needles were stored at room temperature overnight to evaporate the formic acid.

The nylon 6-cellulose (N6-Cel) composites were characterized by infrared (IR) spectroscopy (Bruker Tensor 37, Bruker Optik, Ettlingen, Germany) using the attenuated total reflection (ATR) mode.

2.4. Extraction procedure and analysis by high-resolution AIMS

2.4.1. Isolation of the target analyte from oral fluid samples

For the isolation of the target analyte, the HN hosting 2 mg of N6-Cel composite was attached to a 2 mL syringe (Fig. 1A). The porosity of the N6-Cel composite allows the flow-through of the different solutions involved in the conditioning, sample loading, and washing steps. The conditioning step consisted of 3 strokes (loading and unloading process) of 1 mL of Milli-Q water to release the formic acid that may eventually remain in the N6-Cel, followed by 1 stroke of 1 mL of Milli-Q at pH 9.5. The sample loading step entailed 3 strokes of 1.5 mL of diluted saliva (1:3 dilution factor) at pH 9.5. Afterward, the washing step involved loading and unloading 0.5 mL of Milli-Q water at pH 9.5 to reduce the interferences provided by the matrix, followed by 3 strokes of air to release the remaining drops of liquid in the microextraction device.

2.4.2. Elution online coupled to high-resolution AIMS

The online elution interface in AIMS analysis is schematically presented in Fig. 1B. A 500 μL Hamilton syringe (Bonaduz, Switzerland) was filled with methanol containing 0.1% (*V*/*V*) formic acid, which acted as the eluent and ionizing medium. The syringe pump installed in the MS pumped the methanolic solution at 30 μL/min through a polyether ether ketone tube that was already connected to the N6-Cel-HN by a precut 10 μL pipette tip that acted as an adapter. An electric clamp was connected to the HN, leaving 25 mm between the clamp and the tip of the needle to ensure the mechanical stability of the ESI emitter. The distance between the HN tip and the MS inlet was fixed at 10 mm to prevent electric discharges. The eluent flowed through the N6-Cel sorbent, eluting the analyte that was finally electrosprayed in the tip of the HN.

High-resolution AIMS analyses were carried out in a Thermo LTQ Orbitrap XL hybrid mass spectrometer (Thermo Fisher Scientific Inc., San Francisco, CA, USA), working with the Fourier-transform mass spectrometry analyzer. The ionization parameters of the proposed interface are presented in the Supplementary data.

2.5. Extraction procedure and analysis by DI-MS/MS

The extraction procedure is also compatible with conventional mass spectrometers, including an offline elution step before the final mass spectrometric analysis. To do so, the samples/standards are extracted following the same procedure described in Section 2.4.1. The needles are offline eluted by flowing 200 μL of methanol 0.1% (*V*/*V*) formic acid using a 1000 μL pipette, as indicated in Fig. S1. The eluate was transferred into a high-performance liquid chromatography (HPLC) insert. Finally, 5 μL of the eluate was analyzed by DI-MS/MS (as described in the Supplementary data and Table S1).

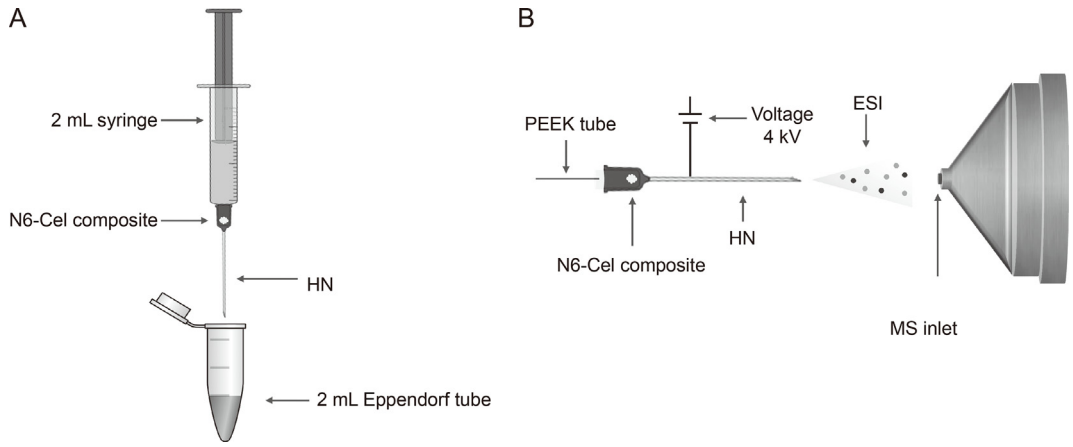

Fig. 1. Schematic diagram of the (A) extraction and (B) ambient ionization mass spectrometry (AIMS) analysis using modified hypodermic needles. N6-Cel: nylon 6-cellulose; HN: hypodermic needle; PEEK: polyether ether ketone; ESI: electrospray; MS: mass spectrometry.

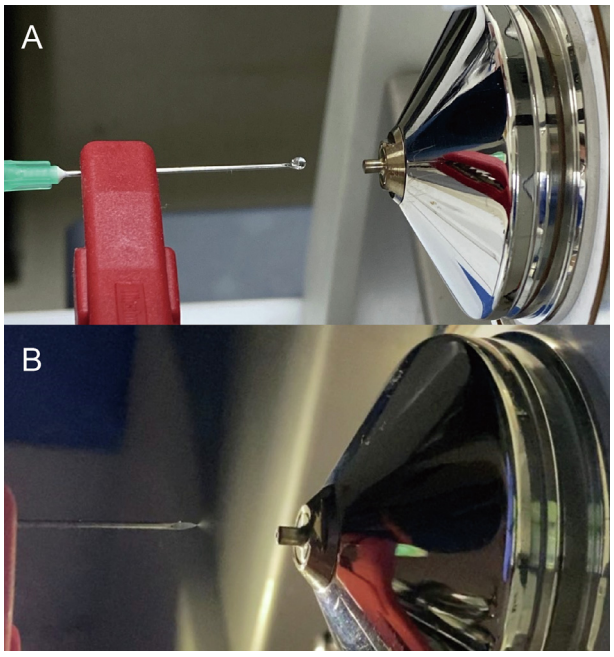

Fig. 2. Electrospray ambient ionization mass spectrometry (ESI-AIMS) analysis of saliva using (A) dilute-and-shot mode and (B) extraction and online elution.

3. Results and discussion

Although saliva presents a simpler matrix than other biosamples (e.g., blood and urine), its inherent viscosity somewhat restricts its direct analysis by ESI-AIMS. This negative effect is also observed when the dilute-and-shot strategy is evaluated, as indicated in Fig. 2A. The diluted saliva forms a drop in the tip of the needle that is ejected without forming a stable electrospray. This phenomenon, which hinders the potential sensitivity of the approach, can be overcome by an extraction procedure. In this sense, as indicated in Fig. 2B, the online elution of the needles forms a stable spray, thus transferring the analytes efficiently to the MS inlet.

The use of sorptive-coated HNs has been recently reported to extract drugs from biosamples [24,43]. In these HNs, a polymer was coated in the inner wall of the needles. However, the geometry of the devices limits the extraction efficiency. On one hand, the sorptive phase is limited to a thin layer of the polymer, reducing the extraction capacity. On the other hand, most of the sample flows through the needle without interacting with the sorptive phase since only the analytes passing close to the walls are efficiently extracted. Using needles with a lower inner diameter may solve the latter problem, but the sample processing would be complex, as higher pressures should be required to pass the sample through the needles. This article hypothesizes that using a fibrous sorbent would solve both limitations since it can provide closer contact with the sample, permitting its flow-through. Although there are several reported techniques for synthesizing polymeric fibers (e.g., electrospinning), we preferred to use a fibrous material (cotton) as the substrate to deposit the sorptive phase over the cellulose fibers.

Cellulose can interact with various compounds, including basic drugs, in aqueous media. This sorption capacity can be improved, both from the extraction capacity and selectivity point of view, by coating cellulose with a polymer. Polyamides are interesting polymers in this sense because they can bind target drugs by hydrophobic and H-bonding interactions. Initially, the role of the polyamide content in the extraction of methadone was evaluated by extracting aqueous standards at pH 9.5 containing the drug at 50 μg/L. Several HNs were prepared with different contents of N6. Initially, a 2 mg cotton bead was placed in the needle hub as support. This amount was selected since it fits easily in the hub without requiring excessive packing of the fibers, which would result in higher backpressure. Twenty microliters of a polyamide solution in formic acid (0%, 0.1%, 0.5%, and 1% (*m*/*V*)) was added to the cotton bead, and the formic acid was evaporated at room temperature. This volume (20 μL) was selected because it wets the whole bead, thus allowing a homogeneous distribution of the polyamide. Higher volumes resulted in the deposition of N6 on the surface of the needle. Fig. 3 compares the sorptive performance of the different composites.

The results demonstrated that raw cotton interacts with the analyte, as has been reported in the literature for other cellulosic substrates [44]. However, the N6-Cel composite exhibited a higher sorptive capacity due to hydrophobic and H-bonding interactions. As the percentage of N6 increased, the reproducibility of the extractions decreased. This effect can be ascribed to the loss of porosity when the polymer is deposited in the pores of the fibrous structure. Therefore, 0.1% N6-Cel was the selected sorbent as the extraction yield increased 2.7 times compared to raw cotton, and its reproducibility was better than that obtained using higher percentages of N6.

ATR-IR measurements were performed to confirm the deposition of N6 on the cellulose surface. Fig. 4 depicts the IR spectra of

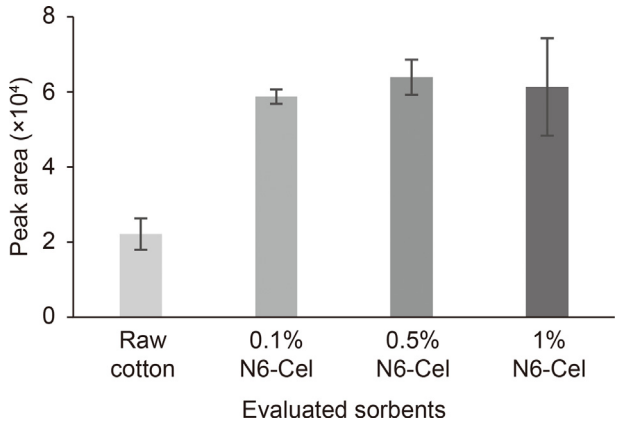

Fig. 3. Effect of the amount of polyamide in nylon 6-cellulose (N6-Cel) composite on the extraction performance. The results are obtained by the offline elution of the analyte and its analysis by direct infusion coupled to tandem mass spectrometry (DI-MS/MS), but the same trend is observed for ambient ionization mass spectrometry (AIMS) analysis. The error bars represent the standard deviation obtained for 3 replicates.

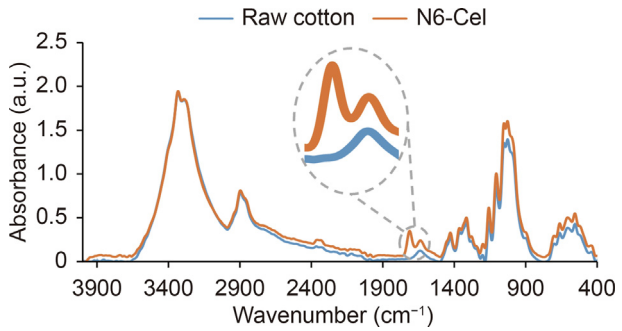

Fig. 4. Attenuated total reflection infrared spectroscopy (ATR-IR) characterization of the sorbent, where the spectra of raw cotton and nylon 6-cellulose (N6-Cel) are compared.

raw cotton in blue and N6-Cel in orange. The carbonyl (C=O) stretching band corresponding to the polyamide at 1713 cm^{-1} only appears in the N6-Cel composite, confirming a good coating.

Finally, the material was characterized by scanning electron microscopy (SEM). SEM images of raw cotton and N6-Cel, acquired at the Central Service for Research Support of the University of Cordoba, are shown in Fig. S2. As expected, raw cotton (Fig. S2A) presents a fibrous structure. This structure is mostly maintained in the N6-Cel material, where the fibers are coated by N6 (Fig. S2B), but some N6 deposits are also observed (Fig. S2C) in some places. Although the material cannot be considered completely homogeneous from the microscopic point of view, the precision of the results (Fig. 3) demonstrated that this microscopic heterogeneity is compensated at the macroscopic level. Additionally, the use of an internal standard in the validation minimizes, as will be shown later, the slight effect on the results.

3.1. Study of the variables affecting the analytical performance

To fully understand the effect of each variable on the extraction of the analyte, a univariant approach was followed using aqueous standards at pH 9.5 containing methadone at 50 μg/L. The term "study" is intentionally used here to make a difference with optimization, which is more appropriately achieved by a multivariate approach. Those variables affecting the extraction (ionic strength, number of strokes, and sample dilution) were studied using DI-MS/MS as the instrumental technique. This strategy was selected because the absolute signals (required for this evaluation) are more precise in DI-MS/MS than in AIMS, where an internal standard is always required for better reproducibility. This strategy does not affect the conclusions since these variables affect the extraction and the trends are independent of the instrumental technique used. The flow rate of the eluent/ionization agent was studied with AIMS since it directly affects its performance.

The ionic strength of the samples was studied in a range between 0% and 1% using NaCl as a model electrolyte. The results are presented in Fig. 5, where the conductance of the standards, instead of the NaCl content, is represented. This approach was selected because conductance (or any related magnitude) is the only variable that could be adjusted in a sample if necessary. As observed in Fig. 5A, the ionic strength negatively affects the extraction of the analytes. This effect can be ascribed to a restriction on the analyte diffusion due to an increased sample viscosity. Interestingly, using an IS (methadone-d_3) normalized the effect of the ionic strength in the extraction process (Fig. 5B).

The number of strokes (times that the sample flowed through the sorbent bead) was studied from 1 to 5, while higher strokes were not considered to guarantee a high sample throughput (Fig. S3A). Although 1 stroke presented the best reproducibility and 5 strokes provided the highest extraction efficiency, 3 strokes were selected as a compromise between precision and sensitivity. The results demonstrated a lower dependence of the sensitivity on the number of strokes compared to the inner coated needles [43].

As previously mentioned, the viscosity of the saliva sample can hinder the extraction of the analyte. Sample dilution before extraction is a common strategy to overcome this limitation. Sample dilution was evaluated between no dilution of the sample and a 1:5 dilution factor. Although the nondiluted samples present a higher extraction efficiency than the diluted samples (Fig. S3B), the backpressure on the needle was higher, making sample processing difficult. Therefore, a dilution factor of 1:3 was selected.

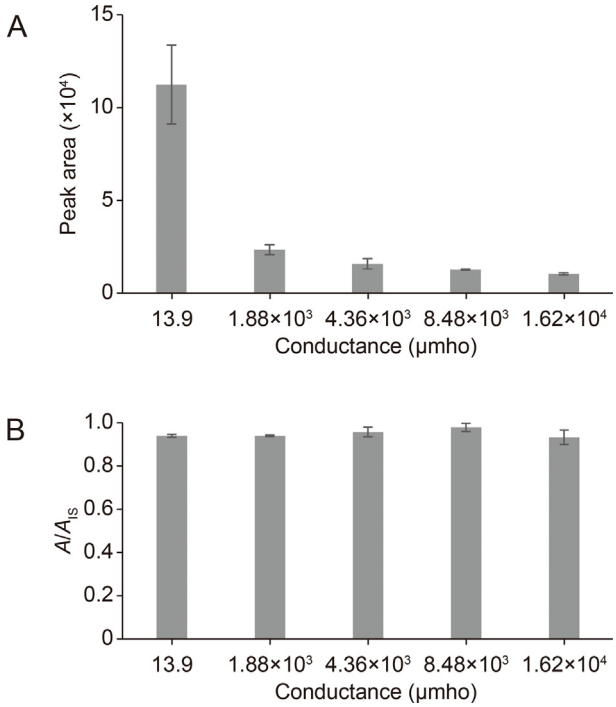

Fig. 5. Effect of the ionic strength (expressed as conductance) on the extraction performance using (A) the absolute signal of the analyte and (B) the signal normalized by the internal standard (IS). The error bars represent the standard deviation obtained for 3 replicates.

Table 1
Comparison of the proposed methods (direct infusion tandem mass spectrometry (AIMS) and direct infusion tandem mass spectrometry (DI-MS/MS)) with other reported methods in the literature.

Analytical procedure	Extraction phase	Ambient ionization	Matrix	LOD (μg/L)	LDR (μg/L)	Multianalyte method	Refs.
TS-MS	VAMS	Yes	Saliva	0.196	0.655–1000	Yes	[22]
NTME-ambient MS/MS	PDA	Yes	Saliva	1.2	4–400	Yes	[24]
TFME-LC-MS/MS	PSP	No	Urine	N.A.	0.05–100	Yes	[45]
Mag-dSPE LC-MS/MS	NP-COF@Mag-PS/DVB/GMA	No	Wastewater	0.23	1–100	Yes	[46]
SPME-DI-MS/MS	N6-WT	No	Saliva	1.5	5–250	Yes	[47]
TDU-DART	SPME-arrow	Yes	Water, air, and aerosol	N.A.	2.5–500	Yes	[48]
SPME-DI-MS/MS	N6-Cel	No	Saliva	2.2	7.3–600	No	This work
SPME-ambient MS/MS		Yes		0.3	0.9–300		

TS: touch spray; MS: mass spectrometry; VAMS: volumetric absorptive microsampling; NTME: needle trap microextraction; PDA: polydopamine; TFME: thin film microextraction; LC: liquid chromatography; PSP: porous sorptive polymer; N.A.: not available; Mag-dSPE: magnetic dispersive solid phase extraction; NP-COF: nano petal-shaped metal organic framework; PS/DVB/GMA: polystyrene-divinylbenzene-glycidylmethacrylate microsphere; SPME: solid phase microextraction; DI: direct infusion; N6-WT: nylon-6 wooden toothpick; TDU: thermal desorption unit; DART: direct analysis in real time; N6-Cel: nylon-6 cellulose.

Finally, the flow rate of the eluent/ionizing agent was studied between 30 and 60 μL/min. Lower flow rates were not considered to reduce the time of each sample analysis due to its high void time. The flow rate negatively affected the signal of the analyte (Fig. S4). Most likely, at lower flow rates, the analyte is eluted in a smaller solvent plug, thus providing better sensitivity. Consequently, the flow rate was selected as 30 μL/min because it presented the highest extraction efficiency.

3.2. Method validation

The method was validated using matrix-matched calibration curves in terms of limit of detection (LOD), limit of quantification (LOQ), and linearity. The LOQ was defined as the lowest concentration with a relative standard deviation (RSD) lower than 15%. Trueness and precision were evaluated using an independent pool of samples. The analytical method was validated using diluted blank saliva with ultrapure Milli-Q water spiked at known concentrations of methadone, using methadone-d_3 as the IS. The linear range, with $R^2 > 0.9925$, was studied at six concentration levels spanning from the LOQ to 300 μg/L by analyzing three replicates at each concentration level (Fig. S5). The precision, expressed as RSD, was calculated at three different concentrations: 3, 150, and 300 μg/L providing RSD values lower than 1.2%, 6.6%, and 9.3%, respectively. Trueness, expressed as relative recovery, was assessed at the same concentration levels, and was 90%, 109%, and 102%, respectively. These results indicate that the method is not affected by the sample matrix. As explained for the ion strength study (Fig. 5), using a deuterated labeled compound is essential to minimize the matrix effect on the results. As an example, Fig. S6 presents the chronogram and the MS transition spectrum of the analyte obtained for the analysis of a spiked sample for the AIMS method.

The feasibility of the method was also evaluated by analyzing single-blind samples. The results are compared in Fig. S7, where an average recovery of 112.9% was obtained.

As high-resolution mass spectrometers are unavailable in many laboratories, the method was also adapted to low-resolution spectrometers. In this case, the needles were eluted offline, and the eluates were analyzed by DI-MS/MS. Fig. S8 presents the chronogram and the MS transition spectrum of the analyte obtained for the analysis of a spiked sample for the DI-MS/MS method. The validation results are presented in the Supplementary data (Fig. S9).

Table 1 [22,24,45–48] compares the two different analytical methods proposed in this article with those previously reported regarding the determination of methadone by AIMS or LC-MS/MS using a microextraction procedure. Using chromatographic separation implies an enhancement in sensitivity and selectivity at the expense of a longer analysis time. In this sense, Azizi et al. [45] and Zhang et al. [46] reported LODs in the range of ng/L. Only Morato et al. [22] and the AIMS method proposed in this work achieved these LOD values in AIMS, which speeds up the sample throughput without negatively affecting the sensitivity of the method. Millán-Santiago et al. [47] reported the elimination of the chromatographic separation maintaining a commercial interface to form the ESI, while other AIMS methods [24,48] and the DI-MS/MS method proposed in this article reported acceptable LODs in the low μg/L range.

4. Conclusions

The sorption performance of inner-wall-coated HNs is somewhat restricted due to thermodynamic (the capacity is limited to a thin layer of polymer) and kinetic (the mass transference takes place through a small surface) issues. This article hypothesizes that changing the geometry of the sorptive phase to a more porous one would improve the performance. In this article, an N6-Cel composite is proposed as an alternative sorptive phase that increases the sorption capacity and permits sample flow-through. The validation results demonstrated that the new configuration allows the determination of methadone in the low μg/L range with good precision (RSD < 9.3%) and good recoveries (90%–109%). The performance of the modified HNs has also been evaluated using triple quadrupole MS. In the latter case, an extraction/offline elution procedure is needed, but this method is more versatile since it can be developed in any MS spectrometer.

Although direct analysis by AIMS would be the perfect strategy in bioanalysis, this approach is sometimes limited by the own biofluid matrix (even when a theoretically clean such as like saliva is used) and the low concentration of the analytes. A sample preparation step can solve both limitations. This additional procedure must be considered not only a tedious step but also an opportunity. In this sense, on-site extractions based on simple devices (such as those described in this article) would permit analyzing samples acquired at different locations with a single AIMS equipment. The samples could be extracted, and the sorptive phases transported to the lab for analysis. This potential, which can extend the applicability of these approaches in governmental health services, will be considered in further studies.

The current approach could be extended to other analytes by selecting the most appropriate sorbent and MS parameters. Determining isobaric compounds could be challenging due to the absence of chromatographic separation. The introduction of a second dimension, such as ion mobility spectrometry, in combination with MS is an interesting strategy to overcome this issue.

CRediT author statement

Jaime Millán-Santiago: Investigation, Methodology, Writing - Original draft preparation; **Rafael Lucena:** Writing - Reviewing and Editing, Conceptualization, Supervision, Funding acquisition; **Soledad Cárdenas:** Writing - Reviewing and Editing, Project administration, Funding acquisition.

Declaration of competing interest

The authors declare that there are no conflicts of interest.

Acknowledgments

The grant “Biopolymer substrates for the determination of opioids in biofluids by ambient mass spectrometry” (Grant No.: PID2020-112862RB-I00) funded by MCIN/AEI/10.13039/501100011033 (Feder “Una manera de hacer Europa”) is gratefully acknowledged.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jpha.2023.06.015.

References

[1] A. Coloma-Carmona, J.L. Carballo, J. Rodríguez-Marín, et al., Use and dependence on opioid drugs in the Spanish population with chronic pain: Prevalence and differences according to sex, Rev. Clin. Esp. (Barc.) 217 (2017) 315–319.
[2] Substance Abuse and Mental Health Services Administration, Behavioral Health Barometer, Vol. 5, Rockville, 2019.
[3] R.N. Xu, L. Fan, M.J. Rieser, et al., Recent advances in high-throughput quantitative bioanalysis by LC-MS/MS, J. Pharm. Biomed. Anal. 44 (2007) 342–355.
[4] E. Bassotti, G.M. Merone, A. D'Urso, et al., A new LC-MS/MS confirmation method for the determination of 17 drugs of abuse in oral fluid and its application to real samples, Forensic Sci. Int. 312 (2020), 110330.
[5] G.M. Merone, A. Tartaglia, S. Rossi, et al., Fast LC-MS/MS screening method for the evaluation of drugs, illicit drugs, and other compounds in biological matrices, Talanta Open 5 (2022), 100105.
[6] R.M. Alberici, R.C. Simas, G.B. Sanvido, et al., Ambient mass spectrometry: Bringing MS into the “real world”, Anal. Bioanal. Chem. 398 (2010) 265–294.
[7] G.A. Gómez-Ríos, M. Tascon, J. Pawliszyn, Coated blade spray: Shifting the paradigm of direct sample introduction to MS, Bioanalysis 10 (2018) 257–271.
[8] M.C. Díaz-Liñán, M.T. García-Valverde, A.I. López-Lorente, et al., Silver nanoflower-coated paper as dual substrate for surface-enhanced Raman spectroscopy and ambient pressure mass spectrometry analysis, Anal. Bioanal. Chem. 412 (2020) 3547–3557.
[9] B.S. Frey, D.E. Damon, A.K. Badu-Tawiah, The effect of the physical morphology of dried biofluids on the chemical stability of analytes stored in paper and direct analysis by mass spectrometry, Anal. Chem. 94 (2022) 9618–9626.
[10] B. Hu, P.K. So, Y. Yang, et al., Surface-modified wooden-tip electrospray ionization mass spectrometry for enhanced detection of analytes in complex samples, Anal. Chem. 90 (2018) 1759–1766.
[11] C. Ling, Q. Shi, Z. Wei, et al., Rapid analysis of quinones in complex matrices by derivatization-based wooden-tip electrospray ionization mass spectrometry, Talanta 237 (2022), 122912.
[12] D.A. Rickert, G.A. Gómez-Ríos, E. Nazdrajić, et al., Evaluation of a coated blade spray-tandem mass spectrometry assay as a new tool for the determination of immunosuppressive drugs in whole blood, Anal. Bioanal. Chem. 412 (2020) 5067–5076.
[13] A. Kasperkiewicz, J. Pawliszyn, Multiresidue pesticide quantitation in multiple fruit matrices via automated coated blade spray and liquid chromatography coupled to triple quadrupole mass spectrometry, Food Chem. 339 (2021), 127815.
[14] S. Jackson, D.J. Swiner, P.C. Capone, et al., Thread spray mass spectrometry for direct analysis of capsaicinoids in pepper products, Anal. Chim. Acta 1023 (2018) 81–88.
[15] D.J. Swiner, S. Jackson, G.R. Durisek 3rd, et al., Microsampling with cotton thread: Storage and ultra-sensitive analysis by thread spray mass spectrometry, Anal. Chim. Acta 1082 (2019) 98–105.
[16] S. Jackson, B.S. Frey, M.N. Bates, et al., Direct differentiation of whole blood for forensic serology analysis by thread spray mass spectrometry, Analyst 145 (2020) 5615–5623.
[17] K. Usui, H. Kobayashi, Y. Fujita, et al., An ultra-rapid drug screening method for acetaminophen in blood serum based on probe electrospray ionization-tandem mass spectrometry, J. Food Drug Anal. 27 (2019) 786–792.
[18] W. Huang, W. Shao, Y. Ji, et al., Covalent organic framework-based solid phase microextraction coupled with electrospray ionization mass spectrometry for sensitive screening and quantitative evaluation of carbamazepine and its metabolite in mice, Talanta 243 (2022), 123341.
[19] G.A. Gómez-Ríos, N. Reyes-Garcés, B. Bojko, et al., Biocompatible solid-phase microextraction nanoelectrospray ionization: An unexploited tool in bioanalysis, Anal. Chem. 88 (2016) 1259–1265.
[20] R. Narayanan, X. Song, H. Chen, et al., Teflon spray ionization mass spectrometry, J. Am. Soc. Mass Spectrom. 31 (2020) 234–239.
[21] T.P.P. Mendes, G.S. Lobón, L.A.S. Lima, et al., Mass spectrometry-based biosensing using pencil graphite rods, Microchem. J. 164 (2021), 106077.
[22] N.M. Morato, V. Pirro, P.W. Fedick, et al., Quantitative swab touch spray mass spectrometry for oral fluid drug testing, Anal. Chem. 91 (2019) 7450–7457.
[23] G.L. de Araújo, D.V.A. de Aguiar, I. Pereira, et al., Polypyrrole-coated needle as an electrospray emitter for ambient mass spectrometry, Anal. Methods 12 (2020) 3235–3241.
[24] C. Vejar-Vivar, J. Millán-Santiago, C. Mardones, et al., Polydopamine inner wall-coated hypodermic needle as microextraction device and electrospray emitter for the direct analysis of illicit drugs in oral fluid by ambient mass spectrometry, Talanta 249 (2022), 123693.
[25] J. Feng, M. Sun, S. Han, et al., Polydopamine-coated cotton fibers as the adsorbent for in-tube solid-phase microextraction, J. Sep. Sci. 42 (2019) 2163–2170.
[26] C. Khamkhajorn, S. Pencharee, J. Jakmunee, et al., Smartphone-based colorimetric method for determining sulfites in wine using a universal clamp sample holder and microfluidic cotton swab-based analytical device, Microchem. J. 174 (2022), 107055.
[27] M.C. Prieto-Blanco, S. Peñafiel Barba, Y. Moliner-Martínez, et al., Footprint of carbonyl compounds in hand scent by in-tube solid-phase microextraction coupled to nano-liquid chromatography/diode array detection, J. Chromatogr. A 1596 (2019) 241–249.
[28] W. Han, N. Shi, X. Wang, et al., Application of natural cotton fibers as an extraction sorbent for the detection of trans-resveratrol in adulterated peanut oils, Food Chem. 339 (2021), 127885.
[29] J. Feng, S. Han, X. Ji, et al., A green extraction material—natural cotton fiber for in-tube solid-phase microextraction, J. Sep. Sci. 42 (2019) 1051–1057.
[30] X. He, G. Zhu, Y. Zhu, et al., Facile preparation of biocompatible sulfhydryl cotton fiber-based sorbents by “thiol-ene” click chemistry for biological analysis, ACS Appl. Mater. Interfaces 6 (2014) 17857–17864.
[31] X. He, G. Zhu, W. Lu, et al., Nickel(II)-immobilized sulfhydryl cotton fiber for selective binding and rapid separation of histidine-tagged proteins, J. Chromatogr. A 1405 (2015) 188–192.
[32] A.R. Ghiasvand, N. Heidari, S. Abdolhosseini, et al., Evaluation of a cooling/heating-assisted microextraction instrument using a needle trap device packed with aminosilica/graphene oxide nanocomposites, covalently attached to cotton, Analyst 143 (2018) 2632–2640.
[33] M. Ghani, K. Haghdoostnejad, Woven cotton yarn-graphene oxide-layered double hydroxide composite as a sorbent for thin film microextraction of nonsteroidal anti-inflammatory drugs followed by quantitation through high performance liquid chromatography, Anal. Chim. Acta 1097 (2020) 94–102.
[34] Z. Liu, W. Zhou, C. Wang, et al., Cotton thread modified with ionic liquid copolymerized polymer for online in-tube solid-phase microextraction and HPLC analysis of nonsteroidal anti-inflammatory drugs, J. Sep. Sci. 43 (2020) 2827–2833.
[35] M.T. García-Valverde, M.L. Soriano, R. Lucena, et al., Cotton fibers functionalized with β-cyclodextrins as selectivity enhancer for the direct infusion mass spectrometric determination of cocaine and methamphetamine in saliva samples, Anal. Chim. Acta 1126 (2020) 133–143.
[36] W. Li, R. Wang, Z. Chen, Zr-based metal-organic framework-modified cotton for solid phase micro-extraction of non-steroidal anti-inflammatory drugs, J. Chromatogr. A 1576 (2018) 19–25.
[37] N. Mehmandost, M.L. Soriano, R. Lucena, et al., Recycled polystyrene-cotton composites, giving a second life to plastic residues for environmental remediation, J. Environ. Chem. Eng. 7 (2019), 103424.
[38] M.T. García-Valverde, R. Lucena, S. Cárdenas, et al., In-syringe dispersive micro-solid phase extraction using carbon fibres for the determination of chlorophenols in human urine by gas chromatography/mass spectrometry, J. Chromatogr. A 1464 (2016) 42–49.
[39] D. Chen, M. Zhang, X. Bu, et al., In-syringe cotton fiber-supported liquid extraction coupled with gas chromatography-tandem mass spectrometry for the determination of free 3-mono-chloropropane-1,2-diol in edible oils, J. Chromatogr. A 1673 (2022), 463081.
[40] R.A. Lugo, K.L. Satterfield, S.E. Kern, Pharmacokinetics of methadone, J. Pain Palliat. Care Pharmacother. 19 (2005) 13–24.
[41] S. Jickells, A. Negrusz, Clarke's analytical forensic toxicology, Ann. Toxicol. Anal. 20 (2008) 233–234.
[42] L. Brunton, J. Lazo, K. Parker, Goodman & Gilman's The Pharmacological basis of therapeutics, eleventh ed., McGraw-Hill, New York, 2005.
[43] C. Vejar-Vivar, M.T. García-Valverde, C. Mardones, et al., Polydopamine coated hypodermic needles as a microextraction device for the determination of

tricyclic antidepressants in oral fluid by direct infusion MS/MS, RSC Adv. 11 (2021) 22683–22690.
[44] M. Cirrincione, R. Lucena, M. Protti, et al., Potential of hydrophobic paper-based sorptive phase prepared by *in situ* thermal imidization for the extraction of methadone from oral fluid samples, J. Chromatogr. A 1675 (2022), 463166.
[45] A. Azizi, F. Shahhoseini, C.S. Bottaro, Biological matrix compatible porous thin film for quick extraction of drugs of abuse from urine prior to liquid chromatography-mass spectrometry analysis, Talanta 241 (2022), 123264.
[46] S. Zhang, H. Cui, Y. Zhao, et al., Preparation and application of nano petal-shaped covalent organic frameworks modified polystyrene-divinylbenzene-glycidylmethacrylate microspheres for the extraction of illicit drugs from wastewater, J. Chromatogr. A 1682 (2022), 463505.
[47] J. Millán-Santiago, M.T. García-Valverde, R. Lucena, et al., Polyamide-coated wooden tips coupled to direct infusion mass spectrometry, a high throughput alternative for the determination of methadone, cocaine and methamphetamine in oral fluid, Microchem. J. 162 (2021), 105843.
[48] R.V. Emmons, E. Gionfriddo, Minimizing transient microenvironment-associated variability for analysis of environmental anthropogenic contaminants via ambient ionization, Sci. Total Environ. 775 (2021), 145789.