Electronic Supplementary Material

Microbeads from recycled polystyrene yogurt cups for the in-syringe micro solid-phase extraction of four opioids from environmental and biological samples

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Optimization of the synthesis of the polymeric microbeads

For the optimization of the emulsion solidification parameters, a reasonable range and some representative values for each involved variable were selected, i.e., volume of the polymeric phase (mL), concentration of the PS in the organic phase (w/v %), ratio of the organic and aqueous phase (o:w) and ratio of the saturated NaCl and sodium dodecyl sulphate solutions in the aqueous phase (NaCl:SDS). A 3 x 4 factorial design, comprising 3 levels and 4 factors (3^4) was employed, leading to the 81 syntheses procedures of PS microspheres, to which a qualitative score was attributed. The final procedure was selected being the one that returns the most satisfactory microbeads, from a morphological point of view, with the lowest consumption of solvents. Table S1 depicts the studied variables and the selected values for each of them.

Table S1. Selected parameters and explored relative values for the optimization study of the synthetic procedure. Underlined values are the selected ones.

Parameter	Lower value	Intermediate value	Higher value
Volume of the organic polymeric phase (mL)	<u>0.25</u>	1	2
Concentration of the organic polymeric phase (w/v %)	1	<u>2.5</u>	5
Ratio of the organic and aqueous phase (o:w)	1:1	1:4	<u>1:7</u>
Ratio of NaCl saturated solution and Na-dodecyl sulphate 1 % solution in the aqueous phase (NaCl:SDS)	5:2	1:1	<u>2:5</u>

Optimization of the extraction procedure

Variables related to the extraction process were optimized with one experimental design and different one-variable-at–a–time analysis (OVAT) studies, grouping variables on the basis of their possible correlations. Firstly, a three-factor three level Box–Behnken design (BBD) methodology was performed, selecting loading volume (X_1), concentration of the loaded spiked solution (X_2) and PS microbeads amount (X_3) as potentially critical variables affecting the extraction of analytes. X_1 ranged between 1 to 5 mL, being the higher limit imposed by the volume of the syringe itself, while the lower limit considers the necessity to maximise the pre-concentration factor. For the X_2 variable, a range of values between 50 to 250 μg L^{-1} was selected. The choice for high concentration values is explained by the aim of defining load quantities limit for the analytes in competitive conditions, determining saturation limits and breakthrough quantities for each of them. For X_3, since the scalability of the synthetic procedure was not studied, the number of synthetic products loaded in the same syringe was chosen as the quantitative parameter, related to the sorbent amount. X_3 was ranged between 1 and 3 loaded synthetic products. The conditions for the other variables were pH 9 and elution with 300 μL of methanol. pH values around 9 were selected, since this value is higher or quite

similar to the pKa of each considered analyte (**Table S2**), promoting interactions between analytes and PS under ionic suppression conditions.

The analytical responses (Y_i) included in the design were the average recovery values. The simultaneous monitoring of X_1 and X_2 allows to control the dependence of the extraction efficiency against the loaded quantity of analytes ($X_1 \cdot X_2$). A similar study permits to define ideal and maximum load quantity for each analyte, being this parameter of essential importance to establish the volume of loaded sample and the dilution level for a selected real sample. Finally, an optimum value for X_3 was established with the experimental model, while simultaneously monitoring the load quantity of the analyte and the saturation limit.

The number of tests of the BBD was calculated using the following equation:

$$N = 2k(k-1) + Cp$$

where N is the number of experiments, k is the number of factors, and Cp is the number of the central points. A total of 30 experiments were performed, carrying out the design by duplicate and including 3 replicates of the central point for the assessment of the test error. The design was built after selecting the range of factors (high and low). Additionally, the multiple linear regression for each response, including linear, quadratic, and interacting terms of independent variables were evaluated following the quadratic polynomial equation:

$$Y = \beta_0 + \sum \beta_i \chi_i + \sum \beta_{ii} \chi_i^2 + \sum \beta_{ij} \chi_i \chi_j + \varepsilon$$

where Y refers to the analytical response; β_0 is the constant coefficient; β_i, β_{ii} and β_{ij} are the regression coefficients of the model; χ_i and χ_j represent the independent variables; and ε is the residual error associated to the experiments.

A desirability function approach was used to find the optimal extraction conditions to maximize the extraction efficiency of the analytes. The global desirability of the experiment (D) is defined by the following equation:

$$D = (d_1(Y_1)d_2(Y_2) \cdots d_n(Y_n))^{1/n}$$

where n is the number of responses studied in each experiment and $d_i(Y_i)$ is the individual desirability of each response in the experiment, calculated as follows:

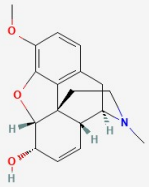
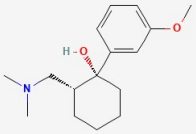
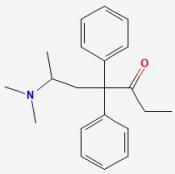
$$(d_i) = \frac{Y_i - Y_{min}}{Y_{max} - Y_{min}}$$

For each response, $Y_i(x)$, a desirability function $d_i(Y_i) = 1$ represents a completely desirable value obtained by the response surface methodology. The statistical analysis was performed using the StatGraphics Centurion XVI (Stat Point Inc., Herndon, VA, USA) software.

In addition, pH was optimized by performing extractions in the range between 3 and 10 at a concentration of 50 μg L^{-1}, loading 1 PS synthesis and passing through 5 mL of aqueous sample. In the same way, dependence of extraction recoveries was studied against the ionic strength, with solutions with conductance ranging from 16.2 μMHO to 0.32 MHO. Electrolyte concentration was controlled by dissolving different amounts of sodium chloride in MilliQ water spiked samples.

Parameters related to the desorption step of the extraction procedure were optimized with OVAT studies. The composition of the eluent phase was optimized by choosing the most effective option from either bare methanol or with acidic or basic organic modifiers (i.e., formic acid and ammonia), at a 1% v/v concentration. Lastly, the volume of the desorption methanolic phase was evaluated, performing the whole extraction with different elution volumes (ranging from 200 to 500 μL) and working in triplicates.

Table S2 Structure and relevant chemical parameters of the selected opioids. Multiple reaction monitoring (MRM) transitions of the analytes.

Common name	Structure	Exact mass (u)	logP	pKa	1st transition (m/z)[a]	2nd transition (m/z)[a]
Morphine		285.1365	0.8	8.21	286.1/201.1	286.1/157
Codeine		299.1521	1.1	8.20	300.1/199	-
Tramadol		263.1885	2.6	9.23	264.2/58.2	264.2/246.1
Methadone		309.2093	3.9	8.95	310.2/265.1	310.2/105.1

[a] The first MRM transition is the most intense one (quantifier), while the other is the second most intense (qualifier).

Data obtained from PubChem [Internet]. Bethesda (MD): National Library of Medicine (US), National Center for Biotechnology Information; 2004. PubChem Compound Summary for CID 5288826, Morphine; [cited 2024 Mar. 4]. Available from: https://pubchem.ncbi.nlm.nih.gov/compound/Morphine; PubChem Compound Summary for CID 5284371, Codeine; [cited 2024 Mar. 4]. Available from: https://pubchem.ncbi.nlm.nih.gov/compound/Codeine; PubChem Compound Summary for CID 33741, Tramadol; [cited 2024 Mar. 4]. Available from: https://pubchem.ncbi.nlm.nih.gov/compound/Tramadol; PubChem Compound Summary for CID 4095, Methadone; [cited 2024 Mar. 4]. Available from: https://pubchem.ncbi.nlm.nih.gov/compound/Methadone.

TGA characterization of the synthetic PS microspheres

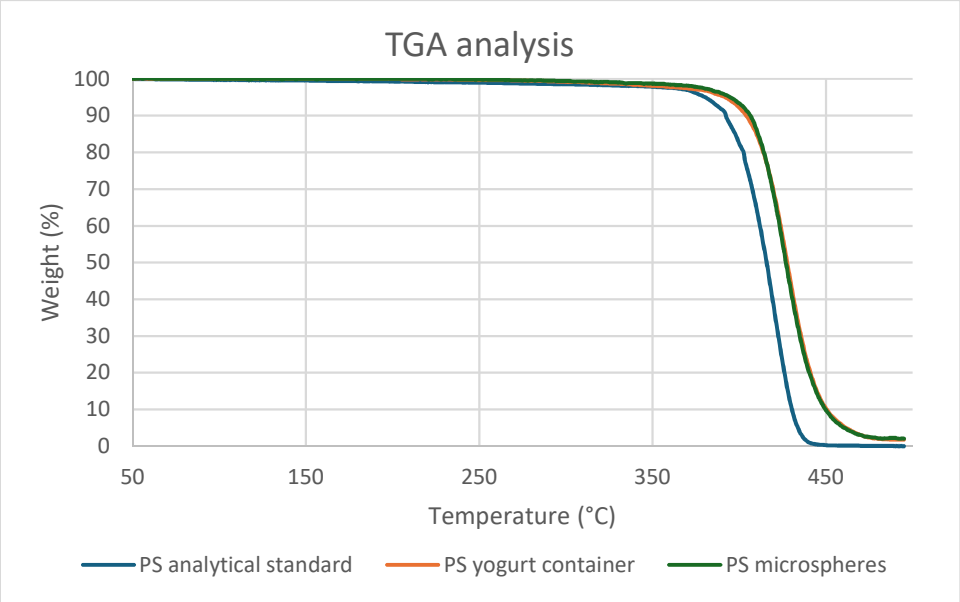


Fig. S1. Thermogravimetric analysis (TGA) curves for polystyrene (PS) analytical standard, commercial PS (recovered from a yogurt container), and synthesized microspheres. All samples exhibit the characteristic thermal degradation profile of polystyrene. Experimental conditions: nitrogen atmosphere (20 mL min^{-1}), temperature range 50–500 °C, heating rate 10 °C min^{-1}.

ATR-IR characterization of the modified paper

The FTIR spectrum of polystyrene exhibits a characteristic absorption band in the range at around 3020 cm^{-1} attributed to =C–H stretching vibrations associated with the aromatic ring. Peaks observed at 2918 cm^{-1} and 2843 cm^{-1} correspond to the symmetric and asymmetric stretching vibrations of CH_2 groups. Additionally, absorption bands at 1604 cm^{-1}, 1490 cm^{-1}, and 1445 cm^{-1} are indicative of the C=C stretching vibrations within the benzene ring. A band at 1024 cm^{-1} is associated with C–O stretching, while the bands between 680 and 800 are attributed to the rocking of C–H of the benzene ring.

On the other hand, the FTIR spectrum of cellulose displays an absorption band at 3345 cm^{-1}, attributed to the stretching vibrations of hydroxyl groups. Bands observed at 2910 cm^{-1} and 1340 cm^{-1} correspond to the stretching and deformation vibrations of the C–H group within the glucose units, respectively. The signal at 1040 cm^{-1} is assigned to the -C–O- group associated with secondary alcohol and ether functionalities present in the cellulose backbone.

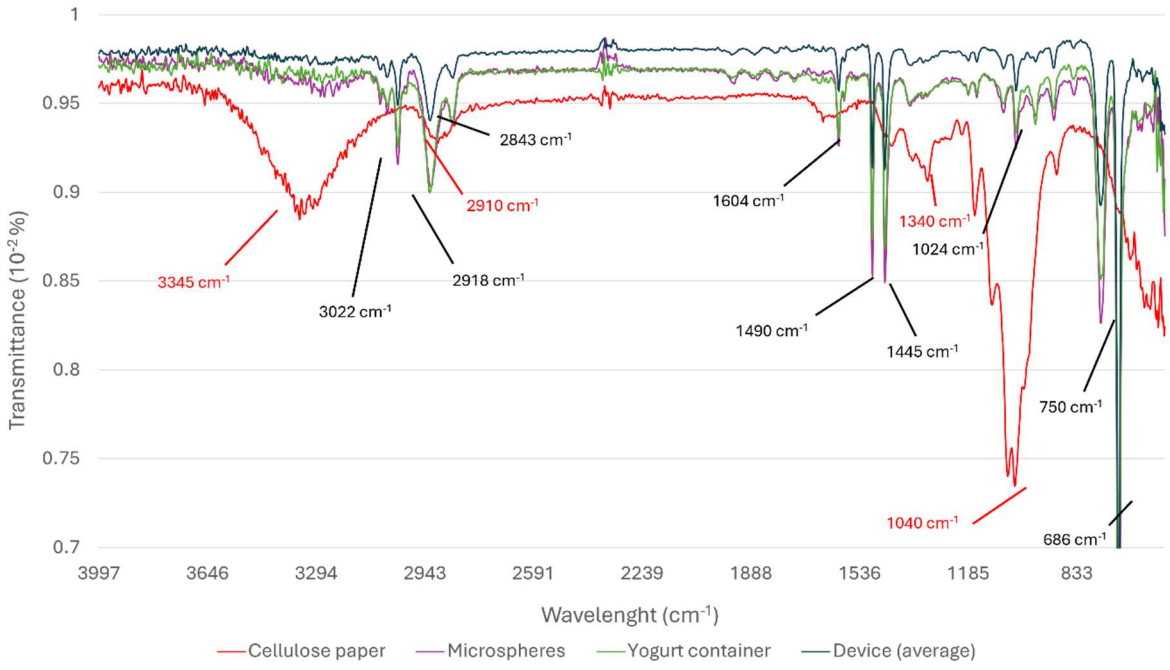


Fig. S2 ATR-FTIR spectra of cellulose paper, with and without functionalization, PS microspheres and the original waste polymeric material.

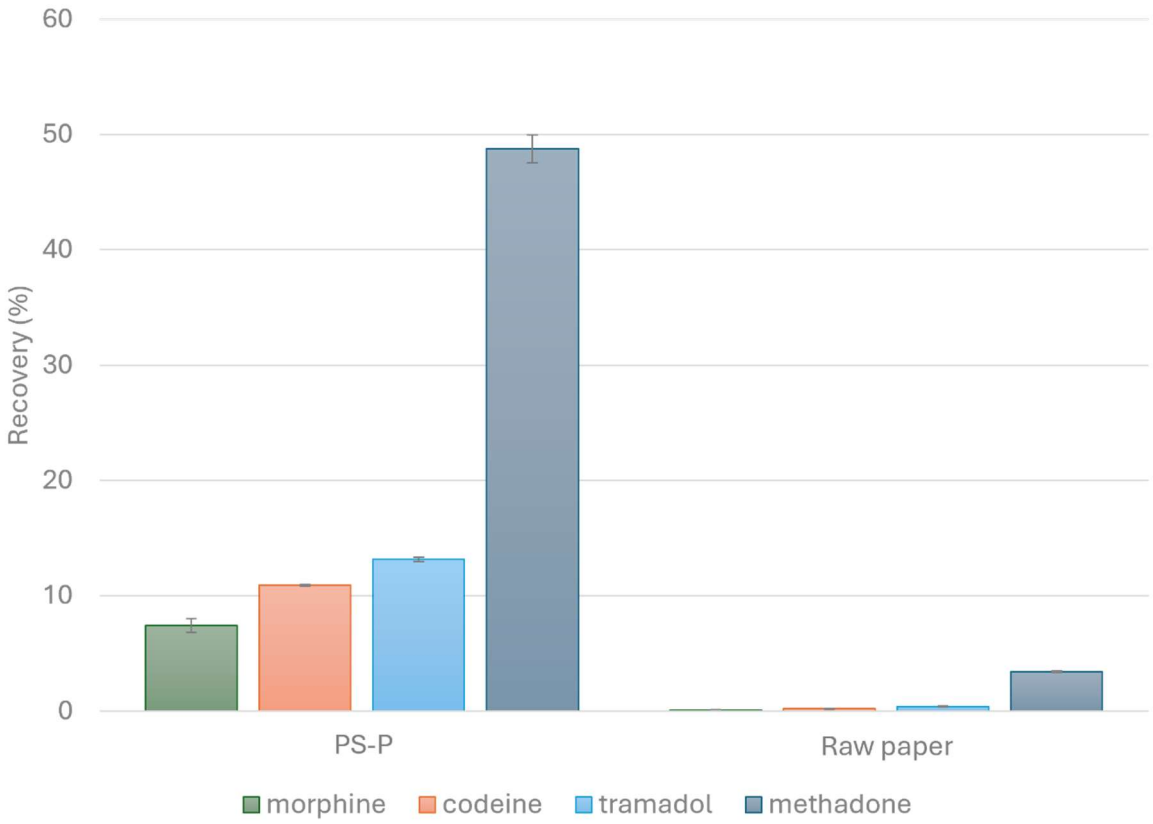


Fig. S3 Comparison between pristine paper and PS-P in terms of extraction performances (average recoveries on three replicates) at the same experimental conditions (5 mL of a 500 µg L^{-1} loading solution at pH 9, 300 µL of MeOH as the elution solvent).

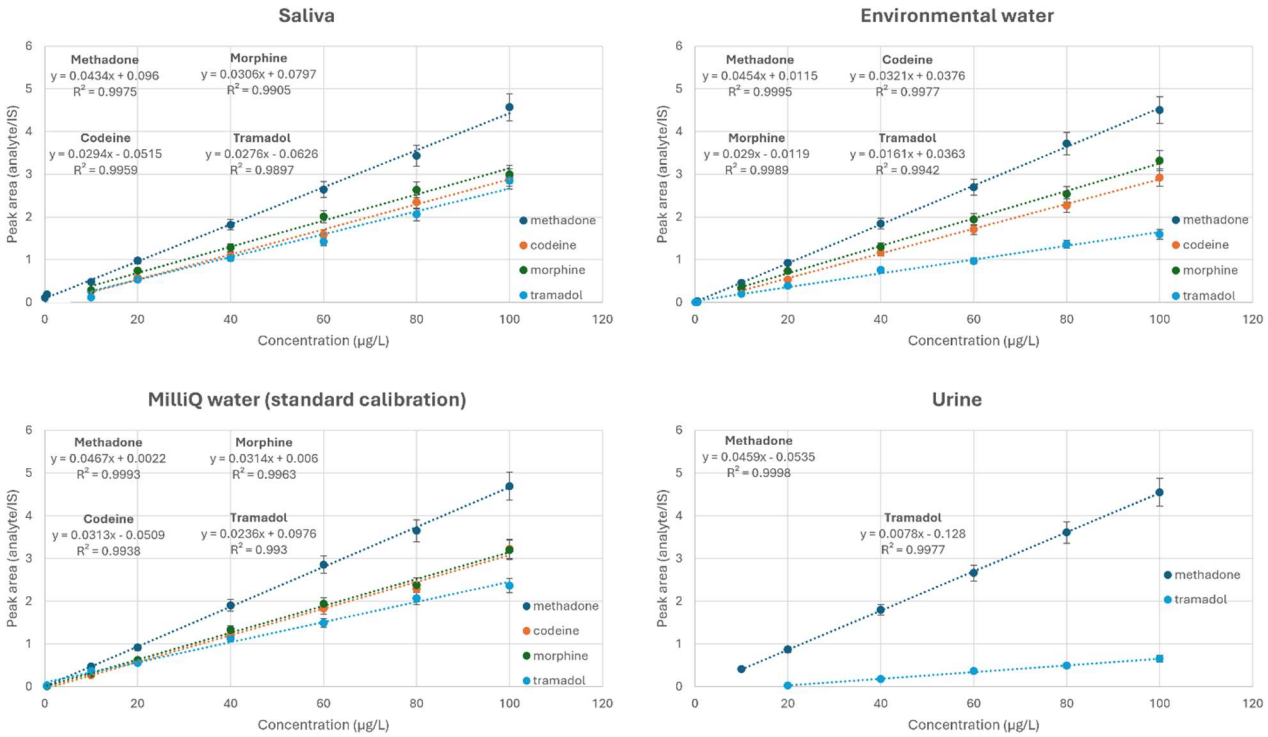


Fig. S4 Matrix matched calibration curves for the analytes in solvent (MilliQ water) and in three different complex matrices are reported in the graphs. The concentration range is included between 0.1 and 100 µg mL^{-1}, taking as the lowest point the LOQ of the analyte in the specific extraction medium. R^2 and linear model equations, extrapolated by the experimental data, are also displayed.

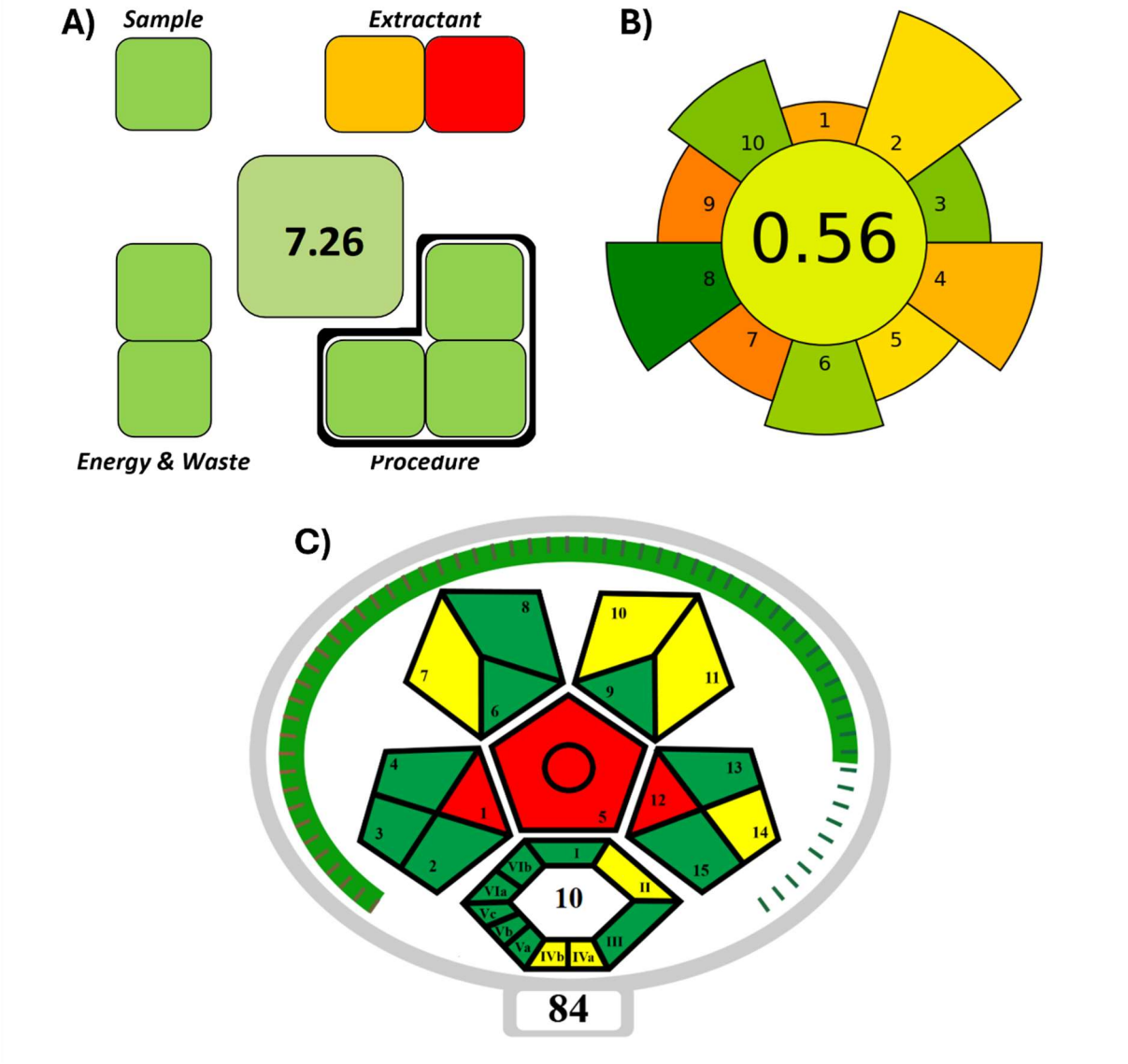


Fig. S5 Evaluation of the sustainability of the proposed method using **A)** Sample preparation metric of sustainability (SPMS), **B)** Analytical greenness metric for sample preparation (AGREEprep) software and **C)** ComplexMoGAPI total scoring system and software.

Table S3 Comparison of the proposed analytical method with other articles reporting the determination of methadone, codeine, morphine and/or tramadol in biological fluids.

Instrumental technique	Extraction Technique	LOQ (µg L^{-1})				Extraction Time (min)	Chromatographic Separation	Matrix	Recycling Material	References
		ME	CO	MO	TRA					
HPLC-UV	DES-LPME	-	1.5	1.5	-	< 2	yes	blood	no	(Binjawhar et al., 2024)
GC-MS	MSPE-DLLME	0.10	-	-	0.11	12	yes	urine	no	(Isazad et al., 2022)
CD-IMS	EME-SFME	2.5	-	-	2.5	17.5	no	urine	no	(Behpour et al., 2022)
DI-MS/MS	TFME	5	5	5	5	> 90	no	saliva	no	(Pedraza-Soto et al., 2024)
HPLC-UV	DMSPE	-	0.3	-	0.15	> 20	yes	saliva	no	(Soltani et al., 2023)
HPLC-UV	IT-G-EME	-	10	-	15	5	yes	plasma	no	(Rahbarian et al., 2023)
GC-MS/MS	MEPS	-	1	1	1	NS	yes	urine	no	(Simão et al., 2022)
DI-MS/MS	TFME	10	-	-	10	> 180	no	saliva	no	(Calero-Cañuelo et al., 2023)
DI-MS/MS	ISµSPE	0.02-2.4	5.9-6.5	5.6-9.1	0.1-14.5	< 5	no	urine, saliva, water	yes	This work

LOQ, limit of quantification.

Pre-treatment: DES-LPME, deep eutectic solvent based liquid phase microextraction; MSPE-DLLME, magnetic-dispersive solid-phase extraction coupled with dispersive liquid–liquid micro-extraction; EME-SFME, electromembrane extraction and slug flow microextraction; TFME, thin film-microextraction; DMSPE, dispersive micro-solid phase extraction; IT-G-EME, in-tube gel electromembrane extraction; MEPS, microextraction by packed sorbent; ISµSPE, in-syringe micro solid-phase extraction.

Instrumental technique: HPLC-UV, high-performance liquid chromatography ultra-violet detection; GC-MS, gas chromatography-mass spectrometry; CD-IMS, corona discharge-ion mobility spectrometry; DI-MS/MS, direct infusion-tandem mass spectrometry; GC-MS/MS, gas chromatography-tandem mass spectrometry.

References

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