

Supporting information

Investigating the drug abuse in hair samples with electrospun nanostructure PEDOT-CNT along with EA-IT-SPME

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Characterization equipment

Fourier transform infrared spectra of the products in the 400–4000 cm^{-1} wave number range were measured by a Thermo Nicolet NEXUS FT-IR spectrophotometer (Madison, WI, USA, www.thermoscientific.com) at a resolution of 4 cm^{-1} using KBr pellet. Particle size and morphology of the synthesized nanocomposite was investigated by field emission scanning electron microscope (FE-SEM, RMRC MIRA3 TESCAN, Kohoutovice, Czech Republic, <https://www.tescan.com>) equipped with an energy-dispersive X-ray (EDX) spectrometer.

Chromatographic conditions

Varian HPLC (Walnut Creek, CA, USA) with a Varian 9050 UV-Vis detector were used for separation and detection of the drugs. A six-port Cheminert HPLC valve from Valco Instruments (Houston, TX, USA) with a 20.0 μL sample loop and a 9012 HPLC pump were other equipment used in analysis. The analyte separations were carried on an ODS-3 column (250 mm \times 4.6 mm, with a particle size of 5.0 μm) from Hector Company (Daejeon, Korea) under isocratic elution conditions at a flow rate of 1.0 mL min^{-1} . The mobile phase contained methanol-acetonitrile-

phosphate buffer (pH 6.0; 20 mM) (16:12:72). The detector wavelength was adjusted to 210 nm. Chromana software (version 3.6.4), developed by Marjaan Khatam (Tehran, Iran), was utilized for data recording and quantification analysis.

Optimization

pH and voltage

The ionization amount of opium in water is affected by pH, which can affect the extraction performance. In addition, the applied potential for extraction can be limited by the pH of the sample solution. Therefore, the applied potential and pH of the sample solution were optimized simultaneously (Fig. S2A). The effect of pH on the extraction efficiency of opium and the potential difference was investigated between 3 and 11 and between 0.0 V and -1.5 V, respectively. According to the pK_a (10.2-13.2) and pK_b (8.7-9.3) values of drugs presented in Table S1, at pH less than 9, the analytes are in a cationic state and migrate towards the cathode by applying negative potential. The best extraction efficiency was obtained at pH = 5 and the potential of -1.2 V (Fig. S2B and C). At this pH, the extraction efficiency increased by decreasing the potential to -1.2V and at more negative potentials, it remained almost constant (Fig. S2C). At more acidic pH (less than 5), the extraction efficiency decreased due to the increased competition between hydronium ions and analytes. At a potential of -1.2 V, the extraction efficiency decreased with increasing pH from 5 to 11. This may be due to the neutralization and charge reduction of the analytes at higher pHs (Fig. S2B). If the potential is not applied, an increasing trend is observed in the extraction at pHs between 5 and 11 (Fig. S2A) because a large part of the analytes in this range of pH are in their neutral state and their extraction occurs in the molecular form. At pH = 11, the extraction efficiency has a slight decrease by applying the potential from 0.0 to -1.5 V. According to the pK_a of the analytes, a

small part of them have a negative charge at this pH and are removed from the negative charge sorbent (cathode) by applying negative potentials. The results showed that the responses obtained in the absence of potential were smaller than those obtained in the presence of applying the potential. Hence, by using EA-IT-SPME, both extraction efficiency and selectivity for ionic analytes (drugs) are clearly improved. In addition, according to the results, this conductive polymer has the ability to adsorb ionic and molecular forms of analytes.¹

Extraction time

To investigate the equilibrium time for the distribution of analytes between the sorbent and the solution, adsorption durations between 5.0 and 25.0 min were chosen. According to Fig. S3A, the extraction efficiencies increased from 5.0 to 15.0 min and then stabilized. Therefore, the EA-IT-SPME reached the equilibrium in 15.0 min, which is the optimal time. In a comparison between methods EA-IT-SPME and IT-SPME for the extraction of analytes under the same conditions (Fig. S3B), it can be seen that, unlike EA-IT-SPME, which has an extraction time of 15.0 min, IT-SPME has not reached the equilibrium up to 25.0 min. In this comparison, the positive and significant effect of applying the electrical potential on reducing the extraction time is clearly evident.

Effect of ionic strength

The effect of ionic strength was investigated by 0.0 to 15.0% w/v solutions of NaCl. As shown in Fig. S3C, with increasing salt percentage, the extraction efficiencies decrease due to the interference effect of salt's cations and salting-in phenomena. Therefore, the rest of the experiments were performed in the absence of salt.

Sample flow rate

The effect of sample flow rate on the extraction efficiency of analytes was optimized in the range of 1.0-0.3 mL min⁻¹. As seen in Fig. S3D, the extraction efficiency of analytes increased with the increase of the extraction flow rate from 1.0 to 2.5 mL min⁻¹. The reason can be attributed to the contact of a larger volume of the sample solution with the sorbent by increasing the flow rate. At higher extraction speeds, the extraction efficiency was reduced. Therefore, 2.5 mL min⁻¹ was chosen as the optimal extraction flow rate. At this speed, the back pressure caused by the high flow rate was not observed. However, since the packed in-tube SPME was used, the decrease in extraction efficiency at higher speeds can be related to the occurrence of back pressure and the decrease in mass transfer from the sample solution to the surface of the sorbent.²

Effect of type of desorption solvent

Desorption solvent play an important role in the recovery of analytes. Therefore, different solvents such as ethanol, acetonitrile, acetone, methanol, and methanol containing 10% w/v of NaCl and 10% w/v of ammonium acetate were used to wash the extracted analytes from the sorbent. As shown in Fig. S4A, it is clear that salt-containing methanol has a higher extraction recovery because the salt ions compete with the analytes and cause their better desorption. In addition, the presence of salt ions helps to improve the conductivity of the organic solvent and makes it easier to apply the potential in the desorption step. Ammonium acetate has better compatibility with HPLC and increases the viscosity of the desorption solvent less than NaCl. Therefore, methanol containing 10% w/v of ammonium acetate was selected as the optimal solvent for desorption.

Effect of desorption applied potential

By applying the appropriate potential in the desorption step, high desorption efficiencies can be achieved along with minimizing the memory effects. Therefore, applying the potential in the desorption step is an effective parameter for increasing the efficiency of the proposed method. In the present work, the effect of desorption potential on the extraction efficiency of the analytes was investigated in the range between 0.0 and 1.5 V. As shown in Fig. S4B, the potential of 1.2 V was chosen as the optimal value. At higher potentials, the extraction efficiencies remained unchanged.

Desorption solvent volume

An adequate volume of desorption solvent is necessary to achieve nearly complete desorption of analytes, reducing the memory effect and washing time between each extraction. Therefore, the effect of desorption solvent volume in the range of 50.0-350.0 μL was studied (Fig. S4C). The extraction efficiency increased up to 250.0 μL and was constant after that. Therefore, 250.0 μL was chosen as the optimal volume. Since the volume of the desorption solvent was fixed at a certain value by performing the solvent evaporation process after desorption, increasing the volume of the desorption solvent did not decrease the preconcentration factor.

Desorption solvent flow rate

The desorption solvent flow rates were explored in the range of 20.0-50.0 $\mu\text{L min}^{-1}$. As shown in Fig. S4D, the extraction efficiencies were almost constant up to 30.0 $\mu\text{L min}^{-1}$, then decreased at a higher flow rates ($> 50.0 \mu\text{L min}^{-1}$), which could be attributed to a significant decrease in the effective contact time between solvent and sorbent. Therefore, 30.0 $\mu\text{L min}^{-1}$ was used as the optimal desorption flow rate.³

The schematic procedure of EA=IT-SPME-HPLC was shown in Fig. S6.

References

- 1 S. S. Hosseiny Davarani, S. Nojavan, R. Asadi and M. H. Banitaba, *J. Sep. Sci.*, 2013, **36**, 2315–2322.
- 2 S. S. Nasrollahi, Y. Yamini and M. Shamsayei, *J. Sep. Sci.*, 2021, **44**, 1122–1129.
- 3 H. Asiabi, Y. Yamini and M. Shamsayei, *Talanta*, 2018, **185**, 80–88.

Table S1 Chemical structures, abbreviations, pK_a, and log P of the abuse drugs.

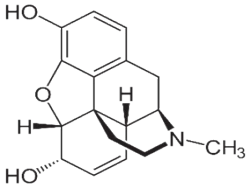
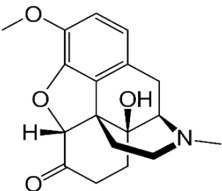
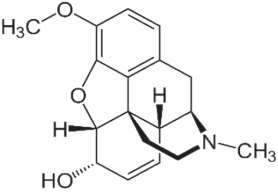
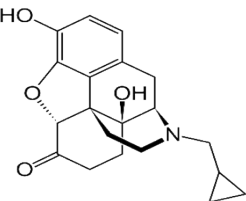
Analyt structure	Component name	Abbreviation	pK _a (Strongest Acidic)	pK _a (Strongest Basic)	Log <i>P</i>
 The structure shows the morphine molecule with a morphine ring system, a hydroxyl group at the 3-position, and a methyl group on the nitrogen atom.	Morphine	MOR	10.26	9.12	0.89
 The structure shows the oxycodone molecule with a morphine ring system, a hydroxyl group at the 3-position, a ketone group at the 6-position, and a methyl group on the nitrogen atom.	Oxycodone HCl	OCD	13.57	8.77	1.04
 The structure shows the codeine molecule with a morphine ring system, a hydroxyl group at the 3-position, a methoxy group at the 3-position, and a methyl group on the nitrogen atom.	Codeine	COD	13.78	9.19	1.19
 The structure shows the naltrexone molecule with a morphine ring system, a hydroxyl group at the 3-position, a ketone group at the 6-position, and a propyl group on the nitrogen atom.	Naltrexone	NAL	10.21	9.35	1.27

Table S2 Determination of the drugs in the hair samples by EA-IT-SPME-HPLC-UV.

Samples	Analytes	C _{initial} ($\mu\text{g L}^{-1}$)	C _{add} ($\mu\text{g L}^{-1}$)	C _{found} ($\mu\text{g L}^{-1}$)	RSD %	RR %
Hair 1	MOR	Nd*	10.0	9.8	4.0	98.4
			500.0	497.5	3.8	104.1
			1000.0	989.5	3.1	98.9
	OCD	Nd	10.0	9.6	3.9	96.4
			500.0	494.3	3.5	95.2
			1000.0	986.0	3.2	98.6
	COD	Nd	10.0	9.5	6.4	95.7
			500.0	489.7	5.6	101.8
			1000.0	992.1	5.4	99.2
	NAL	Nd	10.0	9.5	6.1	95.1
			500.0	497.2	5.2	99.4
			1000.0	1007.3	5.1	100.7
Hair 2	MOR	87.4 (0.2 ₁ $\mu\text{g g}^{-1}$)	50.0	130.0	4.1	91.5
			100.0	177.8	3.7	89.0
			150.0	235.2	3.5	97.4
	OCD	Nd	50.0	45.6	6.7	91.2
			100.0	93.3	6.6	93.3
			150.0	146.4	6.8	97.6
	COD	Nd	50.0	48.2	5.1	96.4
			100.0	92.4	5.9	92.4
			150.0	143.8	5.3	95.8
	NAL	Nd	50.0	47.2	5.4	94.4
			100.0	97.5	5.3	97.5
			150.0	147.5	6.7	98.3
Hair 3	MOR	71.8 (0.1 ₇ $\mu\text{g g}^{-1}$)	50.0	119.1	3.5	96.2
			100.0	168.3	3.2	95.1
			150.0	218.5	3.3	96.4
	OCD	Nd	50.0	48.6	3.1	97.2
			100.0	99.4	3.4	99.4
			150.0	145.5	3.0	97.0
	COD	Nd	50.0	45.5	6.0	91.0
			100.0	90.2	6.6	90.2
			150.0	153.2	6.1	102.1
	NAL	Nd	50.0	46.1	6.7	92.2
			100.0	92.3	6.2	92.3
			150.0	143.5	5.9	95.6
Hair 4	MOR	Nd	50.0	47.3	3.0	94.6
			100.0	97.8	3.3	97.8
			150.0	144.3	2.9	96.2
	OCD	Nd	50.0	48.5	3.3	97.0
			100.0	96.6	3.4	96.6
			150.0	146.8	3.0	97.9

		50.0	43.5	6.7	87.0
COD	Nd	100.0	89.4	6.9	89.4
		150.0	135.1	5.6	90.0
		50.0	110.1	6.0	88.8
NAL	67.7	100.0	156.2	6.6	83.0
	(0.1 ₆ μg g ⁻¹)	150.0	207.5	5.8	84.9

*Nd: not detected

FT-IR spectra

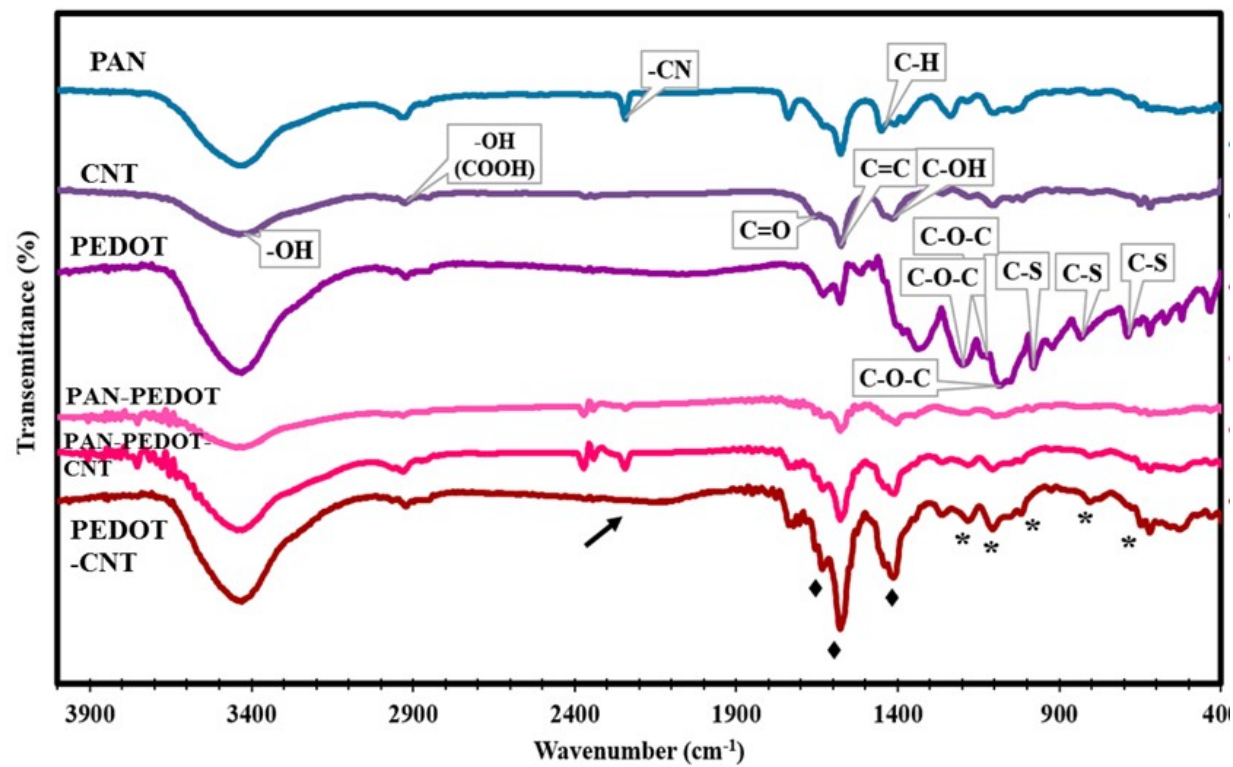


Fig. S1 FT-IR spectra of sorbent at different synthesis steps.

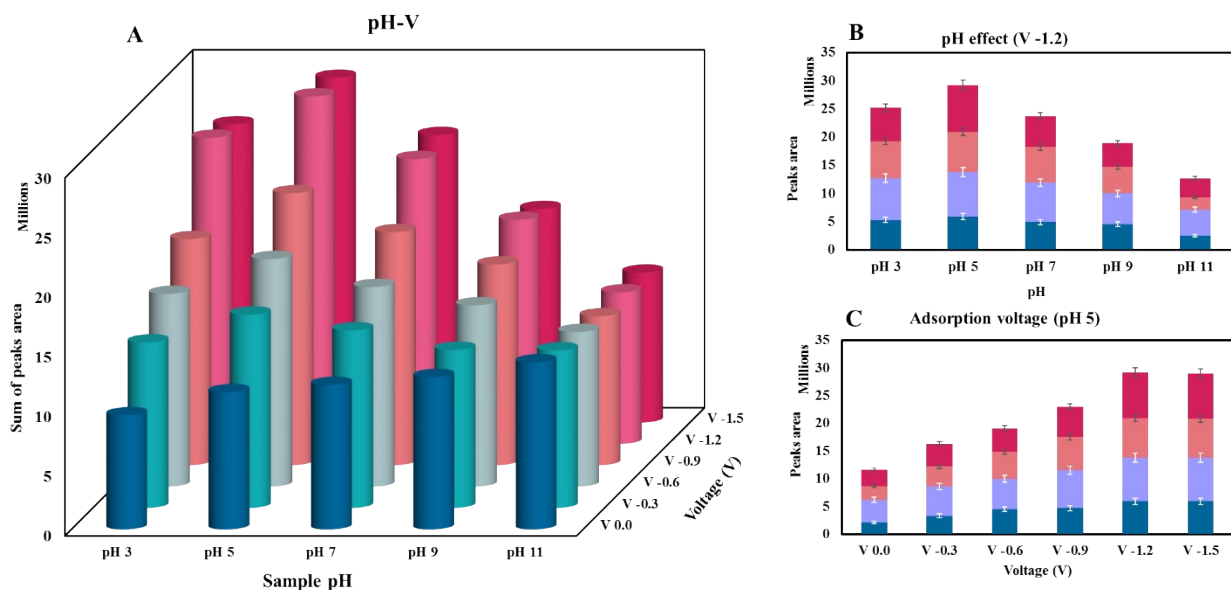


Fig. S2 Optimization of pH and applied potential simultaneously. Optimization procedures were started under constant conditions, 20 mL of sample solution with concentration of $100.0 \mu\text{g L}^{-1}$, pH 7, extraction flow rate of 1.0 mL min^{-1} , and applied potential of -1.0 V and adsorption time of 20.0 min, without the addition of salt. The initial value of each parameter was replaced with the optimal value after optimization.

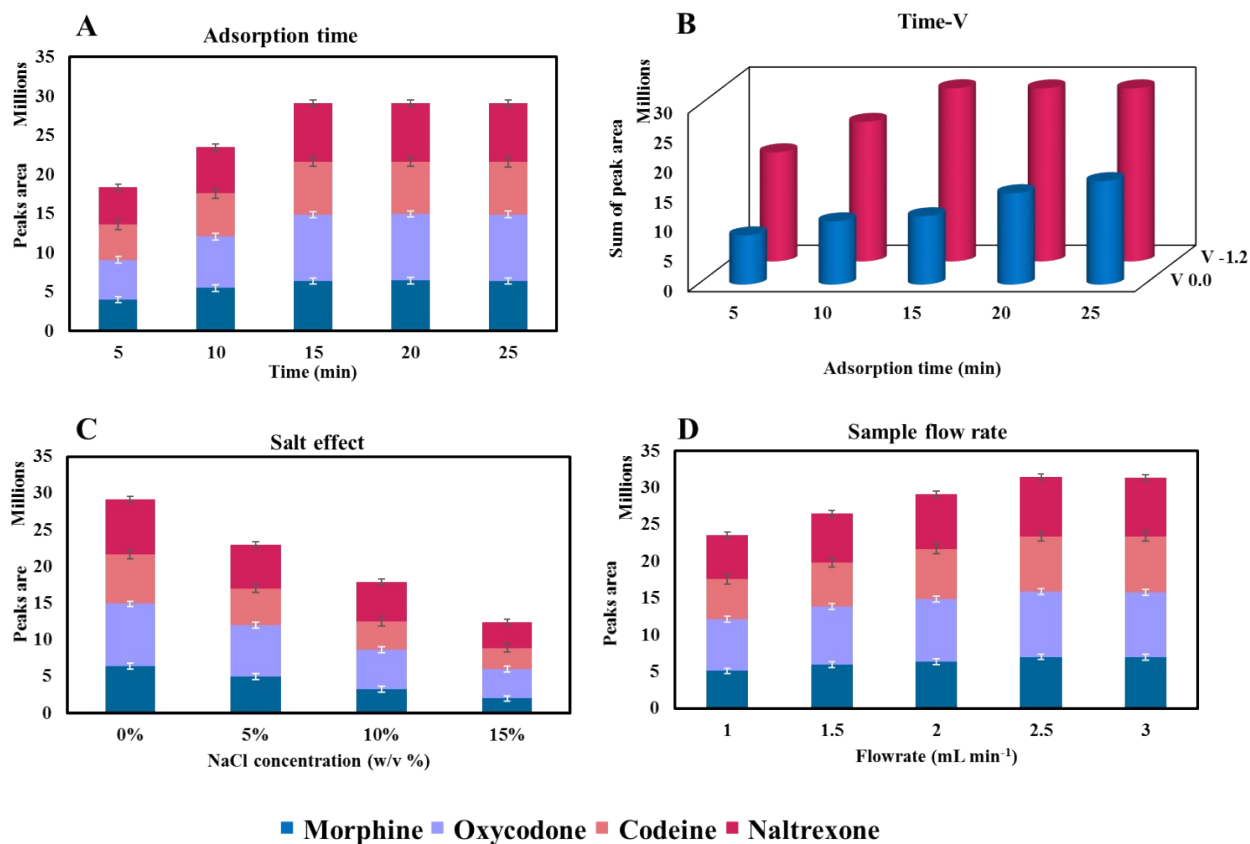


Fig. S3 The effect of (A) adsorption time, (B) comparison between adsorption time of EA-IT-SPME and IT-SPME, (C) salt addition and (D) sample flow rate (mL min⁻¹) on the extraction efficiency of the drugs. Optimization procedures were started under constant conditions, 25.0 mL of sample solution with concentration of 100.0 $\mu\text{g L}^{-1}$, pH 5, extraction flow rate of 1.0 mL min⁻¹, applied potential of -1.2 V and adsorption time of 20.0 min, without the addition of salt. The initial value of each parameter was replaced with the optimal value after optimization.

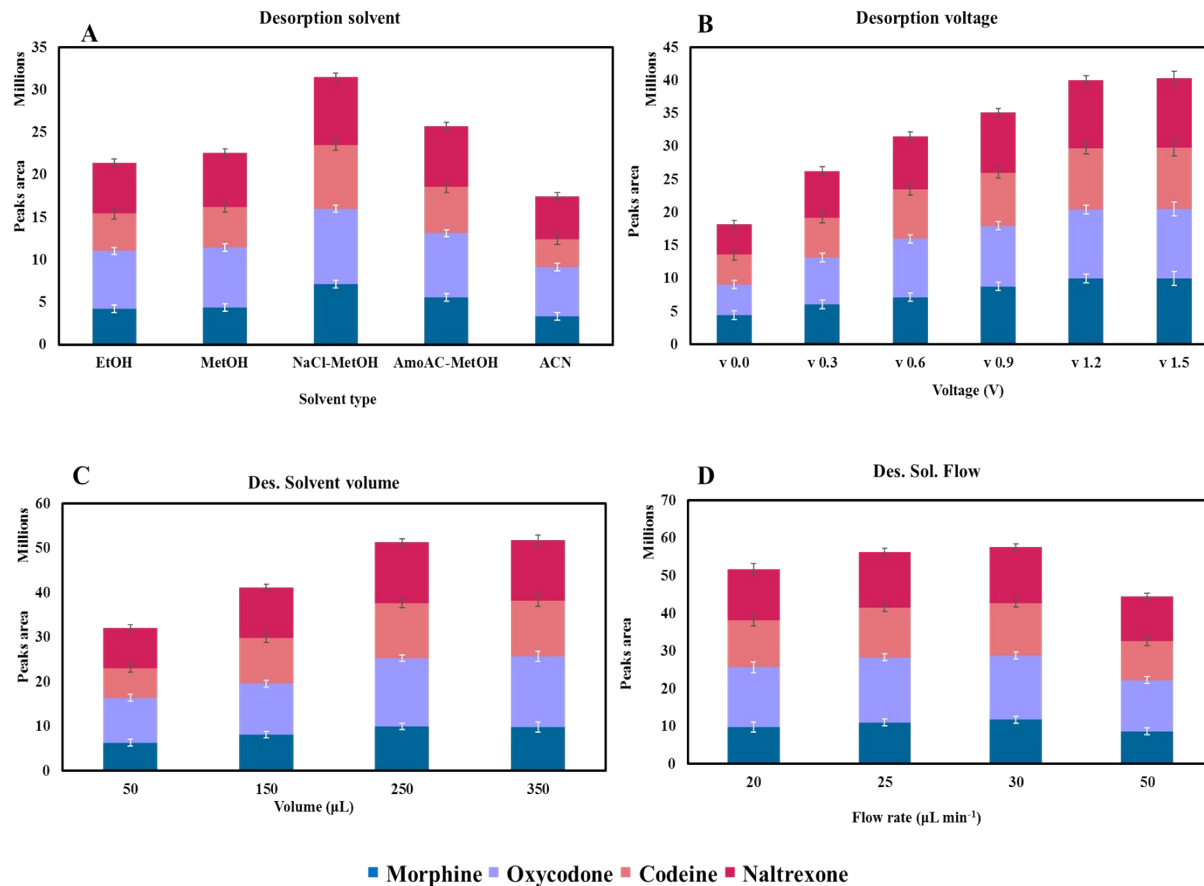


Fig. S4 The effects of (A) the type of desorption solvent, (B) desorption applied potential, (C) desorption solvent volume (μL) and (D) desorption solvent flow rate ($\mu\text{L min}^{-1}$) on the extraction efficiency of the drugs. Optimization procedures were started under constant conditions, 25.0 mL of sample solution with concentration of $100.0 \mu\text{g L}^{-1}$, 400.0 μL of methanol as desorption solvent applied potential of +2.0 V and desorption flow rate of $40.0 \mu\text{L min}^{-1}$. The initial value of each parameter was replaced with the optimal value after optimization.

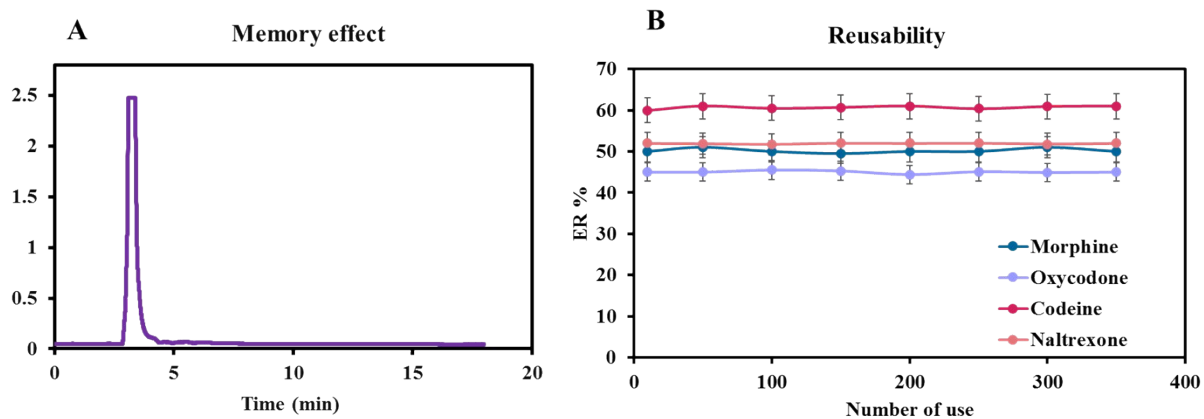


Fig. S5 (A) The chromatogram of the EA-IT-SPME under optimized condition after washing the sorbent with the water-ethanol mixture and ultrapure water and without addition of any analytes to show the lack of memory effect, (B) diagram of extraction efficiency vs the number of times that the sorbent is used.

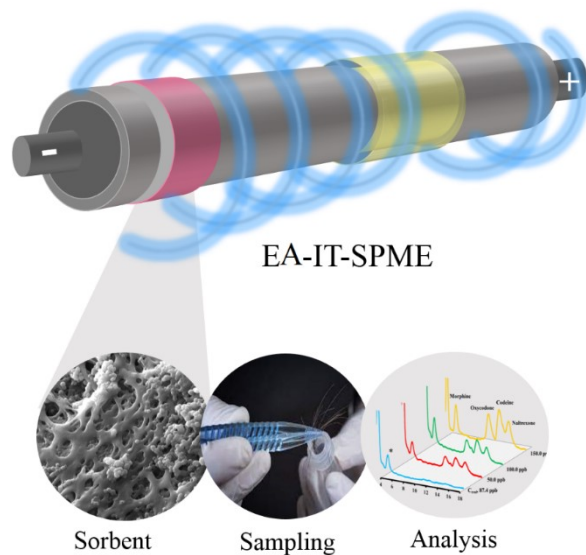


Fig. S6. Schematic procedure of EA-IT-SPME-HPLC