RESEARCH ARTICLE

Determination of Trace Cocaine Based on New Molecularly Imprinted Polymers Combined with Solid-phase Microextraction

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ABSTRACT

This article has developed a novel, sensitive, and cheaper methodology based on a molecularly imprinted polymer (MIP), using different functional monomers as vinyl acetate and 1-vinyl imidazole, with suitable cross-linker and the template (cocaine), to fabricate a monolithic solid-phase microextraction (SPME) fiber with gas chromatography and mass spectrometry (GC/MS); all these analytical methods used for extraction, preconcentration, and selective identification of cocaine and its derivatives. Firmness, stability, and duration of the fabricated fiber give its fundamental and indispensable role in SPME. The purpose of selectivity of the processed fiber is also clarified in detail by the extraction procedure. This study explores the variables that affect polymerization. The template in a solution containing Cocaine The selectivity of related and unrelated compounds under ideal conditions is also optimal. The relative standard deviations (RSD%) for Two patients, repeated experiments for three measurements range from at 20–100 ppm of Cocaine –4–5 %. The relative recoveries obtained for Cocaine in spiked human urine samples are in the range of 96–105 %.

Keywords: Cocaine, Human urine, GC–MS, Molecularly imprinted polymer solid phase extraction.

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INTRODUCTION

Cocaine, also known as coke, is a powerful stimulant most often used as a recreational drug.[1] It is usually snorted, inhaled as smoke, or dissolved and injected into a vein.[2] Mental effects can include an intense feeling of pleasure, lack of interaction with reality, or agitation. A rapid heart rate, sweating, and large pupils can be physical symptoms.[3] High doses may lead to extremely high blood pressure or body temperature.[4] Effects begin within seconds to minutes of use and last between five and ninety minutes.[5] During nasal surgery, cocaine has a limited number of approved medical applications, such as numbing and reducing bleeding.[6] Because of its effect on the reward mechanism in the brain, cocaine is addictive.[7] There is a high risk of dependency arising after a short time of use.[8] Its use often raises the risk of stroke, myocardial infarction, lung complications in those who smoke it, blood infections, and sudden cardiac death.[9] Through inhibiting the reuptake of serotonin, norepinephrine, and dopamine, cocaine acts. This results in higher brain concentrations of these three neurotransmitters.[10] It can quickly cross the blood-brain barrier and can contribute to the barrier breakdown.[11] After cannabis, cocaine is the second most commonly consumed illicit substance globally. Between 14 and 21 million people use the substance every year. In North America, usage is most extensive, followed by Europe and South America.[12] At some stage in their lives, between 1 to 3% of people in the developing world, have used cocaine.

For nasal and lacrimal duct treatment, cocaine is still primarily used. cocaine ‘s propensity for cardiovascular toxicity, glaucoma, and pupil dilation are the main drawbacks to this use.[13] As other synthetic local anesthetics such as benzocaine, proparacaine, lidocaine, and tetra Caine are now used more commonly, medical usage of cocaine has decreased.[14] The anesthetic is paired with a vasoconstrictor such as phenylephrine or epinephrine for a procedure (as it prevents bleeding).[15] Occasionally, some experts in otolaryngology (ENT) use cocaine inside the. Dissolved cocaine is immersed in cotton wool ball and put in the nostril for 10 to 15 minutes immediately before the operation, thereby playing the dual task of both cauterizing and vasoconstriction the region to be cauterized. And when used in this manner, some of the cocaine used can be absorbed and have systemic effects through the oral or nasal mucosa.[16]

Narcotic substance Methamphetamine, estimated by the same method used to estimate Cocaine, and good results were obtained, is a novel method characterized by high sensitivity,

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low cost, and high stability. The template is MAMP to fabricate a monolithic solid-phase microextraction (SPME) fiber. with gas chromatography and mass spectrometry (GC/MS).[17] This study investigates the factors influencing the polymerization and extraction procedures to detail the fabricated fiber's selectivity to the template in a solution containing MAMP.[18] The present work describes a sensitive, selective, accurate, and fully validated procedure for the simultaneous detection and quantification of cocaine (CoC) and its primary metabolites, benzoylecgonine (BEG), in low volume urine samples (15 mL) using MEPS-GC-MS. The procedure is a promising alternative for forensic toxicology concerns, workplace drug testing situations, and improving laboratory throughput.

EXPERIMENTAL

Reagents and Chemicals

Vinyl acetate, 1-Vinyl imidazole, N, N methylene bis acryl amid and benzoyl peroxide were purchased from Sigma–Aldrich (St. Louis, MO, USA, www.sigma-aldrich.com), methanol, chloroform, acetic acid from Merck (Darmstadt, Germany, www.merck.com) and Cocaine was provided from the medico-legal institution (Baghdad, Iraq). Nitrogen gas (99.99) from Arab gulf factory Baghdad.

Instrumentation

Monitoring of the analyses was performed using a GC MC (Agilent technologies (7890A) (USA)) and using UV-Vis (Shimadzu UV spectrophotometer 1800 pc (japan)) and Scanning Electron Microscopy (SEM) (JSM.6390A) (TOKYO JAPAN) and FTIR Shimadzu (FTIR) - 8000 (Japan), heating/ stirring(Germany) and centrifuge (Germany).

Chromatography GC is a process used to break an ingredient mixture into its original components, and the use of three-step separation achieves this approach. The first phase starts with sample injections in the GC, the second step is to divide the sample into separate components. The third stage involves identification using a detector of the sample components. During the polymerization process, pure Cocaine shows an absorption band at 273 nm, and this band can be used to ensure that all Cocaine was removed after washing, then measured by using GC.

MIP Procedure

At the first tube, 0.21 mmol of a template (cocaine) was dissolved in 3 mL methanol, and 0.24 mmol of functional monomer (vinyl acetate) was added. After stirring the mixture for 5 minutes, 0.28 mmol of cross-linker (N, N methylene bis acrylamide), 0.32mg initiator (benzoyl peroxide) were added to the solution. And the second tube 0.091 mmol of the template (cocaine) was dissolved in 3mL methanol, and 0.235 mmol of functional monomer (1-vinyl imidazole)+ was added. After stirring ultrasonically, the resulting mixture for 5 minutes, 2.235 mmol of cross-linker (N, N methylene bis acrylamide), and 0.32 mg initiator (benzoyl peroxide) were added to the solution. Both tube solutions were bubbled with nitrogen for 20 min and used as a pre-polymer solution, sealing the rubber tube. Then both tubes were left in the water bath at 60c overnight. The process of polymerization has been completed, and cocaine-MIPs were formed. The non-molecular imprinted polymer NIP was synthesis precisely the same method of synthesis MIP but without the cocaine. The MIP and NIP tubes were washed a few times with the excess amount of a mixture containing methanol/ acetic acid (32:8, v/v) in the sox let extraction device for 24 hours until template non-reacted compounds were separated and dried for 1 h in a vacuum. The MIP and NIP prepared were left in the oven for drying. Sampling devices before extraction and used as extraction needles. The plastic syringe (column) was packed with prepared MIP by using a plastic syringe. The solution (urine or standard solution) was poured from the upper end of the column; the solution was pushed at 70 rpm by a peristaltic pump.

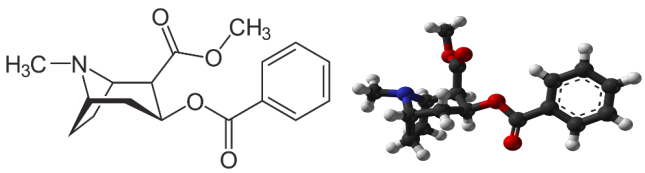

Figure 1: Structure of Cocaine.

Sampling

Stock solutions at concentration (20, 40, 60, 80, and 100 ppm) of cocaine. Cocaine was prepared at pH 8 and at a flow rate of 70 rpm, passed through the column. To minimize the matrix intervention, the extraction column was washed twice using 2 mL distilled water and removed from MIP.

The Sampling Device

The device consists of a 3 mL plastic syringe packed with (0.2 and 0.4 gm) MIP previously grinding and sieving with 0.75 μM bore size.

Samples

Urine samples were collected from cocaine suspected abuser donates sent at the judge's request to medico-legal institution in Baghdad, Iraq. The sample was centrifuged at 5000 rpm for 10 min to get rid of any precipitated material. The supernatant of urine was directly spiked with cocaine, and the spiked and non-spiked samples were extracted by the fabricated column.

The Procedure of Extraction Method

Cocaine was extracted from the urine using a MIP cocaine solid-phase extraction (SPE) column. This column was previously prepared by packing it with MIP (0.2, 0.4 g), its reservoir size, 3 mL. The SPE vacuum was loaded with the supernatant taken from the centrifuged urine sample with flow rate (70 mL.min-1), methanol/ acetic acid (32:8, v/v) column. After elution was collected in a small beaker and the residue was dried in the water bath at 50°C, then 2 mL of methanol was added with stirring to residues, and the sample has been ready to inject in the GC/MS.

RESULTS AND DISCUSSION

Synthesis of MIPs for Cocaine

Two MIP's of cocaine were synthesized by self-assembling (non-covalent) bulk polymerization method. The functional

monomer was a significant purpose in studying the interactions that occur with the template. Two monomers, vinyl acetate and 1-vinyl imidazole were used for the synthesized MIPs and NIPs.

Fourier Transmission Infrared Spectrometry Analysis

To detect the functional groups present in a compound, FTIR is an essential chemical characterization process. The FTIR spectrum of various MIPs and NIPs are shown in Table 1.

The Fourier transmission infrared spectrometry spectra of leached and unleashed Cocaine imprinted polymers MIP and NIP were recorded in the range of 400–4000 cm^{-1} by the KBr pellet method (Table 1). From this table, the FTIR spectrum of the Cocaine showed the following bands: (3419, 2962,2842, 1730, 3024, 1598, 1269, 730, and 752) cm^{-1} for NH^+Cl^-, C-H. Aliphatic stretching, C=O ester stretching, C=C aliphatic Stretching C-O stretching, and out-of-plan bending for the monosubstituted ring. The FTIR spectrum of the Cocaine –MIP (Vinyl acetate) before template removal showed the following bands (3456, 2952,2873, 1718, 3068, 1527, 1224, 1649, 1760, 788 and 705), cm^{-1} for NH^+Cl^- stretching, N-H stretching, C-H aliphatic stretching=O ester stretching, AR-H Stretching C=Aliphatic stretching carbonyl acid stretching, C=C vinyl stretching, C=O Ketone stretching bending, and out of plan bending for the mono ring. The FTIR spectrum of the MIP (Vinyl acetate) after template removal shows the absence of C=Oster stretching, Ar-H stretching and out-of-plan bending for the monosubstituted ring excises in the template (Cocaine) spectrum, which indicates the extraction of drug from the template.

When using the 1-vinyl imidazole as a monomer for the synthesis of another MIPs for Cocaine, the FTIR spectra of MIPs before and after template removal and NIP are shown in Table 2.

From Table 2, the FTIR spectrum of the Cocaine showed the following bands (3419, 2962, 2842, 1730, 3024, 1598, 1269, 730, and 752) cm^{-1} for OH (H_2O) stretching, C-H aliphatic stretching, C=O ester stretching, Ar-H stretching, C=C vinyl stretching, C=N stretching, C-N stretching and out of plane bending for the monosubstituted ring. The FTIR spectrum of the Cocaine –MIP(1-Vinylimidazol) before template removal shows the following bands 3446 cm^{-1} for OH (H_2O) stretching, 2952 and 2873 cm^{-1} for C-H aliphatic stretching amide, 2 927, 2879 cm^{-1} for C-H aliphatic stretching, 1677 cm^{-1} for C=O stretching, 3068 cm^{-1} for Ar-H stretching, 1535 cm^{-1} for C=C aliphatic stretching, 1272cm^{-1} for C-O stretching, 1650 cm^{-1} for C=C vinyl stretching, 1650 cm^{-1} for C=N stretching, 1535 cm^{-1}forC-N stretching and 723, 678 cm^{-1} out of plan bending for monosubstituted ring. After template removal, the FTIR spectrum of the MIP (1-vinyiimidazol) shows the absence of C=O ester stretching, Ar-H stretching, and out-of-plan bending for the monosubstituted ring. Which excises in template (Cocaine) spectrum and which indicates the extraction of drug from the template. Several experiments were carried

Table 1: The most identified peaks of FT-IR spectra (cm^{-1}) for cocaine-imprinted polymer and NIP using vinyl acetate as a functional monomer

	Functional Group	*Cocaine*	*Cocaine-MIP (vinyl acetate) before template removal*	*Cocaine-MIP (vinyl acetate) after template removal*
1	NH^+Cl^-	3419	3456	3467
2	CH-aliphatic.str.(cm^{-1})	2962,2842	2952,2873	2948,2867
3	C=Ostr. ester.(cm^{-1})	1730	1718	
4	Ar-H aromatic.(cm^{-1})	3024	3068	
5	C=C aliphatic.(cm^{-1})	1598	1527	1535
6	C-O str. (cm^{-1})	1269	1224	1267
7	C=C str. vinyl NH^+Cl^-		1649	1652
8	C=O str. Ketone(cm^{-1})		1760	1793
9	Out-of plane-mono-sub	730,752	705,788	

Table 2: The most identified FT-IR spectra peaks (cm^{-1}) for cocaine-imprinted polymer and NIP using (1-vinyl imidazole) as a functional monomer.

	Functional group	*Cocaine*	*Cocaine-MIP 1-vinylimidizol Before template removal*	*Cocaine-MIP 1-vinylimidizol after template removal*
1	OH (H_2O)(cm^{-1})	3419	3446	3467
2	CH-aliphatic.(cm^{-1})	2962,2842	2952,2873	2948,2867
3	C=Ostr. ester.(cm^{-1})	1730		
4	Ar-H aromatic.(cm^{-1})	3024	3068	
5	C=C aliphatic.(cm^{-1})	1598	1535	1529
6	C-O str..(cm^{-1})	1269	1272	1226
7	C=C str. vinyl.(cm^{-1})		1650	1650
8	C=N str..(cm^{-1})		1650	1650
9	C-N.(cm^{-1})		1535	1529
10	Out-of plane-mono-sub	730,752	723,678	

Table 3: The variation ratios of [D: M: C] and progeny used in the preparation of MIPs and NIPs for (MAMP

		Drug cocaine	*Monomer vinyl acetate*	*Cross linker N, N methylene bis acrylamide*	*Initiator*	*Solvent*	*Result*
MIP1	%	8.02	6.01	85.95	0.3	6 mL	Pale
	mmole	0.28	0.21	3	0.32	CH_3OH	yellow
MIP1	%	6.34	3.02	90.6	0.3	6 mL	Pale
	mmole	0.21	0.1	3	0.32	CH_3OH	yellow
MIP1	%	7.75	7.32	83.45	0.3	6 mL	Pale
	mmole	0.21	0.24	2.28	0.32	CH_3OH	yellow
NIP1	%		7.32	83.45	0.3	6 mL	Pale
	mmole		0.24	2.28	0.32	CH_3OH	yellow
		Drug cocaine	*Monomer 1-vinyl imidizol*	*Cross linker N,N methylene bis acrylamide*	*Initiator*	*Solvent*	*Result*
MIP2	%	3.68	2.99	93.36	0.3	6 mL	Pale
	mmole	0.15	0.122	3.8	0.32	CH_3OH	yellow
MIP2	%	2.75	3.029	94.21	0.3	6 mL	Pale
	mmole	0.091	0.1	3.11	0.32	CH_3OH	yellow
MIP2	%	3.56	9.176	87.2	0.3	6 mL	Pale
	mmole	0.091	0.235	2.235	0.32	CH_3OH	yellow
NIP2	%		9.176	87.2	0.3	6 mL	Pale
	mmole		0.235	2.235	0.32	CH_3OH	yellow

Table 4: Standard addition method for drug determination using imprinted polymer method solid-phase extraction used MIP-Vinyl acetate, MIP-1-Vinylimidazole

Wt. of MIP(g)	*Type of MIP*	*No. of patient*	*Conc. taken (ppm)*	*Conc. found (ppm)*	*% Recovery*	*RSD%*	*RE%*
0.2	MIP1	1	60	62	103.3	3.123	3.33
		2	10	9.6	96	4.166	-4
0.2	MIP2	1	20	21	105	2.839	5
		2	40	41.6	104	2.568	4

out using different ratios (D: M: C) to reach the optimum ratio for the preparation of MIPs (Cocaine). Among these experiments of the molar ratios (D: M: C) of (7.75:7.32:83.45), (3.56: 9.176: 87.2) for Cocaine -MIPs have produced polymers suitable characteristics list in Table 3.

Urine Samples Analysis

Under optimal conditions, the MIP-vinyl acetate and 1-Vinylimedazol were applied homogenously to identify Cocaine in urine samples. The sample matrix of the urine was in the first step, and the wash step after the extraction was done. The results are shown in Tables 5 and 6.

The Table 4 show the values of RSD% range from (1.587–4.545) % and the values of RE% range between (2–5)%, A little high because a part of cocaine decomposes to benzoylecgonine in the human body, therefore from the results obtained, it is possible to detect and calculate the cocaine concentration, depending on the presence of benzoylecgonine.

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