SYNTHESIS OF NEW MOLECULARLY IMPRINTED SOLID -PHASE UESD STYRENE AND ALLYL CHLORIDE BASE FUNCTIONAL MONOMER FOR DETERMINATION OF COCAINE BY GC-MASS AND ITS CLINICAL APPLICATIONS

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ABSTRACT
This study was aimed to synthesize a new molecularly imprinted polymer using a functional monomer of styrene(STY) and allyl chloride(ALC) and N, N-methylene bis-acrylamide (MBAA) as a cross-linker for the selective extraction of cocaine from urine samples. Cocaine is used as a template to create a monolithic solid-phase micro extraction (SPME) fiber. All of these analytical procedures were utilized to extract, preconcentrate, and selectively determine Cocaine and its derivatives (SPME) with gas chromatography and mass spectrometry (GC/MS). Samples taken from a suspected cocaine addict who was given to the medico-legal directorate as a donation (Baghdad, Iraq). The analytes were monitored using UV-Vis , (GC MS), FTIR and Scanning electron microscopy (SEM). The RSD percent for two patients' repeated studies for three measurements range from (1.587-4.545) percent cocaine 20-100 ppm .

Keywords: Cocaine/Molecularly imprinted polymer / styrene / allyl chloride

تحضير طبعات بوليمرية جديدة الطور الصلب باستعمال ستايرين واليل كلورايد مونمرات فعالة لتقدير الكوكاين باستعمال طيف الكتلة وتطبيقاتها السريرية

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المستخلص
يهدف هذا البحث الى توليف بوليمر جديد مطبوع جزيئيًا باستعمال مونومر وظيفي من الستايرين (STY) وكلوريد الأليل (ALC) و N، N، ميثيلين ثنائي أكريلاميد (MBAA) باعتباره رابطًا متقاطعًا للاستخراج الانتقائي للكوكايين من عينات البول. يستعمل الكوكايين كقالب لإنشاء ألياف استخراج دقيقة صلبة متجانسة (SPME). تم إنشاء بوليمر غير مطبوع عن طريق تحضير بوليمر بدون مواقع ربط انتقائية (NIP). تم استعمال كل هذه الإجراءات التحليلية لاستخراج الكوكايين ومشتقاته (SPME) والتركيز المسبق وتحديده بشكل انتقائي باستعمال كروماتوجرافيا الغاز وقياس الطيف الكتلي (GC / MS). عينات مأخوذة من مدمن كوكايين مشتبه به تم تسليمه إلى مديرية الطب العدلي كتبرع (بغداد ، العراق). تمت مراقبة التحليلات باستعمال UV–Vis و (GC MS) و FTIR والمسح المجهري الإلكتروني (SEM). تتراوح الانحرافات المعيارية النسبية (النسبة المئوية RSD) لدراسات متكررة لمريضين لثلاثة قياسات من (1.587–4.545) بالمائة من الكوكايين 20–100 جزء في المليون.

الكلمات المفتاحية. الكوكايين، بوليمر مطبوع جزيئيًا، ستايرين، اليل كلورايد

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INTRODUCTION

Cocaine remains the most widely consumed illegal stimulant drug in Europe show figure(1), it is no longer considered a Belite drug due to the notoriety achieved in recent years. Consumption of Cocaine is Numerous health conditions, such as respiratory diseases, neurological deficiency, as well as social challenges and death, are associated with (1, 2). The effects of start was appear within seconds for minutes of use and last anywhere from five to ninety minutes. (1) limited number cocaine has approved medicinal purposes during nose surgery, such the numbing and bleeding decreasing. Cocaine is addictive because of its effect on the brain's reward mechanism. (7) Short period, there is a substantial danger of dependency developing. (8) The risk of stroke increases, infarction myocardial, problems lung, blood infections, and sudden cardiac death in people who smoke it. (9).Polymers have also been manufactured in the same way, and their selectivity has been demonstrated to them when used as high-precision films (10).Since this method is characterized by high selection and economics in consuming biological samples. This method was used in the estimation of other narcotic substances in forensic medicine laboratories such as amphetamine (11-13).

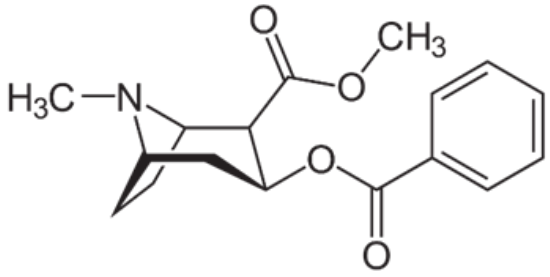

Fig 1. Structure of Cocaine

Instruments and chemicals materials

Styrene(STY) and allyl chloride(ALC) ,N, N-methylene bis-acrylamide (MBAA) and benzoyl peroxide was purchased from Sigma–Aldrich, methanol, chloroform, and acetic acid, were purchased from Merck. Cocaine was provided by the Forensic Medicine Foundation (Baghdad, Iraq).Control was performed using GC MC (7890A) Agilent technologies (USA) and using (UV-Vis Shimadzu 1800 pcs spectrophotometer) and scanning electron microscopy (SEM) (JSM.6390A). 8000 (Japan).

Mips synthesis: One mmol template (Cocaine) was diluted in 5 ml porogen (methanol), and 2.5 mmol (STY) ,3 mmol(ALC) was added. After agitating the mixture with an ultrasonic for 10 minutes, 35mmol of cross linker (MBAA) and 0.34 mg of the initiator (benzoyl peroxide) were added, and the solution mixture was bubbled with N_2 gas for 15 minutes before being placed in a water bath at 55°C overnight. Cocaine-MIPs were generated when the polymerization process was completed. In a drying oven, the synthesized MIPs were left for 1 hour at 30 degrees. After that, the combinations were crushed and ground using a mortar and a 125 mesh filter to obtain 125m particles. Use as extraction needles before extracting from the sampler. The prepared (MIP) material was poured into the plastic syringe (column). The urine or standard solution was poured from the column's higher end, with the solution moving at 70 rpm under vacuum.

Procedure sampling

Prepare a stock solution of cocaine at pH 8 with a concentration of (20, 40, 60, 80, 100 ppm) and a flow rate of 70 rpm through Colum. To remove matrix interference, the column was washed twice with 2 mL distilled water and then removed from the MIPs.

Sampling device

Five ml plastic syringe was utilized, the syringe was filled with different weights of MIPs that had been previously ground and sifted (0.1, 0.3, gm) (0.70 microns).

Real sample

Urine samples of suspected cocaine were collected and forwarded to forensic medicine at the judge's order (Baghdad, Iraq). To remove any precipitated material, the centrifuge sample was spun at 5000 rpm for 10 minutes. Cocaine was immediately impregnated in the urine supernatant, and the non-pointed and squid samples were extracted by column.

Extraction procedure

MIPs Cocaine solid phase extraction (SPE) column was used to extract cocaine from the urine. A 3 ml plastic syringe was previously filled with MIP to make this column (0.2 g). The supernatant from the centrifuged urine sample was poured into the space above the packing of the SPE column at a flow rate of 1

mL/min (75 rpm). The eluent was collected in a small beaker after 1ml of distilled water and 1ml of methanol/acetic acid (32:8, v/v) were added to the column. The eluent was dried for 10 minutes before adding 1 ml (1:100, v/v) acetic acid: methanol, collecting the eluent in the same beaker, and drying the residue in a water bath at 50°C. Later, the solution was cooled to room temperature, the solvent was evaporated to dryness under stream of nitrogen, and the residues and sample were ready to inject into the GC/MS.

RESULTS AND DISCUSSION

Mips cocaine synthesis

Self-assembly (non-covalent) bulk polymerization was used to install two Cocaine Self-assembly (non-covalent) bulk polymerization was used to install two cocaine MIPs. Monomer styrene and allyl chloride were employed to synthesis the MIPs was essential in analyzing interactions with the template. FTIR analysis is an important chemical characterization process Tables 1,2.

Table 1. The most identified peaks of FT-IR spectra for Cocaine-imprinted polymer using STYas a functional monomer

	Functional Group	Cocaine	Cocaine-MIP STY before template removal	Cocaine-MIP STY after template removal
1	N-H str.	-------	3481	3452
2	C-H-aliphatic.(cm^{-1})	2972,2833	2940,2832	2972,2953
3	C=O str. ester.(cm^{-1})	1732	1720	_____
4	Ar-H str.(cm^{-1})	3033	_____	_____
5	C=C str. aliphatic.(cm^{-1})	1596	1554	_____
6	C-O str .(cm^{-1})	1265	1721	1254
7	C=CH_2 str. alkene .(cm^{-1})	_____	1633	1730
8	Out-of plane-mono-sub	733,755	753,712	_____

From Table 1 the FTIR spectrum of cocaine showed the following bands (3481, 3452, 2972, 2833, 1732, 3033, 1596, 1265,733 and 755) cm-1 for N-H stretching , aliphatic CH stretching, C = O ester stretching, ester stretching Ar-H, C = C aliphatic stretching, C = O ester stretching, Ar-H stretching C = C aliphatic CO stretching C = CH2 alkene, and out-of-plane bending of the mono-substituted ring. The FTIR spectrum of Cocaine-MIP (**VIZ**) before de-casting showed the following bands 3481 cm−1 for N-H expansion, 2940 and 2832 cm−1 for aliphatic CH expansion, 1720 cm−1 for C=O ester expansion, 1554 cm− 1 1 for the aliphatic layer C = C, 1721 cm−1 CO, 1633 cm−1 forC = CH2 expansion, and 753, 712 cm−1 for the extrastriatal curvature of the single-substitution ring. The FTIR spectrum of MIP (STY) after de-molding did not show the absence of a C=O stretching ester, AR-H and C=C aliphatic stretching and off-striatal curvature of the single-substituted extrusive ring in the template (cocaine) The spectrum indicating the drug is extracted from Sample.

Table 2. The most identified peaks of FT-IR spectra for Cocaine-imprinted polymer using ALC as a functional monomer

	Functional Group	Cocaine	Cocaine-MIP ALC before template removal	Cocaine-MIP ALC after template removal
1	N-H str.	-------	3425	3433
2	C-H-aliphatic.(cm^{-1})	2872,2855	2930,2820	2975,2963
3	C=O str. ester.(cm^{-1})	1724	1715	_____
4	Ar-H str.(cm^{-1})	3030	_____	_____
5	C=C str. aliphatic.(cm^{-1})	1545	1540	_____
6	C-O str .(cm^{-1})	1252	1221	1258
7	C=CH_2 str. alkene .(cm^{-1})	_____	1633	1730
8	C-Cl	------	596	580

From Table 2 the FTIR spectrum of cocaine shows the following bands (3425, 3433, 2872, 2855, 1724, 3030, 1545, 1252,580 and 596) cm-1 for N-H stretching , aliphatic CH stretching, C = O ester stretching, ester stretching Ar-H, C = C aliphatic stretching, C = O ester stretching, Ar-H stretching C = C aliphatic CO stretching C = CH2 alkene, and

C-CL. The FTIR spectrum of Cocaine-MIP (**ALC**) before de-casting showed the following bands 3425 cm−1 for N-H expansion, 2930 and 2820 cm−1 for aliphatic CH expansion, 1724 cm−1 for C=O ester expansion, 1545 cm− 1 1 for the aliphatic layer C = C, 1221 cm−1 CO, 1633 cm−1 forC = CH2 expansion, and 596cm−1 for the C-CL. The FTIR spectrum of MIP (ALC) after de-molding did not show the absence of a C=O stretching ester, AR-H and C=C aliphatic stretching and off-striatal curvature of the single-substituted extrusive ring in the template (cocaine) The spectrum indicating the drug is extracted from Sample.

Isotherm adsorption

For understanding the mechanism of adsorption template on the polymer surface. The isothermal equilibrium obtained were analyzed to show the isothermal Langmer model or Freundlich models [14]. This is achieved by plotting the binding capacity (Q) against the free concentration of the drug, Q is calculated according to the following equation:

$$Q = [(C_i - C_f) V_s \times 1000] / M_{MIP}$$

Isotherm adsorption experiments were carried out by adding 0.1g and 0.3g of cocaine MIP into 15 mL of cocaine concentration ranged from 10ppm to 40ppm. The solution was kept for several hours around 10 hours at room temperature and the solid phase was separated by centrifugation. The remain concentration of cocaine was measured by Uv-Visible spectrophotometer at wavelength equal to 273 nm and the data for adsorption isotherm were used for a Scatchard analysis. Capacity factor was calculated for two cocaine MIPs mass 0.1g and 0.3g using cocaine concentration range from 10 ppm to 40 ppm. Table (2) shows the values of Q, Q/ Cf of cocaine MIPs based on styrene and allyl chloride as monomers and N, N-methylene bis-acrylamide (MBAA) as a cross-linker.

Table 2. Rebinding values of (cocaine) using cocaine MIP based on (styrene (STY) + MBAA)

Cocaine_MIP(styrene)						
Mass of MIP g	**Constriction ppm**	**Ci mM**	**C_{free} mM**	**Q µMole /g**	**Q/C_{free} L/g**	**T: P (templet:polymer)**
0.1	**10**	**0.033**	**0.00046**	**2.44**	**5304.3**	**1:6**
	20	**0.066**	**0.0010**	**4.88**	**4880.0**	**1:6**
	30	**0.099**	**0.0015**	**7.31**	**4766.7**	**1:6**
	40	**0.132**	**0.0015**	**6.86**	**4713.3**	**1:6**
0.3	**10**	**0.033**	**0.0009**	**1.20**	**1333.3**	**1:6**
	20	**0.066**	**0.002**	**2.40**	**1000.0**	**1:6**
	30	**0.099**	**0.003**	**3.60**	**1200.0**	**1:6**
	40	**0.132**	**0.0031**	**3.40**	**1101.6**	**1:6**

Table 3. Rebinding values of (cocaine) using cocaine MIP based on (allyl chloride (ALC) + MBAA)

Cocaine_MIP(allyl chloride)						
Mass of MIP g	**Constriction ppm**	**Ci mM**	**C_{free} mM**	**Q µMole /g**	**Q/C_{free} L/g**	**T: P (templet:polymer)**
0.1	**10**	**0.2010**	**0.2005**	**0.025**	**0.1246**	**1:4**
	20	**0.4020**	**0.3978**	**0.210**	**0.5279**	**1:4**
	30	**0.6030**	**0.5962**	**0.340**	**0.5702**	**1:4**
	40	**0.8040**	**0.7969**	**0.355**	**0.4454**	**1:4**
0.3	**10**	**0.2010**	**0.1707**	**0.757**	**4.4376**	**1:4**
	20	**0.4020**	**0.3643**	**0.942**	**2.5871**	**1:4**
	30	**0.6030**	**0.5377**	**1.632**	**3.0360**	**1:4**
	40	**0.8040**	**0.7381**	**1.647**	**2.2320**	**1:4**

Isotherm adsorption of cocaine MIP based on allyl bromide monomer was the same pattern which shows one sites connection of cocaine with the polymer with a covalent bond.. Scatchard plot showed only one equilibrium dissociation constant Kd and apparent maximum amount Qmax for the high affinity sites were calculated This behavior indicated that the adsorption was Langmuir isotherm and the binding was homogenies.

Effectofflowreate: To extract cocaine flow rate of the peristaltic pump was used which is

important because it determines the time required for extraction. It should be enough to prevent wasting time by controlling the total analysis time. The effect of sample loading flow rate in the 5-50 rpm range was studied to estimate the effect of contact time between MIP and sample solution on recovery as shown in Table (4 and 5).

Table 4.Effect of flow rate on time of extraction based on Cocaine-MIP (styrene) as function monomer

Cocaine-MIP (styrene)										
C_i mM	0.065									
Mass of MIP g	0.1									
Fiow rate (rpm)	5	10	15	20	25	30	35	40	45	50
Time (min)	85	75	70	65	55	45	40	35	20	20
C_i mM	0.131									
Mass of MIP g	0.1									
Fiow rate (rpm)	5	10	15	20	25	30	35	40	45	50
Time (min)	60	55	45	30	20	10	5	5	5	5

Table 5. Effect of flow rate on time of extraction based on Cocaine-MIP (allyl chloride) as function monomer

Cocaine-MIP (allyl chloride)										
C_i mM	0.045									
Mass of MIP g	0.1									
Fiow rate (rpm)	5	10	15	20	25	30	35	40	45	50
Time (min)	90	85	70	65	50	47	40	35	20	20
C_i mM	0.127									
Mass of MIP g	0.3									
Flow rate (rpm)	5	10	15	20	25	30	35	40	45	50
Time (min)	80	70	65	55	40	35	30	25	20	20

From the following Table 3, the experiment needs a minimum amount of time in order to preserve time. Complete extraction was achieved at any peristaltic pump flow rate from 10 to 70 rpm. The following Figure (2) shows the flow rate per minute with time in minutes. The time decreased as the flow rate increased and we fixed the flow rate at 70 rpm where the time was 5 min and this time was used in the following experiments:

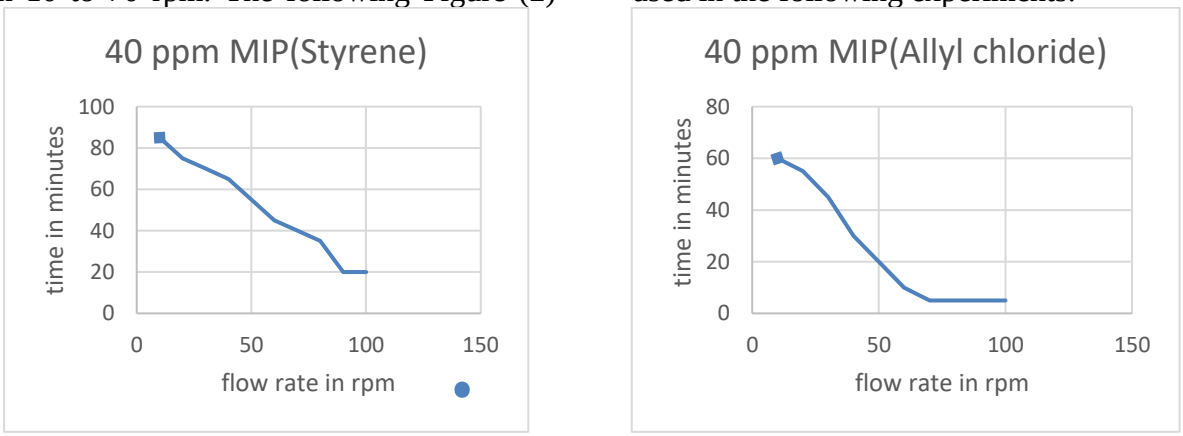

Fig. 2. Relationship between the flow rate and the time of extraction ability based on 0.1 gm of Cocaine-MIPs (styrene and allyl chloride) used 40 ppm from Cocaine

Sampleanalysis

Under optimal conditions, MIP- styrene and allyl chloride were homogeneously applied to the Freundlich isotherm application for the estimation of cocaine in samples of urine. The urine sample matrix was in the first step and the washing step after the extraction procedure. The washing step can be accomplished by allowing the carrier solution, and the solution to flow through the plastic syringe using a peristaltic pump. Poorly absorbed components are expected to be

removed in a homogeneous column by washing time from 60 s to 5 min, the matrix peaks were visibly suppressed. This can be demonstrated by extracting an empty urine sample with a 3-minute wash step. The application of the same washing step to the urine sample increased, and satisfactory results were obtained from cocaine: no reduction in this was found. A plastic syringe containing 0.1-0.3 g MIPs (styrene and allyl chloride) were taken while passing different concentration of cocaine into the urine samples. in the 20-100 ppm range successfully under optimal conditions. The results are shows in Table 6.

Table 6. Standard addition method determination for drug using imprinted polymer method solid phase extraction

Wt. of MIP(g)	Type of MIP	NO.of patient	Conc. Taken (ppm)	Conc. Found (ppm)	% Recovery	RSD%	RE%
0.1	MIP styrene	1	40	41	102.5	3.65	2.5
		2	20	21	95	4.54	-5
0.1	MIP Allyl chloride	1	40	39	102.5	1.86	2.5
		2	20	21	95	1.97	-5

CONCLUSION

In this study, cocaine was extracted from urine samples using a MIP Cocaine Solid Phase Extraction (SPE) column. Which used styrene and allyl chloride as a monomers. The drug can be estimated based on small concentrations and multiple mixtures. The first step was the preparation of molecularly imprinted cocaine polymers, in which small proportions of the drug and at different times of drug metabolism could be concentrated and quantified. The second step was to obtain a concentration using solid-phase extraction, thus combining a molecularly printed polymer with solid-phase micro-extraction (SPME) to obtain a pre-concentration and a one-step estimation process for better accuracy, sensitivity and selectivity . UV-Vis used to identification the results of extraction parameters . MIP fibers have been successfully applied in the selective extraction of cocaine in urine samples with relative recovery ranging (95-102.5).

REFERENCES

1. Alvear E, D von Baer, C Mardones, Hitschfeld A. Determination of cocaine and its major metabolite benzoylecgonine in several matrices obtained from deceased individuals with presumed drug consumption prior to death. J Forensic Legal Med.;23:37–43. doi:10.1016/j.jflm.2014.01.003

2. Ali. R. Mahdi, Y. K. Al-Bayati, S. T. Ameen 2019, Preparation of anew molecularly imprinted polymers and its use in the selective extraction for determination Bromhxine Hydrochloride at pharmaceuticals, Iraqi Journal of Agricultural Sciences – 2019:50(3):886- 900

3. Brunetto Mdel R, Y Delgado, S Clavijo, Contreras Y, Torres D, Ayala C, et al. 2010. Analysis of cocaine and benzoylecgonine in urine by using multisyringe flow injection analysis-gas chromatography– mass spectrometry system. J Sep Sci.;33(12):1779–86. doi:10.1002/jssc.200900833

4.Dowling G, L Regan, J Tierney, and M. A Nangle.2010. Hybrid liquid chromatography-mass spectrometry strategy in a forensic laboratory for opioid, cocaine and amphetamine classes in human urine using a hybrid linear ion trap-triple quadrupole mass spectrometer. J Chromatogr A.; 1217 (44):6857–66. doi:10.1016/j. chroma.2010.08.043

5. Lerch O, O Temme, and T Daldrup.2014. Comprehensive automation of the solid phase extraction gas chromatographic mass spectrometric analysis (SPE-GC/MS) of opioids, cocaine, and metabolites from serum and other matrices. Anal Bioanal Chem.;406(18): 4443–51. doi:10.1007/s00216-014-7815-7

6. Langman L. J, M. W. Bjergum, C. L Williamson, and F. W. Crow. 2009. Sensitive method for detection of cocaine and associated analytes by liquid chromatography-tandem mass spectrometry in urine. J Anal Toxicol.;33(8):447–55

7. Jagerdeo E, M. A. Montgomery, M. Sibum, T.A. Sasaki, and M.A. LeBeau. 2008. Rapid

analysis of cocaine and metabolites in urine using a completely automated solid-phase extraction- high- performance liquid chromatography -tandem mass spectrometry method. J Anal Toxicol.;32(8):570–6.

8. Johansen S. S, and H. M Bhatia 2007. Quantitative analysis of cocaine and its metabolites in whole blood and urine by high-performance liquid chromatography coupled with tandem mass spectrometry. J Chromatogr B Analyt Technol Biomed Life Sci. ;852(1–2): 338–44. doi:10.1016/j.jchromb.2007.01.033.

9. Muhamed F., A., Yehya and K. Al-Bayati 2019., Determination of methamphetamine by GC-Mass based on molecularly imprinted solid-phase used 2-hydroxy ethyl methacrylate as functional monomer, eurasian Journal of Analytical Chemistry 14 (2): 71-83.

10. Reid M.J, K.H. Langford, M. Grung, Gjerde H, E.J. Amundsen, J Morland,and et al. 2012Estimation of cocaine consumption in the community: a critical comparison of the results from three complibrsmentary techniques. BMJ Open. 2012; 2(6).doi:10.1136/bmjopen-2012-001637.

11. Ruiz M. J, D Paolieri, L. S Colzato, and M.T. Bajo. 2015. Chronic and recreational use of cocaine is associated with a vulnerability to semantic interference. Psychopharmacology. 2015;232(10):1717–26.doi:10.1007/s00213-014-3806-9.

12. Strano-Rossi S, A. M. Bermejo, X. Torre, F. Botre. 2011. Fast GC-MS method for the simultaneous screening of THC-COOH, cocaine, opiates and analogues including buprenorphine and fentanyl, and their metabolites in urine. Anal Bioanal Chem.;399(4):1623– 30. doi:10.1007/s00216-010-4471-4

13. Valente M. J, F Carvalho, M. L. Bastos, M. Carvalho, and Pinho PGd 2012. Chromatographic Methodologies for Analysis of Cocaine and its Metabolites in Biological Matrices. In: DBS, Editor. Gas Chromatography - Biochemicals, Narcotics and Essential Oils. CC By, pp :163–167. doi:10.5772/32225.

14. Yaha. K.l Al-Bayati, Muhamed Farhan Abd 2017., Determination of methamphetamine drug by GC-MS based on molecularly imprinted solid-Phase used meth acrylic acid and acryl amide as functional monomers, Journal of Science, Vol. 58, No.4B, pp: 2022.