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Preparation and Application of a Novel Nanocomposites Membrane for Extracting the Primary Metabolite of Cannabis in Urine

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ABSTRACT

The updated Nanostructure materials and nano technological tools are predicted to enable the synthesis of novel polymers, as well as polymer–inorganic composites to manufacture higher qualified membranes possess attractive physical and chemical properties with high performed sensitivity and selectivity. An accurate and simple method for drug analysis is required for forensic toxicological investigation. In this study new carboxylated poly (vinyl chloride) (CPVC) nanocomposites membranes were prepared and characterized. In the comparison between the peak areas resulted from application of pure CPVC, CPVC /silver nanocomposites and CPVC /palladium nanocomposites membranes for extraction of primary metabolite of cannabis in urine; 11-nor-Δ^9-tetrahydrocannabinol-9-carboxylic acid (THC-COOH) indicated that incorporating palladium nano particles in the CPVC membrane improved the extraction capacity of the membrane to a high extent. The method was optimized to study the effect of different extraction and desorption conditions such as pH, ionic strength and membrane thickness, on enhancing sensitivity of the method. The extracted analytes were tested by gas chromatograph–mass spectrometer (GC–MS) in a selected ion monitoring (SIM) mode. Projected lower limit of detection (LOD) and lower limit of quantitation (LOQ) were 0.01 and 0.03µg/ml respectively, with relative standard deviations lower than 20%. The linearity range of the procedure was between the LOQ and 15 µg/ml, with correlation coefficient of 0.9818. The method satisfactory absolute recovery of 104.46 %. Finally, the proposed procedure was effectively tested to determine (THC-COOH) via 20 urine samples collected by the Forensic Toxicology lab.

Key words: Carboxylated poly (vinyl chloride)**,** nanocomposites membranes**,** enhancing sensitivity

Introduction

Throughout the world, cannabis is the most commonly used recreational drug. Delta-9-tetrahydrocannabinol (THC) is the psychoactive compound in cannabis. 11-nor-Delta-9-tetrahydrocannabinol-9-carboxylic acid (THC-COOH) is the primary metabolite of THC. The detection of THC- COOH in a urine sample is an indicator of cannabis use (Jamerson *et al.,* 2005). The most conventional techniques used for determination of the concentration levels of these drugs in biological samples are Liquid/liquid extraction and solid-phase extraction (SPE). Due to the low polar structure of THC and its metabolites, it posses a low solubility in aqueous media and partitioning of the drug between hydrophobic and lipophilic interfaces, such as polymeric tools (Welsh *et al.,* 2009).

A competition between the medium and material surface appear to be the main factor to determine which has the higher affinity toward THC-COOH. The more polar materials showed small affinity, whereas nonpolar materials gave rise to the largest one. The inclination of THC-COOH to be adsorbed by hydrophobic substances may be referred to a hydrophobic interaction as the main factor for binding (Roth *et al.,* 1996).

Jamersons *et al*., (2005) have proposed that adsorption of THC-COOH is affected by type of adsorbent material, adsorption temperature, adsorption time. These studies demonstrated that adsorption of THC -COOH is an observable fact creates up to 56% of the primary concentration of the drug in acidic medium at pH 4.6 and is vanished in neutral or basic urine solutions (Jamerson *et al.,* 2005).

According to molecular modeling; the surface area of a THC-COOH molecule was expected to be about 125 Å^2 (8.5 Å X 14.7 Å,). Accordingly, the least quantity of THC-COOH needed to form monolayer on the adsorbent surface was intended to be just about 46 ng/cm^2. If the determined concentration of THC-COOH was less than the mentioned value, it proposed that adsorption of a single layer is occurred (Welsh *et al.,* 2009).

The talented new extraction methods via membranes offers a lot of improvements more than other tools, for example, they can provide a supreme cleanup efficiency, high selectivity, nearly solvent less extraction without emulsion problem and high enrichment factors (Jonsson, 2008).

Earlier studies for the possibility of interaction between several types of polymers such as polyvinyl chloride, polyethylene, and nylon against some sort of drugs, as a result, an adsorption of the drugs to the polymers were recognized (Chantelau *et al.,* 1987). The results indicated, that the interaction between drugs and the polyvinylchloride tube show a noticeably higher adsorption compared to other polymers (Treleano *et al.,* 2009).

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At higher temperatures, the quantity of adsorbed drugs is increased, which indicates that PVC has an open structure at higher temperatures (Perret *et al.,* 1972).

Carboxylatedpoly (vinyl chloride) (CPVC) is a synthetically prepared article has the ability of facilitate adsorption to surface via the carboxylic group (Cosofret *et al.,* 1994). Properties of the carboxylated polyvinyl chloride relative to neutral PVC are better due to the relatively higher bulk conductivity and carrier loading selectivity for CPVC membranes (Rezk *et al.,* 2012).

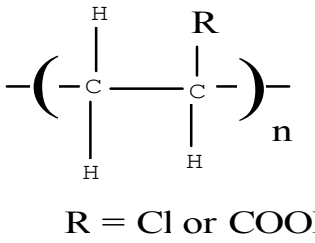
Nanostructure materials attracted a huge payment of awareness as a result of their prospective for improving specific properties such as sensitivity and selectivity. The newly accessible nano structure materials and nanotechnological tools can facilitate the production of new polymers, as well as a composite between polymers and inorganic materials to produce higher quality membranes with improved sensitivity and selectivity (Jamerson *et al.,* 2005).

In the previous studies, Polyamide and Tenax compounds were used as a new kind of solid phase micro-extraction membrane to extract some cannabinoids from biological fluids, and the resulted extract has been determined using LC–MS. Characteristics of the membrane, extraction conditions and desorption setting were studied and optimized (Yang and Xie, 2006).

THC-COOH must be protected by derivatization because it decarboxylates above 80°C. In a study for preference between the most suitable derivatizing agent from five different derivatization trials for three major acidic metabolites of Δ^1 -tetrahydrocannabinol, the most booming derivatives concerned using trimethylsilyl (TMS) derivatives for mass spectrometry (Szirmai *et al.,* 1996).

A screening extraction technique of THC-COOH from urine samples involving derivatizationg has been done without the need of previous sample cleaning steps- using a permeable hydrophobic polypropylene hollow fiber membrane. The carboxylic acid metabolite was derivatizedvia a combination between N, O-bis (trimethylsilyl) trifluoroacetamide and octane (Kirsten *et al.,* 2001).

In this work, a new polyvinylchloride (PVC) and carboxylated polyvinylchloride (CPVC) nanocomposites membranes were prepared and used as solid phase micro-extraction membrane tools (Figure1). Membranes were characterized and optimization of extraction conditions were done in terms of membrane thickness, pH, ionic strength, desorption solvents, desorption time and dealing tools supported desorption were also deliberated and optimized.



PVC (R=Cl) or PVC-COOH (R=COOH).

Fig. 1: Chemical structure of the repeated unite of polyvinylchloride (PVC) and carboxylated polyvinylchloride (PVC-COOH).

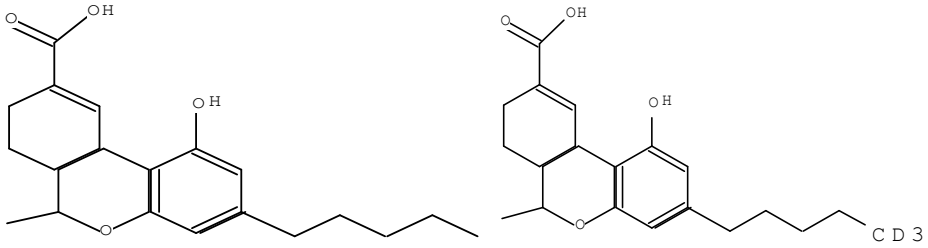
According to their chemical structures, THC-COO Has a major metabolite of abused Δ^9-THC and its deuterated form (Figure 2), were predicted to form a hydrophobic bonds to the polymer membrane and coordination bonding between their O atoms and the impregnated nano-palladium particles. THC-COOH, was spiked in urine samples and extracted using the proposed membranes. The analytical procedure using the nano composite membranes was validated with respect to limit of detection, limit of quantitation, linearity, precision and accuracy.

To conclude, the method has been applied to detect and determine THC-COOH in a 20 cases collected by Egyptians Forensics Toxicology lab.

Experimental:

Chemicals and reagents. For membranes preparation, high molecular weight polyvinyl chloride (PVC), carboxylated poly (vinyl chloride) (CPVC), dioctylphthalate, and tetrahydrofuran (THF) were used as received from Fluka. Silver nitrate ($AgNO_3$), palladium chloride ($PdCl_2$), poly ethylene glycol, sodium chloride, sodium citrate, hydrochloric acid, sodium hydroxide and methanol HPLC-grade were purchased from Merck and were of analytical reagent grade. Ultrapure water obtained via Ultra clear Direct SG was used to prepare all the solutions. The further chemicals and substances were of analytical mark and obtained via trade suppliers. Methanolic solutions of THC-COOH and its deuterated form (THC-COOH-d_3) as internal standard (IS) (1 mg/mL, for each) were obtained from Promochem/Radian (Wesel, Germany). N,O-bis (trimethylsilyl) trifluoroacetamide (BSTFA)

obtained by Pierce Chemical (Rockford, IL).



THC-COOH d_3-THC-COOH

Fig. 2: Molecular structure of 11-nor- Δ^9 -tetrahydrocannabinol-9-carboxylic acid (THC-COOH) and deuterated 11-nor-Δ^9 tetrahydrocannabinol-9-carboxylic (d_3-THC-COOH)

Stock and working solutions:

Working solutions of THC-COOH and its deuterated form (IS) have been prepred separately using the obtained concentration level of the two analyts 1.0 mg/mL to final concentrations of 1, 2, 10, 20, 100, 200 and 300 µg /mL, for THC-COOH and the working standard solution of IS at concentration 50 µg/mL in methanol and both hoard at -20° C.

Instruments:

Ultraviolet-visible Spectroscopy (UV-Vis.) was performed in a Perkin-Elmer Lambda Spectrophotometer.

Transmission Electron Microscopy (TEM): For characterization of size, morphology and composition of the nanoparticles, a drop of colloid holding solution of Pd and Ag nanoparticles, individually, were deposits on surface of a copper grid into a JEOL model 1200EX device worked at an accelerating voltage of 120 kV. After that, images of the nanoparticles were obtained.

Gas Chromatograph–Mass Spectrometer (GC–MS). An Agilent 6890 Network capillary gas chromatograph connected to a mass selective detector (MSD) Agilent 5973 Network was used in the chromatographic analyses. The capillary column was DB-5 (L=15m, I.d 0.25mm, film thickness =0.25 µm). The thermal program of GC oven was as shown: 120°C stand for 2 min, 50 °C /min to 200 °C and stand for 3.6 min, then 15 °C/min to 280 °C and stand for 10 min. Situation of the mass detector imply using a splitless injection manner with transfer line temperature of 250 °C. The carrier gas used was Helium and the injection port pressure was kept at 72 kPa. The mass spectrometer was managed using electron impact (EI) manner with 70 eV. Scan mode was used to optimize the retention times and the resolution of chromatogram for identification of the substances. Selected ion monitoring (SIM) manner was used for substances quantification. Three definite ions were chosen for studying each analyte as follows; *m/z* 371, 473, and 488 for THC-COOH and *m/z* 374, 476, and 491 for IS respectively (quantification ions underlined).

Development of Carboxylated nanocomposites membranes:

Preparation of PVC and CPVC Membranes: In a two glass petri dishes (5 cm diameter), two portions of 0.9 g CPVC and PVC, separately, were carefully mixed with 0.4 g dioctylphthalate for each, and then dissolved in 15 ml tetrahydrofuran(THF) (Ramadan *et al.,* 2012). The solutions was stirred well and heated continuously at 50 ^oC for half an hour until turn into homogeneous viscous solution. Each Petri dish was enclosed with filter paper and left to situate for 1 h to allow slow solvent vanishing.

Preparation of silver nanoparticles:

Silver nitrate solution (1mmol/ml) was prepared and used as a metal salt precursor for preparing silver nanoparticles. Solution of sodium citrate with the same concentration of $AgNO_3$ was used as reducing and stabilizing mediator. Conversion of the transparent colorless solution to characteristic pale yellow is a good marker for creation of nano sized silver. Centrifugation were used for the purification process of the produced nanoparticles, after that The colloids containing nanoparticles were washed at least three times using deionized water and nitrogen flow to get rid of excess silver ions. A freeze-drying method was used to obtain a dry dust of nanoparticles. To use the nanoparticles and perform its characterization, the dried dust of the nano sized silver were suspended in deionized water and a Fisher Bioblock Scientific ultrasonic cleaning container was used to homogenize the suspension (Guzman *et al.,* 2009).

Preparation of palladium nanoparticles:

Ethylene glycol is a good reagent for reduction, stabilization and dispersion during formation of palladium

nanoparticles; in brief, 1 mL of 0.0565 M aqueous solution of $PdCl_2$ was mixed with 20 mL of ethylene glycol exclusive of any exterior mediator for stabilization. For full reduction, the solution was kept for 1 h under temperature of 100 ^{o} C (Arora *et al.,* 2010).

To evaluate the characteristics of Pd nanoparticles, the solution was centrifuged at 15,000 rpm for 30 min by a Sigma 3K30C-Kubota centrifuge, secluded and extracted by acetone. After that, the solution was centrifuged and dispersed in 3 ml ethyl alcohol by sonication to create the nano sized Pd. Palladium nanoparticles were washed using 9 ml of hexane, then; the mixture was centrifuged at 15,000 rpm for 5 min. A mixture of hexane and ethanol was used to wash the produced nanoparticles three times; lastly, the product was hold to dry for 12 h at 110 ^{o}C (Nguyen *et al.,* 2010).

Characterization of the nanoparticles:

Samples were deposited on carbon-coated copper TEM grids for transmission electron microscopy (TEM) investigation. The deposited layer on the TEM grids was situated for 2 min. Then, blotting paper is used to get rid of the excess solution, then; the grid was permitted 2min for drying previous to its determination. Particle size distribution was evaluated using the TEM histograms via determining the size of at least 50 particles.

Preparation of carboxylated PVC nanocomposite membranes:

For the preparation of a nanocomposites membrane, either the nanoparticles were created ex situ and subsequently mixed with the polymeric blend or created within the polymeric medium using the polymer solvent to reduce the ionic metal in situ (Savage *et al.,* 2009). Two types of CPVC nanocomposites membranes have been prepared, CPVC/nano- Agand CPVC/nano-Pd. All the amount of nanoparticles resulted from the previous preparation steps was singly suspended in THF with sonication for 1 h, and then each type separately added to the casting CPVC/THF solution and the steps to form the CPVC membrane were completed as mentioned above.

Adsorption and desorption procedure (extraction method):

For extraction method, the method was proceeds in a 10 mL screw capped glass vial. A 2mL of fortified blank urine was spiked with the appropriate drug concentration and then, the sample was vortex-mixed for 30s. THC-COOH is conjugated with glucoronic acid prior to urinary excretion, producing THC-COO-glucuronide which is an ester-linked. For accurate validation results, the plank urine samples were treated as a urine case, so that; the samples must be hydrolyzed prior to its adsorption. For hydrolysis process; 2 mL of urine sample is placed in a glass tube, and 100 µl of THC-COOH-d_3 (IS) with a concentration of 50 µg /mL, and then vortexed with 0.25 mL of 10N KOH. Then the tube was capped and incubated for 15 min at 50-60°C and then cooled at room temperature(Stout *et al.,* 2001), after that the suitable pH was sustained using solutions of HCl and NaOH. Sodium chloride was added for salting out process at appropriate molarities. Subsequently, the membrane is applied to the mixture to extract the analyte at the optimum temperature and time of extraction with rotation. PVC and CPVC membranes with area 1cm^2 and thickness 0.5 mm for each were employed for comparison between the performances of the two membranes. Each membrane was soaked, separately, for 5 min by n-hexane: ethyl acetate 7:3 to improve its adsorption capacity, after that 1cm^2 of the membrane was placed into the spiked urine sample for 30 min under rotation, and was then secluded out, rinsed and dried using filter paper, then put into a 5 ml test tube with 1 ml n-hexane: ethyl acetate 7:3, respectively. The least amount of desorption solvent is 1 ml, that let the membrane to be immersed completely. Each membrane was desorbed by sonication for 5 min, at 60 ^{o}C. The extracts were evaporated under nitrogen at 40°C, reconstituted with 25 µL acetonitrile and 75 µL BSTFA, vortex, for 5 min. Vials were capped and derivatized at 80 °C for 20 min (Ellison *et al.,* 2009); finally 2 µL was injected into the GC/MS.

Method validation:

Validation of the mentioned method was carried out in stipulations of linearity, limit of detection (LODs), limit of quantification (LOQs), specificity, recovery, stability, precision and trueness along with international guidelines on the bioanalytical method validation.

Linearity and quality control samples:

For Calibration curve creation; 100 µL from working solution of analyte were spikedto 2 mL of blank urine to attain final concentrations of 0.05, 0.1, 0.5, 1, 5, 10 and 15,µg/mL and 100 µL of IS (50 µg/mL) was added. Optimization of the method was approved by quality control samples of 0.1, 1 and 5µg/mL (5 replicates). These samples were extracted in the same approach as mentioned above. Calibration curve is obtained by plotting peak areas against concentrations (µg/mL) of the analyte, from which, parameters of the least square regression were calculated, and consequently, concentrations of the test samples were interpolated from the regression parameters. The rule $Y = mX + b$ is used to find out sample concentrations, where Y = peak area, X = standard's concentration in µg/mL, m = the slope of the curve and b = the intercept with Y axis. Correlation coefficient (r^2) for the calibration curve was calculated.

Curr. Sci. Int. 3(4): 390-402, 2014

Recovery, Specificity, Accuracy and Precision:

Recovery was calculated by dividing the ratio of peak areas obtained from Solid phase micro extraction membrane (SPMEM) using spiked urine sample to that formed by the pure analytes of the quality control samples (n=5).

Specificity and any co-eluting components interferences were calculated via the comparison between the chromatograms of various groups of free urine to those from spiked urine.

Accuracy is expressed as the percent deviation of the mean of five replicate QC samples analyses from the respective target concentration.

Precision is expressed as the CV (%) of 5 replicate QC samples. Precision was evaluated under optimum conditions with 5 replicates were analyzed on the same day in a short period of time (within-day precision) and the replicates were analyzed on different days (between-day precision)

LODs and LOQs were calculated in urine as analyte concentrations producing signal-to-noise ratios of 3 and 10, respectively.

Stability of the analytes was assessed by analyzing QC samples under short-term room temperature conditions, long-term storage conditions (–20°C) and freeze-thaw treatment.

Method applicability:

About 20 urine samples were collected by forensic toxicology lab which gave positive results for cannabinoids by immunoassay tests. These samples firstly undergo hydrolysis process prior to the extraction method to obtain the free THC-COOH (Figure 3). After that the adsorption and desorption procedures were done as mentioned above.

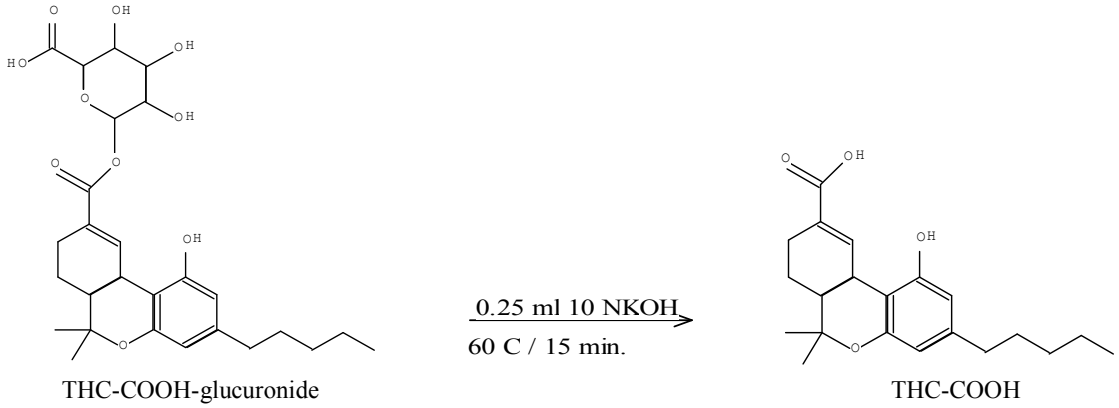


Fig. 3: Chemical hydrolysis process of THC-COOH-glucuronide.

Results and Discussion

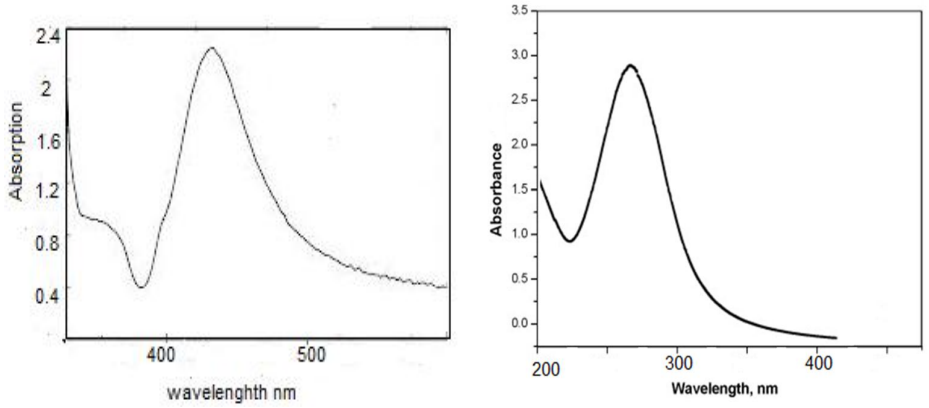
Characterization of nanoparticles:

UV–visible spectroscopy is approved to be a very valuable tool used to investigate and characterize the nanoparticles. On our studying for the created silver nanoparticles; a strong, broad peak, observed at around 430 nm in the spectrum obtained from UV–visible instrument (He *et al.,* 2013). Inspection of this peak (Figure 4a), consign to a surface plasmon, is well familiar for diverse of metal nanoparticles with size ranging from 2 to 100 nm (Kurita, 1998). The creation of Pd nanoparticles investigated by the same technique; a sharp and strong peak observed at 265 nm from the obtained spectrum (Figure 4b). These results were a good sign for the formation of Pd nanoparticles with size in the range of 2–30 nm on starting with a mixture of aqueous solution of $PdCl_2$ and poly ethylene glycol as reducing, dispersing and stabilizing agent (Nguyen *et al.,* 2010).

The transmission electron micrographs of silver nanoparticles were characterized in Figure 5 a, the bit size distribution was ranging from 10 to 80nm with an average particle size of 50 nm.

In Figure 5 b, different forms of Pd nanoparticles were spheres and further irregular shapes that were characterized by the formation of Pd nanoparticles by alcohol reduction (Gniewek *et al.,* 2005). The particle size allocation was found to range from 2 to 20 nm with average of 8 nm.

Curr. Sci. Int. 3(4): 390-402, 2014



(a) (b)

Fig. 4: (a, b). UV-Visible absorption spectrum of silver and palladium nanoparticles, respectively.

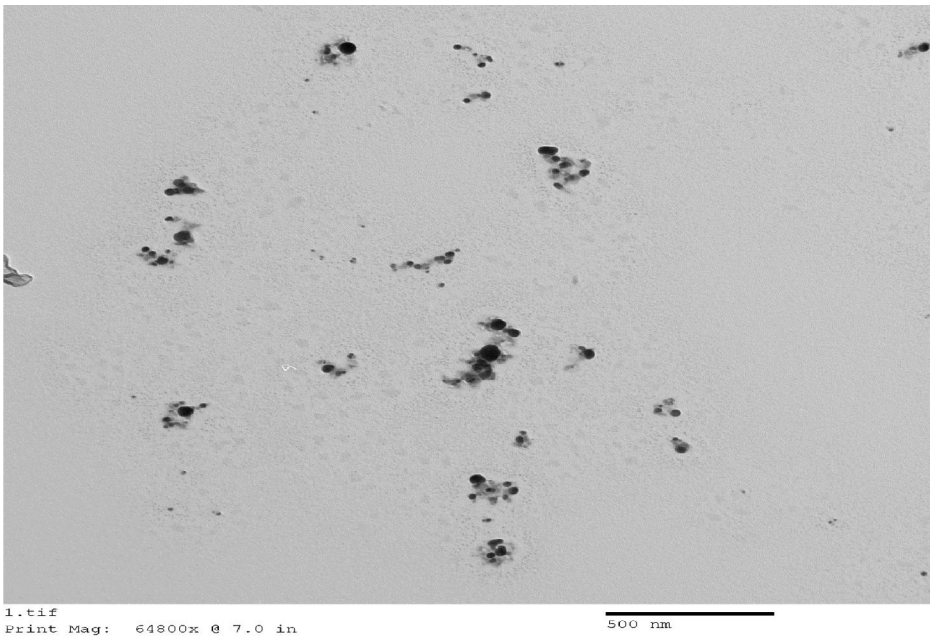


Fig. 5a: Transmission electron microscopic image for the silver nanoparticles.

Optimization of the extraction conditions:

Parameters affect on the recovery of the extraction process were investigated using a quality control sample5µg/mL (n=5). The plan was to obtain an optimized method to attain high performance, sensitive and selective extraction efficiency.

Efficiency of the extraction procedure was studied by evaluating the response of GC–MS after injecting the extracted analyte, under the greatest selected extraction conditions, the extraction competence, selectivity and sensitivity of SPMEM was enhanced as presented in subsequent sections.

Property of the membrane:

The property of the self-prepared SPMEM was studied in two stages; the first by comparing the adsorption capacity of PVC and CPVC, this comparison is clearly showed that the carboxylated PVC membrane created higher signal intensities than the PVC one.

In the second stage, three types of membranes: CPVC, CPVC /nano-Agand CPVC /nano-Pd, were compared to top quality the most efficient one for the extraction of the target molecules.

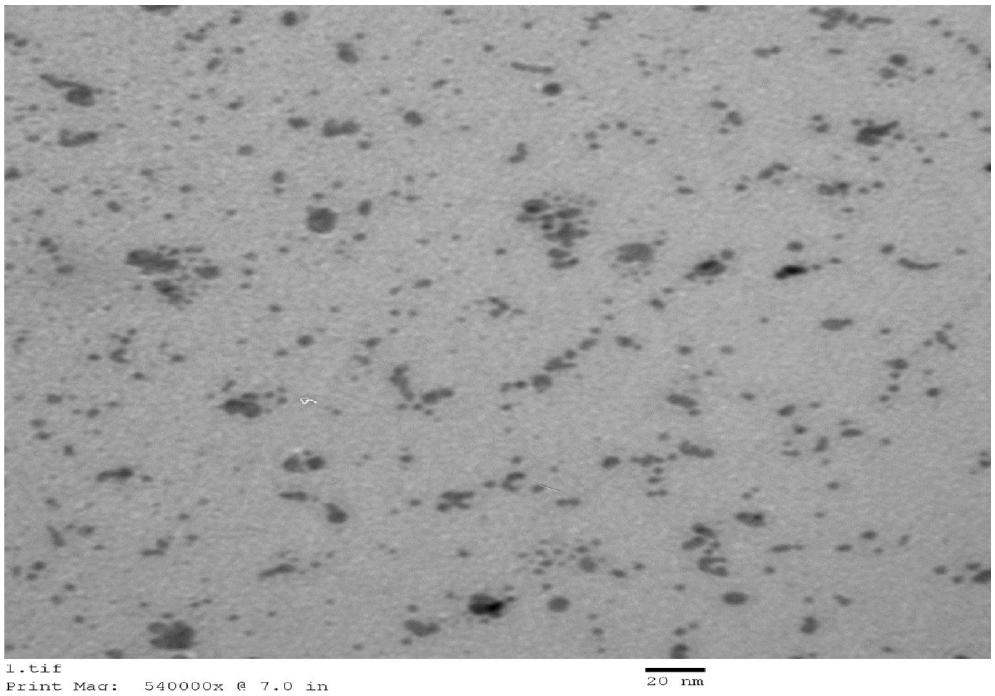


Fig. 5b: Transmission electron microscopy for the palladium nanoparticles.

In requisites of the recovery, the CPVC/nano-Pd composite membrane was selected because its recovery nearly 4 and 2 times more than CPVC and CPVC/nano-Ag, respectively (Figure 6). This may be attributed to that; The properties of carboxylated polyvinyl chloride relative to neutral PVC are better due to the gain of improved bonding to surface through the COOH group (Cosofret *et al.,* 1994),further more the previous studies approved that Palladium forms stable complexes with a extensive variety of organic ligands with O atom, due to its distinct coordination properties (Liu *et al.,* 2009).The inclination of THC-COOH for binding to the hydrophobic materials suggests some sort of hydrophobic communication as the dynamic force for adsorption (Welsh *et al.,* 2009). Surface area and volume are another important concepts to accepting why nano-palladium is more effective than nano-silver composite membranes, the tiny size of nano-palladium particles with respect to that of the nano-silver gives them a greatly bigger surface area to volume ratios which is an essential feature for the reactivity, that is, the rate at which the chemical reaction will proceed, because more surfaces are vacant to react and assist chemical processes. In addition, the experimental results approved that in case of CPVC/nano-Pd composite membrane the membrane swelling ratio was reduced, resulting in enhancement on selectivity and strength of the membrane.

Optimum time of extraction:

Since SPMEM is an equilibrium extraction approach, the highest quantity of analyte that can be extracted by the membrane is attained at equilibrium time (Chen, 2004). Therefore, in our study, 30 min were adequate to give the optimum recovery and sensitivity of SPMEM (Figure 7).

Adjustment of pH:

The extraction process is mainly affected by the molecular-form of analytes and the pH value of matrix, therefore, extraction of alkaline analytes should be under alkaline condition, and vice versa. The pH was optimized within range from 1 to 10 pH values. The acidic pH value 3.5 gives the optimal extraction (Figure 8).

Ionic strength:

Adjusting of the ionic strength by adding up a salt is branded as ‘‘salting out” effect. In our search, sodium chloride (NaCl) used to augment the extraction recovery of organic analytes (Kuo *et al.,* 2003). On adding NaCl with a certain concentration to the analyte solution, the polar compounds is greatly manipulated by providing

Curr. Sci. Int. 3(4): 390-402, 2014

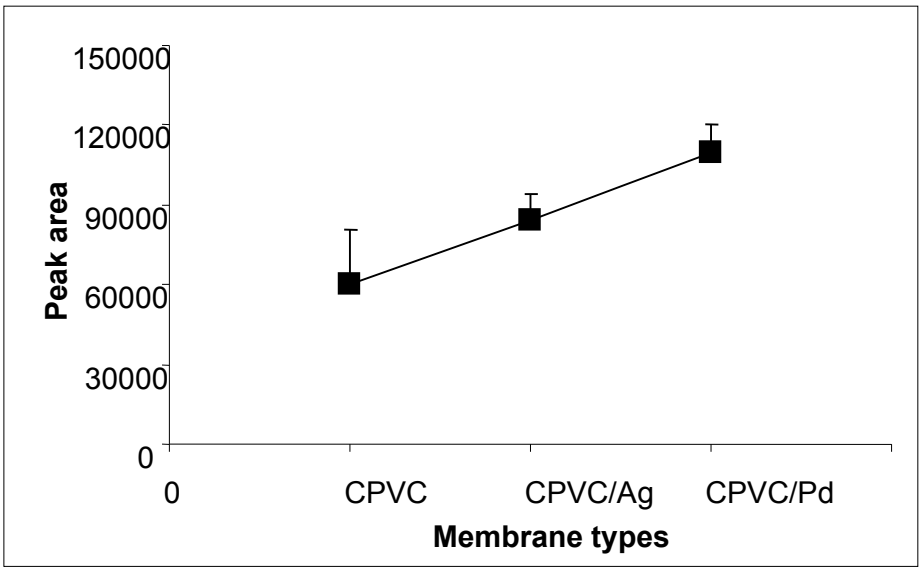


Fig. 6: Comparison of the types of membranes; CPVC, CPVC/nano-Ag and CPVC/nano-Pd in SPME of 5 µg/mL of THC-COOH in terms of peak area.

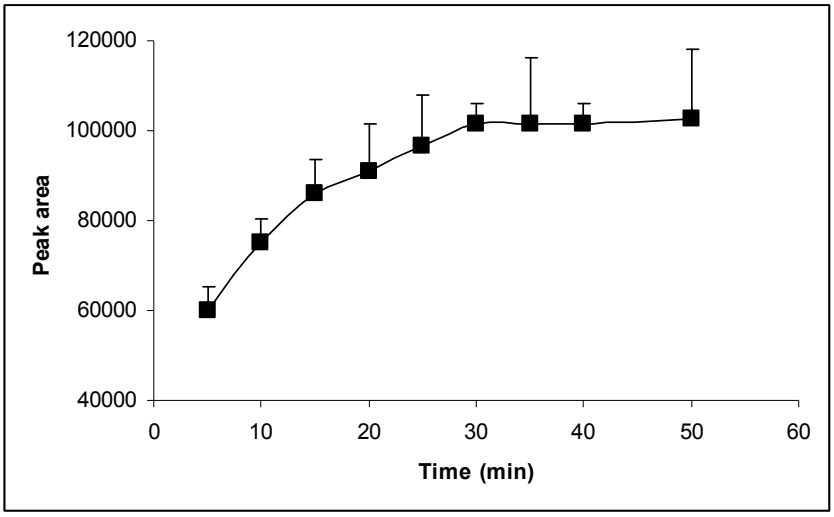


Fig. 7: Effect of time of SPME of 5 µg/mLof THC-COOH versus Peak area.

alkaline cations to swap or exchange the adsorption of original cations (Chen, 2004). The optimum extraction concentration of THC-COOH can be reached by the adjustment of the salt concentration at 1 M (Figure 9). On this condition, not only the solubility of target analyte in the aqueous media decreased, but also the partition coefficients of the analyte increased, consequently, the adding of sodium chloride enhanced the hydrophobic character of the analyte, supporting its movement from the aqueous media to the membrane.

It is recommended to immerse the membrane in ultrapure water for a minute previous to desorption, is an attempt to get rid of the salt from the membrane. This action did not reduce the drugs on the membrane as reported (Zelda, 1998).

Membrane thickness:

To study the effect of membrane thickness on recovery, a series of membranes were prepared with a different thickness varies from 0.1 to 1.0 mm. hypothetically; membranes capacity is predicted to be improved on increasing the thickness of the applied membrane. But in point of fact, the pore aspect would be blocked so that the membrane capacity is reduced and as a result, the analyte were binded only on the surface of membrane and will be was able throughout preceding the method. This behavior is different in case of the nano composite membrane;

the membrane capacity will be optimum at 0.75mm thickness (Figure 10), which may be attributable to the formation of some nano pores which will increase the surface area of the membrane. But there will be some drawback of the higher thickness such as; the membrane will require a longer time to be soaked before the adsorption process, and longer time of sonication for the desorption process.

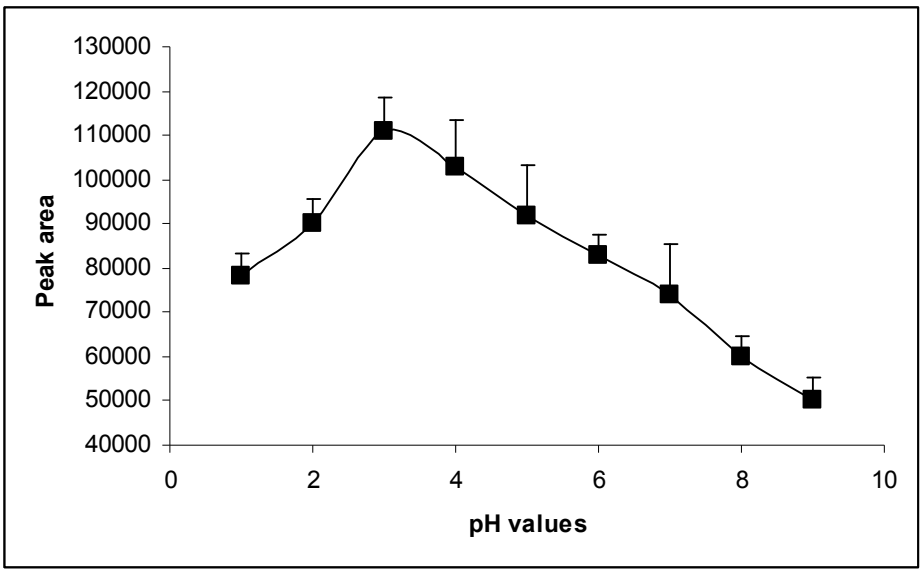


Fig. 8: Effect of pH on SPME of 5 µg/mL of THC-COOH in terms of peak area.

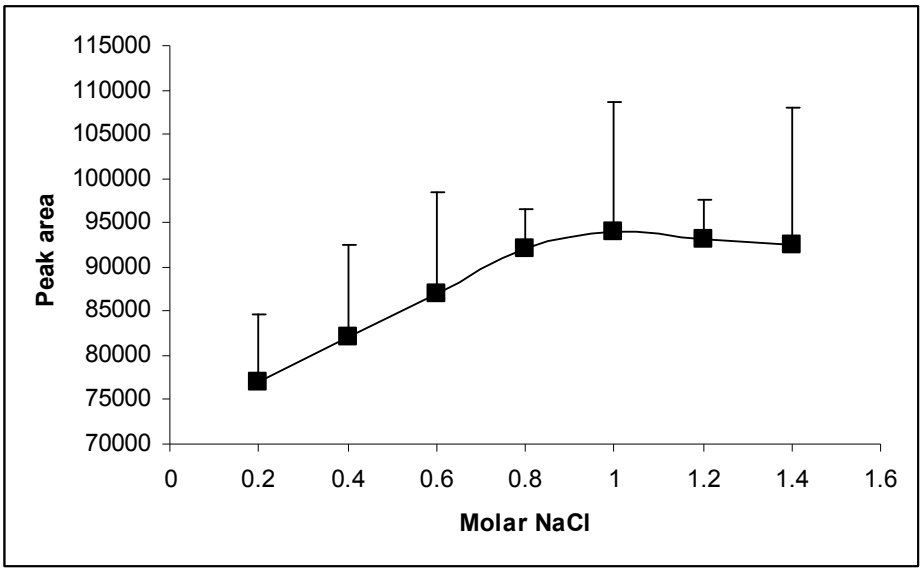


Fig. 9: Ionic strength profile of SPME of 5 µg/mL THC-COOH.

The desorption conditions:

The experimental studies approved that the organic solvent mixture (n-hexane: ethyl acetate 7: 3 respectively) is the superlative solvent that can be used as the desorption mediator and desorption competence can attain 99–100%. The physical treatment like temperature and ultrasonic can hasten desorption outcome and reduce the time of desorption. Moreover, the study approved that the above two mentioned dealing tools have little effect on the membrane characteristics. The investigation all studies approved that on applying the ultrasonic and heat treatment, 100% desorption can be attained in only 5 min at 60 °C.

Validation of the method:

Subsequent to optimization, the methodology was validated in accordance with international guidelines on

Curr. Sci. Int. 3(4): 390-402, 2014

the bioanalytical method validation (Food and drug administration, 2006 and Guidline on bio analytical metod validation, 2009).

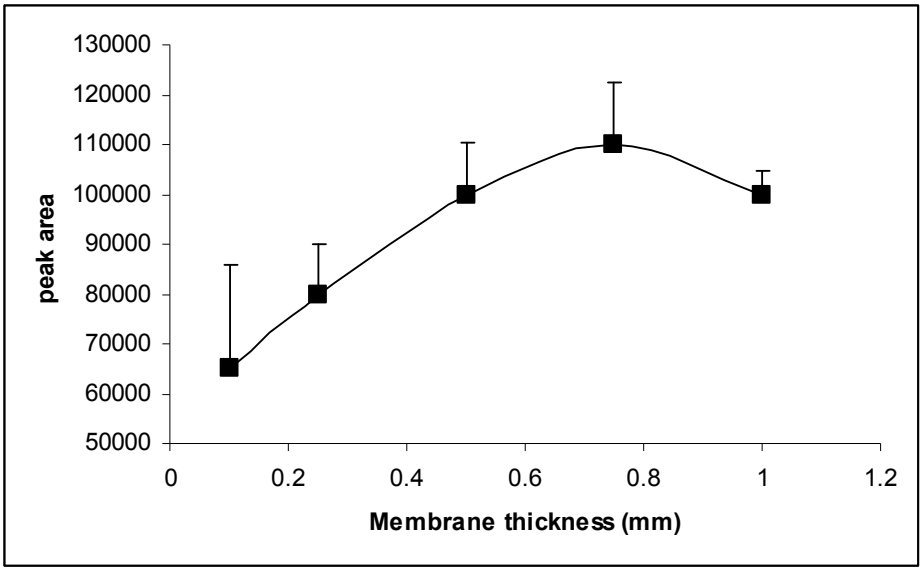
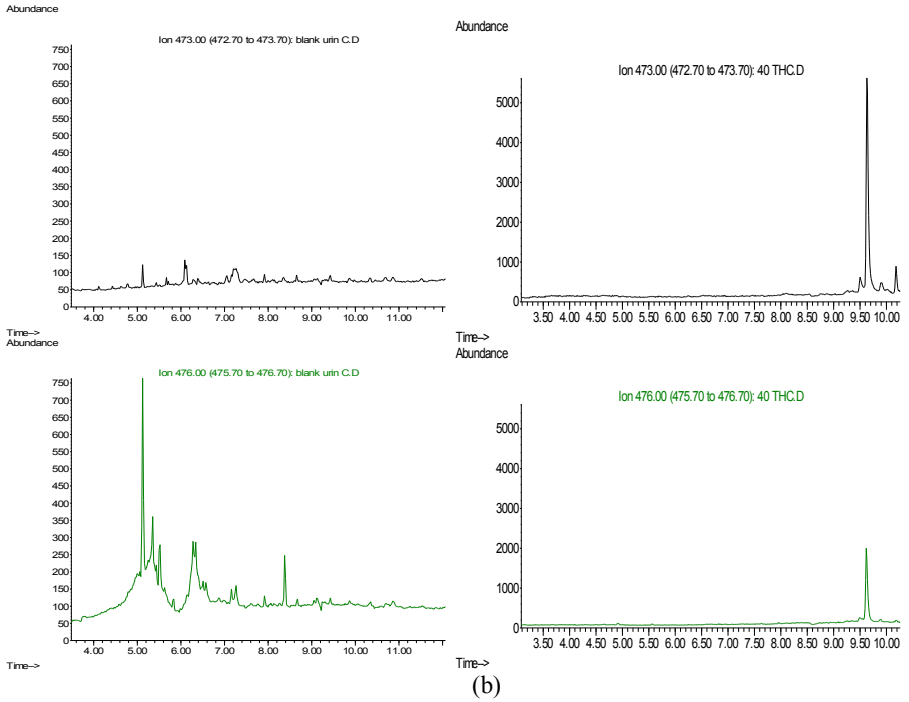


Fig. 10: The effect of membrane thickness on the SPME of 5 µg/mL of THC-COOH using CPVC/nano-Pd membrane.

Selectivity of the method:

A distinct six blank urine samples were analysed to evaluate the Selectivity. On comparing the chromatograms obtained from this samples with those resulted from urine samples spiked with THC-COOH, well separated peaks were resulted without observing any interference. Moreover, the interference produced from matrix that involved within the samples could be expelled due to unlike retention times and/or mass spectra (Figure 11).



(a) (b)

Fig. 11: SIM chromatograms of a) blank urine. And b) spiked urine with 5 µg/mL THC-COOH (*m/z* 473) and 0.25 µg /mL THCCOOH-d3 (*m/z* 476).

Method Linearity:

A good linear calibration curve was attained on plotting the peak area ratio between the analyte and IS versus analyte concentration using five points ranging from 0.05 to 15.0 µg/mL in urine with Correlation coefficient (r^2) of 0.9818, and the calibration data is presented in Table 1.

Table 1: Summary of validation parameters for SPME of THC-COOH using CPVC/nano-Pd membrane.

Property	THC-COOH
Linear range (µg/mL)	0.03-15
Calibration equation	y=17860x+10360
Correlation coefficient (r^2)	0.9818
Absolute recoveries (%) for QC samples (n=5)	104.46±13.45
LOQ (µg/mL)	0.03
LOD (µg/mL)	0.01
Accuracy (%)	91.8±6.3
Precision (%)	
Intraday for QC samples (n=5)	10.51±0.47
Interday for QC samples (n=5)	9.64±0.57
Specificity	Specific

Limits of detection and Quantitation:

In accordance with EMEA strategy, the LOD and LOQ for the analyte was considered as the lowest concentration yielding signal-to-noise ratios of at least 3:1 and 10:1, respectively, with correct relative ion ratios and a retention time within ±0.2 min of the average calibrator retention time. LOD and LOQ were 0.01 and 0.03µg/mL, respectively.

Accuracy and Precision:

Accuracy and precision were found in acceptable range according international guidelines on the bioanalytical method validation. Where accuracy was found 91.8±6.3%, and intra- and inter-day precision were found 10.51±0.47 and 9.64±0.57 respectively less than 15% (Table 1).

Absolute recoveries:

Recovery of the analyte was 104.46±13.45 using QC samples (0.1, 1 and 5 µg/mL)(5 replicates)(Table 1).

Stability:

Stability studies were performed to ensure good reproducibility of the method. The stability was discussed in the processed samples using QC concentration levels (n=3) via the mentioned method. Though, subsequent to extraction the membrane was withdrawn, but instead of desorbing it at once in the proposed solvent, the membrane was left to stand at room temperature for restricted time periods (5, 10, 15, 20 and 30 min) prior to its desorption. The resultant concentration values for each measured time did not diverge more than 5% from the supposed concentration, and as a result the drug was stable on the membrane for no less than 30 min at room temperature.

For Short-term stability, methanolic solutions of analyte at QC concentration levels (n=3) were studied. These solutions were stand for 24 h at room temperature, and then analysed. The resulted data compared with those prepared and analysed immediately, and the acquired values for precision were less than 10%. For long term stability, the mentioned solutions were stored at both 20°C for 24 h and–20°C for 30 days and then analysed. The resulted data compared with those prepared and analysed immediately, and the acquired values for precision were less than 13%

Freeze and thaw stability was in addition assessed in triplicate at QC concentration levels. The QC samples were kept at the planned storage temperature for 24 h, following that they were thawed spontaneously at room temperature. When wholly thawed, the samples were frozen again for 12–24 h within identical conditions. This freeze/thaw sequence was doubled further, and the samples were tested using the mentioned method after the third sequence. The resulted data was evaluated with those obtained from samples which prepared and analysed at once. The results proved that the analytes were stable for not less than three freeze/thaw sequences and the resulted concentration values did not diverge beyond 15% from the supposed concentration for each drug.

Adsorption capacity of the membrane:

The membrane adsorption capacity was studied under the planned optimum conditions with solutions of 50 µg /ml for analyte. The obtained peak areas were calculated from the calibration curve of the analyte, and the results indicated that the membrane can carry over analyte up to 25 µg /cm^2within the linear part of the calibration curve, whereas, the membrane can carry over up to 35 µg /cm^2 out of this area.

Curr. Sci. Int. 3(4): 390-402, 2014

Applicability of the method:

The prepared membrane can be mainly used for the quality and quantity analysis of THC-COOH due to its good adsorption capacity. To reveal the applicability of this method, samples collected from 20 cases of Human urine specimens that were determined to give positive results for cannabinoids via immunoassay screening instrument were analyzed as mentioned in the experimental part and the obtained results were within the prepared calibration curve range. The compound was identified by its retention time and by the relative amounts of the selected ions.

Conclusions:

In the present work, CPVC/nanoparticles composite membranes were successfully prepared and used to extract THC-COOH from urine. The suitable conditions for preparing composite membranes with homogeneously dispersed nanoparticles could be fabricated. It has also been found that the embedding of nanoparticles in membranes improved noticeably the adsorption capacity of the membrane. The proposed method in this search provided selective and accurate procedure for determination of the main urinary metabolite of cannabis THC-COOH, devoid of costly cleaning tools, solvent-intense run or complexed devices. The LOQs were within acceptable limits and showed that the method could be applied for forensic toxicological analysis on abusers. This method (extraction, separation and applied techniques) is simple and efficacious for the determination of analytes in urine samples. From the results above, the self-prepared nano composite membrane has high adsorption and desorption capacity, and is more environmental friendly because less organic solvents are consumed. Finally**,** this method highlighted the role of nanoparticles in enhancing the physical properties of composite membranes which elevates its adsorption capacity toward some drugs of abuse.

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