ORIGINAL ARTICLE

Analytical methods for diagnosis a mixture of narcotic substances in seized materials

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Abstract

Introduction: In the present study, reversed-phase high-performance liquid chromatography-ultraviolet (HPLC-UV) method was developed and validated for the three narcotic substances in grains tablets. The process of analysis used in the detection and qualitative assessment of narcotic substances includes three stages. Materials and Methods: The first detection uses the spot color under with the help of microscope (20% $HC_2H_3O_2$ and platinic chloride) as detector where it interacts with the molecule and gives a specific color of that molecule which could be detected clearly by an optical microscope with strength Zoom 200 Mega pixels and comparing the obtained images with photos of standard models. Results: This method was most important in the detection of the first narcotic substances. The other detection involves HPLC-UV technique using the Arcus EP-C18; 5 µm, 4.5 × 250 mm column with a flow rate 1.2 ml/min at 25°C and wavelength 275 nm where the number of samples in the mixture of narcotics is isolated and diagnosis of the initial detection is confirmed by the number of peaks in this chromatogram. Hence, the number of peaks in this method is three peaks indicating clearly the number of materials in the mix. The third detection was conducted by gas chromatography (GC)-mass technology and included the separation of chromatography in the first phase and then estimation of the mass spectrum of each material in the mix using the instrument (GC-mass spectrum [GC-MS], MSDCHEM\1\METHODS\MUAFAQ.M) for the determination of M/Z negative ions at range temperature (70–375°C). **Conclusion:** The results of the microscopic analysis showed the appearance of three forms of the three studied compounds that are very similar to the standard images of the same compounds. The HPLC analysis showed the appearance of three clear peaks of the compounds in the mix. The GC-Mas analysis showed three compounds in the mix. All results of the analysis obtained indicate the accuracy and sensitivity of the method used in the analysis and measurement.

Key words: Narcoticraw grains, psychotropic materials, three method diagnoses

INTRODUCTION

Diagnosis of a mixture of narcotics is due to the fact that forensic analysis must be trusted. The methods used in the analysis show a high sensitivity in the detection of trace quantities of materials in the matrices that have been analyzed, high-performance liquid chromatography (HPLC) and gas chromatography–mass spectrum (GC-MS) are most widely used analytical methods to identify compounds. Quantitative assessment of narcotic substances requires high-precision techniques and the analytical methods that has global credibility.[1,2]

Methods are major part in the interpretation of the data. The most important opioids, which are derivatives of raw opium, are caffeine, amphetamine and ether, and methyl diphenylmethyl. The last compound possesses a very moderate psychological effect, which is a semi-industrial opioid and has many varieties, including oxycodone.[3,4]

Most types of various analytical techniques and methods used for the measurement and determination of narcotics were GC-MS, HPLC. GC-MS and HPLC are practically and technically deemed as the most common techniques used to determine the presence of narcotic substances, despite the various techniques used to obtain such determination.[5,6]

A hyphenated technique, GC-MS specifically, standardizes the separation power and sensitivity of a GC with the analyte

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Received: 13-8-2018
Revised: 31-8-2018
Accepted: 08-09-2018

specificity of a spectroscopic technique. It can reflect high-specific spectral data on individual compounds in a complex mixture without prior isolation.[7] GC-MS occupies the highest degree of specificity and is the recommended reference method for the analysis of cocaine. However, the use of GC-Mass is peculiar to volatile analytes, and some substances of pre-analytical derivatization are frequently required.[8] The present Manuscript as other various studies and researches which have shown how closely HPLC methods compared with GC-Mass for the quantitative analysis of Narcotic substances specifically psychotropic effect materials and sensory receptors.[9]

High-sensitive HPLC methods have been significantly developed for the detection and quantitation of narcotic substances.[10] The determination of narcotic substances is an issue that has a key importance from the healthy, social, and economic point of view. HPLC entertains a major rank among other various available separation techniques that it is deemed as one of the methods used largely for the purpose of narcotic substances analysis. The reversed-phase chromatography is fairly recommended for the analysis of narcotic substances due to the ease of sample preparation, best reproducibility and detectability, lower cost, and less sample preparation. The most universal and versatile column is a bounded octadecyl silica column (C18).[11-13]

The use of HPLC has been promoted to be more familiar as the advent of diode array where multiwavelength detectors have improved the selectivity of the method by giving ultraviolet (UV) absorption profiles and derivative spectral data for each peak in the chromatogram.[14]

The analyst may use the quantitative HPLC method - described below - despite the availability of various stationary and mobile phases. This method is applied for its own best performance. All methods should be properly validated and/or verified before routine application.[15,16]

The selection of the analytical tool relies on the question being asked and the problem being solved. Traditional techniques such as GC-MS are usually applied as analytical methods to determine unknown narcotics, but the analyst would encounter certain difficulties such as small sample volume/mass seen in these alternative specimens or the target analyte may react differently compared to traditional specimens used in the toxicological analysis. This study compares three analytical methods used to determine the sensitivity and specificity in an unknown mixture.[17-19]

Researches and studies conducted in this regard seek seriously to demonstrate the throughput screening. To obtain the highest throughput screening, methods such as thin-layer chromatography, HPLC, and electrokinetic can be applied. Yet, GC-MS has been still used widely to maintain such type of screening when results must be confirmed. Due to GC-MS advantages such as its lower cost and the absence of a derivatization step before analysis, it has been turned to be the method of choice. On the other hand, HPLC is noticeably more affordable method to determine narcotics substances than other ones and has begun to spread widely as an alternative technique to achieve such purpose as a result of such advantages. Moreover, it has become more common even though GC-MS provides more sensitivity and is less susceptible to matrix interferences compared to HPLC and GC-MS appears to be as a useful method for quantification and routine analysis.[20,21] GC-MS and HPLC-UV both require the same extensive sample clean-up, but the latter requires a derivatization step. The study of the two techniques involves separation methods to shorten the time spent on sample cleanup to be used again by such traditional methods. To maintain a proper detection of narcotic substances, the present study employs the use of separation for sample clean-up before analysis.[22]

In recent years, extensive attention in clinical and forensic toxicology has focused on the increasing abuse of caffeine, amphetamine, and methyl diphenylmethyl ether. Figure 1 shows the structural formula of caffeine, amphetamine, and methyl diphenyl methylether. A number of services and even fatal intoxications attributable to these drugs have been reported.[23]

Consequently, detection and identification analyses for these compounds are routinely performed in clinical and forensic laboratories.

Amphetamine and its derivatives belong to the common drug of abuse in many countries.[24] The first amphetamine pharmaceutical was benzedrine, a brand which was used to treat a variety of conditions. Currently, pharmaceutical amphetamine is prescribed as racemic amphetamine, Adderall, dextroamphetamine, or inactive prodrug lisdexamfetamine. Amphetamine increases monoamine and excitatory neurotransmission in the brain, with its most pronounced effects targeting the norepinephrine and dopamine neurotransmitter systems.[25,26]

Caffeine is a central nervous system stimulant of the methylxanthine class.[27] It is the world's most widely consumed psychoactive drug. Unlike many other psychoactive substances, it is legal and unregulated in nearly all parts of the world. There are several known mechanisms of action to explain the effects of caffeine. The most prominent is that

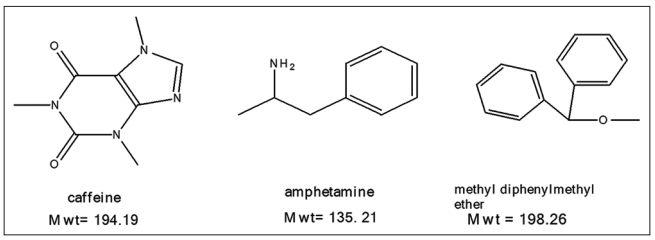


Figure 1: The structural formula of caffeine, amphetamine, and methyl diphenylmethyl ether

it reversibly blocks the action of adenosine on its receptor and consequently prevents the onset of drowsiness induced by adenosine. Caffeine also stimulates certain portions of the autonomic nervous system.

Caffeine is a bitter, white crystalline purine, a methylxanthine alkaloid, and is chemically related to the adenine and guanine bases of deoxyribonucleic acid and ribonucleic acid. It is found in the seeds, nuts, or leaves of a number of plants native to Africa, East Asia, and South America[28] and helps to protect them against predator insects and to prevent germination of nearby seeds.[29] The most well-known source of caffeine is the coffee bean, a misnomer for the seed of coffee plants. Beverages containing caffeine are ingested to relieve or prevent drowsiness and to improve performance. To make these drinks, caffeine is extracted by steeping the plant product in water, a process called infusion. Caffeine-containing drinks, such as coffee, tea, and cola, are very popular; as of 2014, 85% of the American adults consumed some form of caffeine daily, consuming 164 mg on average.[30]

Caffeine can have both positive and negative health effects. It can treat and prevent the premature infant breathing disorders bronchopulmonary dysplasia of prematurity and apnea of prematurity. Caffeine citrate is on the WHO Model List of Essential Medicines.[31] It may confer a modest protective effect against some diseases,[32] including Parkinson's disease.[33] Some people experience sleep disruption or anxiety if they consume caffeine, but others show little disturbance. Evidence of a risk during pregnancy is equivocal; some authorities recommend that pregnant women limit consumption to the equivalent of two cups of coffee per day or less.[34,35] Caffeine can produce a mild form of drug dependence - associated with withdrawal symptoms such as sleepiness, headache, and irritability - when an individual stops using caffeine after repeated daily intake.[36-38] Tolerance to the autonomic effects of increased blood pressure and heart rate and increased urine output develops with chronic use (i.e., these symptoms become less pronounced or do not occur following consistent use).[39]

At therapeutic doses, amphetamine causes emotional and cognitive effects such as euphoria, change in desire for sex, increased wakefulness, and improved cognitive control. It induces physical effects such as improved reaction time, fatigue resistance, and increased muscle strength. Larger doses of amphetamine may impair cognitive function and induce rapid muscle breakdown. Drug addiction is a serious risk with large recreational doses but is unlikely to arise from typical long-term medical use at therapeutic doses.

Very high doses can result in psychosis (e.g., delusions and paranoia) which rarely occurs at therapeutic doses even during the long-term use. Recreational doses are generally much larger than prescribed therapeutic doses and carry a far greater risk of serious side effects.[40]

Electron impact sources introduced a vapor into a beam of electrons. The electron transfers energy to the vaporizing molecule, and this energy can often result in fragmentation of the molecule. The detector used in this experiment is a mass spectrometer, which ionizes samples to produce molecular ions (possibly fragmenting the molecule in the process), and then measures the mass-to-charges ratio of the molecular (fragment) ions. Mass spectrometry is used to detect many organic and pharmaceutical compounds, and is a good chromatographic technique for many highly sensitive pharmaceutical analyzes. Mass spectrometry results are verified by the appearance of the values of the mother parent molecular ions and the values of the fragmented ions. The fragmentation pattern which results is reproducible, allowing these patterns to be used as an identifying fingerprint. Several gas chromatographic methods to analyze samples or mixtures in doping control and to toxicological analysis have been reported.[41] Due to their relatively low molecular weights, high polarity and volatility derivatization are necessary when using GC. GC-MS using electron ionization (EI) mode is a widely used technique in drug analysis, as it leads to a number of fragment ions providing structural information.[42] In this paper, we utilized a GC-MS to identify the quantities of unknown samples from narcotic substances. Furthermore, we present mass spectra to detailed fragmentation for caffeine, amphetamine, and methyl diphenylmethyl ether using GC-MS in EI mode. The aim of this study is to find a high efficient, simple, sensitive, easy, and fast method to analyze and qualitative diagnosed a mixture of narcotics that are mixed with grinded powder.

Equipment

1. Optical microscope equipped with camera (200 megapixel magnifying glass).
2. IC-UV system including:
   - LKB Bump 2150–HPLC, Bromma.
   - IonPac column Arcus EP-C18; 5 µm, 4.5 × 250 mm *(P/N* 11051194 L).
   - Metrohm electric injection valve with 100 µL loop inject in system before flow cell quartz.
   - APD 303 UV Detector equipped with 18 µl flow cell (Helma, UK).
3. GC-mass system (MSDCHEM\1\METHODS\ MUAFAQ.M) to determine M/Z negative ions.

MATERIALS AND METHODS

All solvents and reagents were of analytical grade unless indicated otherwise, and all experiments were performed with deionized water (18.2 Ω cm) resistivity at 25°C.[43]

Reagents and Standards

- Dichloromethane (DCM) BDH Chemical Ltd.
- 20% $HC_2H_3O_2$ and platinic chloride ($PtCl_2$)

- Ethanol and methanol, BDH Chemical Ltd.
- Acetonitrile, BDH Chemical Ltd.
- Helium gas (very purity).

Methods

Qualitative estimation of narcotic substances in the studied samples

The particles of the narcotic substances, which are small blocks with different forms of fibrosis, were isolated from some tablets which could be grinded into powder by manual grinder, and then, 15 g sample was taken to conduct three kinds of tests.

1. 5 g of the sample was well washed with deionized water, and the presumptive color test was performed with 20% $HC_2H_3O_2$ and platinic chloride ($PtCl_2$) as reagent and the samples were diagnosed under the microscope which compared with the standard forms of substances that previously measured.[44]
2. 5 g of the sample was taken to be tested and dissolved in 100 ml of 1:1:1 ethanol:methanol:acetonitrile underwent the testing process by applying HPLC method under standard conditions and strictly determine the number of materials in the sample. This can eliminate the inaccurate thin-layer chromatography technology and avoid potential interference for proving the number of substances in the mixture. HPLC has clearly demonstrated the number of narcotics within the sample through the number of peaks in the chromatogram.[45]
3. 5 g of the sample was washed with deionized water and dissolved well in DCM solution, filtering the sample with filter paper type Millipore USA (45 μm), and the filtrate was taken to the testing process in the GC-Mass instrument.[46]

RESULTS

Analyze Method by Presumptive Color Test

Color tests simply required the addition of a reagent or reagents to the unknown substance and observing a color change which may reflect the presence of a drug or a class of drugs. The mechanism of chemical reagents added to narcotic molecules shifts the optical absorption of the visible colored spots by an auxochrome group that shows in Figure 2.[47]

Analyze Method by HPLC-UV System (Qualitative Measurement)

Chromatograms of narcotic substance samples as well as the comparison of peaks and retention time [Figure 3] allow the identification of some narcotics and recoveries of the standard sample ranged from 98% to 100% which suggests that the analysis method is accurate. All results were obtained by optimum conditions which are shown in Table 1.

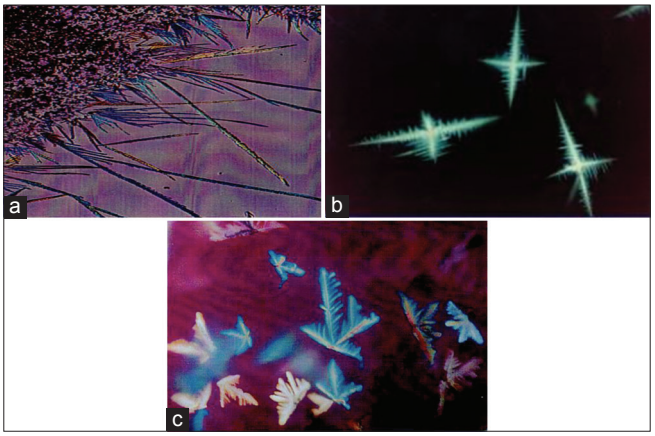


Figure 2: For three microcrystalline narcotics substances images under optical microscope. (a) Amphetamine, (b) ether, methyl diphenylmethyl, (c) caffeine

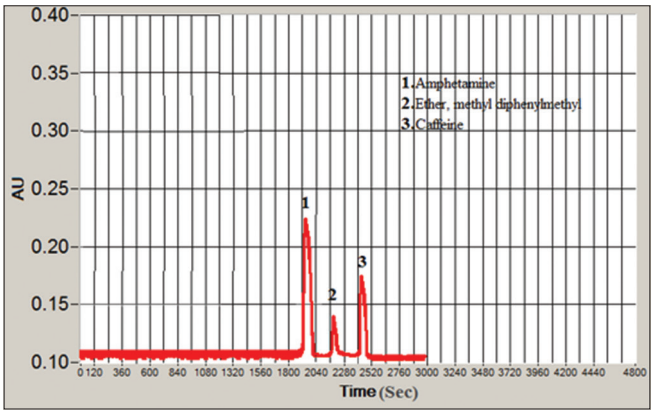


Figure 3: High-performance liquid chromatography peak's for three microcrystalline narcotic substances

Table 1: Parameters table of specific GC-MS for amphetamine

Name	Amphetamine
Cas number	000300-62-9
Entry number	15370
Molecular formula	$C_9H_{13}N$
Misc information	NIST MS#313162, Seq# R3384
Match quality	90
Company ID	NIST 2008
Retention index	0
Melting point	-
Boiling point	203°C
Molecular weight	135.10

GC-MS: Gas chromatography–mass spectrum

Analyze Method by GC-MS

The compounds were studied through GC-MS [Table 2] to create the molecular ion for each compound, and it was found that the molecular ion is equal to formula weight minus one

or more as shown in Figure 4a that confirms the narcotic molecules weight which gives a good indication for the isolation and identification of amphetamine, ether, methyl diphenylmethyl, and caffeine.[48]

DISCUSSION

Three cases in which the results obtained can be discussed as follows:

By Presumptive Color Test

The color tests are not fully dependable because there are more than one compound which can give the same results. Moreover, such tests cannot conclusively identify the presence of a compound; however, they are practically considered as a good preliminary testing tool for this reason. Since they are relatively easy to perform compared with other tests applied in this respect, color tests are widely used by law enforcement agencies as an initial testing when such agencies encounter a suspected drug. Figure 2 shows the narcotic substances images under optical microscope.[49]

By HPLC-UV System

To obtain pure material narcotic substances (detection) by reversed-phase HPLC with UV detection at 275 nm, a flow rate of 1 ml/min was used and the injection volume is 100 µl, pre-fractionated the sample on a C18 solid phase column was used for quantification of narcotic substances at a constant temperature (25°C) using an elution gradient with methanol:ethanol:acetonitrile (1:1:1) V/V/V, retention information about the throat-irritating principal HPLC method allowed to determine thereof. A new HPLC gradient was thus developed, and only three well-resolved peaks were throat-irritating, shown in Figure 3.

By GC-MS

In general, studies demonstrate that all narcotics can be analyzed more precisely by applying GC-MS technique. The reaction conditions may have to response to the caffeine, amphetamine, and ether, methyl diphenylmethyl. The fragments allow for easy identification by MS. For the purpose of shortening GC analysis time in the analysis of these compounds, a recommended chromatographic column with a studied global specification such as the capillary column is used for 30 mm × 0.250 µm. × 0.25 µm, SS., Inlet He is recommended. Figure 4a shows the separation chart by GC-MS, and the optimum conditions for the separation and diagnosis of all Narcotic substances are mentioned in Table 3.

To identify the quantity of the seized narcotic mixture, an analysis using GC-MS is carried out. The GC/MS spectrum [Figure 2] exhibits three peaks at 134.1 [M/Z]+ for the amphetamine $[C_9H_{13}N]$+, [M/Z]+198.0 for the methyl diphenylmethyl ether $[C_{14}H_{14}O]$+, and [M/Z]+ 194.0 for the caffeine $[C_8H_{10}N_4O_2]$. The GC/MS data can be considered as an evidence that the seized mixture is forbidden narcotics.

The mass spectrum of amphetamine shows several peaks due to the fragmentation of mass- to-charge of this compound and suggested mechanism of fragments pattern of amphetamine.

Figure 4b and Scheme 1 that show the mass spectrum of amphetamine show a peak at m/z 134.1 which corresponds to molecular ion $[C_9H_{13}N]^+$. A peak at m/z 120. 1 was also observed which correspond to $[C_9H_{11}]^+$ ion and attributed to the loss of NH_2 group from molecular ion $[C_9H_{13}N]^+$. The peak at m/z 103.1 is due to the loss of CH_3NH_2 molecule from molecular ion $[C_9H_{13}N]^+$ to give ion with formula $[C_8H_8]^+$. The fragment at *m/z* 91.1 can be attributed to $[C_7H_7]^+$ ion after losing $CH_3CH_2NH_2$ molecule from molecular ion $[C_9H_{13}N]^+$. The fragment with m/z 77.1 attributed to $[C_6H_6]^+$ ion due to the loss of $(CH_3)_2CHNH_2$ molecule from molecular ion $[C_9H_{13}N]^+$. The other peaks at m/z 65.1, 56.1, 44.1, and 28.1 attributed to $[C_5H_5]^+$, $[C_4H_7]^+$, $[C_3H_7]^+$, and $[C_2H_4]^+$ ions, respectively.

Table 2: Parameters table of specific GC-MS for ether, methyl diphenyl methyl

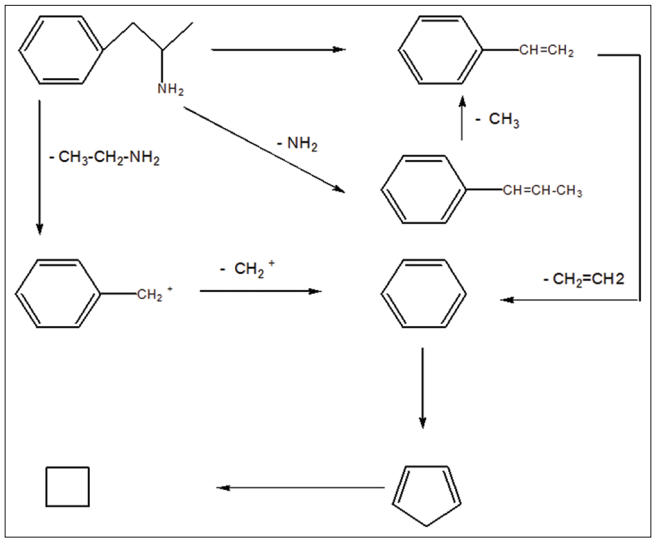
Name	Ether, methyl diphenyl methyl
Cas number	001016-09-7
Entry number	58064
Molecular formula	$C_{14}H_{14}O$
Misc information	NIST MS#156513, Seq# M84165
Match quality	98
Company ID	NIST 2008
Retention index	0
Melting point	-
Boiling point	-
Molecular weight	198.10

GC-MS: Gas chromatography–mass spectrum

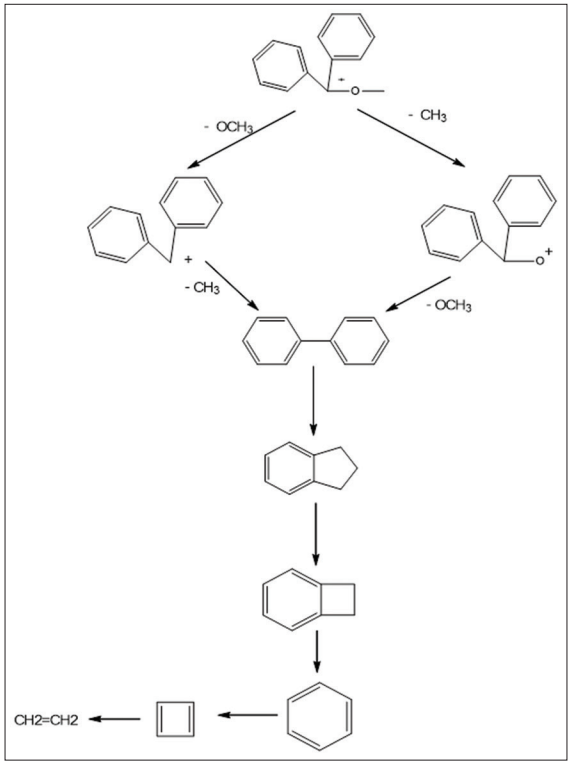
Table 3: Parameters table of specific GC-MS for caffeine

Name	Caffeine
Cas number	000058-08-2
Entry number	55120
Molecular formula	$C_8H_{10}N_4O_2$
Misc information	NIST MS# 335205, Seq# R23880
Match quality	97
Company ID	NIST 2008
Retention index	0
Melting point	-
Boiling point	-
Molecular weight	194.08

GC-MS: Gas chromatography–mass spectrum

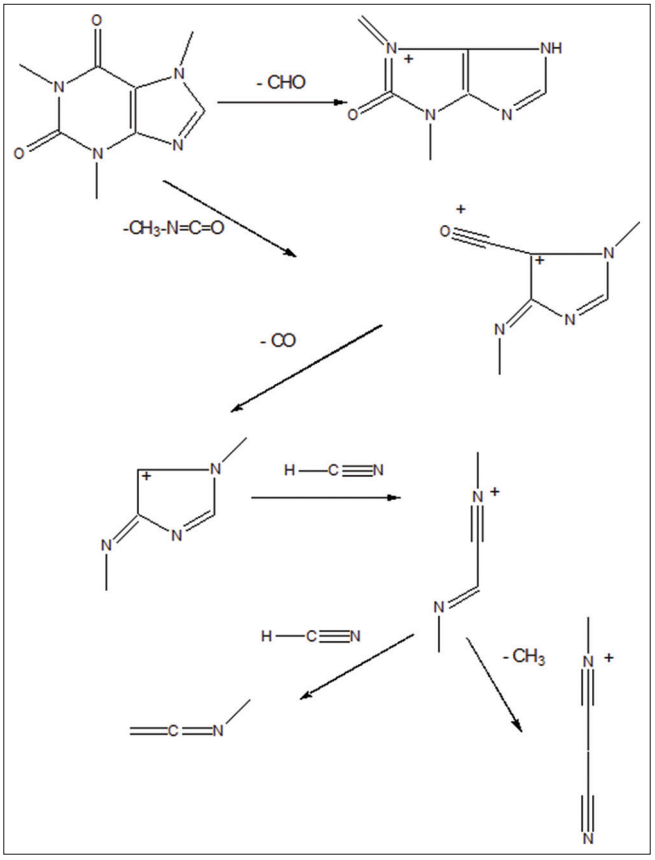


Scheme 1: The suggested mechanism of fragment pattern of amphetamine



Scheme 2: The suggested mechanism of fragment pattern of methyl diphenylmethyl ether

Figure 4.C and Scheme 2, the MS for methyl diphenylmethyl ether, Scheme 2, by m/z 198 originating formed the molecule ion [C14H14O]+ that resulted from methyl diphenylmethyl ether molecule. Molecular ion of $[C_{14}H_{14}O]^+$ experiences radical fragmentation by releasing CH_3 radical and produces fragments by m/z 182 originating from $[C_{13}H_{12}O]^+$ ion,while the high-intensity peak lies in m/z 167 originating from $[C_{13}H_{12}]^+$ ion then undergoes releasing OCH_3 group. The low-intensity peak of m/z 152 originates from biphenyl ion



Scheme 3: The suggested mechanism of fragment pattern of caffeine

$[C_{12}H_8]^{++}$ ion resulting by coupling reaction of two phenyl groups. The high-intensity peak of m/z 121 originates from $[C_9H_{10}]$ ion, while m/z 105 originates from $[C_8H_8]^+$. The other peaks of m/z 77, 51, and 29 attributed to $[C_6H_5]^+$, $[C_4H_4]^+$, and $[C_2H_4]^+$ ions, respectively.

Figure 4d and Scheme 3 show the mass spectrum of caffeine including several peaks due to the fragmentation of mass- to-charge of this compound, and Scheme 1 shows the suggested mechanism of fragments pattern of caffeine. The mass spectra of caffeine were recorded at 70 eV and provided further support for their suggested structure. The first electron removed from caffeine under electron impact probable originates from the nitrogen atom to form the first molecular ion peak at [M-1]+ 194 due to $[C_8H_{10}N_4O_2]$ + ion. The mass spectra of caffeine show two low-intensity peaks due to liberated CHO or CH_3-N=C=O groups from caffeine pointed by at m/z at 165 $[C_7H_9N_4O]$ +ion and 137$[C_6H_7N_3O]$ +ion, respectively. These occurred due to α-cleavage from the amide nitrogen. The mass spectra of caffeine show that an intensity medium peak at m/z 109$[C_5H_7N_3]$ +ion can be attributed due to the loss of CO group from $[C_6H_7N_3O]$ +ion. The mass spectrum of caffeine shows that a low-intensity peak at m/z 82 ion can attributed to the $[C_4H_6N_2]$ +ion due to the loss of HCN group from $[C_5H_7N_3]$ +ion. The mass spectrum of caffeine shows two low-intensity peaks at m/z

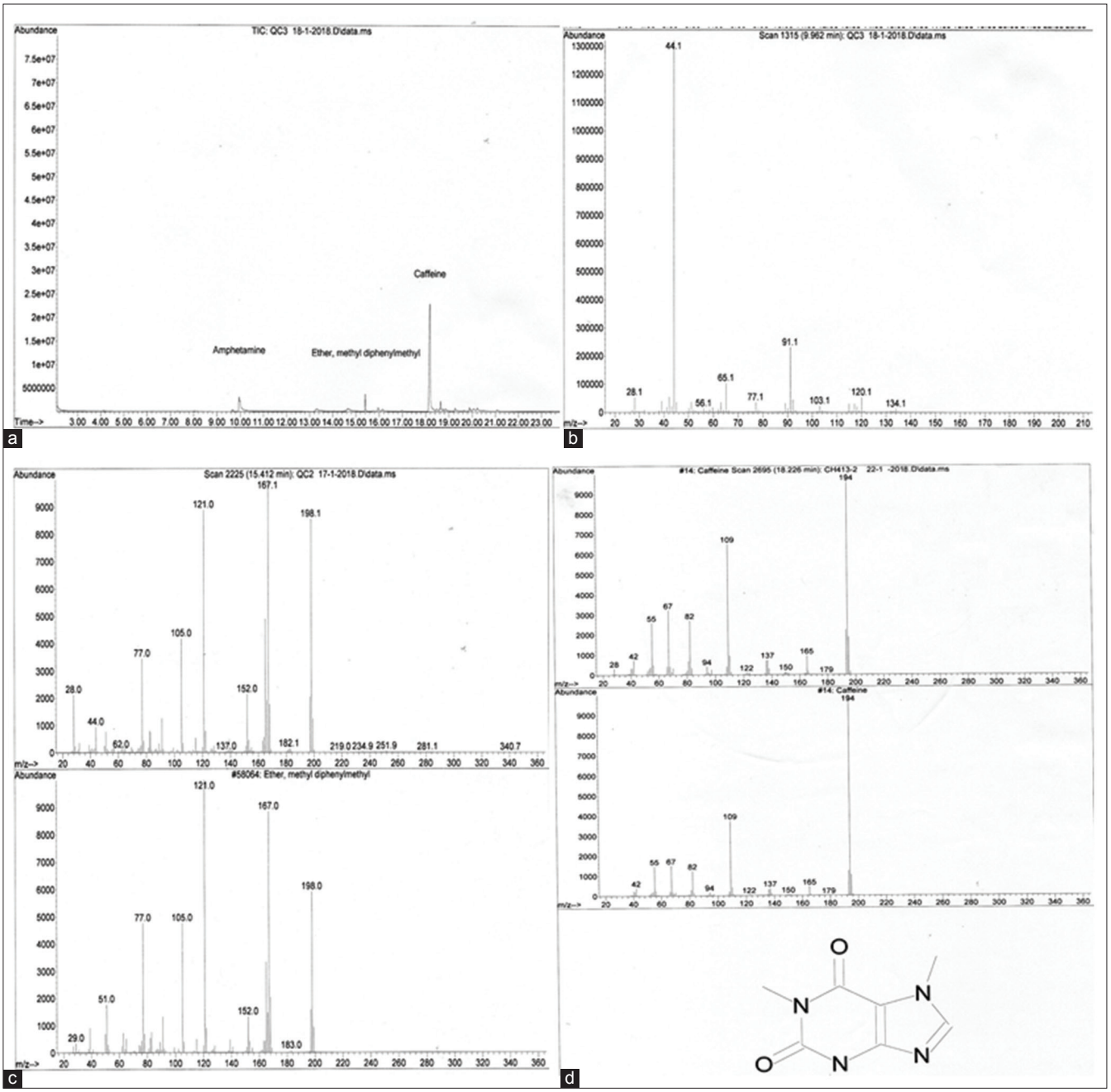


Figure 4: Gas chromatography–mass spectrum (GC-MS) for three microcrystalline narcotics structures. (b) GC-MS for amphetamine. (c) GC-MS for ether, methyl diphenylmethyl, (d) GC-MS for caffeine

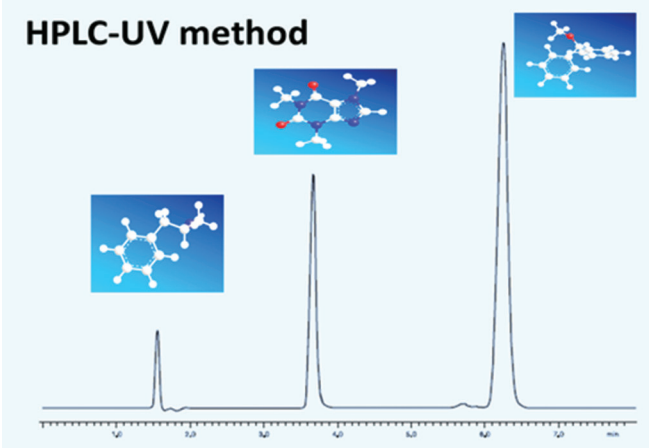
67 [$C_3H_3N_2$] + and 55 [C_3H_5N] + ions due to loss of CH_3 and HCN groups from [$C_4H_6N_2$]+ ion. The other peaks at m/z 67.0, 55.0, 42.1, and 28.0 attributed to [C_5H_5]+, [C_4H_7]+, [C_3H_7]+, and [C_2H_4]+ ions, respectively.

CONCLUSION

Based on the above results, this study divulges with three important analytical methods used to determine the presence of narcotics. The first method included a preliminary qualitative examination to determine whether or not the substance to be evaluated is narcotic and is performed using color reagents that are mixed with the narcotic substances and examined under the optical microscope and thus determine the category and type of narcotic. This process is compared to the images revealed under the microscope with images previously taken of narcotic substances standard. The other second analytical method was completed using an IC technique that separates three active components in the mixture of the narcotic substances using presumptive color test before separating compounds using C18 column 4.6 × 250 mm, 5 µm, and mixture eluent (methanol:ethanol:acetonitrile (1:1:1) as mobile phase. This method can be used for the quality control

of narcotic compounds. It is superior to the IC method that measures only three purported active components compared with the separated method that requires low separation times and has sufficient and very obvious peaks. The third method, the most important one compared to the aforesaid analytical methods, the work in the GC-MS method demonstrates clear results and determines the substance category accurately, depending on the specific molecular ion of each material and the number of fragments, thus calculating the total mass of each compound.

The three modern methods used in this study have also been used extensively in the analysis of narcotic substances and drugs and have yielded excellent results in the identification of the drug category with high accuracy.

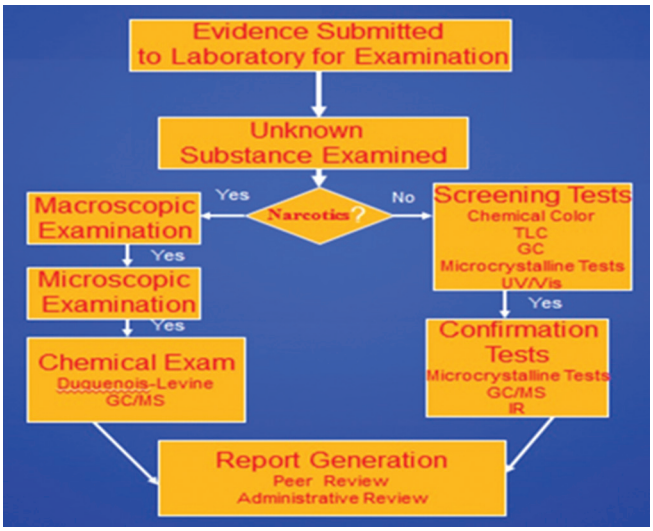


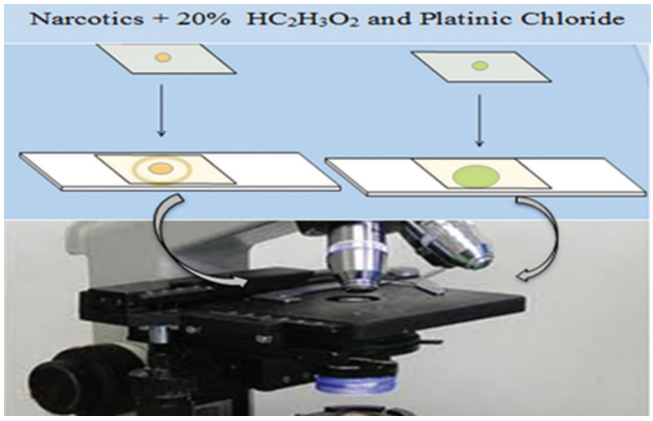
ACKNOWLEDGMENTS

The author is heartily thankful to all staff who spent a great deal of efforts to accomplish this research in propitiate manner to be published in the journal. Authors would mention that this work was supported by Basrah Police Directorate/ Criminal Evidence Laboratories and in collaboration with Pharmacy College/Basrah University.

Highlights

- The spot color tests under the microscope were initially defined as the microcrystalline narcotic substances.
- An important work is to diagnose the microcrystalline narcotic substances using HPLC-UV technology.
- The GC-MS technology is essential in determining the molecular mass of each microcrystalline narcotic substances.
- The possibility of identify three microcrystalline narcotic substances in a mixture.
- The values of the reference library of the instrument, where found a recovery 95–100%.





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Source of Support: Nil. **Conflict of Interest:** None declared.

SUPPLEMENTARY MATERIAL

1 - Additional file.
2 - Figures of chemical structures.
3 - Parameters tables for three narcotics (Specific GC-MS).

Additional File

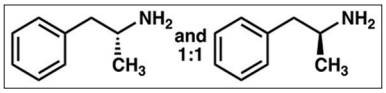
Number of opioid receptors such as alpha, beta, and gamma are receptors for opioid such as ORL1 receptor. Opioid system which controls pain and addictive behaviors can be controlled through the sensory cells in the brain. Opioid receptors are more abundant in the brain and are found in the cells of the nervous system, respiratory tract, and spinal cord. Fun activities such as laughter and abnormal joy are caused by endorphins, dynorphine, and enkephalins, which are produced by nerve cells in the brain that activate the opioid receptors and thus improve their metabolism. Opioid receptors can also be activated by external compounds such as narcotic analgesics that work on alpha receptors in the brain and are very effective in relieving pain, but unfortunately the activation of addiction reward routes and the need to use excessive and increase the doses taken over time. There are a number of chemical and biological medical properties of the five narcotic components which have been still under research that they need to be mentioned to benefit the research procedure as follows:

1. Amphetamine [Figure 2:2:A] is a methyl homolog of the mammalian neurotransmitter phenethylamine with the chemical formula $C_9H_{13}N$. The carbon atom adjacent to the primary amine is a stereogenic center, and amphetamine is composed of a racemic 1:1 mixture of two enantiomeric mirror images. This racemic mixture can be separated into its optical isomerslevo-amphetamine and dextro-amphetamine. At room temperature, the pure free base of amphetamine is a mobile, colorless, and volatile liquid with a characteristically strong amine odor, and acrid, burning taste. Frequently prepared solid salts of amphetamine include amphetamine aspartate, hydrochloride, phosphate, saccharide, and sulfate, the last of which is the most common amphetamine salt. Amphetamine is also the parent compound of its own structural class, which includes a number of psychoactive derivatives. In organic chemistry, amphetamine is an excellent chiral ligand for the stereo selective synthesis of 1,1-Bi-2-naphthol.
2. Ether, methyl di-phenyl methyl [Figure 2:2:B] is the organic compound with the formula $C_{14}H_{14}O$. The molecule is subject to reactions typical of other phenyl rings, including hydroxylation, nitration, halogenation,

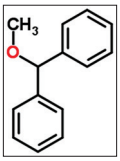
sulfonation, and Friedel–Crafts alkylation or acylation. Ether, methyl diphenyl methyl and many of its properties were first reported as early as 1901. It is synthesized by a modification of the Williamson ether synthesis, here the reaction of phenol and bromobenzene in the presence of base and a catalytic amount of copper. The main application of diphenyl ether is as a eutectic mixture with biphenyl, used as a heat transfer medium. This mixture is well suited for heat transfer applications due to the relatively large temperature range of its liquid state. A mixture of fusion, commercially. The proportion of 73.5% is diphhenyl ether (diphenyl oxide BDE) and 26.5% biphenyl (diphenyl PCB).

3. Caffeine which shows in Supplementary Figure 2:2:C has a molecular formula: $C_8H_{10}N_4O_2$ and molecular mass 194.19 g/mol, IUPAC name [1,3,7-trimethylpurine-2,6-dione]. It is a sub-alkaline substance from the group of Zantines which is classified as psychotropic drugs of the group of stimulants. The caffeine acts as a stimulant for the central nervous system in humans, prevents drowsiness, and temporarily renews activity.

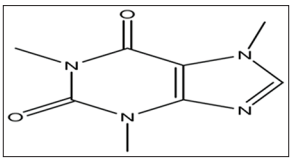
Figures of Chemical Structures



Supplementary Figure 2:2:A: Chemical structure of amphetamine



Supplementary Figure 2:2:B: Chemical structure of ether, methyl diphenyl methyl



Supplementary Figure 2:2:C: Chemical structure of caffeine