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Study Of Methamphetamine Abuse Among Drug Abuse Positive Cases Attending Sohag Clinical Toxicology Laboratory by New Validated HPLC-DAD Method

Rania A. Radwan[1], Nour F. Abouzied[1], Ahmed Mora[2], Ali M. Hassan [2] and Mai M. Abdelkader[1]

1 Forensic Medicine and Clinical Toxicology Department, Faculty of Medicine, Sohag University, Egypt.
2 Chemistry Department, Faculty of Science, Al-Azhar University, Cairo, Egypt.

Abstract

Introduction: Substance abuse becomes a global challenge with alarming increase in use of crystal meth (shabu) in Egypt. For that, there is an urgent need to develop a reliable analytical method in routine operations. **Aim:** The current study aimed to develop and validate of a sensitive and accurate method for methamphetamine (meth) determination in human urine to evaluate prevalence of meth in Sohag population. **Methodology**: The study was applied on subjects attending Sohag Clinical Toxicology Laboratory for drug abuse testing, which exhibited primary positive results for amphetamine by rapid immunoassay dipstick (ABONtm). Amphetamine positive cases were analyzed for meth by validated method on HPLC-DAD**.** Tert-butylmethyl ether (MTBE) in alkaline media was used to extract meth from urine and back extracted into hydrochloric acid where propranolol was employed as the internal standard with extraction efficiency 77.77 %. **Results:** The calibration curve was linear (r^2> 0.99) in the concentration range from0.25 to 3 µg /mL for methamphetamine. Limits of detection and quantification were 0.1 and 0.25 µg/mL, respectively. Intra- and inter-assay precisions for meth were within 2.75& 9.70 and 1.90 & 4.87%, respectively. Intra- and inter-assay accuracies were within (-6.67) &9.33 and (-2.78) & 6.67 %., respectively. The method revealed 51 cases of meth abuse in studied subjects. Their risk assessment and drug usage association were studied. **Conclusion:** The current method low limit of quantification (LOQ) of 0.25 µg/mL provides a sensitive and adequate confirmatory physical evidence for the presence of meth in the urine. **Recommendations:** Routine meth screening is recommended viewing its increasing abuse and its hazardous health effects.

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Key words Drug of abuse, methamphetamine, HPLC-DAD

Introduction

Substance abuse is a global challenge with detrimental effects on health, livelihood and security of nations and individuals' physical and mental health. Stimulants such as amphetamines and cocaine have worldwide increasing rate of addiction (Abd Eldayed and Abd Elaziz, 2018; Kabbash et al., 2022).

Methamphetamine (meth) (2S)-N-methyl-1-phenylpropan-2-amine misuse, is one of the fatal health crises that have spread over the world. It is the second most often misused substance all over the world. Methamphetamine is known by its strong effect on central nervous system. It is called in market by several names such as crystal meth, chalk, crank, and ice. One of the most powerful forms of meth is crystal meth (shabu in Egypt) (Hashisha et al., 2022; Kim et al., 2023).

The World Health Organization estimated that more than 35 million individuals regularly use meth worldwide. Methamphetamine is a highly addictive substance and its use is associated with a range of health harms, including psychosis, depression and other mental disorders, cognitive and neurologic deficits, cardiovascular and renal dysfunction, transmission of human immunodeficiency virus, viral hepatitis, and sexually transmitted infections, overdose and increased mortality (Jones et al., 2022; Yamamoto et al., 2022).

Psychostimulants were the second most common drug class involved in overdose deaths in 2019 as their percentage of overdose deaths increased from 8.2% in 2013 to 22.9% in 2019 (after opioids as a class) (Jones et al., 2022). Over the past several years, there has been an alarming growth in crystal meth abuse among Egypt population, especially among younger ages, stressing the significance of checking for co-morbid conditions with its misuse (Hashisha et al., 2022). For this reason, the need for a simple and rapid analytical method for meth detection has become necessary.

Methamphetamine was determined using various biological samples like blood, urine, saliva, sweat, nails and hair but urine sample is the most suitable sample for meth detection as 40% of meth intake is found unchanged in urine which make it the best matrix to be used. Also, it has a long window of detection and non-invasive sample collection (Saito et al., 2023).

The gas, liquid and high performance liquid chromatography are used routinely for the detection of the meth and derivatives especially with addition of other methods including supercritical fluid

chromatography (SFC)/tandem mass spectrometry (MS-MS), However, one of the most commonly used techniques for detecting drugs in biological samples is still high-performance liquid chromatography–diode array detection (HPLC–DAD) because of its full scan ultraviolet (UV) spectra for common drugs and its affordable consumables (Hilal and Mohamed, 2014; Khorablou et al., 2021).

The purpose of this study was to develop and validate simple analytical method for determination of meth from human urine to study the prevalence of meth abuse among drug abuse positive cases attending Sohag Clinical Toxicology Laboratory to point out importance of its routine screening in legal and suspected clinical cases.

Subjects and Methods

Study design:

This study is a cross-sectional study.

Subjects and sampling:

The study was applied on subjects attending the Sohag Clinical Toxicology Laboratory from January 2021 to December 2023 for drug abuse testing (The information was collected from the Sohag Clinical Toxicology Laboratory database after written consent from the director. Consent patient form was included in this database. According to the commitment standard operating procedure guidelines, ethical approval was obtained from the Medical Research Ethics Committee of the Faculty of Medicine, Sohag University. The ethical approval was obtained on 8/8/2022 under IRB Registration number: Soh-Med-22-08-19. The information which includes personal history, drug or polydrug intake, medical examination, and analytical toxicology results was recorded.

The sample size was calculated by equation for the human part of open epi software (http://www. Open epi. com/) and according to reference (Barati et al., 2014; Rawson & Kintsch, 2005) with study power 80%, significance level 95%, and confidence interval 95% (Charan & Kantharia, 2013).

Chemicals

Methamphetamine hydrochloride (purity>99 %), propranolol hydrochloride, triethylamine liquid (TEA) 99% were purchased from Merck, Germany. HPLC grade solvents [methanol (99.8%), hexane (96 %), ethyl acetate, isopropanol, chlorobutane, methyl-tert-butyl ether (MTBE), diethyl ether, acetonitrile (ACN), chloroform and dichloromethane] and Potassium dihydrogen phosphate powder and ortho-phosphoric acid liquid were purchased from Sigma-Aldrich company, Germany. Sodium hydroxide powder and hydrochloric acid (HCl) were purchased from Egyptian Company for chemicals and pharmaceuticals. Egypt, Ammonium hydroxide 33% was purchased from El-Nasr pharmaceutical chemicals company. Multi-drug screen panel dipstick (amphetamine (amp), barbiturates, opiate, Delta 9-tetrahydrocannabinol (THC), tramadol and cocaine) ABONtm (Abon Biopharm, Hangzhou) CO., Ltd, China.

Instruments:

High Performance Liquid Chromatography (HPLC) 1200 series, (quaternary pump, photodiode array detector, vacuum degasser, an autosampler injector and Zorbax - C8 (250 mm ×4.6 mm, 5 µm) column, Agilent, United States of America (USA) were used for chromatographic separation. CDx90 drug analyzer, Thermo Fisher Scientific Company, Germany

Methodology:

Screening test:

Cases were examined using polydrug screen panel dipstick (amp, barbiturates, opiate, THC, tramadol and cocaine) which is based on immunoassay technique. Data were interpreted as shown in the panel, figure (1). Positive cases of drug use, except for amphetamine cases, are confirmed by CDx90 drug analyzer device, while amphetamine cases are examined by an HPLC-DAD to determine methamphetamine cases.

Methamphetamine detection from urine by HPLC:

A stock solution of Meth at a concentration of 1mg/ml in methanol is prepared and kept stored at – 20°C. Working solutions of Meth in methanolic HCl is prepared at concentrations of 10, 15 and 100 µg/ml. A stock solution of Propranolol, used as internal standard (IS), at a concentration of 1mg/ml in methanol is prepared and kept stored at – 20°C.

Working solution of IS at 5 µg/ml is prepared by distilled water dilution.

Working IS was 5 µg/mL which was prepared from 100 µg/mL of propranolol by distilled water dilution. Six calibration standards were made (0.25, 0.5, 1, 1.5, 2 and 3 µg/mL) by a serial dilution of 10 µg/mL of meth with blank urine.

Three quality control (QC) samples [low (L), middle (M) and high (H)] were prepared by a serial dilution of 100 µg/mL of meth with blank urine. They were 0.75 µg/mL, 1.8 µg/mL and 2.4 µg/mL respectively.

Extraction Procedure

Different solvents with different alkaline media have been used in liquid extraction methods of analytes from urine, such as hexane, diethyl ether, ethyl acetate, chorobutane, isopropanol, chloroform, dichloromethane and MTBE were studied.

HPLC conditions

In HPLC separation conditions, several isocratic and gradient elution conditions were tested with different percentage of eluent. The best optimized method was thoroughly and completely validated in accordance with United States Food and Drug Administration (USFDA) requirements (specificity, linearity, limits of detection (LOD) and quantification (LOQ), intra- and inter-assay precision, accuracy and extraction efficiency) (Deshpande et al., 2019).

Methamphetamine detection in urine samples:

Urine samples of amphetamine positive cases among drug abuse positive cases (amphetamines, barbiturates, opiate, benzodiazepines, tramadol and cocaine) were analyzed for meth by validated method.

Statistical analysis:

Data was entered and analyzed using SPSS (Statistical Package for the Social Sciences) version 26. Data was expressed as mean ± SD.

Statistical analysis of the recorded data was performed using independent T-test for quantitative data and chi-square test X2 for qualitative data. Significance was considered at a P-value < 0.05. Person's correlation was also performed.

Results

Optimization and validation of the method:

Propranolol (C16H21NO2) was discovered to be the chemical of choice as an IS for meth in UV scan; 205 nm was chosen wavelength because it displayed the optimum peak properties for both meth and IS, figure (2).

The effect of eluent pH changes on the separation of analyte and IS phase was tested using phosphate buffer of pH from 2 to 6, figure (3) illustrate the peak changes with pH values (2-6). Phosphate buffer (0.01 M potassium dihydrogenphosphate with 0.1% TEA adjusted at pH 3 by 0.1 M sodium hydroxide to achieve the required pH for best separation.

A gradient condition was performed to achieve the best separation between analyte and IS with total run time was 10 minutes.

The mobile phase was initially composed of Acetonitrile (ACN) to phosphate buffer by ratio (25:75, v/v). The gradient elution was performed by decrease in aqueous eluent from 75 % to 50 % after eight minutes from run beginning also rise in flow rate from 1ml\min to 1.5 ml\min after five minutes from run beginning led to base line separation with adequate resolutions. And detector was set had a discrete channel set at 205 nm. Retention times of meth and propranolol were 3.884 and 9.100 minutes respectively, figure (4).

The optimum solvents for methamphetamine extraction at constant condition were MTBE, hexane, ethyl acetate and diethyl ether respectively. Methamphetamine and IS from urine samples were extracted using optimum organic solvent and returned back to aqueous using HCl. Add 100 µL (5 µg/mL) of IS to 1 mL of urine sample and mixed vigorously. 150 µL of 33% ammonium hydroxide and 6 mL of MTBE were added. The tubes were then vortexed for five minutes before centrifugation at about 4000 rpm for five minutes. The upper organic layer was transferred into a tube containing half mL of 1M HCl and mixed by vortex. Following a five minutes centrifugation at 4000 rpm, the upper organic layer was discarded. 200 µL of 33% ammonium hydroxide and two mL of MTBE were added to the remaining aqueous solution. The samples were then vortex mixed for 60 seconds and centrifuged for five minutes. The organic layer was transferred and evaporated. The dried extract was, then, reconstituted in 200 µL 0.05 M HCl

The chromatograms indicate excellent peak properties for amphetamine and IS. There was no endogenous noise detected at the meth and IS retention periods in the negative urine samples.

The limit of detection was 0.1 µg/mL while the limit of quantification was 0.25 µg/mL. The meth standard calibration curve was linear along dynamic concentration range of 0.25-3 µg/mL. Figure (5) showed the peak area ratio, which represents the ratio area response of meth and IS.

Precision and accuracy of the method were evaluated at three concentrations (LQC, MQC, HQC) over the linear dynamic range are presented in table (1). Five replicates at each concentration were assayed to determine intra-assay accuracy and precision. Intra and inter-assay precisions for meth were in between 2.75 & 9.70 and 1.90 & 4.87 %, respectively.

Intra- and inter-assay accuracies for meth were in between -6.67 & 9.33 and -2.78 & 6.67 %, respectively. Mean extraction recovery of meth was 77.77 %.

Methamphetamine and IS were stable for one day after extraction especially after reconstitution in 0.05 M HCl to convert meth and propranolol from free base form to the more stable base salt.

Determination of meth in urine samples of studies subjects:

The current study was a cross-sectional study carried out on 7981 urine sample examined on Sohag Clinical Toxicology Laboratory from January 2021 to December 2023 for drug abuse. The positive drug abuse cases were 233 cases.

The amphetamine positive cases (n=65) by the dipstick method were tested for meth by HPLC and revealed meth positivity in 51 out of 65 cases (78.46%). Limit of quantification (LOQ) of 0.25µg/ml was used in the quantitative analysis of Meth positive samples.

Methamphetamine concentrations were ranging from 0.75 to 31.35 µg/ml (the median peak concentration was 5.37 µg/ml).

Table (2) showing risk assessment for meth positive compared to other positive drug of abuse cases in the studied duration.

Tables (3) showed polydrug usage in studied drug of abuse cases while Table (4) showed association between meth intake and other drugs of abuse in studied cases.

Table (1): Intra- and inter assay precision and accuracy for meth in spiked blank human urine samples.

Nominal concentration (µg/mL)	Average concentration (µg/mL)	Precision (RSD%)	Accuracy (Bias%)
Intra-assay (n = 5)			
LQC (0.75)			
Day 1	0.80	2.75	6.67%
Day 2	0.79	3.88	5.33%
Day 3	0.82	2.82	9.33%
LQC (1.8)			
Day 1	1.65	3.58	-8.33%
Day 2	1.78	8.45	-1.11%
Day 3	1.80	9.15	0.00%
LQC (2.4)			
Day 1	2.24	3.50	-6.67%
Day 2	2.36	9.70	-1.67%
Day 3	2.46	9.15	2.50%
Inter-assay (n = 15)			
LQC (0.75)	0.80	1.90	6.67%
MQC (1.8)	1.75	4.87	-2.78%
HQC (2.4)	2.35	4.68	-2.08%

RSD = Relative Standard Deviation, low quality control (LQC), middle quality control (MQC) and high quality control (HQC)

Table (2): Chi-square test for risk assessment for meth positive cases (51 cases) compared to other positive drug of abuse cases (233 cases) in studied duration.

Analytical method			HPLC [a]		Rapid screening immunoassay kit [b] 182 (78.1)										P-value
Drug Variables		N (%)	Meth- amp		Amph only		Barbiturate		Opiate		Benzo		THC		Tramadol		
			51 (21.89)		14 (27.9)		4 (1.72)		33(14.16)		30(12.88)		114(48.93)		54(23.18)		
			N	%	N	%	N	%	N	%	N	%	N	%	N	%	
Age	< 20	32(13.73)	6	2.58	1	0.4	2	0.86	2	0.86	0	0.00	16	6.87	7	3.00	0.011 (s)
	20-29	58(24.89)	18	7.73	2	0.9	1	0.43	10	4.29	4	1.72	37	15.88	22	9.44	
	30-39	67(28.76)	18	7.73	4	1.7	0	0.00	10	4.29	6	2.58	36	15.45	20	8.58	
	40-50	58(24.89)	7	3.00	4	1.7	0	0.00	8	3.43	10	4.29	18	7.73	4	1.72	
	> 50	18(7.73)	2	0.86	3	1.3	1	0.43	3	1.29	10	4.29	7	3	1	0.43	
Sex	M	14(6.01)	51	21.89	10	4.3	4	1.72	31	13.30	28	12.02	114	48.93	48	20.60	0.013 (s)
	F	219(93.99)	0	0.00	4	1.7	0	0.00	2	0.86	2	0.86	0	0.00	6	2.58	
Employment	Yes	60(25.75)	31	13.30	13	5.6	3	1.29	22	9.44	26	11.16	74	31.76	47	20.17	0.041 (s)
	No	173(74.25)	20	8.58	1	0.4	1	0.43	11	4.72	4	1.72	40	17.17	7	3.00	
Year	2021	42 (18.03)	3	1.29	2	0.9	3	1.29	12	5.15	5	2.15	18	7.73	11	4.72	0.000 (s)
	2022	61(26.18)	6	2.58	8	3.4	0	0.00	7	3.00	20	8.58	14	6.01	16	6.87	
	2023	130 (55.79)	42	18.03	14	6	1	0.43	14	6.01	5	2.15	82	35.19	27	11.59	

Meth: Methamphetamine, Amp: Amphetamine, THC: Tetrahydrocannabinol, Benzo: Benzodiazepines, N: Number of cases, %: Percentage of cases, P-value: Probability value, (s): Significant P-value < 0.005, a Urine samples positive for amphetamine by new validated method, b from analytical reports

Table (3): Chi-square test comparing polydrug usage in studied positive drug of abuse cases (233 cases).

Polydrug usage	One drug		Two drugs		Three drugs		Four drugs		P-value
	N	%	N	%	N	%	N	%	
Meth-amph	0	0	17	33.3	27	52.7	7	13	0.000 (s)
Amph only	10	71	3	21.4	1	7.1	0	0	0.000 (s)
Barbiturate	2	50	2	50	0	0	0	0	0.28(NS)
Opiate	16	48.5	8	24.2	4	12.1	5	15.2	0.00 (s)
Benzo	26	86.7	4	13.3	0	0	0	0	0.06
THC	68	59.6	13	11.4	27	23.7	6	5.3	0.00 (s)
tramadol	37	68.5	10	18.5	4	7.4	3	5.6	0.3

P-value: Probability value, (s): Significant P-value < 0.005, (NS) non-significant.

Table (4): Pearson's Correlation coefficient (r) between meth intake and other drugs of abuse in studied cases (233).

Drug correlation	Pearson's coefficient (r)	P-value
Barbiturate (4)	-0.7	0.2 (NS)
Opiate (33)	-0.007	0.9 (NS)
Benzo (30)	-0.2	0.002 (s)
THC (114)	0.1	0.1 (NS)
Tramadol (54)	-0.19	0.003 (s)

P-value: Probability value, (s): Significant P-value < 0.005, (NS) non-significant.

Table (5): Comparison of some previous analytical methods developed for determination of methamphetamine.

Technique	Sample type	LOD (µg/ml)	LOQ (µg/ml)	References
Built -in digital camera of iPhone 4.0	Meth tablet (yaba)	11	44	(Choodum et al., 2014)
Built -in digital camera of iPhone 4.0	Meth tablet (yaba)	207 to 590	-----	(Choodum et al., 2015)
Built -in digital camera of iPhone 4.0	Amphetamine street sample	1010	1010	(Choodum & Nic Daeid, 2011)
UV-Vis Spectrophotometer	Ecstasy tablet	21.99	66.66	(Bachri et al., 2021)
HPLC -fluorescence detection	Urine	0.015	0.05	(Saito et al., 2023)
GC- mass spectrometry	Urine	0.36	1.09	(Aulia et al., 2023)
GC-mass spectrometry	Urine	0.007	0.023	(Yudiana et al., 2023)
HPLC-ultraviolet detector	Odor-adsorbent Material	1	3	(Sun et al., 2024)
UV-Vis Spectrophotometer	Manufactured Meth sample	50	50	(Munawar et al., 2024)
UV-Vis Spectrophotometer	Urine	0.5	1	(Abouzied et al., 2024)
HPLC-DAD	Urine	0.100	0.250	Current study

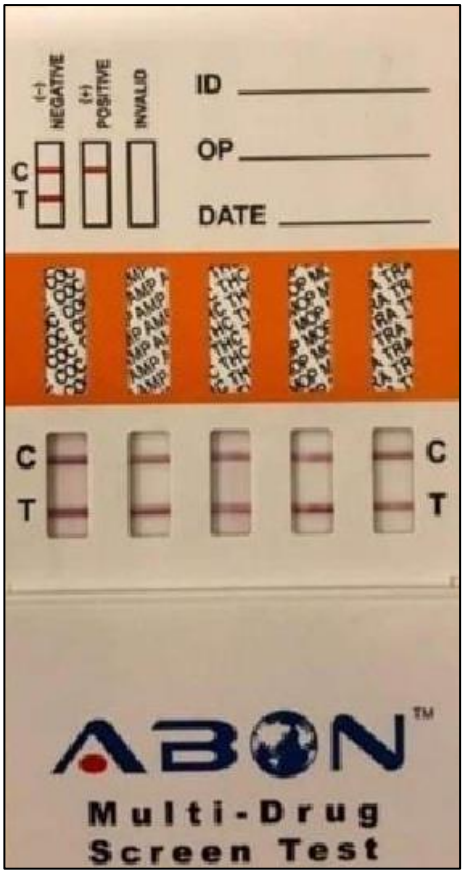

Figure (1): Polydrug rapid immunoassay screening panel dip steak (amp, barbiturates, opiate, THC, tramadol and cocaine).

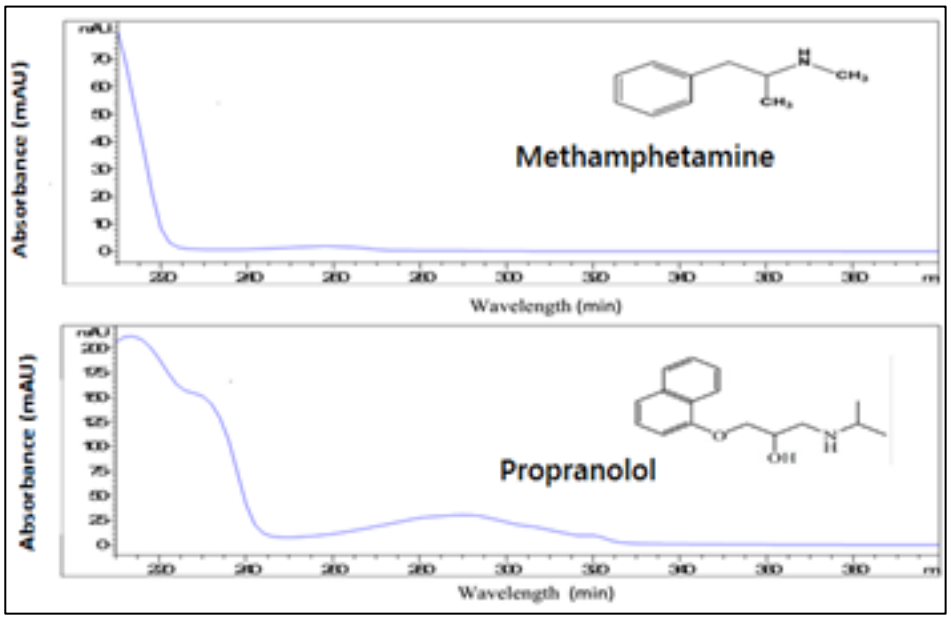

Figure (2): UV-Spectra scan (200 to 800 nm) for meth and propranolol (IS).

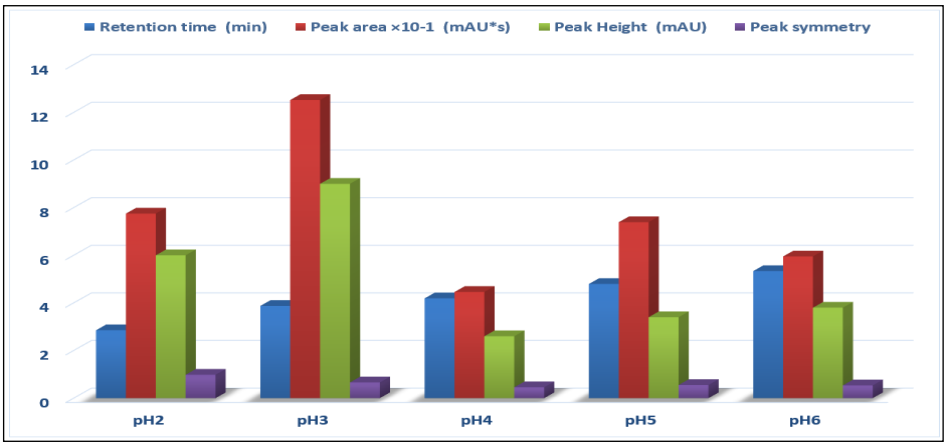

Figure (3): Effect of pH values on the peak responses and peak shape for methamphetamine.

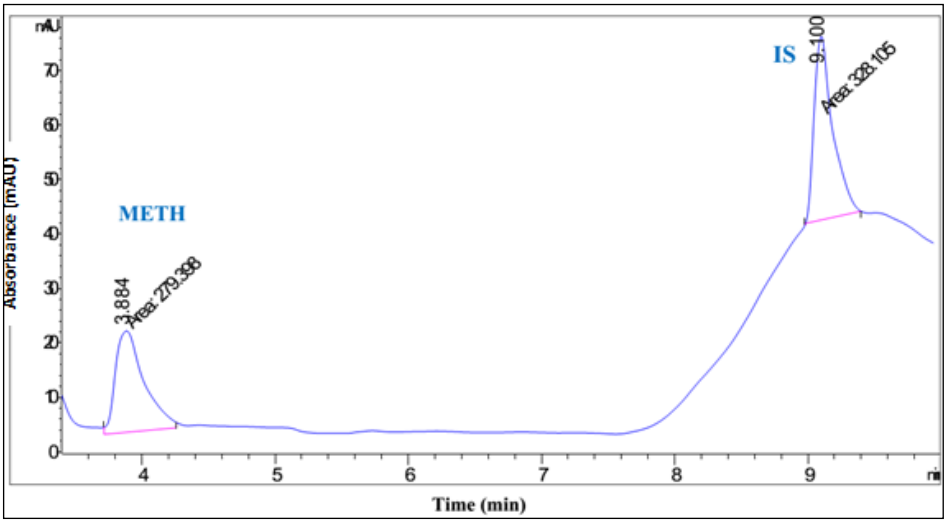

Figure (4): HPLC chromatograms of meth (3.88 min) and IS (9.1 min) obtained at 205nm.

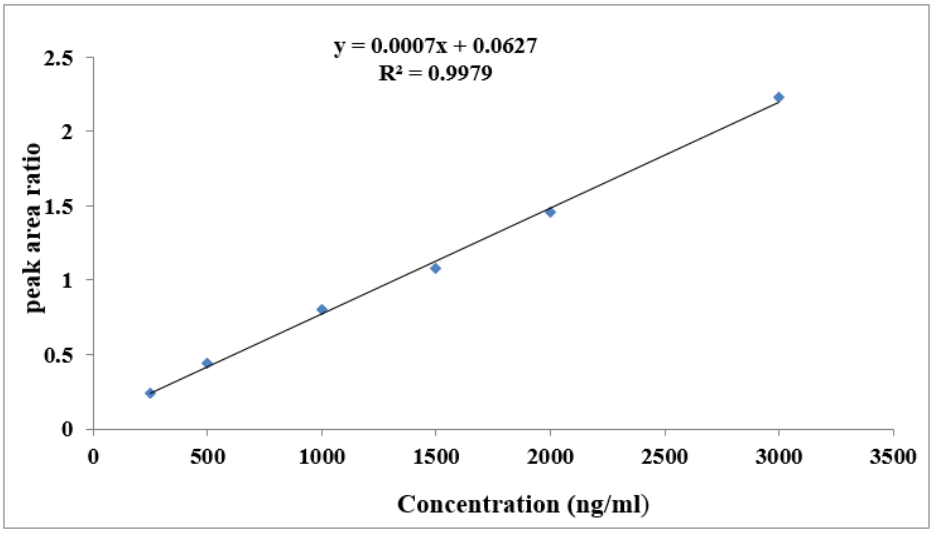

Figure (5): Standard calibration curve for meth quantification in urine samples.

Discussion

Drug abuse is an extremely serious prevalent phenomenon that increases year after year; around thirty million people worldwide presented with drug addiction bad health effects with considerable growth of drug abuse in Egypt. Recently, the Egyptian market for illegal drug misuse has changed substantially, with obvious growth in the abuse of Shabu among population Hashisha et al. (2022).

The current study described an analytical procedure for the detection of meth in human urine. The dependence of retention times of meth on mobile phase pH changes come in agreement with Reuhs, (2017). Table (5) showed that the current method more sensitive than Abouzied et al. (2024); Aulia et al. (2023); Bachri et al. (2021); Choodum et al. (2014, 2015); Choodum and Nic Daeid (2011); Munawar et al. (2024); Sun et al. (2024). On the other hand, the present method using low-cost and high availability instrument for quantification of meth compared with Saito et al. (2023); Yudiana et al. (2023) who use other technique need further derivatization to detect meth by HPLC FLD or GC MS, also use solid phase extraction technique higher cost than liquid-liquid extraction that use in current method.

Analysis of amphetamine positive samples for meth based on that it is the primary metabolite of meth Chen et al. (2010); Oyler et al. (2002). Quantitative results for meth in the current study agreed with Chung and Choe (2019) who revealed that the concentration of meth in urine sample of chronic abusers were 1-90 µg/ml.

In the present study, there was significant difference in age group use of meth (the most cases were in the age range 20-39), P-value< 0.05, this is similar to Hamdi et al. (2016) who revealed that most of cases were between ages 26-35, in contrary to a study done by El-Sherbiny. (2015) who found that cases were significantly higher between 18 and 25 years of age with 42.9% than those between 26 and 40 years of age, between 41 and 60 years, and above 60 years of age with 6.0, 7.5, and 9.4% respectively. In current work, the most of methamphetamine cases were in the age range of 20-39 which agreed with Barati et al. (2014) as the mean age was 30.4 years and most of cases were between 21 and 30years (52.8%). While, Yamamoto et al. (2022) stated that most of cases were age group of 40–49 years. These results can be referred to the fact that young adulthood in Egypt is a time of social, cultural, and economic change, making it a desirable period for drug use that disrupts social norms. It is also a time of considerable peer pressure and influence Hashisha et al. (2022). Regarding sex there was male predominance by 93.99% with no recorded cases for meth abuse between females which is similar to El-Sherbiny (2015); Su et al. (2018); Hashisha et al. (2022). In the present study there is significant increase in meth use among non-employees, P-value< 0.05, this come in agreement with El-Sawy et al. (2010) who stated that 26.47% of positive drug abuse cases was unemployed. Also, Rohmanika et al. (2022) stated that 35.8% of meth abusers were unemployed. In contrary Su et al. (2018) revealed that 52.04% of meth abusers were unemployed. There was significant increasing rate in meth usage throughout studied duration, P-value< 0.05. THC still the most frequently used drug of abuse (48.93%) followed by meth (21.89%) as its rate increased from year to year. These results agreed with Yamamoto et al. (2022) and Rohmanika et al. (2022) who revealed that meth is the second most often used substance after cannabis. Also, in Hamdi et al. (2016) Cannabis was the commonest drug of abuse representing 77% followed by alcohol and opiates. Although the exact causes of the rise of meth usage are unclear, researches had identified a number of potential contributing variables, such as increased product purity, reduced cost, and easier availability of crystal meth Papamihali et al. (2021).

In the current study, there was significant negative correlation, P-value < 0.05, with benzodiazepines (benzo) and tramadol which may be explained by their depressant effect which counter the

action of meth while it failed to approve any positive correlations with other drugs, P-value > 0.05 which disagree with the study of Rohmanika et al. (2022) who found that most abusers used meth in conjugation with other addictive substances including cannabis followed by alcohol, tranquillizers and opioids. Also, Papamihali et al. (2021) stated that meth use was strongly associated with opioid use, cannabis use, alcohol use and use of other substances.

Conclusion

Methamphetamine usage had been increased in the last few years and became in the second place after THC. It is more in males between 20-39 years and mostly used with other drugs of abuse like THC, opiate and tramadol. The suggested procedure is simple, rapid, reliable and sensitive enough to determine meth in urine. The limit of quantification for this procedure was 0.25 µg/mL that adequate to provide confirmatory physical evidence of the presence of meth in the urine after detection by a rapid drug screening test, especially its concentrations of in the urine of chronic abusers were 1-25 µg/mL.

Recommendations

Routine meth screening is recommended viewing its increasing abuse and its hazardous health effects. Positive cases should be confirmed by current method to exclude false positive results.

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دراسة حالات ادمان الميثامفيتامين بين حالات ادمان المخدرات المترددين علي معمل السموم الاكلينيكية بسوهاج باستخدام طريقة جديدة متحقق من صحتها علي جهاز HPLC-DAD

رانيا أحمد رضوان[١] و نور فاضل زيد[١] و احمد مرة[٢] و على مصطفى حسن[٢] و مى مصطفى عبد القادر[١]

الملخص العربي

المقدمة: أصبح تعاطي المخدرات تحديًا عالميًا مع الزيادة المزعجة في استخدام الميثامفيتامين البلوري في مصر. لذلك، هناك حاجه ماسة الي تطوير طريقة تحليل موثوقة في العمليات الروتينية. **الهدف:** تهدف الدراسة الحالية إلى تطوير وإثبات صحة طريقة حساسة ودقيقة لتحديد الميثامفيتامين (الميث) في البول البشري لتقييم انتشار الميثامفيتامين في سكان سوهاج. **المنهجية:** تم تطبيق الدراسة على الأشخاص الذين يرتادون معمل السموم الاكلينيكية في سوهاج لاختبار تعاطي المخدرات. يتم استهداف الحالات التي تعطي نتيجة ايجابية للامفيتامين في الاختبار الاولي ومن ثم يتم الكشف عن وجود الميثامفيتامن- بعد معالجة عينات البول – بطريقة معتمدة على ,HPLC-DAD تم استخراج الميثامفيتامين من عينات البول باستخدام مذيب عضوي (tert-butylmethyl ether) في وجود هيدروكسيد الأمونيوم كوسط قلوي واستخلاص عكسي بحمض الهيدروكلوريك ١.٠ مولاري. تم استخدام بروبرانولول كمعيار داخلي. كانت كفاءة الاستخلاص ٧٧.٧٧٪. **النتائج:** كان منحنى المعايرة خطيًا (r2> 0.99) في نطاق التركيز من ٠.٢٥ إلى ٣ ميكروجرام/مل ميكروجرام/مل للميثامفيتامين. كانت حدود الكشف والقياس الكمي ٠.١ و٠.٢٥ ميكروجرام/مل على التوالي. كانت دقة التحليل (Precision) داخل وخارج التحليل للميثامفيتامين في حدود ٢.٧٥-٩.٧٠ و١.٩٠- ٤.٨٧% على التوالي. كانت دقة التحليل (Accuracy) داخل وخارج التحليل في حدود (- ٦.٦٧) – ٩.٣٣ و (- ٢.٧٨) و ٦.٦٧ على التوالي. كشفت الطريقة عن ٥١ حالة من تعاطي الميثامفيتامين في الأشخاص محل الدراسة . تمت دراسة العوامل التي تؤثر علي تعاطي المخدرات لديهم. **الخلاصة:** كان حد القياس الكمي لهذا الإجراء ٠.٢٥ ميكروجرام/مل وهو ما يكفي لتوفير دليل مادي تأكيدي على وجود الميثامفيتامين في البول. **التوصيات:** يجب أن يكون الفحص الروتيني للميثامفيتامين أمرًا ضروريًا للحد من استخدامه المتزايد مع تأثيراته الصحية الخطيرة.

١. قسم الطب الشرعي والسموم الاكلينيكية ، كلية الطب، جامعة سوهاج، سوهاج، مصر.

٢. قسم الكيمياء، كلية العلوم، جامعة الازهر، القاهرة، مصر.