Malaysian Journal of Chemistry, 2024, Vol. 26(5), 488-497
(Special Issue: *4th IKMPB Online Symposium 2024 - Development of Chemistry to Accomplish a Sustainable World*)

Analysis of Amphetamine-Type Stimulants (ATS) Drugs and their Metabolites in Hair Samples of Drug Abusers using GC-FID

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Hair has become a fundamental biological sample, as an alternative to the common samples of blood and urine for drug testing. Chemicals from drugs will move from the bloodstream into the follicle base and bind to the growing hair cells. Once the drugs are incorporated into hair, they will remain with high stability in hair allowing the detection of drugs up to several months after intake. The objective of this study is to determine the presence of Amphetamine-Type Stimulants (ATS) drugs from the hair samples and to validate the method of ATS drug analysis in hair samples of drug abusers by using Gas Chromatography-Flame Ionization Detector (GC-FID). In this study, hair samples from 97 male drug abusers were collected from Narcotic Addiction Relief Centre in Sabah and Rehabilitation Centre in Johor. The ATS drugs in the hair samples were analysed by using alkaline digestion method with GC-FID instrumentation. Method for hair analysis were successfully analyzed and validated with the assessment of the following parameters: linearity of calibration curve, limit of detection (LOD) and limit of quantification (LOQ). The results revealed that AMP analyte was the most detected ATS drug in hair, followed by MAMP, MDEA, MBDB, MDA and MDMA. MAMP analyte showed the lowest LOD and LOQ values which were 0.0394 ppm and 0.1195 ppm respectively. Meanwhile the highest LOD and LOQ was from MDEA analyte with the value of 0.1316 ppm and 0.3989 ppm respectively. The method of alkaline digestion was successfully applied for the quantification of ATS drugs in hair samples of drug abusers. Therefore, hair also can be used as a biological specimen in drugs of abuse procedure in workplace drug testing, drug rehabilitation and treatment centres and in forensic investigation of drug-related crime and fatalities.

Keywords: ATS drug; drug abusers; hair samples; GC-FID; LOD; LOQ

Received: January 2024; Accepted: July 2024

Amphetamine-type stimulant (ATS) is a synthetic stimulant drug that can increase the central nervous system's activity. In other words, ATS drugs can increase a person's energy, alertness and focus [1]. ATS drugs appear in various forms like coloured powders, crystalline powders, liquid form, capsules as well as tablets and are usually administered through injection, smoking, snorting or taken orally [2]. In Malaysia, recent statistics have shown an increase in ATS usage based on their market demands, police seizures, consumption levels, and manufacturing data [3]. Drug abuse is a persistent global health concern with societal and individual implications [4]. In recent years, aside from blood and urine samples, hair has become a fundamental biological sample for drug analysis especially in the workplace testing, medico-legal sector, forensics, research, treatment monitoring and driving licensing [5].

Hair samples offer several advantages in drug analysis, one being, it allows the detection of drugs in a wider time-window (months to years) compared to usual biological samples, such as blood and urine (hours to days) [6]. Previous research of drug analysis in hair has been carried out under many circumstances due to its advantages such as less-invasive collection and easy storage in comparison to blood and urine [7]. There are various types of analytical instruments used in drug of abuse analysis such as Liquid Chromatography (LC), Gas Chromatography (GC) and Capillary Electrophoresis (CE) [8]. In this study, we focus on the investigation of ATS drugs that can be identified in the human hair using GC-FID.

Under Malaysia's drug laws, any individual with a positive urine screening for substances classified as illicit by the Dangerous Drug Act (1952) and the Drug Dependence (Treatment and Rehabilitation) Act 1983 and deemed to be a drug addict by a government medical officer can be mandated to two years of detention and two years of community supervision following release [9]. There is no guarantee from drug abusers who were undergoing community supervision that they would abstain from taking drugs illicitly. Therefore, it is proposed that during the monthly urine tests, hair analysis should also be considered due to its longer time window of drug detection compared to traditional media as blood and urine. In addition, this project will be the first study conducted in Malaysia using hair samples from drug abusers as part of an ongoing effort to combat drug abuse in Malaysia.

†Paper presented at the 4th IKMPB Online Symposium 2024 – Development of Chemistry to Accomplish a Sustainable World

EXPERIMENTAL

Chemicals and Materials

The chemicals used in this study were methanol, dicholoromethane, sodium hydroxide (NaOH), ethyl acetate and pentafluoropropionic anhydride (PFPA). Six amphetamine-type stimulants (ATS) standards were used in this study which were amphetamine (AMP), methamphetamine (MAMP), N-methyl-1-(1,3-benzodioxol-5-yl)-2-butanamine (MBDB), 3,4-methylenedioxyamphetamine (MDA), 3,4-Methylene-dioxy-N-ethylamphetamine (MDEA), 3,4-methylene-dioxymethamphetamine (MDMA). All standards were purchased from Cerilliant (USA) with concentration of 1000ppm for each standard. All glassware's used in this study were sterilized with an autoclave machine (Hirayama HVE-50). The apparatus used for sample preparation were weighing balance (A&D HR-250AZ), shaking water bath (Memmert WNE 14) and Eppendorf AG22331 centrifuge.

Instrument

The analytical instrument used to analyse ATS drug in hair sample was Gas Chromatography (Agilent Technologies 7890A) equipped with Flame Ionization Detector (FID) and HP-5MS capillary column (Agilent J&W Scientific, Folsom, CA, USA).

Validation of Analytical Method

A method validation was established and evaluated according to the guidelines of Standard Practices for Method Validation in Forensic Toxicology by Scientific Working Group for Forensic Toxicology (SWGTOX) [10] before being applied to the real samples. Accuracy, linearity of calibration model, limit of detection (LOD) and limit of quantification (LOQ) were the validation parameters that have been evaluated to validate the analytical method of drug analysis in hair samples. The accuracy of the method was calculated as percentage recovery (R%) and was assessed in concentration level of 30 ppm. About 300 µL of each ATS was spiked into drug-free samples (blank hair samples) and extracted for the accuracy determination. The percentage recovery for AMP was found to be at 97%, while MAMP is 88%, MDA is 95%, MDMA is 79%, MDEA is 78% and MBDB is at 92%.

The calibration curve of peak area of blank or drug-free samples (x-axis) versus concentration of ATS drugs in ppm (y-axis) was plotted based on five concentrations levels of standard ATS drugs that had been injected into GC-FID. Five levels of concentrations that begin with 2, 5, 10, 30 and 50 ppm of each ATS drug were established with three replicates at each concentration. The linear regression of equation $y = mx + c$ and coefficient of determination (R^2) were established based on the calibration curve.

Limit of detection (LOD) is the lowest concentration of the analyte that can be distinguished from the background noise. In this study, the method was validated by using Signal-to-Noise (S/N) ratio technique. In chromatography, the LOD is the injected amount that results in a peak with a height at least three times higher as the baseline noise level (S/N: 3/1). Limit of quantification (LOQ) is the lowest concentration of an analyte in the sample that can be determined with acceptable precision and accuracy under stated conditions of test. Both LOD and LOQ are two basic elements of method validation that define the limitations of an analytical method. LOQ was determined based on the standard concentration of ATS drugs with a peak of ten times higher than the baseline noise level (S/N: 10/1).

Standard Preparation

All six ATS standards were prepared as 1000 ppm in methanol and stored at 4°C. A mixture of ATS standard stock solution (100 ppm) was prepared from six individual ATS standards each with 1000 ppm. The spiking samples were prepared from 100 ppm stock solution into 1 mL vial by spiking 20, 50, 100, 300 and 500 µL for calibration standards of 2, 5, 10, 30 and 50 ppm, respectively. The extraction step was performed immediately after the spiking procedure. Finally, a calibration curve was plotted to quantify the amount of ATS drugs present in drug abusers' hair samples.

Sample Collection and Storage

Drug-free hair samples or blank hair samples were collected from ten drug-free volunteers by using sterilized scissors. The blank hair was prepared for the control and calibration samples. 97 hair samples were collected from drug abusers. The samples were collected from male drug abusers of 19 to 44 years old with a history of ATS drug usage. The hair samples were collected by officers from Narcotic Addiction Relief Centre in Sabah and Rehabilitation Centre in Johor. The hair samples were wrapped in an aluminium foil to maintain integrity and to avoid contamination [11]. Hair samples were stored under dry conditions at room temperature. Hair samples preparation was initially performed with decontamination or washing method, followed by extraction of ATS drugs and finally with derivatization step. The samples then were ready to be injected into analytical instrument of GC-FID.

Decontamination of Hair Samples

Decontamination process is the first step of drug analysis in hair samples. The purpose of decontamination step is to remove external interferences without extracting the analyte of interest from sample matrix [12]. Hair samples were decontaminated by a gentle mixing of samples in two glass tubes containing 5 mL

distilled water for 2 min and followed by 2 min baths in 5 mL of methanol. Then, between the different washing steps, samples were dried using sheets of absorbent paper.

Extraction of ATS Drugs in Hair Samples

After decontamination step, extraction of ATS drug in hair samples were carried out using the method described by [13]. Washed hair were finely cut to 1-2 mm length and were weighed about 30 mg as shown in Figure 1. Next, hair samples were introduced into a 10 mL glass tube.

Then, hair samples were dissolved in 1 mL NaOH (1M) and incubated in shaking water bath for 3 hours at 50°C. Hair samples were then cooled to room temperature and extracted with 3 mL ethyl acetate (Figure 2). After 10 mins, the samples were centrifuged at 3000 rpm for 10 min. Two layers formed after the centrifugation step and the upper organic layer was transferred to a new test tube. Next, the extracted hair samples were concentrated by drying under nitrogen steam before proceeding to the derivatization process.

Derivatization of Extracted Hair Samples

As for ATS drug analysis using GC, derivatization procedure is needed because ATS drugs are not volatile enough to be analysed in GC. Therefore, derivatization step was performed by adding 50 µL of ethyl acetate and 50 µL PFPA into the extracts followed by heating at 50°C in shaking water bath for 30 mins. Next, the extracts were dried under nitrogen steam and reconstituted with 50 µL of ethyl acetate. Finally, 1 µL of the extracts were transferred into 1 mL vial and injected into the GC-FID.

Quantitative Analysis of Hair Samples

The presence of ATS drugs and metabolites in hair samples were identified by using GC-FID. Six ATS drugs including AMP, MAMP, MDA, MDMA, MDEA and MBDB were identified based on the retention time of each analyte eluted in standard ATS mixture. Detection of six analytes of ATS drugs in hair samples were identified based on the eluted retention time of ATS standards in standard solution mixture.

Figure 1. Hair Sample.

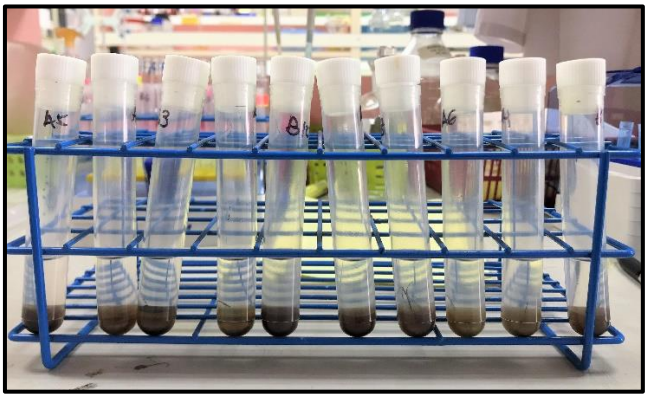

Figure 2. Hair samples extraction using ethyl acetate.

The extracted hair samples were analysed using Gas Chromatography (Agilent Technologies 7890A) equipped with Flame Ionization Detector (FID). Chromatographic separation was carried out on HP-5MS capillary column (Agilent J&W Scientific, Folsom, CA, USA) with helium as carrier gas at a flow rate of 3.1599 mL/min. Splitless injection mode was used with injector temperature at 250°C. The oven temperature was set at 90°C for 2.0 min, and then was increased to 150°C at a rate of 20°C /min and held for 17 min. The retention time of each ATS analyte obtained from standard mixture solution was recorded to construct the calibration curve of peak area of blank samples (x-axis) versus concentration of ATS drugs in ppm (y-axis). Each sample was injected in triplicate to get the average peak area. The calibration curve then was used to quantify the amount of ATS drugs present in the hair samples.

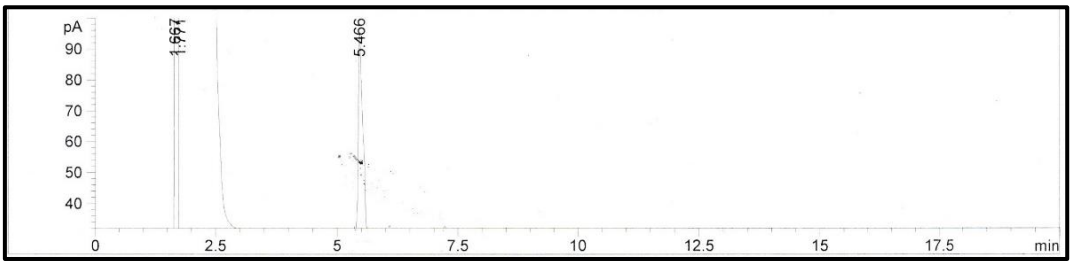

Figure 3. Chromatogram of Individual Standard AMP.

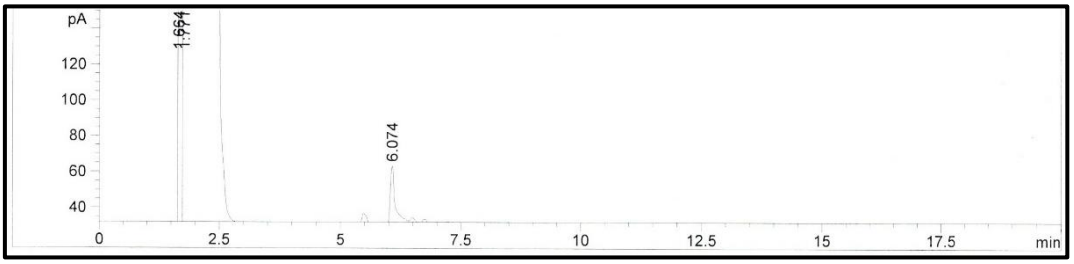

Figure 4. Chromatogram of Individual Standard MAMP.

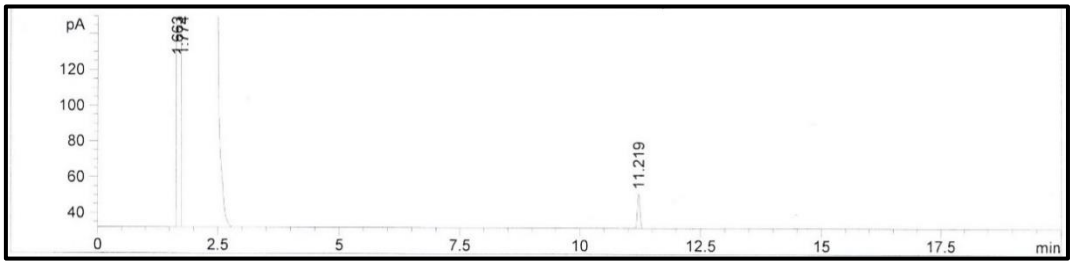

Figure 5. Chromatogram of Individual Standard MDA.

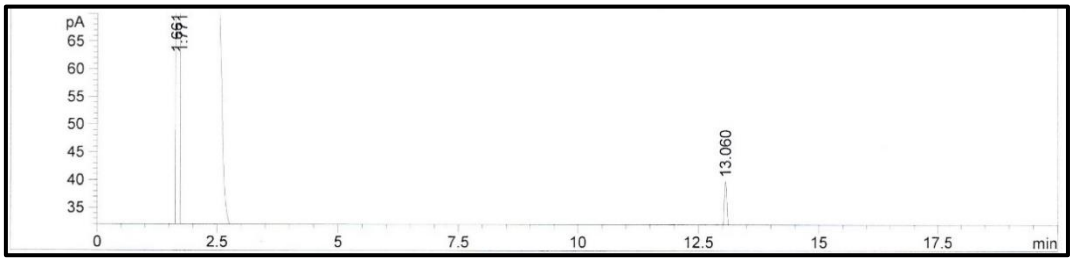

Figure 6. Chromatogram of Individual Standard MDMA.

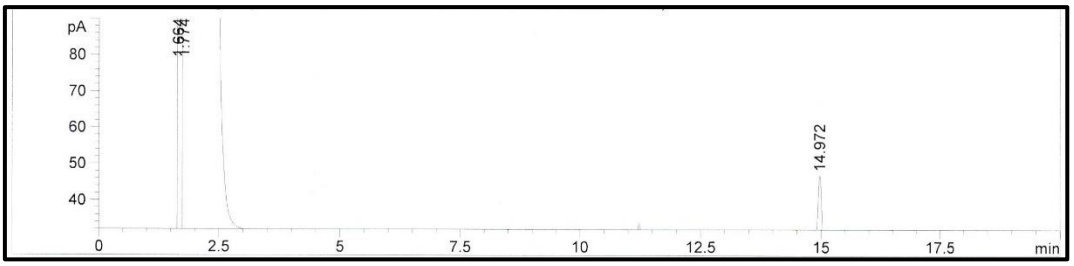

Figure 7. Cromatogram of Individual Standard MDEA.

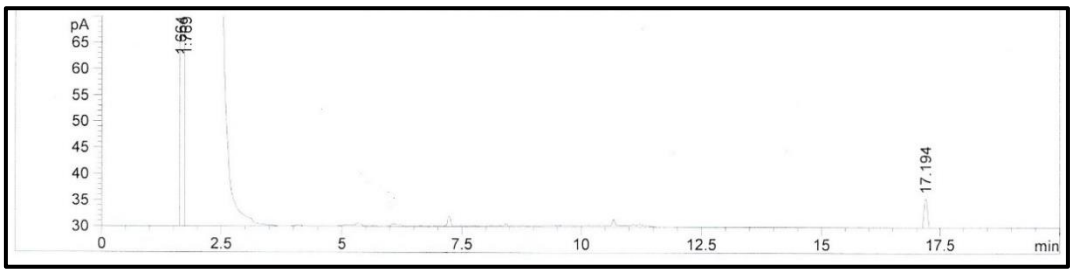

Figure 8. Chromatogram of Individual Standard MBDB.

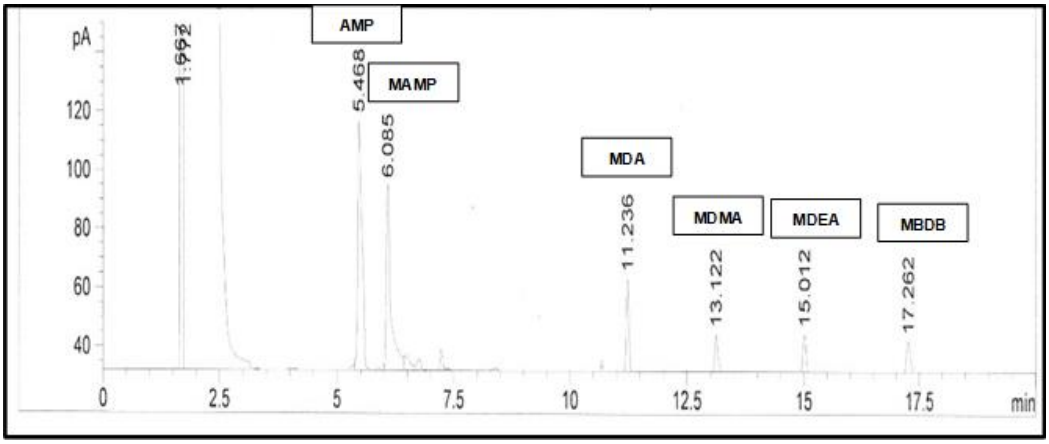

Figure 9. Chromatogram of Standard Mixture of ATS Drugs.

Table 1. Retention Time and Molecular Weight for Each Analyte in Mix Standard of ATS Drugs.

Analyte	Retention time, RT (min)	Molecular weight (amu)
AMP	5.468	374.22
MAMP	6.085	149.23
MDA	11.236	72.06
MDMA	13.122	193.25
MDEA	15.012	119.16
MBDB	17.262	207.27

RESULTS AND DISCUSSION

GC-FID Analysis of Individual Standard of ATS Drugs

The individual standard of ATS drugs was injected in triplicate into the GC-FID to determine the average retention time of each analyte. There were six retention times recorded for six different analytes of standard ATS drugs. The retention time for individual standard AMP was eluted at 5.466 min while standard MAMP was at 6.074 min. The standard MDA appeared at 11.219 min. Meanwhile the retention time for standard MDMA was at 13.060 min, standard MDEA was eluted at 14.972 min and standard MBDB was at 17.194 min as shown in Figure 3 to 8.

GC-FID Analysis of Mix Standard of ATS Drugs

Standard mixture of ATS drugs was established by mixing all six individuals standard of ATS drugs into one vial. The retention time for six standard ATS drugs are shown in Figure 9 and Table 1 were used to analyse the ATS drug content in hair samples. The retention time of each ATS drugs in standard mixture solution is slightly different from their individual retention time due to differences in matrix concentration. Chromatogram of ATS drugs analysed in this study show a typical chromatogram separation pattern type of ATS drugs in hair samples. The retention time was used to analyse the ATS drugs content in drug abusers' hair samples. The first analyte eluted was standard AMP which was at 5.468 min, followed by standard MAMP at 6.085 min. At retention time 11.236 min, standard MDA was eluted, MDMA appeared at 13.122 min, followed by MDEA at 15.012 min. Finally, standard MBDB was the last analyte to be eluted with a retention time of 17.262 min.

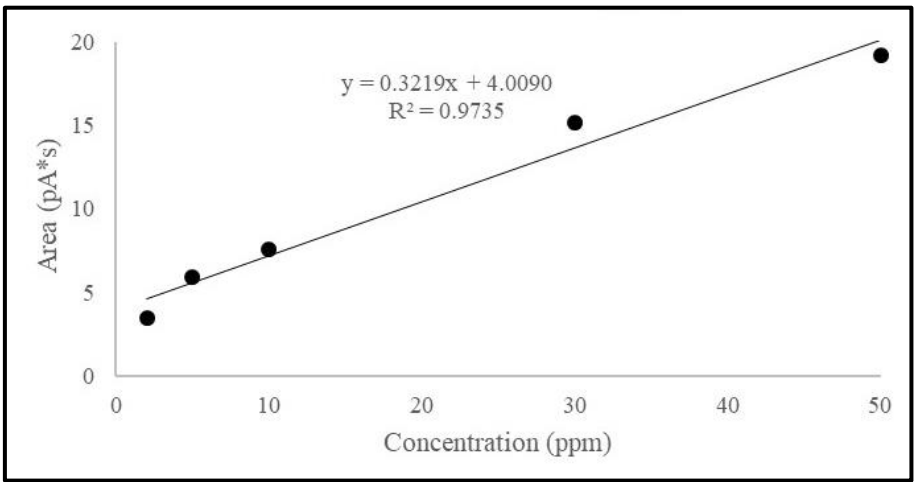

Figure 10. Calibration Curve of Standard AMP in Blank Hair Samples.

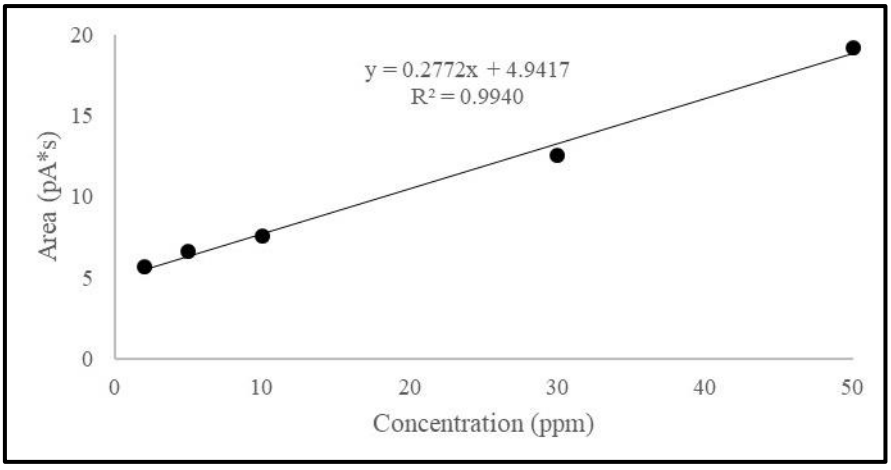

Figure 11. Calibration Curve of Standard MAMP in Blank Hair Samples.

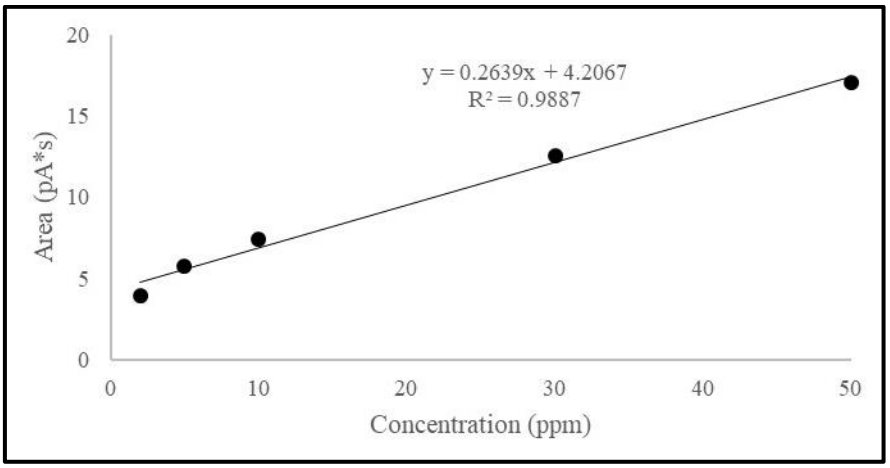

Figure 12. Calibration Curve of Standard MDA in Blank Hair Samples.

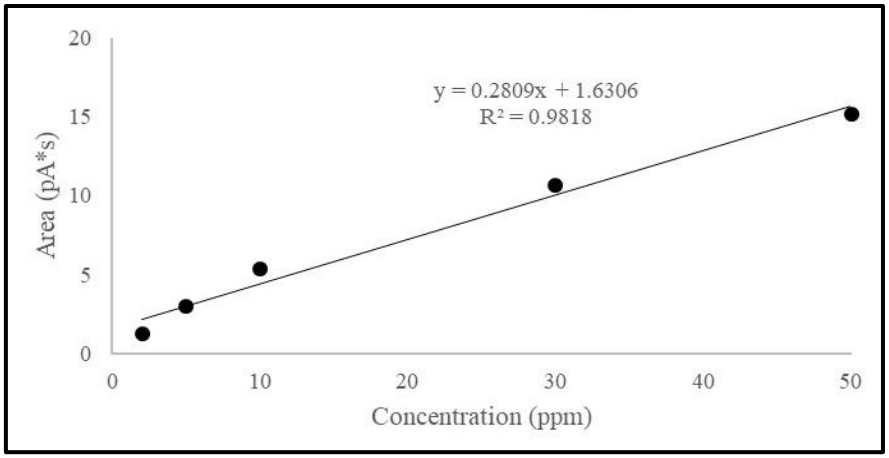

Figure 13. Calibration Curve of Standard MDMA in Blank Hair Samples.

Calibration Curve of ATS Drugs in Blank Hair Samples

The calibration curve of peak area of blank hair samples (x-axis) versus concentration of ATS drugs in ppm (y-axis) was plotted based on five concentrations of standard ATS drugs that had been injected into GC-FID. Five levels of 2, 5, 10, 30 and 50 ppm concentrations of each ATS drug were established with three replicates at each concentration. From all the calibration curves, the method was found to be linear in the range of 2 to 50 ppm.

The calibration curve for AMP analyte showed a good linear regression with the equation of y = 0.3219x + 4.0090. Good linearity with correlation coefficient (r) of 0.9867 was obtained from the calibration curve as shown in Figure 10. While for MAMP analyte the equation for linear regression was y = 0.2772x + 4.9417 with correlation coefficient (r) of 0.9970 as shown in Figure 11. The calibration curve for MDA analyte as shown in Figure 12 formed a linear regression of y = 0.2639x + 4.2067 with r value of 0.9943. Figure 13 reveals that the linear regression of MDMA analyte was y = 0.2809x + 1.6306 with r value of 0.9908, meanwhile the linear regression and r value of MDEA analyte was y = 0.3181x + 2.3992 and 0.9678 respectively as plotted in Figure 14. Finally, calibration curve at Figure15 indicates that the linear regression of MBDB analyte was y = 0.3049x + 4.904 and the r value was 0.9874.

Limit of Detection (LOD) and Limit of Quantification (LOQ)

The LOD and LOQ of each analyte of ATS drugs for hair analysis is recorded in Table 2. MAMP showed the lowest LOD and LOQ values of 0.0394 ppm and 0.1195 ppm respectively. Meanwhile the highest LOD and LOQ was from MDEA with the value of 0.1316 ppm and 0.3989 ppm respectively.

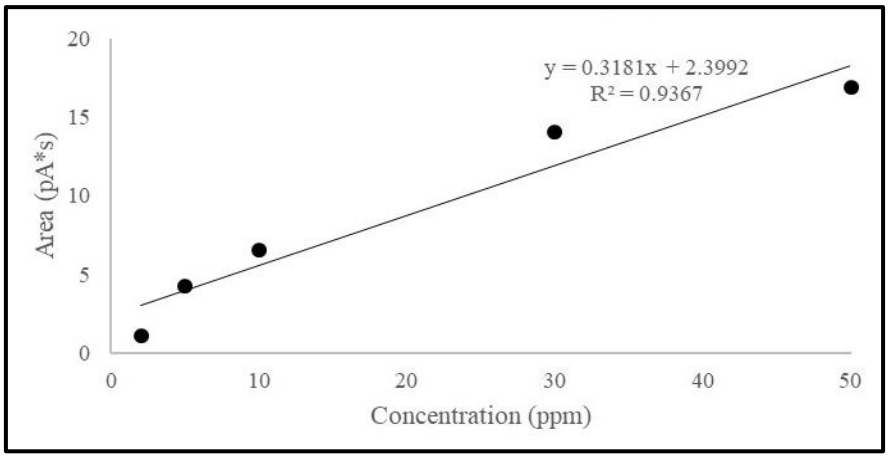

Figure 14. Calibration Curve of Standard MDEA in Blank Hair Samples.

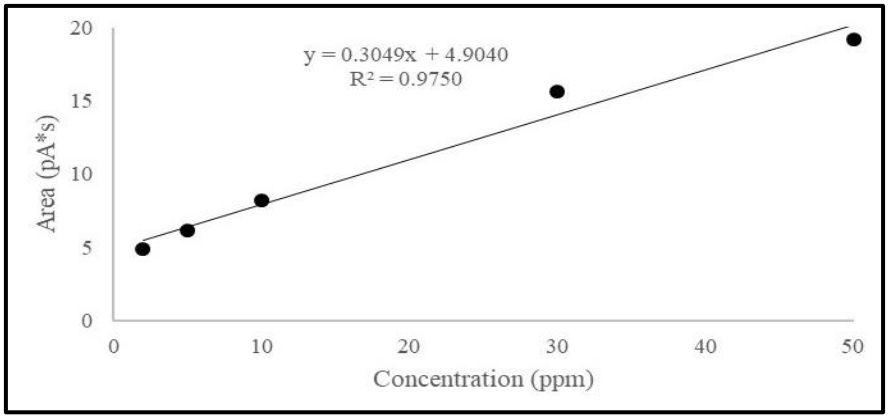

Figure 15. Calibration Curve of Standard MBDB in Blank Hair Samples.

Table 2. Calibration Curve Details, LOD and LOQ for Hair Analysis.

Analyte	Slope	y-intercept	Coefficient of determination (R^2)	LOD (ppm)	LOQ (ppm)
AMP	0.3219	4.0090	0.9735	0.0834	0.2529
MAMP	0.2772	4.9417	0.9940	0.0394	0.1195
MDA	0.2639	4.2067	0.9887	0.0542	0.1642
MDMA	0.2809	1.6306	0.9818	0.0689	0.2087
MDEA	0.3181	2.3992	0.9367	0.1316	0.3989
MBDB	0.3049	4.9040	0.9750	0.0811	0.2458

Table 3. Number of ATS Drug Detected and Not Detected in Hair Samples.

ATS Drug	AMP	MAMP	MDA	MDMA	MDEA	MBDB
Detected	72	61	55	44	56	53
Not Detected	25	36	42	53	41	44

Table 4. Range Concentration of ATS Drug Contents in Hair Samples.

ATS drug	Range concentration in hair samples (ppm)
AMP	0.0070 – 44.4715
MAMP	0.3149 – 33.2907
MDA	0.2385 – 28.4055
MDMA	0.2385 – 28.4055
MDEA	0.0224 – 32.7124
MBDB	0.2493 – 24.3450

ATS Drug Contents in Hair Samples of Drug Abusers

The analytical data derived from hair samples of 97 subjects is tabulated in Table 3. AMP analyte was the most detected ATS drug in hair samples with 72 out of 97 subjects, followed by MAMP, MDEA, MBDB and MDA contents while the least detected ATS drug detected in hair samples was MDMA analyte with detection only in 44 subjects.

The result from this study revealed that the range concentration for AMP analyte in hair samples was between 0.0070 – 44.4715 ppm while for MAMP analyte was in the range of 0.3149 – 33.2907 ppm. MDA analyte has the range concentration of 0.2385 – 28.4055 ppm, MDMA analyte was in the range of 0.0046 – 30.9796 ppm, meanwhile for MDEA and MBDB analytes, the range of concentrations were between 0.0224 – 32.7124 ppm and 0.2493 – 24.3450 ppm respectively, as shown in Table 4.

According to a previous study conducted by [14], the data were obtained from 2355 hair samples showed that the concentration range of AMP analyte was from 0.1 – 41.4 ppm, while for MAMP was between 0.5 – 608.9 ppm. In addition, previous research that applied to real case also indicated that AMP has the concentration of 0.06 ppm and MAMP was in the range of 0.24 – 0.63 ppm [5]. In another study of 15 subjects of ATS drugs users, discovered that the range concentration of AMP and MAMP were 0.1 to 3.5 ppm and 0.9 to 56.4 ppm respectively [15]. All the previous studies mentioned above produce a diverse range of concentration because the researchers used different instruments with different levels of sensitivity and selectivity for their study.

Furthermore, other factors that may contribute to these differences were probably the efficiency of the derivatizing agents. According to studies done by [7,13], the stronger and more powerful derivatizing agent for derivatization steps such as heptafluorobutyric anhydride (HFBA) were more preferable because they provide more specific mass spectrometric information and better selectivity and recovery for all analytes compared to pentafluoropropionic anhydride (PFPA) which was used in this present study. It is important to choose the proper derivatizing agent to increase the detection sensitivity. Moreover, a possible problem in hair analysis could be the different hair growth, different hair melanin content and the use of hair treatments such as dyeing or bleaching could produce individual differences in drug uptake into hair among different population around the world [16].

CONCLUSION

As a conclusion, the proposed Gas Chromatography-Flame Ionization Detector (GC-FID) method for the ATS drugs determination from hair samples of drug abusers was successfully analysed and validated with the assessment of the following parameters: linearity of calibration curve, limit of detection (LOD) and limit of quantification (LOQ). The simple and reliable alkaline digestion extraction technique by using NaOH digestion to extract ATS drugs from hair samples have been achieved and successfully applied for the quantification of ATS drug in hair samples of drug abusers. Therefore, hair also can be used as a biological specimen in drugs of abuse procedure in workplace drug testing, drug rehabilitation and treatment centres and in forensic investigation of drug-related crime and fatalities. In addition, this project will be the first study conducted in Malaysia using hair samples from drug abusers as part of an ongoing effort to combat drug abuse in Malaysia.

ACKNOWLEDGEMENTS

The authors would like to thank the National Anti-Drugs Agency (NADA) for their kind assistance during sample collection and the Faculty of Applied Sciences for the provided facilities throughout this study. This study was supported by a Universiti Teknologi MARA grant (RAGS/1/2014/SG05/UITM//2). The authors declare no conflicts of interest with respect to the authorship or publication of this article. Ethical approval was obtained from the Institutional Research Ethics Committee [Ref: UiTM 600-IRMI (5/1/6)].

REFERENCES

1. United Nations Office on Drugs Crime, World Drug Report 2020 (2020) Contract No.: Sales No. E.20.XI.6.

2. George, J., Baker, J., Allsop, S. & Makkai, T. (2008) National amphetamine-type stimulant strategy background paper (National Drug Strategy Series No. 69). *Canberra: Australian Government Department of Health and Ageing.*

3. Kanato, M., Sarasiri, R. and Leyatikul, P. (2022) Asean Drug Monitoring Report 2021. *Bangkok: ASEAN Narcotics Cooperation Center (Eds.)*.

4. Hu, J., Liu, W., Zhang, S., Xiang, P., Wu, H. and Chen, H. (2022) Retrospective analysis of laboratory data from 19,275 hair samples submitted for drug abuse/misuse analysis from February 2019 to June 2021. *Forensic Science International*, **337**, 111350.

5. Imbert, L,. Dulaurent, S., Mercerolle, M., Morichon, J., Achatre, G. & Gaulier, J. (2014) Development and validation of a single LC–MS/MS assay following SPE for simultaneous hair analysis of amphetamines, opiates, cocaine and metabolites. *Forensic Science International*, **234**, 132–138.

6. Chooi, W. T., Mohd Zaharim, N., Desrosiers, A., Ahmad, I., Yasin, M. A. M., Syed Jaapar, S. Z., Sharifah, Z., Schottenfeld, R. S., Vicknasingam, B. and Chawarski, M. C. (2017) Early Initiation of Amphetamine-Type Stimulants (ATS) Use Associated with Lowered Cognitive Performance among Individuals with Co-Occurring Opioid and ATS Use Disorders in Malaysia. *Journal of Psychoactive Drugs*, **49(4)**, 326–332.

7. Hung, T. V., Hein, H. M., Chinh, P. Q., Ha, P. T. T., Nhu, N. D. and Tuan, N. D. (2023) Development and validation of a GC-MS method for determination of amphetamine-type stimulants and ketamine in human hair. *Pharmaceutical Sciences Asia*, **50(1)**, 24–33.

8. Vogliardi, S., Tucci, M., Stocchero, G., Ferrara, S. D. & Favretto, D. (2014) Sample preparation methods for determination of drugs of abuse in hair samples: A review. *Analytica Chimica Acta*, **857**, 1–27.

9. Kamarulzaman, A. (2009) Impact of HIV Prevention Programs on Drug Users in Malaysia. *Journal of Acquired Immune Deficiency Syndromes*, **52(1)**, 17–19.

10. SWGTOX, X. (2013) Scientific Working Group for Forensic Toxicology (SWGTOX) standard practices for method validation in forensic toxicology. *Journal of Analytical Toxicology*, **37**, 452–474.

11. Cooper, G. A. A., Kronstrand, R. & Kintz, P. (2012) Society of Hair Testing guidelines for drug testing in hair. *Forensic Science International*, **218(1)**, 20–24.

12. Lin, D. L.,Yin, R. M., Liu, H. C., Wang, C. Y. and Liu, R. H. (2004) Deposition Characteristics of Methamphetamine and Amphetamine in Fingernail Clippings and Hair Sections. *Journal of Analyytical Toxicology*, **28(6)**, 411–417.

13. Kim, J. Y., Shin, S. H. & In, M. K. (2010) Determination of amphetamine-type stimulants, ketamine and metabolites in fingernails by gas chromatography-mass spectrometry. *Forensic Science International*, **194**, 108–114.

14. Lee, S., Han, E., Park, Y., Choi, H. & Chung, H. (2009) Distribution of methamphetamine and amphetamine in drug abusers' head hair. *Forensic Science International*, **190**, 16–18.

15. Miki, A., Katagi, M. & Tsuchihashi, H. (2003) Determination of methamphetamine and its metabolites incorporated in hair by column-switching liquid chromatography– mass spectrometry. *Journal of Analytical Toxicology*, **27**, 95–102.

16. Musshoff, F. & Madea, B. (2007) Analytical pitfalls in hair testing. *Analytical and Bioanalytical Chemistry*, **388(7)**, 1475–1494.