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DETECTION AND IDENTIFICATION OF ILLICIT DRUGS IN VAPE LIQUID SAMPLES USING THE ENZYME MULTIPLIED IMMUNOASSAY TECHNIQUE, THIN LAYER CHROMATOGRAPHY AND GAS CHROMATOGRAPHY-MASS SPECTROMETRY

(Pengesanan dan Pengenalpastian Dadah Terlarang dalam Sampel Cecair Vape Menggunakan Kaedah Imunoasai Berganda Enzim, Kromatografi Lapisan Nipis dan Kromatografi Gas Spektrometri Jisim)

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Abstract

Vaping is a common phenomenon in the world including in Malaysia, and one that involves all strata of society. Recently, vaping has become an unintended mode of delivery for various illicit drugs of abuse. The objective of this study is to detect and identify the presence of illicit substances including opiates, tetrahydrocannabinol (THC), amphetamine-type stimulants (ATS), benzodiazepines (BZD), barbiturates, phencyclidine, ketamine and mitragynine in three vape liquid samples provided by the National Anti-Drugs Agency (NADA), Kelantan, Malaysia. Some of these drugs have been linked to fainting episodes after vaping. The samples were analysed using enzyme multiplied immunoassay technique (EMIT), thin layer chromatography (TLC) and gas chromatography-mass spectrometry (GC-MS). One of the samples was found to contain ketamine (the band was seen on the TLC plate and the result was further confirmed using GC-MS, with 99% match) with an estimated concentration of at least 25000 ng/mL by linear extrapolation of the calibration curve. Two other samples were noted to contain nicotine (bands were seen on TLC plate and also confirmed by GC-MS). Ketamine abuse is not widely reported because the vast majority of drug-screening setups do not offer testing for ketamine. Ultimately, vape liquid samples in this study were found to contain illicit drugs such as ketamine and nicotine. This suggest that tighter regulation would help curb this health bane on society.

Keywords: vape, illicit drugs, enzyme multiplied immunoassay technique, thin layer chromatography, gas chromatography mass spectrometry

Abstrak

Vape atau rokok elektronik merupakan fenomena yang lazim di dunia termasuklah di Malaysia melibatkan semua lapisan masyarakat. Kebelakangan ini vape telah menjadi salah satu cara penggunaan dadah terlarang. Objektif kajian ini untuk mengesan dan mengenalpasti dadah terlarang termasuk opiat, tetrahidrokanabinol (THC), amphetamine-type stimulant (ATS), benzodiazepin (BZD), barbiturat, phencyclidine, ketamin and mitragynine yang terdapat di dalam 3 sampel vape daripada pihak Agensi Anti Dadah Kebangsaan (AADK), Kelantan, Malaysia yang mana telah dikaitkan dengan keadaan tidak sedarkan diri pengguna vape tersebut. Sampel-sampel tersebut telah dianalisa dengan menggunakan kaedah imunoasai berganda enzim, kromatografi lapisan nipis dan kromatografi gas spektrometri jisim. Salah satu sampel didapati mengandungi ketamin (Jalur yg berkenaan kelihatan pada plat TLC dan disahkan oleh GC-MS dengan 99% kepastian) dengan kepekatan yang dianggarkan sekurang-kurangnya 25000 ng/mL daripada unjuran lengkung kalibrasi. Dua sampel yang lain didapati mengandungi nikotin (jalur yang berkenaan kelihatan pada plat TLC dan disahkan melalui GC-MS). Penyalahgunaan ketamin kurang dilaporkan kerana kebanyakan pusat saringan untuk pengesanan dadah tidak menawarkannya. Sebagai kesimpulan, sampel cecair vape dalam kajian ini didapati mengandungi dadah terlarang (ketamin) dan nikotin. Penguatkuasaan undang-undang yang lebih ketat mungkin diperlukan untuk membantu menangani kemudaratan kesihatan ini dalam masyarakat.

Kata kunci: vape, dadah terlarang, teknik imun berganda enzim, kromatografi lapisan nipis, kromatografi gas-spektrometer jisim

Introduction

Vape or electronic cigarettes (EC) are perceived by the smokers to be healthier alternatives to tobacco cigarettes [1]. These devices are used to deliver nicotine, flavourings and other chemicals via aerosolization [2, 3]. Vape liquids containing flavours, solvents and other unknown ingredients, are often heated in e-cigarettes and vape rigs, and creating an aerosol that users inhale.

Vaping is a common phenomenon globally. In Malaysia, the prevalence of EC uses increased from 0.8% in 2011 to 4.9% in 2019 [4]. Alarmingly, a cross-sectional study conducted in 2018 showed 73% of the students from a secondary school in Kuala Lumpur were EC users [5]. Unlike tobacco cigarette, ECs and vapes have been not regulated in Malaysia to date: there is no regulation; neither towards the group of users, the electronic device used (safety or design) nor the liquid contents. Therefore, the sale of these devices and related products to users including underage children or youth is possible which could lead to health risk problems and also criminal abuse of the vape liquid.

Daily vaping has been reported to increase the risk of developing myocardial infarction and E-cigarette or Vaping Use-Associated Lung Injury (EVALI). These problems may arise through various mechanisms, including oxidative stress and inflammation, DNA damage, altered haemodynamic and platelet activity [6-8]. Vape liquid’s flavour has been reported to contain illegal psychoactive drugs which was adulterated in addition to the four primary components of vape liquids (propylene glycol, glycerol, various flavourings, nicotine). Previous literatures have reported the detection of amphetamine-type stimulants (ATS) [9], tetrahydrocannabinol (THC) [10, 11] and even various new psychoactive substances (NPS) [12] in vape liquids. These substances may cause significant health and development effects, especially among young users due to the are exposure to drugs of abuse via vaping itself and other side effects of vaping.

The presence of illicit drugs in vape liquids is factual. These have also been reported in other studies [13,14]. Analysis of drug of abuse in vape liquid was not implemented due to the absence of regulation and enforcement on vape liquid ingredients. To date, there is no standard procedure for the analysis of vape liquid, or suspected adulterated vape liquids when those samples are submitted for analysis to government laboratories. Therefore, this study reports on in-house exploratory screening and confirmatory analysis method of illicit substances in vape liquid. Three vape liquid samples were collected from National Anti-Drugs Agency (NADA), Kelantan, Malaysia and were tested for the presence of illicit drugs including opiates, THC, ATS, BZD, barbiturates, phencyclidine, ketamine and mitragynine. The samples were assessed following Pharmacology Laboratory, Hospital USM procedures and forensic narcotic screening protocols.

Materials and Methods

Chemicals and reagents

Methamphetamine (MA) hydrochloride, methyl enedioxymethamphetamine (MDMA) hydrochloride, nitrazepam, nimetazepam, diazepam, flunitrazepam and mitragynine drug standards were purchased from Lipomed (via Labchem, Malaysia). Amphetamine sulphate, alprazolam, ketamine hydrochloride and nicotine were purchased from Sigma Aldrich, (USA). Morphine, codeine, oxazepam and midazolam were purchased from Cerilliant (Texas, USA).

Thin layer chromatography (TLC) reagents used in liquid-liquid extraction and as mobile phase components, ethyl acetate, methanol, ammonia 28%, chloroform, iso-propanol and petroleum benzine, were acquired from Merck, USA. The visualization reagents, ninhydrin, hexachloroplatinic acid, potassium iodide, sulphuric acid, naphthyl ethylenediamine, glacial acetic acid and bismuth nitrate were purchased from Merck, USA. Fast Black K, dimethylaminobenzaldehyde were acquired from Sigma Aldrich (USA). Sodium nitrite and ammonium sulphamate were bought from Acros Organic (New Jersey, USA).

Sample preparation: Drug standard

The drug standards methamphetamine (MA) hydrochloride, methylenedioxymethamphetamine (MDMA) hydrochloride, nitrazepam, nimetazepam, diazepam, flunitrazepam, mitragynine amphetamine sulphate, alprazolam, ketamine hydrochloride, nicotine, morphine, codeine, oxazepam and midazolam were prepared at 100 ng/mL concentration.

EMIT

For immunoassay analysis, vape liquid samples (sample 1, sample 2 and sample 3) were subjected to analysis without further sample preparation.

TLC

For TLC, the samples were subjected to liquid-liquid extraction (LLE). For benzodiazepine analysis, neutral extraction was performed by adding 0.6ml petroleum benzine to 0.3 ml of vape liquid sample and was centrifuged at 1000 rpm for 5 min. The top layer of the mixture that was the organic layer was removed. The sample was dried at 50 ^{o}C.

For ATS, mitragynine and for the analysis of other slightly alkaline substances such as ketamine and nicotine, the samples were first alkalinized to about pH 10 by adding dilute NaOH to 0.3 mL of vape liquid sample each. Basic extraction was then performed by adding 1ml of chloroform to the flask as the extraction solvent.

Extraction procedure

1 mL of vape liquid sample and 2 mL of chloroform were added to the tubes. The tubes were shaken on a rotator for 10 min. This was followed by centrifugation at 3000 rpm for 30 min. After centrifugation, the chloroform phase was transferred into V-tubes and evaporated to dryness under a stream of nitrogen at 45 °C. The dried extract was reconstituted with 70 µL of methanol, vortex-mixed and injected into the GC-MS system.

EMIT screening

The vape liquid samples were screened by a Viva-E drug analyser (Siemens, Germany) for the presence of opiates, tetrahydrocannabinol (THC), amphetamine-type stimulants (ATS), benzodiazepines (BZD), barbiturates and phencyclidine which uses Enzyme Multiplied Immunoassay Technique (EMIT).

Thin layer chromatography

Thin layer chromatography was performed for analytes that had significant readings on EMIT screening, as part of confirmation. 20µL of extracted vape liquid samples were spotted onto TLC silica plates, alongside drug standards to determine presence of substance of abuse using a TLC spotter.

For ATS TLC plate, the bottom of the spotted plate was placed in ethyl acetate/methanol/ammonia (8.5: 1.0: 0.5) for development and sprayed with visualization reagent (10% ninhydrin and Fast Black K). The spot retention factor (R_{fs}) values were compared against ATS standards including methamphetamine, amphetamine and 3,4-methylenedioxy-methamphetamine (MDMA). While this plate was specifically optimized for the ATS detection, it also can detect other amines, such as ketamine and mitragynine.

For BZD TLC plate, acetone/hexane mixtures (1:3) were used as developing solvent system. Both unhydrolyzed and hydrolysed vape samples were spotted onto the TLC plates. The spot Rfs were compared against benzodiazepine standards including oxazepam, nitrazepam, nimetazepam, flunitrazepam, midazolam. Dragendorff's reagent and Bratton-Marshall's reagent were used as visualization reagents for unhydrolyzed and hydrolysed samples respectively.

For mitragynine plate, ethyl acetate/hexane (7:3) was used as a mobile phase. Spot R_{fs} were compared against mitragynine standard. Ehrlich's reagent (5% dimethylaminobenzaldehyde in 95% ethanol) was used as the visualization reagent.

Gas chromatography mass spectrometry

Drug standard mixtures and all the vape liquid samples (diluted and extracted) were analysed using GC-MS method to confirm the presence of the substances found during TLC. Narcotic scan program parameters were able to resolve all drug standards of interest. An Agilent 7890B Gas Chromatography System and 7683 Autosampler Agilent (USA) paired with Agilent 5977B Mass Spectrometry detector was used. Enhanced Chemstation software (Version F.01.03.2357) Agilent Technologies Inc. (USA) was used for instrument control and data acquisition. Spectral library comparisons for compound identification are referred to National Institute of Standards and Technology, NIST 14 Mass Spectral Library and Search Software (Version 2014).

Separation was carried out using HP5 MS capillary column (30 m x 250 m x 0.25 m) with helium carrier gas at a constant flow of 1.0 mL/min and injection in split mode ratio of 50 mL/min. The injector was heated to 27 °C and the transfer line is heated to 280 °C. Initial oven temperature is 80 °C then ramped at 20 °C/min to 150 °C and hold for 2 minutes. Second ramp is at 20 °C/min to 300 °C and hold for 14 minutes. The mass spectra were collected after 3 minutes solvent delay time. The ionisation voltage was 70 eV and the mass range were 40-500 m/z.

Results and Discussion

EMIT

EMIT screens were negative for opiates, THC, barbiturates and phencyclidine in all three examined vape liquid samples. Conversely, EMIT recorded readings for ATS in sample 1 and sample 2 whilst trace readings for BZD compounds were detected in all three samples.

TLC

None of the samples contains ATS. However, one band with higher Rf was noted on the ATS plate (Table 1).

ATS TLC plates revealed that none of the sample contains ATS compounds. In sample 1, the R_f values of the two bands were not comparable to the ATS standards. Interestingly, in vape liquid sample 2, bands for methamphetamine, amphetamines or MDMA were detected on ATS plate despite EMIT screening for ATS recorded detection of 6323.5 ng/mL. Nonetheless, a brownish band at R_f 0.75 and a large violet coloured band at R_f 0.93 were observed on the ATS plate. Although for the screening for MDMA revealed a violet band, the violet band in sample 2 may not be the same compound as MDMA since the R_f was different (R_f 0.93) compared to the Rf for MDMA was 0.57.

For BZD TLC plate there was a band that was compatible with flunitrazepam in sample 1, while there was one vague band compatible with diazepam is in sample 2 and 3. The presence of these unknown bands on both plates required further analytical evaluation to determine the compound. TLC for mitragynine was also performed to sample 1, however the results was negative. The amounts in sample 2 and 3 were insufficient for testing, thus was not performed.

Table 1. Summary of TLC analysis of ATS and BZD plate

Standards and Samples	**ATS Plate**			**BZD Plate**		
	Number of Band	**Colour of Band**	**R_f Value**	**Number of Band**	**Colour of Band**	**R_f Value**
Methamphetamine standard	1	Grey	0.56			
MDMA standard	1	Violet	0.57			
Amphetamine standard	1	Light Yellow	0.61			
Nimatezepam standard				1	Yellow	0.18
Nitrazepam standard				1	Yellow	0.12
Flunitrazepam standard				1	Yellow	0.15
Diazepam standard				1	Yellow	0.26
Sample 1	2	Pink Violet	0.44 0.75	1	Yellow	0.15
Sample 2	2	Brownish Violet	0.75 0.93	1	Pale yellow	0.26
Sample 3	0	Not available	Not available	1	Pale yellow	0.26

GC-MS

The chromatogram of the mixed drug standards showed that ATS standards eluted earlier than the BZD substances (Figure 1). All drugs were well separated and adequately resolved, despite there being similar chemical properties among the ATS group. Similarly, all four BZD standards separated adequately within analysis run time.

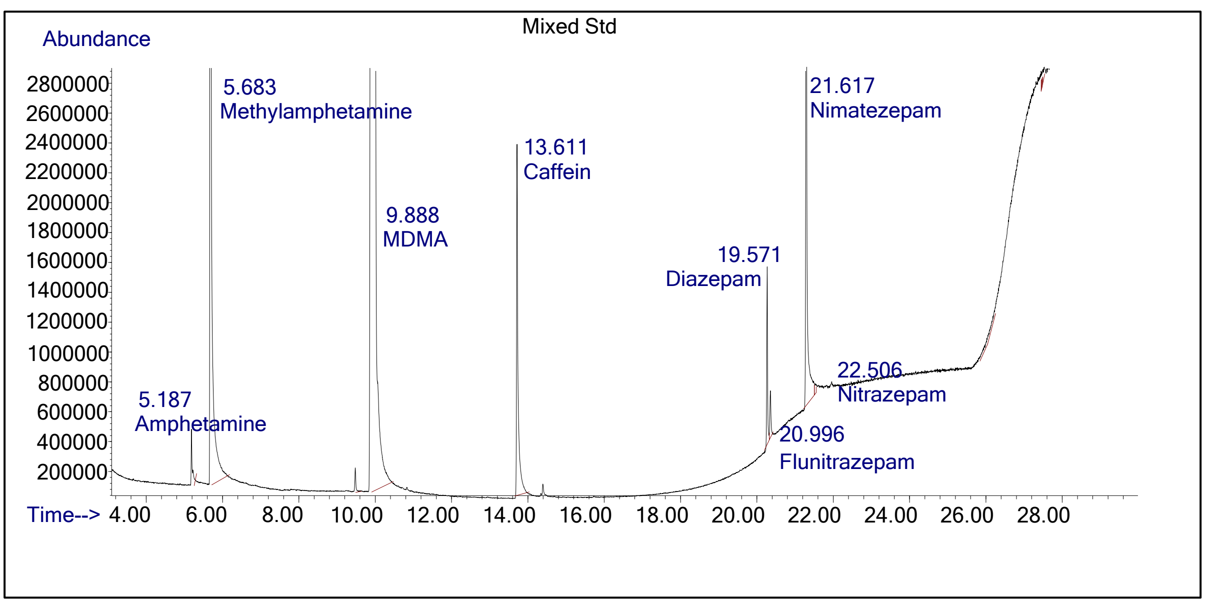

Figure 1. GC-MS total ion chromatogram (TIC) of mixed ATS and benzodiazepine (BZD) drug standards for cross reference with neat and extracted vape liquid samples

A large peak with retention time (R_t) ranging from R_t 3.553 to R_t 5.643 in mixtures of glycerine, L-arabinatol and ethyl acetate was noted in GC-MS total ion chromatogram (TIC) of diluted vape liquid samples (Figure 2). These compounds could be carriers for the vape liquid active compounds.

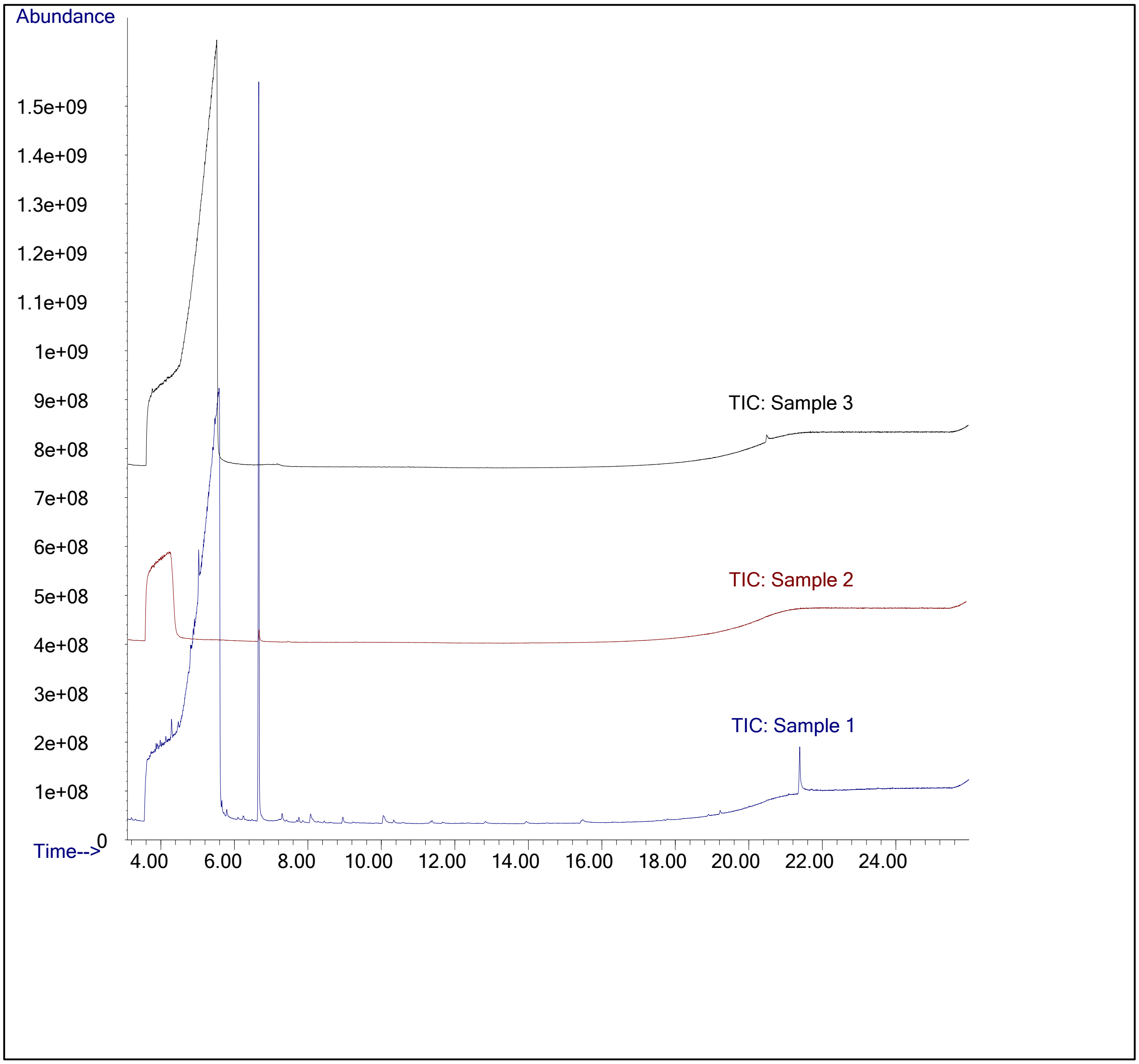

Figure 2. GC-MS total ion chromatogram (TIC) of neat vape liquid samples (2% vol/vol methanol).

Since amphetamine and methamphetamine compounds appeared at R_t 5.185 and R_t 5.679 respectively as shown in Figure 1, the presence of carrier peaks (shown in Figure 2) could have obscured the peaks of ATS compounds. Therefore, extraction was further performed on the samples in order to eliminate the effect of carrier. As a result, better TIC profiles were observed in the extracted vape liquid sample 1, 2 and 3.

Sample 1

GC-MS analysis was unable to detect the presence of the ATS compounds in sample 1. However, two prominent compounds were present at high levels peaks of R_t 6.674

and R_t 21.591 as shown in Figure 3. A mass spectral library search tentatively identified the peaks as 2-Heptenoic acid, heptadecyl ester (25% Qual) and 2,3-Diphenylbenzo-1,4-dioxin (27% Qual) respectively. Because the elution time of Sample 1 peak at R_t 21.591 is very close to the retention time of nimatezepam standard, there was a possibility of the GC chromatographic shift. The shift was checked out by comparing the mass spectrum of the compound and it was confirmed that neither nimatezepam nor lunitrazepam were present in sample 1 (Figure 4).

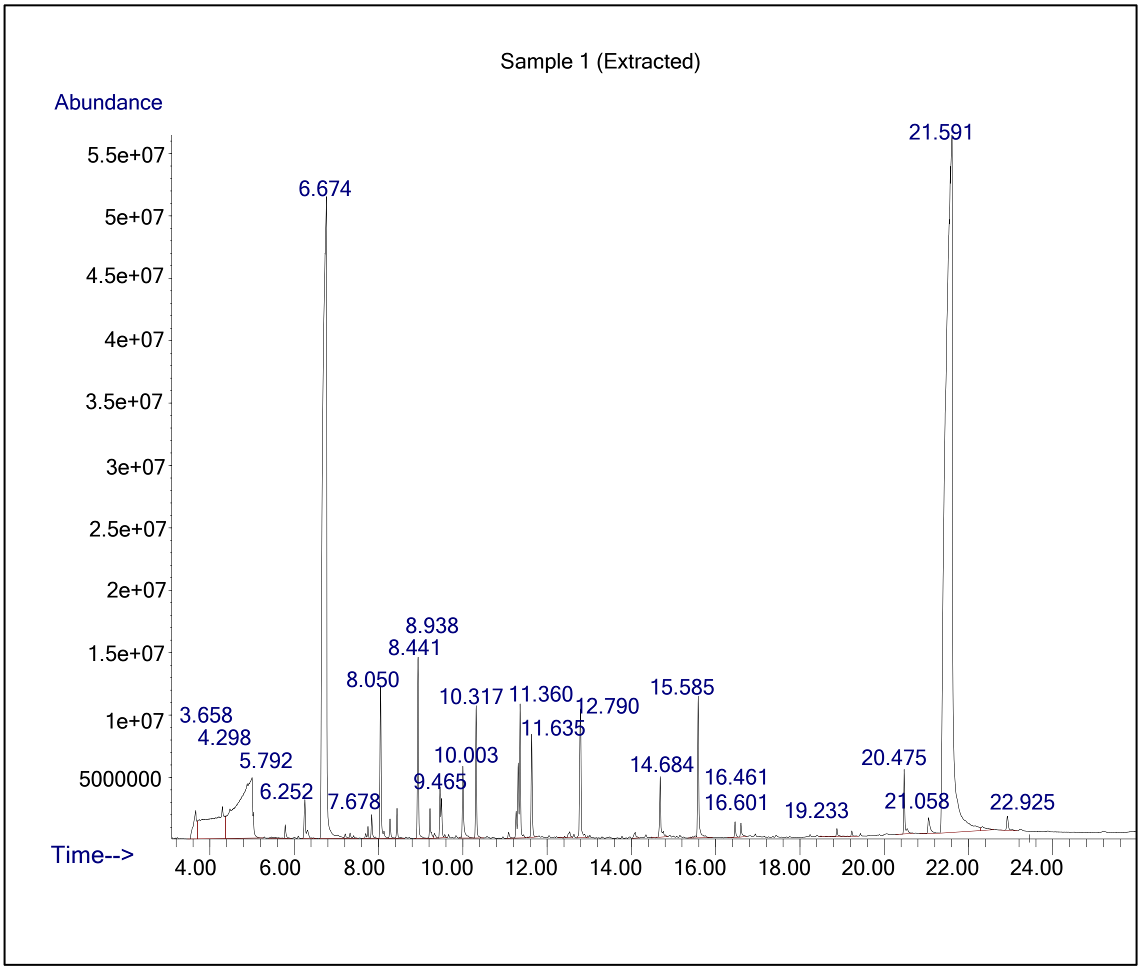

Figure 3. GC-MS total ion chromatogram (TIC) of extracted vape liquid sample 1

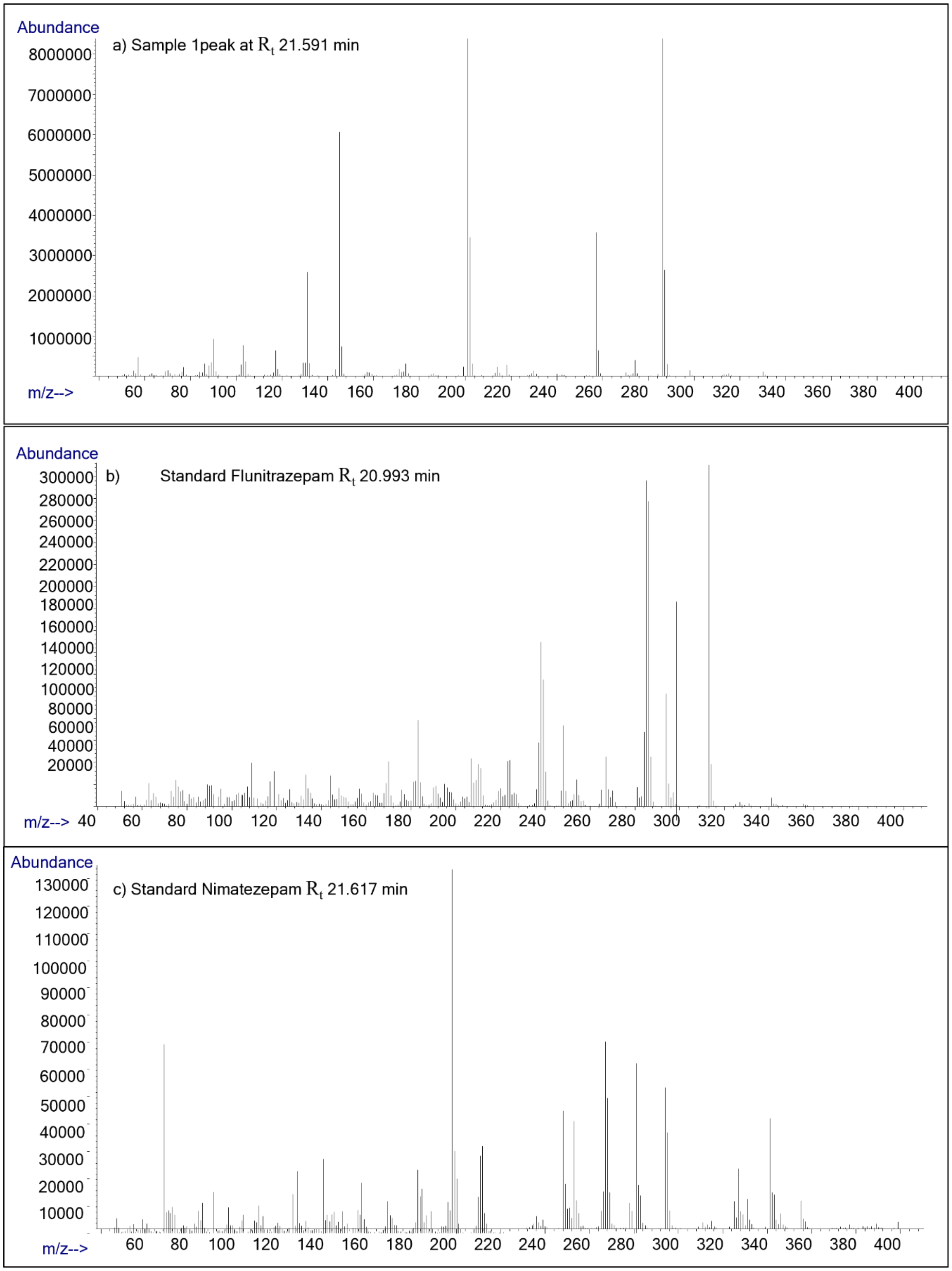

Figure 4. Mass spectrum of benzodiazepines in comparison to sample 1 peak R_t 21.591

Sample 2

The analysis of sample 2 using GC-MS identified peaks for nicotine and ketamine from the extracted sample (95-99% certainty according to NIST14 database), eluted at Rt 7.6 and Rt 13.9 respectively (Figure 5). Nicotine was identified with major fragments at 84 and 133 m/z as seen in Figure 6. The molecular ion, M+, appears at m/z = 162 and M-1 peak (m/z = 161,) was characteristic for cyclic amines [15]. Ketamine was identified at 179 and 220 m/z as shown in Figure 7.

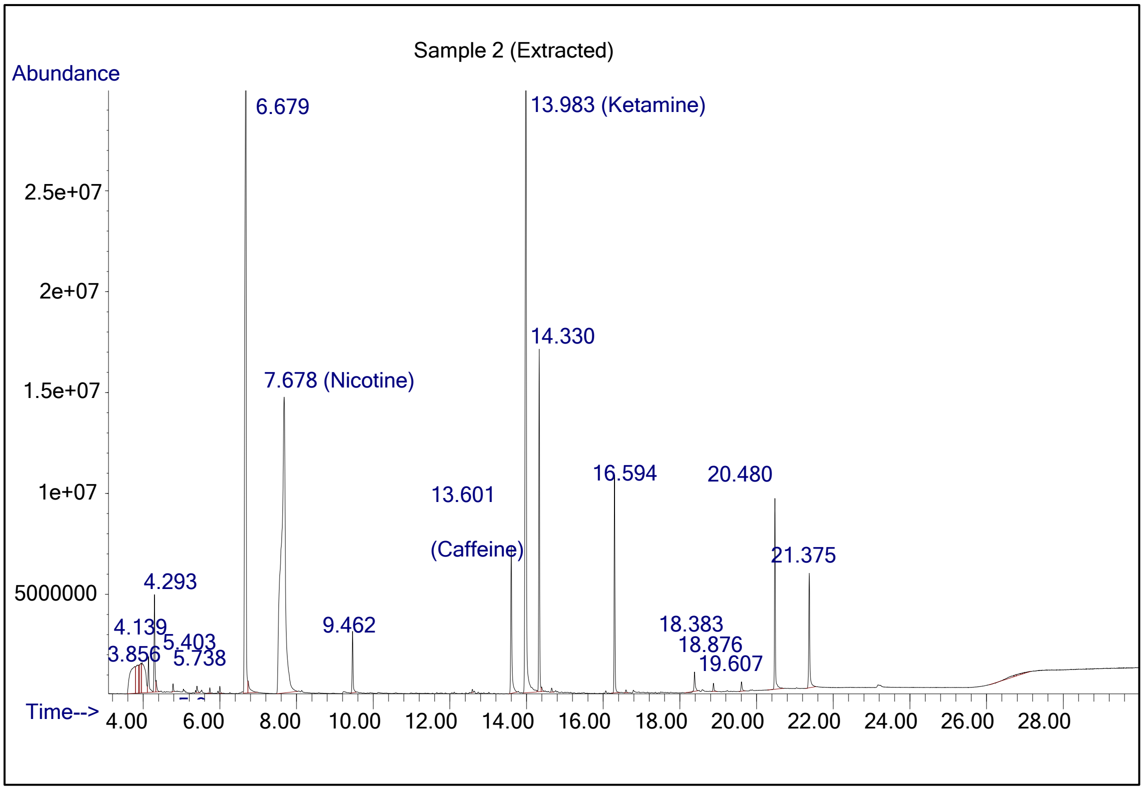

Figure 5. GC-MS total ion chromatogram (TIC) of extracted vape liquid sample 2

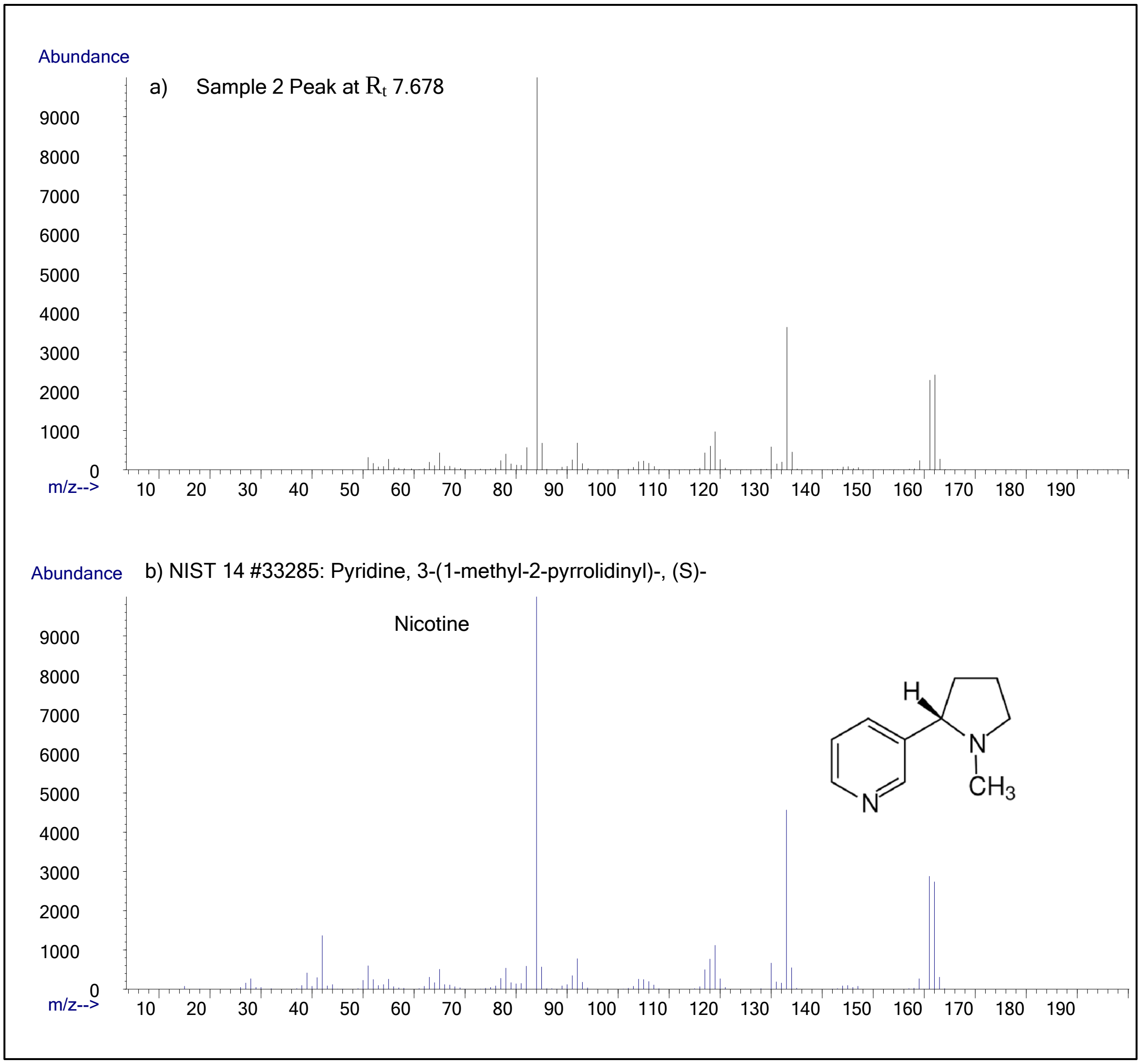

Figure 6. Mass spectrum of peak RT 7.675 identified as Pyridine, 3-(1-methyl-2-pyrrolidinyl)-, (S)-, generally known as nicotine.

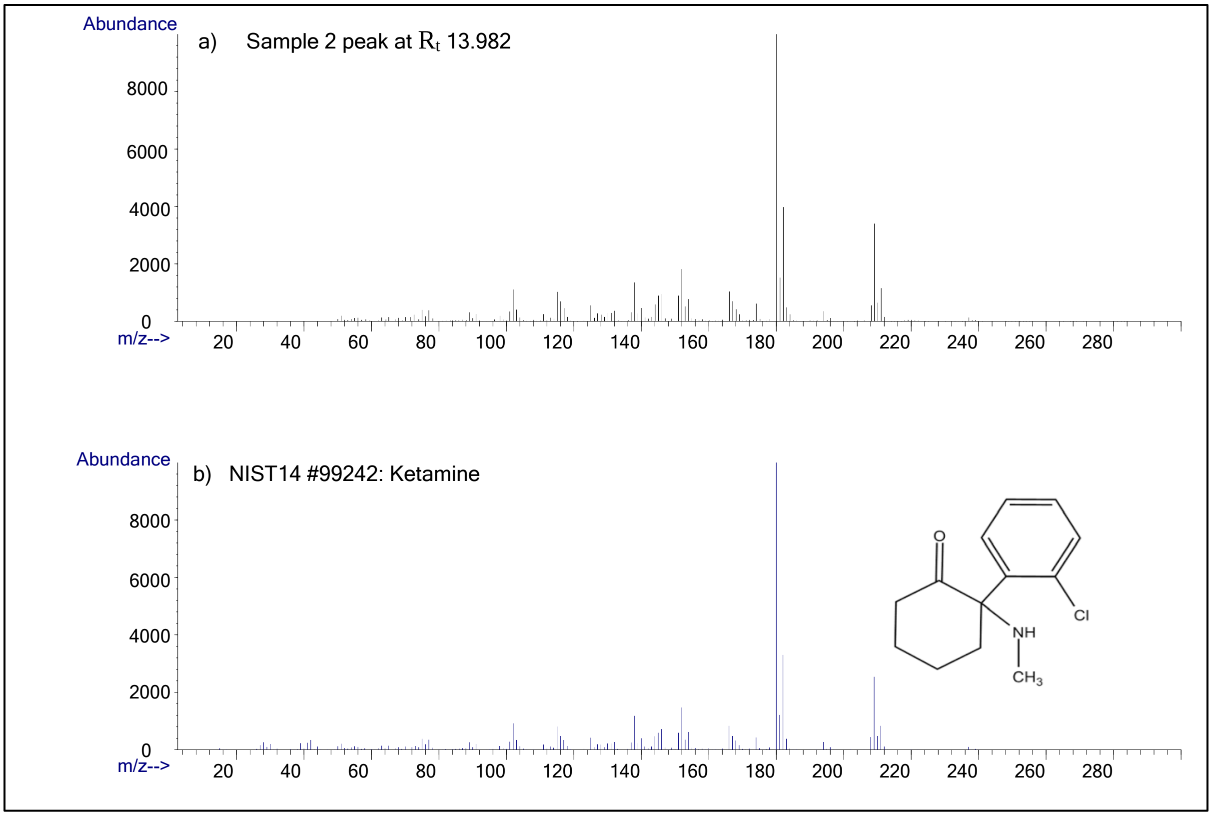

Figure 7. Mass spectrum of peak RT 13.983 identified as ketamine

Sample 3

TIC confirms the absence of ATS compounds in sample 3 in GC-MS analysis (Figure 8). In comparison to the other two samples, nicotine and ketamine were not detected. To confirm the BZD plate which obtained a band consistent to the diazepam standard, the mass spectrum of sample at RT 19.615 compared to diazepam peak RT 19.575 as presented in Figure 9.

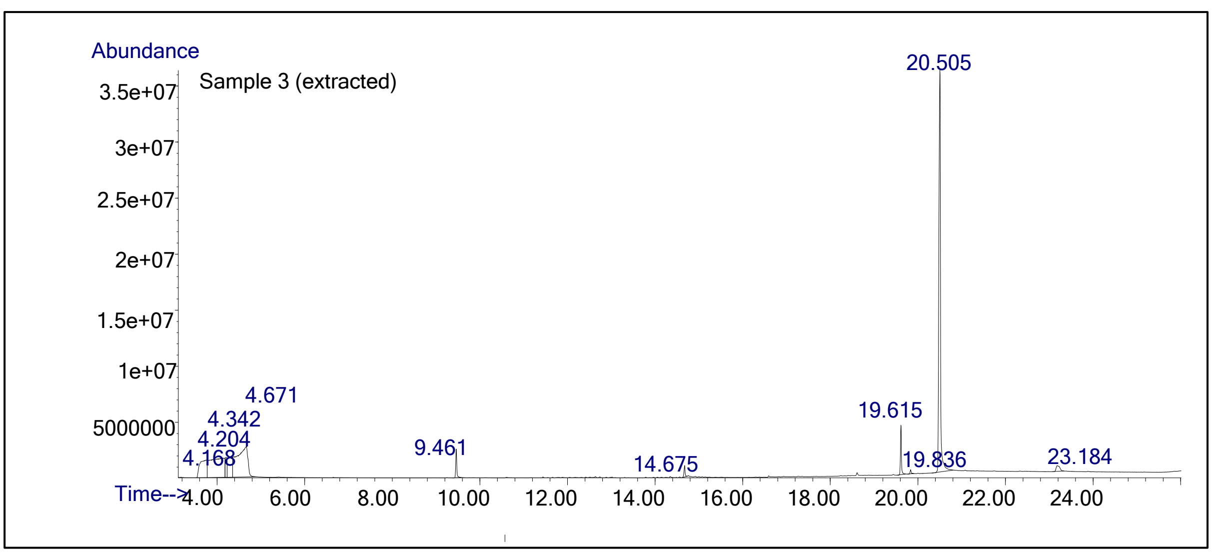

Figure 8. GC-MS total ion chromatogram (TIC) of extracted vape liquid samples sample 3

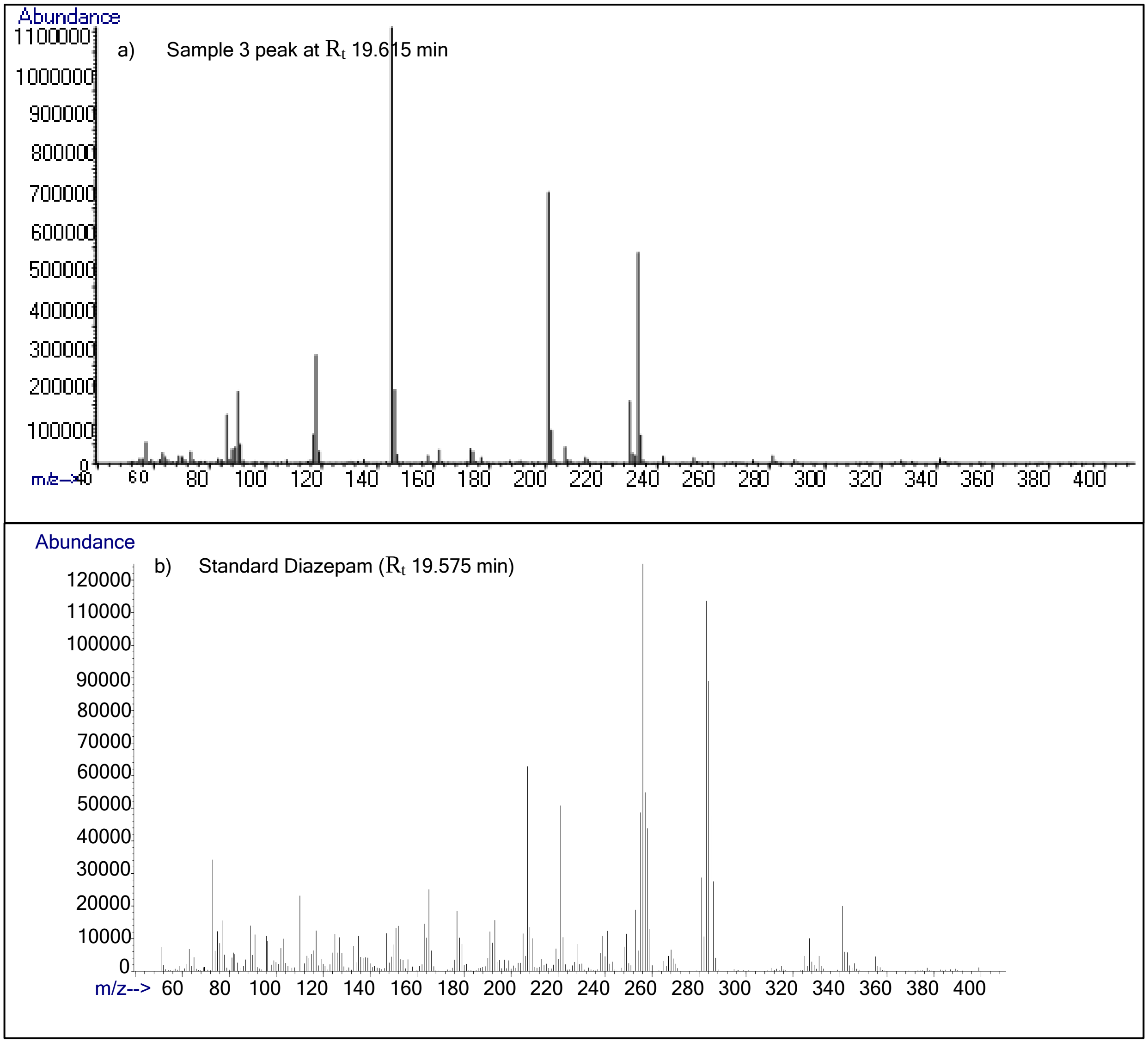

Figure 9. Mass spectrum of sample 3 peak R_t 19.615 in comparison to diazepam standard R_t 19.575

Vaping was originally intended to be a substitute to smoking, primarily serving as a nicotine delivery tool and reducing the number of harmful substances and carcinogens found in regular cigarettes. However, it has become an emerging system of illicit drug delivery [16] for various drugs of abuse, exacerbated by poor regulation from the authority. Ketamine has been discovered in recent raids in Malaysia [17] and also been reported in vape in previous surveys [12], but is relatively uncommon, most likely due to gross under detection since ketamine was not a drug that is routinely screened.

Abuse of methamphetamine dominates current cases in Kelantan [18], alongside benzodiazepines (nimetazepam, nitrazepam) and to a lesser extent opiates and THC. Ketamine on the other hand is infrequently reported, as it is not officially part of drug screening in district hospitals, or even in most tertiary referral centres in Kelantan. No exact history was available from subjects who used these vape samples. However, on further questioning, NADA did reveal general phenomena of light-headedness and fainting after using this vape. The symptoms could be either due to ketamine or high levels of nicotine in the vape liquid.

Ketamine is a dissociative hallucinogen used clinically for the induction and maintenance of anaesthesia, acting via NMDA receptor blockade which explained the effects of light-headedness and fainting experienced by those using this vape liquid. Ketamine was not detected during screening since EMIT screening for ketamine was not available. The sample volume provided was also inadequate for a ketamine dipstick. While the ATS TLC plate revealed suspicious bands, we could not definitively confirm the presence of ketamine until GC-MS analysis was performed.

Even at lower doses, ketamine can cause changes in sensory perception, including visual or auditory hallucinations and a sensation of feeling detached from oneself and one's surroundings, hence its categorization as a dissociative. Other adverse effects include disorientation, confusion, or loss of motor coordination, dizziness, nausea, or vomiting. Increased blood pressure, heart rate, breathing, or body temperature are commonly seen. At high doses, ketamine induces loss of consciousness and unresponsiveness with respiratory depression (shallow or irregular breathing). The lethal dose for ketamine for a 70 kg adult is estimated to be about 4200 mg. LD_{50} in rats are established at 447 mg/kg. Typical doses used for anaesthesia range around 450-750mg and recreational use (via snorting) ranges around 75-125 mg.

Nicotine was detected in two of the vape samples. This result is alarming since nicotine use may result in dependence and abuse. With greater delivery, faster rate of absorption and higher nicotine concentration, the risk of dependence may be higher. Furthermore, the pharmacological effects of nicotine in the brain are influenced by the mode of administration and dose [19]. Since vaping has the potential of high and rapid nicotine delivery, vaping can cause or maintain nicotine dependence – this is why vaping as a method for smoking cessation is controversial [20]. It has been reported that a cigarette contains between 6.17-28.86 mg of nicotine, varying between cigarette brands [21]. Nicotine concentration in vape liquid varies considerably, ranging from 0-87.2 mg/mL [22]. The amount of nicotine delivered and the way in which it is delivered influence the addictiveness of a tobacco product [23]. As the vapers under the impression that vaping is safer than cigarette smoking, they may not control the amount of vape used. In addition, the heating process for vapes may lead to the generation of hazardous compounds [24]. Drugs may be abused freely in future through vaping since it is not obvious that the person took drugs using vape compared to "chasing the dragon", one of the current modes of administration using aluminium foils adopted by the drug users.

Conclusion

We detected ketamine in 1 of the 3 vape samples and nicotine in 2 samples collected from NADA. The presence of psychoactive substances in vape is a concerning issue as these products are sold to school going children. Here, we would like to ask for a reassessment of the risks and dangers of vaping and potentially the enactment of stricter laws and regulations to cover its use (essentially to ensure it does not contain dangerous psychoactive substances) and trade (limiting its availability to underaged children).

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