DETECTION OF ETIZOLAM AND CLOZAPINE AS ACTIVE INGREDIENT IN ILLICIT ERIMIN-5 TABLETS

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ABSTRACT: In this study, six different batches of illicit Erimin-5 tablets seized by Royal Malaysia Police in Penang State, Malaysia were analysed. A simple and rapid gas chromatography-mass spectrometry (GC-MS) method was used to identify active ingredients in the illicit Erimin-5 tablets. An attenuated total reflection Fourier-transform infrared spectroscopy (ATR-FTIR) and Raman Spectroscopy were used to identify the tablet excipients. As a result of the analysis, five different compounds have been identified as main active ingredients, which were nimetazepam (n=2), phenazepam, nitrazepam, etizolam and clozapine. Etizolam and clozapine were newly detected in illicit Erimin-5 tablets in Penang State, Malaysia. This is the first reported identification of etizolam and clozapine as active ingredients in illicit Erimin-5 tablets.

Keywords: Illicit Erimin-5 tablets, nimetazepam, phenazepam, nitrazepam, clozapine, etizolam.

INTRODUCTION

Nimetazepam, a type of benzodiazepine (Figure 1) that is legally manufactured under the brand name Erimin by Sumitomo in Japan. It is sold as a tablet of 5 mg which gives it street name of "Erimin-5" or "Happy-5". They were generally orange colour with the imprints of Sumitomo's logo and "028" at one side, and "5" on another side of the tablet. Nimetazepam is an intermediate acting hypnotic drug which is generally prescribed for the short-term treatment of severe insomnia in patients who have difficulty of falling asleep. However, the manufacturing of Erimin-5 has been ceased in Japan since early November 2015.

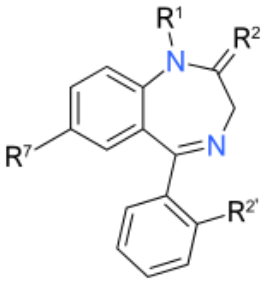


Figure 1. Chemical structure of Benzodiazepine.

In Malaysia, Erimin-5 tablets are commonly used as recreational drugs in nightclubs. "Erimin-5" is popular among methamphetamine abusers because it worked as come down pill to relieve insomnia. Besides that, overdose of benzodiazepines in combination with alcohol are also frequently found in suicidal cases.

In 2014, most of the Erimin-5 tablets seized in Penang state contain nimetazepam as main ingredient while only small amounts of tablets contain phenazepam, another type of benzodiazepine drug. Phenazepam and nitrazepam were considered as "traditional drug" in illicit Erimin-5 tablets as it appeared in global drug market many years ago. Erimin-5 tablets also is a common drug of abuse in Singapore. Singapore reported detection of nitrazepam and phenazepam in illicit Erimin-5 tablets in 2006 and 2016 respectively [1,2].

In this paper, we present a total of six different types of Erimin-5 tablets encountered in Penang state, Malaysia. Gas chromatography mass spectrometry (GC-MS) analysis of tablets confirmed the presence of nimetazepam, phenazepam, nitrazepam, etizolam and clozapine respectively. Etizolam and clozapine are new compounds detected in illicit Erimin-5 tablets.

MATERIALS AND METHODS

Materials

Six illicit Erimin-5 tablets seized samples (Sample 1 to Sample 6) and four aluminium foils were used in this study. These seized samples were submitted by the Royal Malaysia Police to Narcotics Section, Department of Chemistry, Penang State in the course of investigation. All chemicals and solvents used were of analytical grade.

Physical characteristics study of tablets and foil aluminium

The stamped logos on the front and rear sides of each tablet were photographed and recorded. The nett weight of tablet was weighed using Mettler Toledo ME204 analytical balance. The diameter and thickness of the tablets were measured using digital calliper. The stamped words on the front and rear side of each aluminium foil were photographed and recorded.

Extraction procedure for Erimin-5 tablets

For qualitative analysis, each Erimin-5 tablet was crushed into homogenised fine powder by using mortar and pestle. Approximately 50 mg of homogenised powder was sonicated in 5.0 ml of chloroform/methanol mixture (1:1, v/v) for 10 minutes. The mixture was filtered and used for GC-MS analysis.

Gas chromatography mass spectrometry (GC-MS) conditions

The GC-MS instrument used was an Agilent 7890A gas chromatograph equipped with an Agilent 5975C mass selective detector and an DB-5MS capillary column (30 m×0.25 mm×0.25 μm film thickness). The injector was operated in split mode (50:1) at 270ºC. The injection volume was set at 1 μL. Helium gas was used as carrier gas at a flow rate of 1.0 mL/min. The oven temperature was initially held at 220ºC for 2 minutes, then increased to 300ºC at a heating rate of 20ºC/min, and held at that temperature for 6 minutes. The MS was operated in scan mode, and the acquisition range was set to 50–500 m/z. The temperature of GC-MS interface, ion source and quadrupole were 280ºC, 230ºC and 150ºC respectively. The mass spectra of samples were compared with NIST 2014 and SWGDRUG MS Version 3.4 Library.

ATR-FTIR and Raman Spectroscopy analysis of tablet excipients

Infrared spectroscopy measurements were conducted in a Thermo Scientific Nicolet iS10 FTIR Spectrometer equipped with Thermo Scientific Smart iTR ATR sampling accessory equipped with a diamond crystal. Tablets were homogenised with a pestle and mortar without any sample treatment. The measurements were made on the sample that deposited on ATR crystal. Spectra were recorded from 4000-525 cm^{-1}, with a resolution of about 4 cm^{-1} and 16 scans. ATR crystal was cleaned with a tissue soaked in ethanol after each sample to remove any residue. Identification of tablet excipients were done by matching the IR spectra of samples with in-house library by using OMNIC 8.0 software.

Raman spectra were obtained by using Rigaku Progeny 1064 nm handheld Portable Raman analyzer. Sample spectra were acquired in the point-and-shoot mode with the sample positioned in front of the adjustable focal point nose cone. Auto timing and average spectrum were used for data acquisition for approximately 30 seconds. The obtained sample spectra were searched against Rigaku In-House library.

RESULTS

Physical characteristics study of tablets and foil aluminium

The photo, weight, diameter and thickness of each tablet are shown in Table 1. The weights of tablets were in the range of 164.1 mg to 196.4 mg and showed considerable variation. The diameter and thickness of tablets range from 8.02 mm to 8.06 mm and 2.58 mm to 2.95 mm respectively. Besides that, the size of font of imprints “5” and “028” and also Sumitomo logo were found different among these samples. The imprints of Sample 4 and Sample 5 are similar because there were seized from the same clandestine laboratory that produce illicit Erimin-5 tablets in 2018.

Aluminium foils from Sample 1, Sample 2, Sample 4 and Sample 5 were available in this study. Figure 2 shows the front and rear view of each aluminium foil. In this study, each aluminium foil had different imprint on it which are “A6000A”, “A5000A”, “A5000K” and “A5000AA” respectively. The aluminium

foil with imprint "A5000K" and "A5000AA" (Sample 4 and Sample 5) were found in a clandestine laboratory that manufactured illicit Erimin-5 tablet in 2018. The Erimin-5 tablet in all aluminium foil "A5000K" was found to contain nitrazepam while Erimin-5 tablet in aluminium foil "A5000AA" contain clozapine. In this clandestine laboratory case, it is believed that the imprints of "A5000K" and "A5000AA" on aluminium foil function as specific code to differentiate between Erimin-5 tablet that contain nitrazepam and clozapine, respectively.

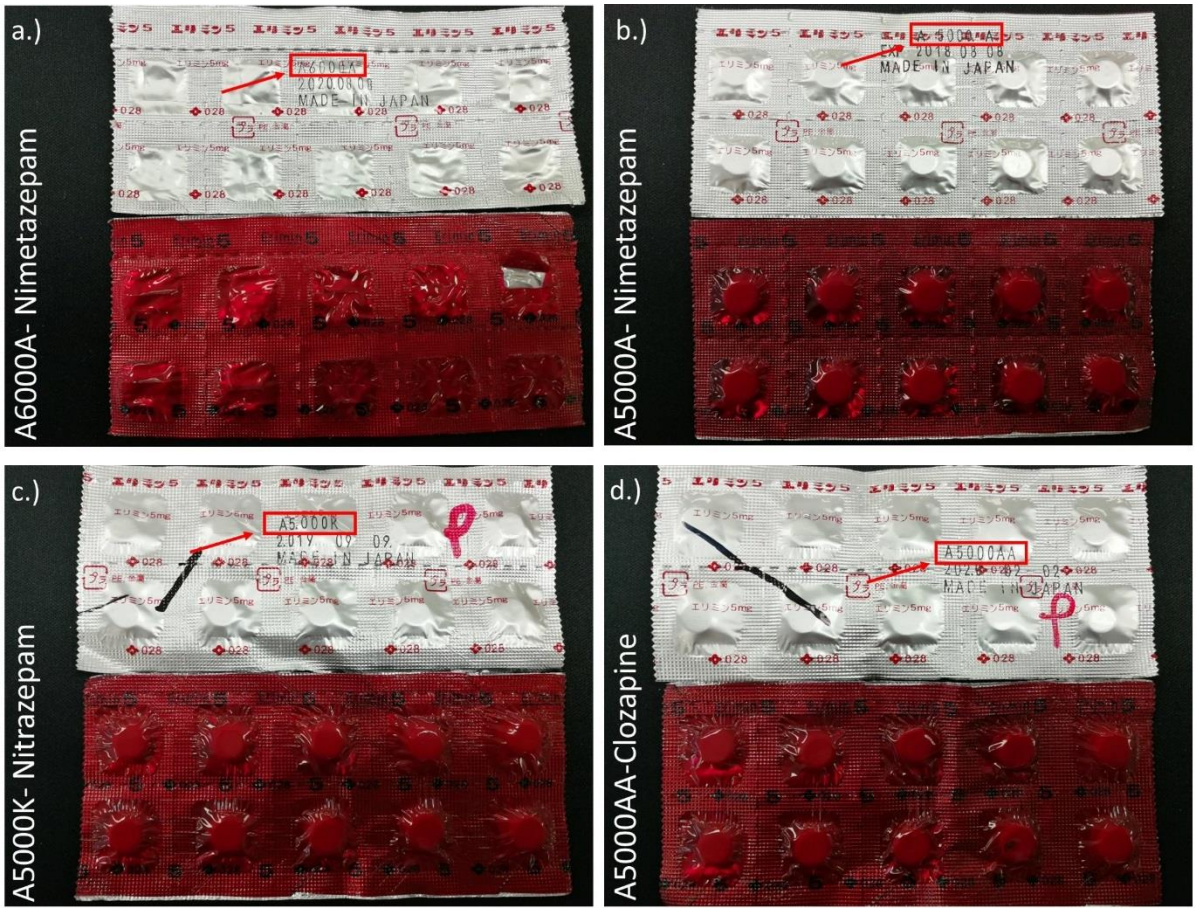
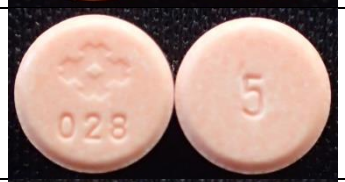
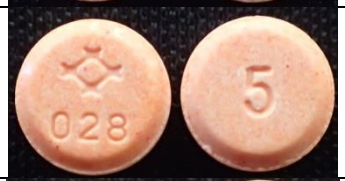


Figure 2. Aluminium foil of: (a) Sample 1, (b) Sample 2, (c) Sample 4 and (d) Sample 5.

Gas chromatography mass spectrometry

To identify active ingredients in Erimin-5 tablets, a total of six different batches of seized samples were analysed using the GC-MS technique described above. As the result of analysis, five different compounds were identified in the seized samples. The five identified compounds were nimetazepam (n=2), phenazepam, nitrazepam, clozapine and etizolam. GC-MS total ion chromatogram (TIC) and mass spectra of all samples are shown in Figure 3. Nimetazepam, phenazepam, nitrazepam, clozapine and etizolam were identified at retention times of 6.029 mins, 6.164 mins, 6.475 mins, 6.916 mins and 7.819 mins, respectively. Nimetazepam, nitrazepam and clozapine were identified using SWGDRUG (version 3.4) and in-house GC-MS (NIST 14) libraries. However, phenazepam and etizolam can only be identified through SWGDRUG library.

Table 1. Photo, physical characteristics, and analytical result of six illicit Erimin-5 tablets.

Sample	Photo	Weight (mg)	Diameter (mm)	Thickness (mm)	GC-MS	FTIR	Raman	Code on aluminium foil
1		179.7	8.06	2.58	Nimetazepam	Lactose	Nimetazepam + Alpha-D-Lactose Monohydrate	A6000A
2		183.4	8.02	2.70	Nimetazepam	Mannitol	Mannitol + Nimetazepam	A5000A
3		196.4	8.03	2.94	Phenazepam	Lactose	Alpha-D-Lactose Monohydrate	n.a.[a]
4		179.2	8.06	2.95	Nitrazepam	Mannitol	Mannitol + Clonazepam	A5000K
5		164.1	8.04	2.85	Clozapine	Mannitol	Mannitol	A5000AA
6		179.4	8.05	2.93	Etizolam	Mannitol	Mannitol	n.a.

[a] Not available.

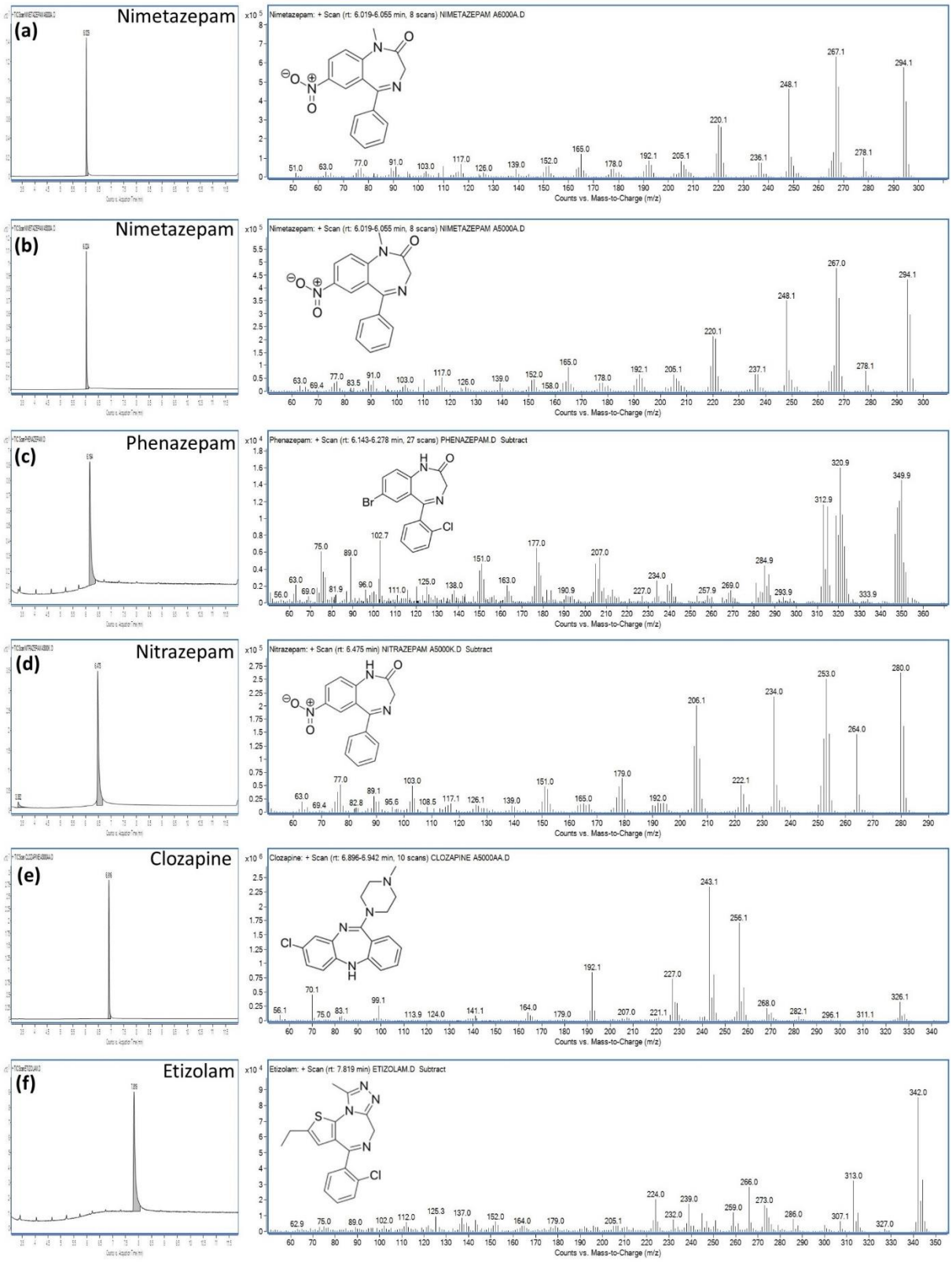


Figure 3. GC-MS total ion chromatogram (TIC) and electron ionization (EI) mass spectra of: (a) Sample 1-Nimetazepam, (b) Sample 2-Nimetazepam, (c) Sample 3- Phenazepam, (d) Sample 4 - Nitrazepam, (e) Sample 5- Clozapine and (f) Sample 6- Etizolam.

ATR-FTIR and Raman Spectroscopy analysis of tablet excipients

All tablets were found by FTIR to contain either lactose (n=2) or mannitol (n=4) as the major excipients (Table 1). Identification of lactose and mannitol were accomplished by matching the IR spectra of samples with in-house library spectra. Figure 4(a) shows the spectrum of Erimin-5 tablet containing phenazepam which has been bulked with lactose. Similarly, Figure 4(b) and Figure 4(c) show Erimin-5 tablet that containing clozapine and etizolam respectively, which have been bulked with mannitol. The major excipient in sample 1 (lactose) and sample 2 (mannitol) were found to be different, although both samples were found by GC-MS to contain nimetazepam.

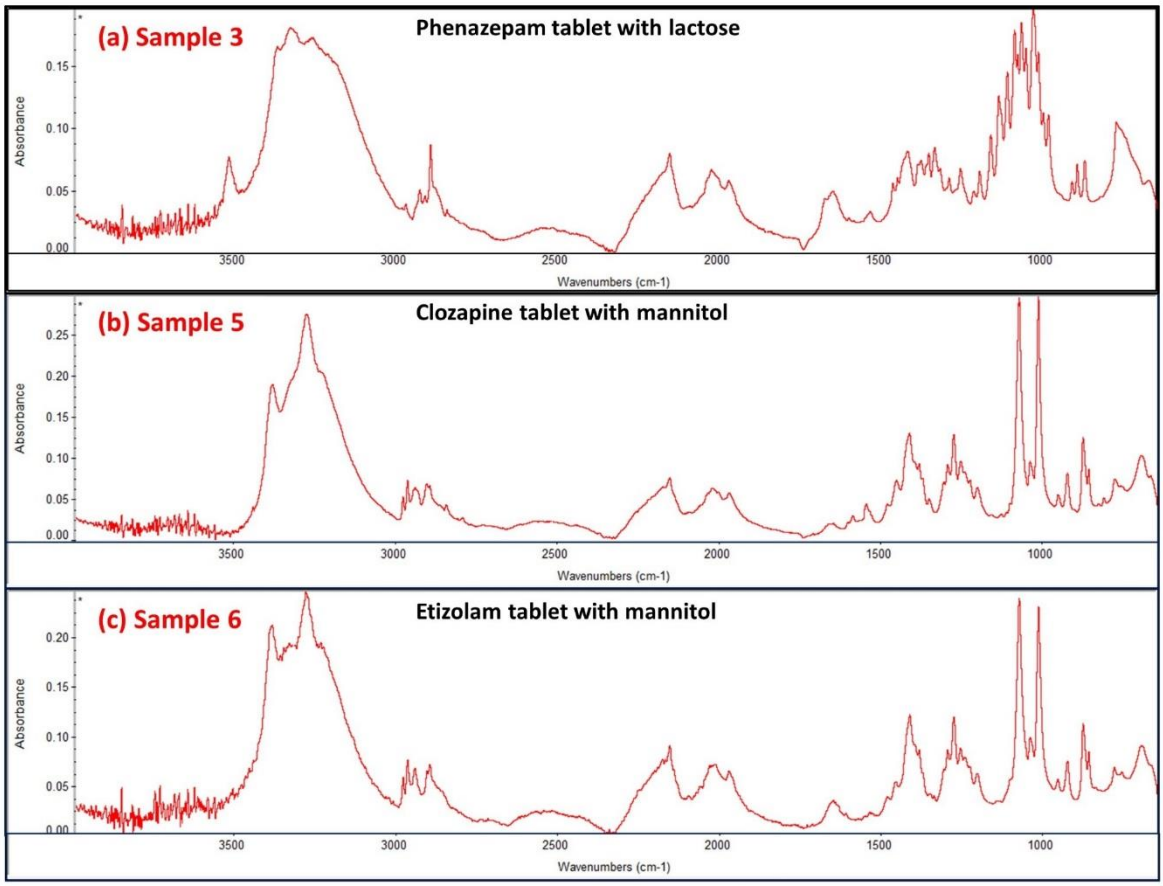


Figure 4. ATR-FTIR spectra of: (a) Sample 3- Phenazepam tablet with lactose, (b) Sample 5- Clozapine tablet with mannitol, and (c) Sample 6 – Etizolam tablet with mannitol.

Some of the complex drug mixtures were prepared by mixing one drug with up to few excipients. Figure 5 shows the Raman spectra of Sample 1 to Sample 6. Identification of lactose or mannitol as tablet excipients by Raman Spectroscopy were identical to FTIR results (Table 1). In this study, Raman Spectroscopy identified a mixture of excipient and active ingredient in Sample 1, Sample 2 and Sample 4, while FTIR results showed the presence of excipient only. Raman spectrum of Sample 1 shows the presence of a mixture of nimetazepam and lactose (Figure 5a). Similarly, raman spectrum of Sample 2 shows the presence of a mixture of nimetazepam and mannitol (Figure 5b). However, raman spectrum of Sample 4 shows a mixture of mannitol and clonazepam (Figure 5d), but GC-MS result of Sample 4 confirmed the presence of nitrazepam only. In general, portable Raman Spectroscopy of seized samples is a reliable, rapid, non-contacting and non-destructive technique for the identification of both the drug and the excipients.

DISCUSSION

Among these detected compounds (Figure 6), etizolam and clozapine were newly detected in illicit Erimin-5 tablets to our knowledge. In Penang state, etizolam was firstly detected in Erimin-5 tablets in September 2017, while clozapine was detected in April 2018. It is believed that etizolam and clozapine type of illicit Erimin-5 tablets had emerged as new replacement to continue supplying drug market in Malaysia after Sumitomo ceased to manufacture the commercial Erimin-5 tablets.

Etizolam is a thienodiazepine derivative, with high affinity for the benzodiazepine site in GABAA receptors [3]. Etizolam is currently a prescription medication in Japan, India, and Italy to treat insomnia and anxiety [4]. However, etizolam has emerged as a recreational substance in the illicit drug market in Europe and United States. It is usually encountered in powder, in tablets form or spiked onto blotter paper [4]. Etizolam is classified as new psychoactive substances (NPS) belonging to the benzodiazepine class. In March 2020, United Nations Commission on Narcotic Drugs has decided to control etizolam under Schedule IV of the Convention on Psychotropic Substances of 1971 [5].

In 2014, a case report of etizolam dependence has been documented. A 23-year-old male was prescribed etizolam at dose of 0.25 mg/day to treat social anxiety disorder. Gradually, his intake increased to 2.5 mg/day over a period of month. The patient experienced characteristic benzodiazepine withdrawal symptoms (palpitations, tremulousness, and impaired sleep) if taking lower dose of etizolam [6]. In Scotland, etizolam was the third most frequently detected substance reported among all drug-related deaths in 2016, after heroin/morphine and methadone [7]. The number of death cases relating to etizolam increased from 58 in 2015 to 270 in 2016, with more female than male [8].

Unlike other benzodiazepine derivatives, clozapine is a dibenzodiazepine class of medicine that is used as anti-psychotic medication. Clozapine is mainly used to treat schizophrenia as it can reduce hallucinations and suicidal behaviour among schizophrenia patients. However, there is insufficient toxicological study and information on their unknown side effects, physical and psychological dependence that associated with misuse of clozapine.

In Malaysia, nimetazepam was controlled as dangerous drug under First Schedule of Dangerous Drug Act 1952. Phenazepam and nitrazepam were classified as 1,4-benzodiazepines derivatives while etizolam as benzodiazepine analogue under First Schedule of Poison Act 1952. Although in Malaysia clozapine is used as prescription medication for schizophrenia patients, it is still controlled under First Schedule of Poison Act 1952.

CONCLUSION

The abuse of Erimin-5 tablets among drug users in Malaysia is alarming. Long term and chronic abuse of benzodiazepines show high potential of tolerance and drug dependence. The situation is getting worse when the legal prescription medication for schizophrenia was abused by drug users. Moreover, multidrug administration of these new substances mostly involved interaction with alcohol and methamphetamine which is bearing unpredictability of effects and serious health risks for consumers. In this concern, we believe that forensic chemists play a fundamental role in constant monitoring and rapid identification of abused substances in seized samples to supply useful information for enforcement agencies.

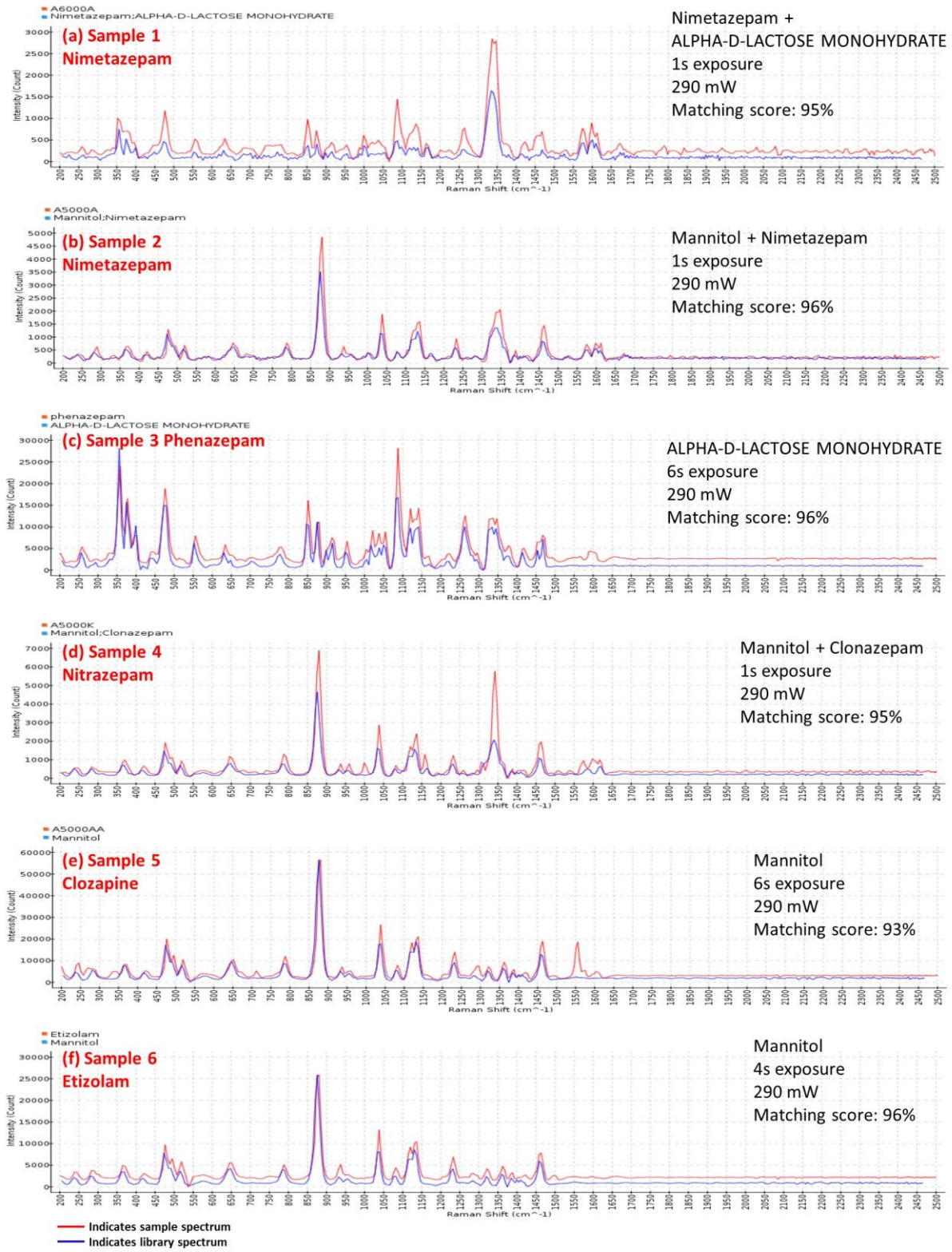


Figure 5. Raman spectra of Sample 1 to Sample 6, over the 200-2500cm^{-1}.

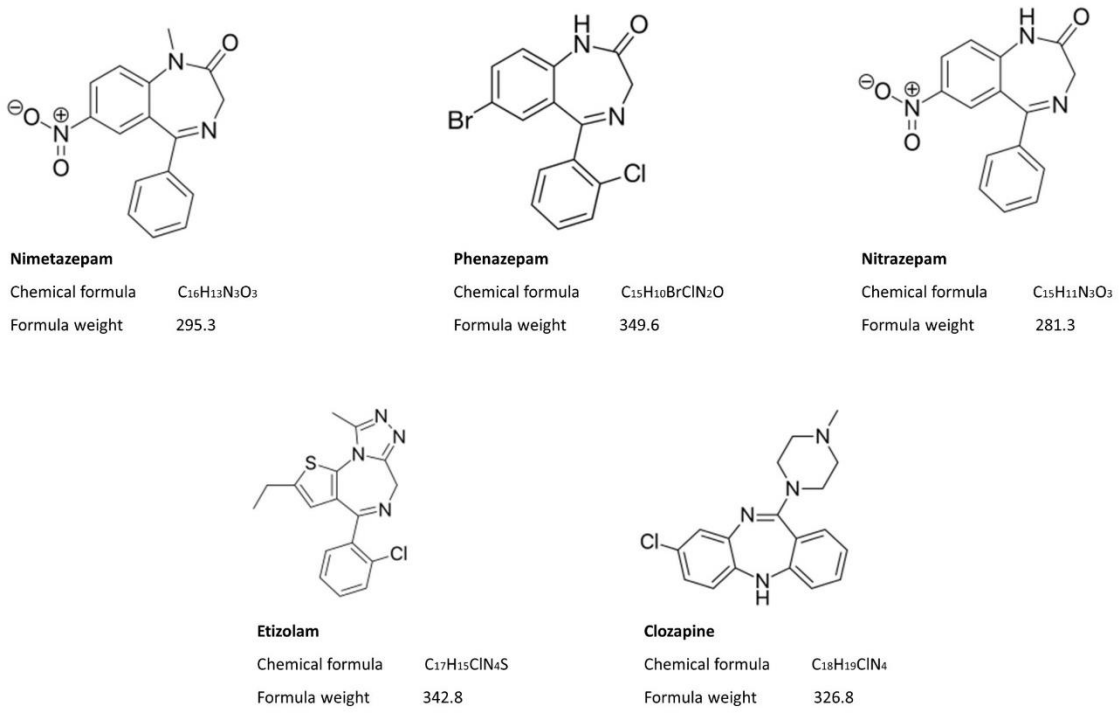


Figure 6. Benzodiazepines identified in the seized Erimin-5 tablets.

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