An Analytical Study on Erimin-5 Tablets For Forensic Profiling

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ABSTRACT: A substance classified under benzodiazepines is sold as Erimin-5 which might contain nimetazepam or other substitutes. Due to potential of abuse, many benzodiazepines listed in the Schedule IV of the International Convention on Psychotropic Substances 1971 are banned in many countries, including Malaysia. In routine forensic analysis, gas chromatography-mass spectrometry (GC-MS) and high-performance liquid chromatography (HPLC) techniques are utilised for qualitative and quantitative determination of benzodiazepines, respectively. However, efforts on source tracking and sample-to-sample comparison of such drug substance have not been prioritised thus far. Therefore, forensic profiling of Erimin-5 tablets through physical and chemical means warrants further investigation. In this study, 101 Erimin-5 tablets collected from casework were visually observed, and evaluated in term of their imprints, shape, and colour. Subsequently, the choice of adulterants and colourants in their composition were determined using attenuated total reflectance-Fourier transformed infrared (ATR-FTIR) spectroscopy and thin layer chromatography (TLC), respectively. GC technique was then applied to confirm the presence of benzodiazepine related substances, and the percentage of nimetazepam was quantified using HPLC. Physical examination demonstrated the unique characteristics of Erimin-5 tablets. ATR-FTIR coupled with principal component analysis grouped the Erimin-5 samples into the clusters similar to those adulterated with mannitol or lactose. TLC analysis determined sunset yellow dye as the common colourant added, while GC-MS detected the presenec of either nimetazepam or etizolam among the tested samples. Nimetazepam was subsequently quantified and reported with percentage range between 0.62 – 4.49%. To conclude, forensic discrimination of Erimin-5 tablets was successfully carried out, at least allowing for sample-to-sample comparison for drug related investigation and intelligence.

Keywords: forensic science; benzodiazepines; Erimin-5; profiling; intelligence

Introduction

Misuse of drug continues to be a challenging issue confronting the law enforcement authorities worldwide. According to the United Nation Office of Drugs and Crime (UNODC), the number of drug users had achieved nearly 269 million people used drugs worldwide, and this figure was reported to be 30% higher than in 2009 [1]. In the context of drug control and legislation, due to drug abuse potential of benzodiazepine, majority of the related substances under this group are classified under Schedule IV of the International Convention on Psychotropic Substances 1971 and banned in many countries [2,3]. In Malaysia, nimetazepam and flunitrazepam are scheduled under the Dangerous Drugs Act 1952 [4].

According to World Drug Report, majority of the sedatives and tranquilisers seized worldwide in 2019 were gamma hydroxybutyrate (GHB). However, the benzodiazepines were mostly seized in Malaysia, indicating the seriousness of the drug issue in the country [3]. Benzodiazepines, mainly contain nimetazepam as the common active composition, collectively sold as Erimin-5 in the black market [5,6]. Erimin-5 is commonly in the form of tablets. These drugs are sold in relatively cheap price, and they are easily accessible as compared to other conventional drugs such as heroin and cocaine. It also makes Erimin-5 one of the commonly abused sedatives [7,8]. Similar use and distribution patterns were also reported in neighbouring countries, including Singapore and Indonesia [9-12]. The severity of these drugs had been frequently reported in media.

Literature search indicated several works reported on gas chromatography (GC) determination of the compounds. Gjerde et al. [13] have attempted to simultaneously determine common benzodiazepines in blood using capillary gas chromatography. For forensic toxicological application, Moore et al. [14] confirmed the presence of benzodiazepines in urine

upon trimethylsilyl derivatisation and detected on gas chromatography-mass spectrometry (GC-MS). To address the potential of spiking benzodiazepines in drug-facilitated crime setting, Famiglini et al. [15] reported a rapid measurement of benzodiazepines in a beverage GC-MS analysis, and found that the quick extraction can detect drugs in milk-based drinks fortified with different commercial drugs. The sensitivity of GC-MS was also demonstrated by the work done by Yegles et al. [16]. The work showed the ability to detect benzodiazepines and other psychotropic drugs in human hair by GC-MS. It was also noted that the hair testing was complementary to the conventional post-mortem analysis in forensic toxicology cases. In a forensic profiling of Erimin-5 samples, GC-MS was used to determine the active ingredients reported in 64 case samples in a study perform in Malaysia. The authors found that compounds other than nimetazepam were present in Erimin-5 tablets, indicating the possibility of substituting the active compounds with other similar ingredients [5].

Besides GC, HPLC is perhaps another key technique in forensic laboratories. UNODC [17] noted that due to the diversity in chemical structures of different benzodiazepines, a single HPLC method might not be able to separate all benzodiazepines. A method for the qualitative and quantitative analysis of benzodiazepine derivatives was proposed under international control. In brief, the method suggested a 250 mm by 5 mm i.d. column of octadecyl-silica HPLC 5 µm diameter (ODS-Hypersil or equivalent). A flow rate of 1.5 mL/min was suggested and an injection volume of 20 uL with the peak areas being detected by UV at 240 nm or diode-array detector (DAD) for the presenec of benzodiazepines. It was also important to note that the selection of column and solvent system would also depend upon the benzodiazepines encountered by the laboratories. Abdul Rahim [18] also quantified the amount of the target compound upon identification, specifically the nimetazepam, using HPLC.

As part of analytical studies on Erimin-5 tablets, discrimination of Erimin-5 tablets using various techniques including both the physical and chemical means are worth to be explore with aim to establish potential clustering of the seized Erimin-5 samples. Drug profiling is a procedure involving the systematic characterisation of seized sample by physical and chemical approaches to support the intelligence and operational works by the law enforcement authorities. The potential linkage among samples could be established, and samples from different seizures could also be classified into different groups of related samples. Subsequently, drug profiling could also provide information on the possible linkages with the suppliers, distributors, and users, as well as the distribution networks or patterns of the drug substances [19]. It is hoped that the classification of the Erimin-5 tablets encountered in this study could assist forensic investigation and intelligence in linking the street level seizures, as well as in facilitating sample-to-sample, case-to-case, and seizure-to-seizure comparisons.

Materials and Methods

Erimin-5 tablet samples

A total of 101 Erimin-5 tablets samples were seized and collected for forensic analysis. Since the number of samples in each case was different, at least two tablets were kept and separated labelled with specific identification number as S001 to S101. Actual case number for all the samples were kept confidential.

Physical examination of Erimin-5 tablets

Physical examination was initiated prior to any further chemical analyses. All the tablets were visually observed, and any special observation was recorded. They were photographed using digital camera from the front, rear, and side views of each tablet. The criteria concerned during the physical examination included the presence of any logo or imprint on the surface of tablets, as well as the colour in every tablets.

Attenuated total reflectance-Fourier transformed infrared (ATR-FTIR) spectroscopy

Using pestle and mortar, drug samples were grounded into powder form. Subsequently, the finely powdered samples were separately kept. Standards of drug substances were also analysed to compare with the ATR-FTIR spectra of tested samples with unknown composition. With any additional sample treatment, at approximately 10 mg powdered samples was placed onto the ATR crystal of an ATR-FTIR instrumentation (Bruker Corporation, Billerica, MA) set with wavenumbers ranging from 4000 to 600 cm^{-1}. Sixteen scans were done at a resolution of 4 cm^{-1} and spectra were acquired using the built-in software, namely the OPUS 7.0.122 software (Bruker Corporation, Billerica, MA). All ATR-FTIR spectra were compared, evaluated, and observed for any similarities and differences.

Thin layer chromatography (TLC)

Dye standards was run by TLC to determine the dye that have been added to the composition of Erimin-5 tablets. 100 mg of the powdered sample was transferred into a GC vial and added with 5% acetic acid. Then, a piece of white knitting wool of 5 cm and knotted together was added into the vial. Then, the mixture was warmed on a heating plate to allow for the dye originated from the tablets to be transferred onto the wool. Upon the completion of heating process, the wool was removed from the acid solution. The wool was washed several times with distilled water and allowed at room temperature to dry before inserted into another vial. 1 ml of solution with of equal volume of acetone and 3 N ammonia was added for alkaline treatment and heated for another 15 min, or until the colour of the solution turned into the colour of the dye. After heating procedure, the wool was removed from the vial using tweezers. To maximise the dye to be applied on the TLC plate in the latter stage, the wool was depressed on the side of the vial to transfer the solution into the vial. Subsequently, the dye solution was gently warmed in a water bath. This was done to evaporate the solvent away and to obtain a more concentrated solution for TLC analysis.

Spotted TLC plate was placed in TLC solvent tank filled with solvent system made up of isopropanol: ammonia (S.G. 0.880): Water (7:2:1 v/v/v). All visible bands upon completion of TLC separation were marked. The TLC plates was also illuminated under ultraviolet light to observe the presence of any fluorescent spot. The number of spots and their colours were documented. R_f values of each spot were also calculated. The spots developed from the drug samples were compared to the standard reference colour spots to determine the potential dye that found in the drug samples. Any differences among the drugs samples in term of the dye that have been added into the respective composition was evaluated.

Gas chromatography-mass spectrometry (GC-MS) analysis

Standards solutions were prepared independently by dissolving small amount of standards (*i.e.* nimetazepam, etizolam, and nitrazepam) in 2 mL vial and mix with analytical grade methanol:chloroform (1:1 v/v), in order to achieve a final concentration of 1.0 mg/mL for each compound. Each standard was accurately weighed using an analytical balance at 10 mg (± 0.1 mg). On the other hand, drug samples in tablet form were firstly crushed using pestle and mortar until the samples became powder form. An amount of the powdered samples (approximately 10 mg) was transferred into 2 mL vial containing 1.5 mL of solvent [methanol: chloroform (1:1 v/v)]. The vials were then thoroughly mixed to dissolve the sample and subjected to GC-MS analysis.

A Perkin Elmer TurboMass Software (Version 5.4.2.1617) (Perkin Elmer, Waltham, MA) was used for GC-MS automation and data interpretation. The chromatographic separation was achieved using a cross-linked methyl silicone gum, HP-5 capillary column (30 m × 0.25 mm i.d., 0.25 µm film thickness). Purified helium gas (99.9%) was chosen as the carrier gas with a column flow of 1.0 mL/min. Liquid injection was performed using GC-MS through a split mode (45:1) into the injector set at 270˚C. The initial temperature of oven was set at 220˚C, ramp to 300˚C at 20˚C/min and lastly hold for 6 mins. The transferline was set at 280˚C, and the acquisition mode was set to scan mode for 50 - 500 amu. The total run time was 12 mins.

In this study, a volume of 1 µL of respective standard solution and drug sample was separately introduced into the injector port. The resulting peaks in the chromatograms produced were identified by comparison to the retention time of standards as well as the NIST mass spectral library 14 (National Institute of Standards and Technology, Gaithersbury, MD).

High Performance Liquid Chromatography (HPLC) analysis

Upon identification of drug content in the seized samples, quantitative analysis was subsequently conducted. In the routine testing in Malaysia, only the presence of nimetazepam was further quantified with its scheduling in the Dangerous Drug Act of Malaysia. The standard stock solution was prepared at a concentration of 1.0 mg/mL where 50 mg of nimetazepam standard was weighed accurately into a 50 mL volumetric flask and dissolved with a solution of methanol:chloroform (5:1 v/v). Five calibration points of the nimetazepam standard was prepared at concentration levels ranging from 0.02 to 0.240 mg/mL to construct a calibration curve.

Prior to HPLC analysis, an amount of the powdered tablet (measured at 40-60 mg) was weighed in replicate in a 10 mL of volumetric flask. The taken amount was recorded to calculate drug content per weight of tablet. The drug samples were then dissolved using a solution of methanol:chloroform (5:1 v/v) to the levelling of volumetric flask. The mixture was also sonicated for 10 min to allow for complete dissolution of drug sample.

All the standards and samples were injected into a Waters HPLC system (Waters Corporation, Milford, MA) through liquid injection. A C18 column (5 µm particle size, 15 cm × 4.6 mm i.d.) was used for chromatographic separation. The ultraviolet detector set at a wavelength of 265 nm was used for detection of target compound. A combination of methanol and water (65:50) was used as the mobile and its pH was adjusted to 4.0 with phosphoric acid. The injection volume and the flow rate were set at 10 mL and 0.8 mL/min, respectively. Water Empower Pro version 6.20.00.00 (Waters Corporation, Milford, MA) was used for data acquisition. The standards and samples were injected separately and in replicate. A blank (mobile phase) was run before the sample analysis to avoid carry over. Nimetazepam was detected and quantified against the reference standard. Each sample run took 12 min.

Upon analysis, the percentage of nimetazepam per gram within a tablet was calculated by considering the dilution factor and sample weight. The HPLC instrument was calibrated to determine the concentration of nimetazepam in unit of mg/mL. Subsequently, the % nimetazepam was calculated and compared.

Results

Physical examination

For physical examination, the imprints, colours, and any specific appearance were considered. Imprint on the tablets is an important indicator on the identity of a drug, both legal medicinal and illicit drugs. Figure 1 illustrates the representative samples of Erimin-5 tablets with logo imprints. All Erimin-5 tablets have specific marking with a number “5” on one side and “028” with a four-leaf clover logo on the other side, serving as specific indication of Erimin-5 tablets. However, it was noted that the font size of the wordings, in certain samples, was varied. Additionally, the logo was also slightly different in size among the samples, as demonstrated in Figure 2. Such observation could be due to the different tabletting machine with built-in embossed marking to produce the tablets of imprinted on the double sides.

Figure 1: Physical observation on the imprint of Erimin-5 tablet.

Figure 2: Variations in the logo and colours on the Erimin-5 tablets.

Colour examination was carried out through direct and straightforward observation. Erimin-5 tablets tested in this study were dominated by peach-like colour, as it was common colour encountered elsewhere in such drug substance. However, their colours were found varied in term of their respective hues, ranging from very light to darker orange colour as demonstrated in Figure 2. This could be due to the different dyes or combinations of dyes added during the manufacturing process. Literature suggested that erythrosine, tartrazine and ponceau 4R were among the dyes introduced into the drug composition [5]. Direct colour

observation did not allow the determination of dyes which can be performed through TLC in subsequently section.

ATR-FTIR analysis

As a part of the study, ATR-FTIR analysis was used as an initial screening in the forensic laboratories prior to subsequent confirmatory tests for identification and quantification. An ATR-FTIR analysis followed by PCA, reported in our previous study in Mail et al. [20] suggested that the seized samples tested in this study had belonged to two different groups based on their cutting agent composition. PCA had enabled a more objective clustering and discrimination of the drug samples especially when they were added to different adulterants.

TLC analysis

In this study, TLC has successfully separated various dye standards with characteristic R_f values as listed in Table 1. Some dyes fluoresced when they were visualised under UV light, and they were noted as ( ) in the table. With fluorescence characteristic of certain dyes, they had added discriminative value to differentiate them from dye with similar R_f values but did not fluoresce. In this case, two dye standards fluoresced under the ultraviolet illumination with longwave radiation. Rhodamine B showed very intense fluorescence behaviours upon illumination, as such behaviour can be easily distinguished this dye from most of the dye standards which did not fluoresce. Besides rhodamine B, erythrosine BS also showed very weak fluorescence behaviour which was useful to differentiate it from other dyes carrying very similar R_f values during the comparison.

All TLC plates spotted with samples in their respective spots were dipped into solvent system containing isopropanol: ammonia: water (7:2:1 v/v/v) and allowed for separation. This study examined 101 peach-like coloured samples. Figure 3 summarises the percentages of different combination of dyes found in the Erimin-5 samples tested in this study. Amongst, 93 samples (92.08%) showed the presence of sunset yellow dye in their composition, suggesting it as the most common the dye added during the manufacturing process. Four samples reported with the presence of tartrazine alone at 3.96%. In these samples, only one type of dye was detected in contrary to another four samples where more than one spots were observed indicating the presence of more then one dye. Different combinations of colour dyes were detected to give their peach-like colour in their physical appearance. Three samples (2.97%) showed a combination of sunset yellow and tartrazine, while another sample (0.99%) showed unique dye combination, reported with the presence of sunset yellow and ponceau 4R upon TLC development, as indicated by sample S84.

Table 1: R_f values of dye standards upon TLC analysis.

Standard	R_f values
Tartrazine	0.62
Sunset Yellow	0.68
Egg Yellow	0.62
Fast Green	0.68
	0.64
Green S	0.62
Brilliant Blue	0.68
Apple Green	0.68
	0.62
Amaranth	0.62
Carmoisine	0.68
Erythrosine BS	(0.78)
Ponceau 4R	0.61
Rhodamine B	(0.81)
	(0.78)
	(0.72)
Red 2G	0.70

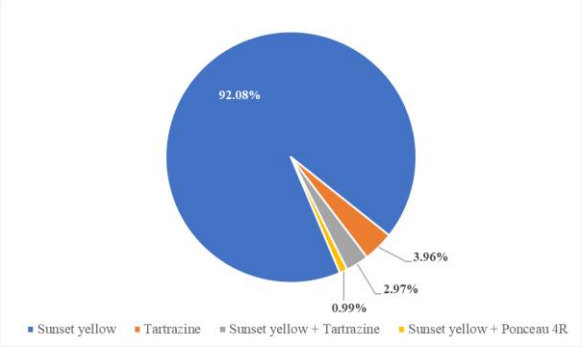

Figure 3: Percentages of dye composition in 101 Erimin-5 samples.

No fluorescence behaviour was noticed in all samples tested under the illumination of ultroviolet light, suggesting that the dye added in the composition of Erimin-5 tablets did not fluoresce. According to a previous study [5], sunset yellow was the dye frequently encountered in Erimin-5 samples, reporting with 66.04% from a total of 53 samples tested in that study. In this study, a greater percentage of sunset yellow dye alone was reported, which had supported the common of this dye to be added into Erimin-5 tablet samples. In the previous study [5], it was also noted with the detection of a combination of dyes including tartrazine, ponceau 4R and erythrosine in the Erimin-5 samples. However, such combination was

not found in our tested samples, which could suggest that such dye combination might no longer utilised in the Erimin-5 tablets, at least among the 101 samples in this study.

GC-MS analysis

The identities of target compound were confirmed by GC-MS. Within a 12 min run, the retention times of nimetazepam, etizolam and nitrazepam were determined as 2.78 min, 6.16 min and 9.25 min, respectively. Figures 4 illustrate the representative chromatograms of the above-mentioned compounds. Additionally, the presences of nimetazepam, etizolam and nitrazepam were also confirmed through the comparison of mass spectrum generated from GC-MS analysis with those obtained from NIST spectral library.

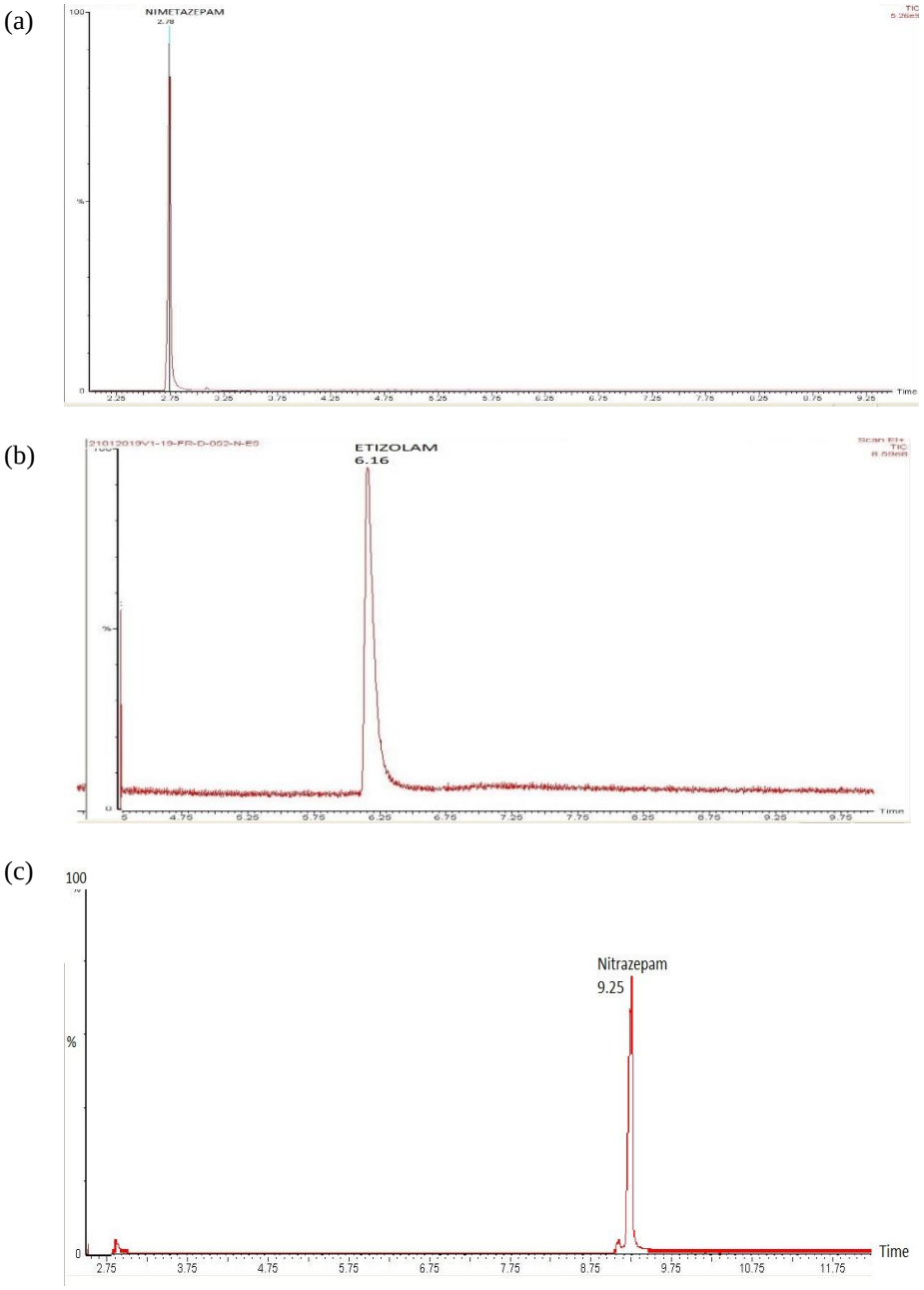

Figure 4: GC chromatogram showing the presence of (a) nimetazepam, (b) etizolam, and (c) nitrazepam.

Majority of the samples were detected with etizolam at 71.3% (72 samples), followed by nimetazepam, (27.7%, 28 samples). No nitrazepam was detected in the 101 seized samples tested in this study. Additionally, one sample (0.99%) did not show any presence of drug under the classification of benzodiazepines. In other words, etizolam was found to the most common active ingredient detected in Erimin-5 tablets tested in this study. However, it was also noted that etizolam is not listed under Dangerous Drug Act in Malaysia at present. Etizolam is a new substance which had been scheduled in the Schedule IV of the Convention on Psychotropic Substances of 1971 in March 2020 followed recommendations by WHO. It had been associated with 40 post-mortem cases, at least 90 driving under the influence of drugs cases as well as 19 clinical admission cases [21]. In Malaysia, the international controlled nimetazepam was also scheduled under the Dangerous Drug Act.

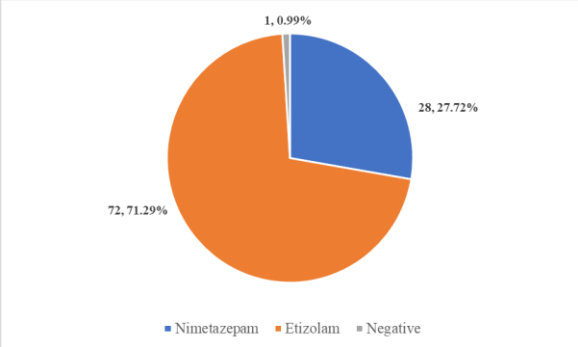

Figure 5: Percentages of benzodiazepine groups detected in the seized samples tested in this study.

As compared to other profiling studies conducted in Malaysia, Chong et al. [22] reported the presence of diazepam in Malaysian Erimin-5 samples in addition to nimetazepam early in 2004. In 2012, forensic analysis of 64 Erimin-5 tablets by Abdullah et al. [5] reported that 53 out of 64 samples (82.8%) were detected with nimetazepam and the remaining contained diazepam as the active ingredient. A profiling study by Abdul Rahim [18] showed that nimetazepam dominated the seized samples where 91% of the tested Erimin-5 tablets were found with this controlled substance from a total of 46 samples. Beside nimetazepam, 7% and 2% of the samples were detected with nitrazepam and diazepam, respectively. From this study, it was found that the main active ingredient had been gradually shifted from the controlled substance nimetazepam to etizolam which is currently not listed under the Dangerous Drug Act. Moreover, the compounds detected in previous Malaysian studies such as diazepam and nitrazepam, however, were absent. The study implied that there was a shift on the choice of active compound usage in the composition of Erimin-5 samples. A thorough profiling study of Erimin-5 tablets encountered across the country shall be carried out periodically to investigate the prevalence of benzodiazepines added into these drug substances.

HPLC analysis

HPLC was chosen to quantify the concentration of nimetazepam. Note that only those samples detected with the presence of nimetazepam were subjected to HPLC analysis due to the scheduling of nimetazepam in the Dangerous Drug Act in which the exact quantity of the controlled substance was crucial. In this case, 28 samples were analysed by HPLC in coupled with PDA. HPLC-PDA was found to be a good technique for quantitative analysis as it did not interfere by the presence of excipients in the tablets, such as adulterants and binders [18]. It was shown to provide good separation and the presence of nimetazepam was determined through the evidence of peak at 7.83 min. Figure 6 demonstrates the presence of nimetazepam peak upon HPLC analysis.

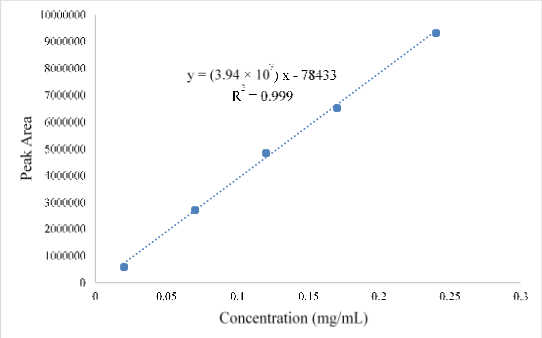

Figure 7: Calibration curve of peak area versus concentration curve for nimetazepam standard.

Calibration curve for nimetazepam was generated through the analysis of a series of calibration standards ranging from 0.02 to 0.240 mg/mL. From the calibration curve, the linear equation and regression coefficient (R^2) value were determined as demonstrated in Figure 7. Through the assessment of linearity, the best fit regression line was achieved at 0.999, sufficiently good for quantitative analysis.

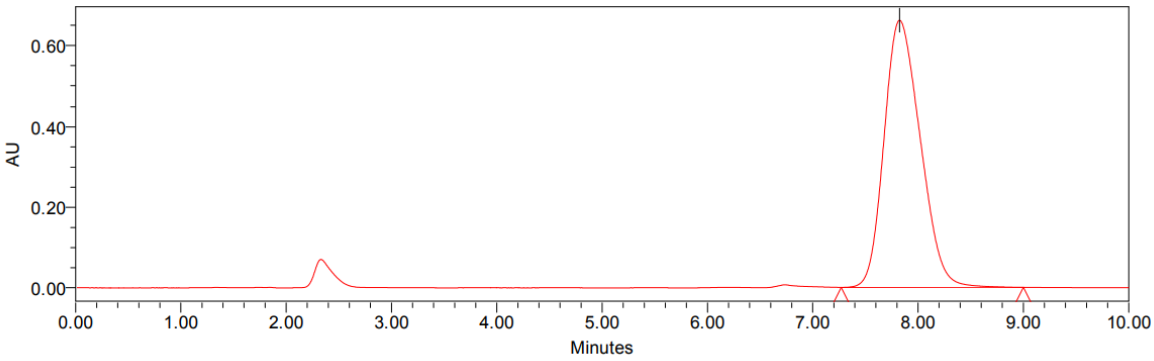

Figure 6: HPLC chromatogram showing the elution of nimetazepam standard at 7.83 min.

Figure 8 displays the frequency of % nimetazepam in 28 drug samples. The the % nimetazepam in each individual case was varied. Majority of the drug samples (14 samples, 50.0%) were found to have contained 3-4% of nimetazepam per weight of the tablets. More than 80% of the samples contained at least 3% of the controlled substance by weight of the tablets. In other words, all the Erimin-5 tablet samples have been adulterated to increase their respectively bulk size. The maximum percentage of nimetazepam was recorded at 4.49% in S014, while S015 contained the lowest percentage of nimetazepam at only 0.62%.

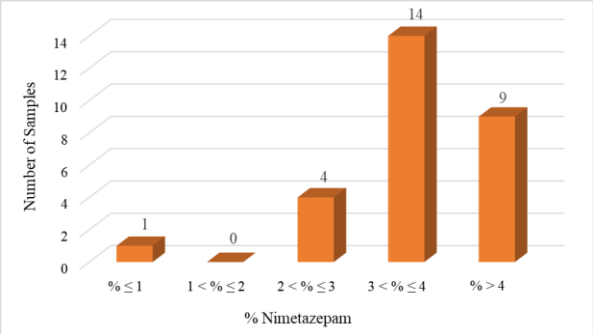

Figure 8: % nimetazepam of Erimin-5 tablets analysed in this study.

The findings from this study were found almost similar from a study conducted by Abdul Rahim [18] in which most samples (14 samples, 33%) contained nimetazepam in a percentage range of 2.5 and 2.7%. Erimin-5 samples tested in this study were found to have slightly greater percentage of nimetazepam by comparison; however, all the samples tested in both studies were reported at percentage not more than 5%.

Discussion

Physical and chemical examination of drug samples allowed the gathering of information related to the characteristics and composition. Various physical examination and chemical analyses allowed the characterisation of Erimin-5 tablet samples in this study. Variations were reported among the seized samples in term of their respective imprints and colours. It was noted that the physical examination might only restricted to sample-to-sample comparison, where those samples possessed great variation had probably been undergone different tableting procedure. Additionally, the colour variation was also one criterion to be considered for discrimination between Erimin-5 tablets.

Subsequent chemical analyses had contributed to greater chance for discrimination of Erimin-5 samples. ATR-FTIR coupled with chemometrics demonstrated the formation of two groups of drug samples, either adulterated with mannitol or lactose [20]. TLC had grouped the samples into four separate groups due to their respective dye composition. GC-MS showed that the active ingredient that made up the tablets was either nimetazepam or etizolam. Note that one sample showed no presence of any benzodiazepine, suggesting its uniqueness from the others. The percentage of nimetazepam determined through the HPLC analysis had also allowed the sample-to-sample comparison. One sample which contained significantly lower percentage of nimetazepam could also be discriminated from other samples which contained at least 2% or more percent of nimetazepam in the tablets. Figure 9 demonstrates the grouping of Erimin-5 samples upon a series of chemical analyses, where nine groups were formed.

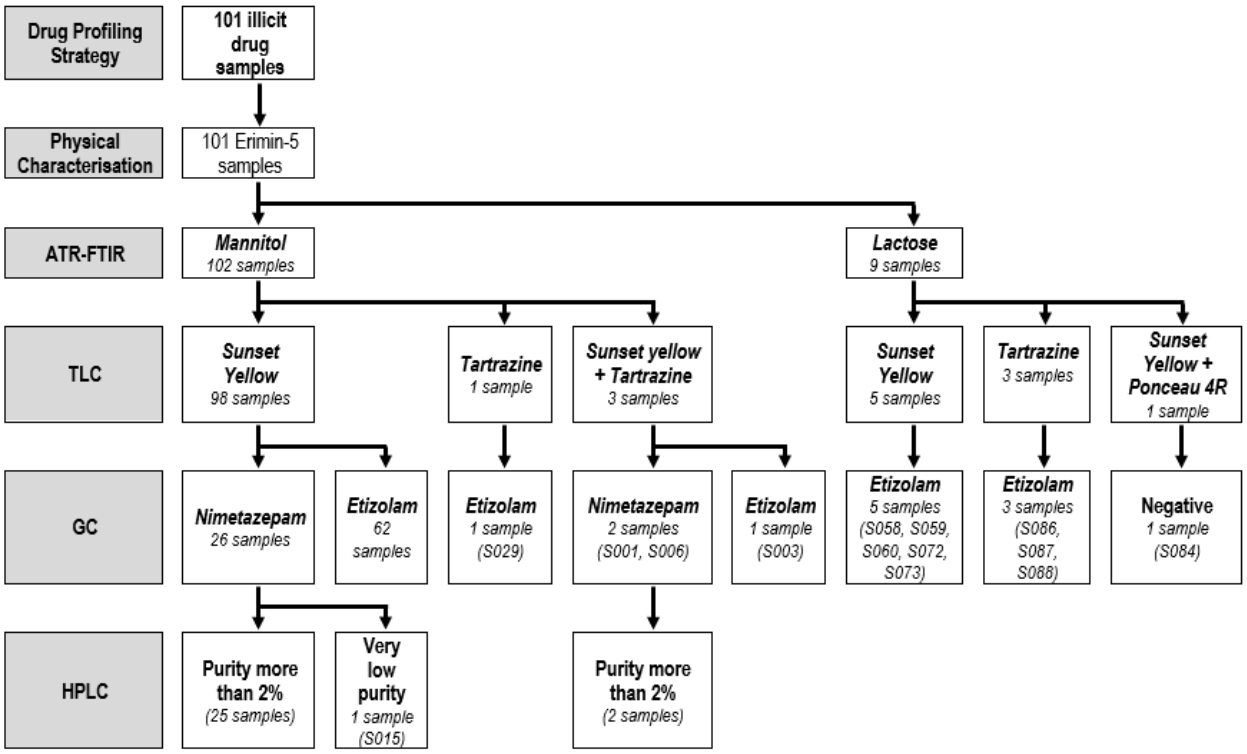

Figure 9: Groups of Erimin-5 samples upon a series of physical characterisation and chemical analyses.

The production of drugs involved multiple stages, including the manufacturing, adulterating, tableting, packaging, and finally the distribution. Each step could contribute to certain variations in the physical appearance and chemical profiles that allowed the comparison between the samples. This study found that the Erimin-5 tablets submitted to the Forensic Laboratory were of different profiles, and the information shall be utilised for forensic intelligence, especially to trace the possible sources and distribution channels of these drug substances.

The significant differences among the samples would allow the discrimination of a specific drug sample from the others, suggesting their different tracks. If two or more samples showed very similar profiles from both the physical and chemical perspectives, they could not be excluded from originating from the same sources and deserving further investigation by the law enforcement agencies.

Conclusion

Useful information was successfully retrieved through the physical characterisation and chemical analyses of Erimin-5 tablets from 101 selected cases. The profiling study was initiated with physical examination, where the marking with a number "5" on one side and "028" with a four-leaf clover logo on the other side, as well as the peach-like colour were unique to Erimin-5 tablets. ATR-FTIR as a non-destructive technique had suggested the presence of adulterants, either mannitol or lactose. Although sunset yellow was found to be the most common dyes used in manufacturing the tablets, eight samples were added with different combinations of dyes, either with tartrazine alone, a combination of sunset yellow and tartrazine, as well as a combination of sunset yellow and ponceau 4R. GC-MS techniques used in this study confirmed the identity of active compounds as either nimetazepam or etizolam. Subsequent HPLC analysis reported that samples detected with nimetazepam carried a percentage of the controlled substance at least 2% but less than 5%, except one sample with only 0.62%. The chemical characteristics of Erimin-5 tablets were successfully established. With a great amount of drug samples, a comparative study could aid in linking the samples. Erimin-5 samples tested in this study were found to be varied, depending whether it allowed discrimination of certain samples from the others. Based on the experimental results, the analytical profiles of Erimin-5 samples were compared, and nine separate groups were formed through a series of analytical methods.

Erimin-5 is expected to be continue threatening our society, especially with recent seizure and dismantled of clandestine laboratories in Malaysia. Therefore, apart from solely detecting and quantifiying the controlled substances in the seized drugs, forensic profiling through physical characterisation and chemical analyses of drug substance is a strategy for intelligence purposes. Studies on the forensic profiling of Erimin-5 tablets shall continue to be conducted periodically to investigate the trend and prevalence of such drugs from time to time. To date, there is yet a Malaysian database on the profiles of Erimin-5 tablets. In fact, the availability of such a database shall provide important information on the possible sources, as well as trafficking and distribution chains of the drug substances. Such information shall also assist the law enforcement authorities for forensic investigation of drug related cases, and also for forensic intelligence. Similar studies are also recommended to be conducted in other regions of Malaysia, including Sabah dan Sarawak. Due to the borderless trafficking and smuggling activities, the establishment of probable trafficking routes domestically, or even internationally would aid in tackling the drug issue. Additionally, comprehensive profiling involving combination of various analytical technique such as elemental analysis and impurity studies is also recommended to produce more data and information for the attribution or exclusion of common source of drug samples.

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