PHYSICAL CHARACTERISATION AND DETERMINATION OF COLOURING AGENTS IN ERIMIN-5 TABLETS

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ABSTRACT: Benzodiazepines possess hypnotic and sedative effects, making them to be widely used as depressants on human central nervous system. A substance classified under benzodiazepines is sold as Erimin-5 in the market which might contain nimetazepam or other substitutes. In routine forensic analysis, gas chromatography-mass spectrometry and high-performance liquid chromatography techniques are utilised for qualitative and quantitative determination of benzodiazepines, respectively. However, the physical characteristics and the colouring profiles of such illicit drug could also contribute important information for forensic intelligence. In this study, 101 Erimin-5 tablets collected from case work were visually observed, and evaluated in term of their imprints, shape, colour, diameter, thickness, and weight. Subsequently, the choice of colourants in the composition of Erimin-5 tablets were determined using thin layer chromatography (TLC). Physical examination demonstrated the unique characteristics of Erimin-5 tablets, allowing certain degree of sample-to-sample comparison. TLC analysis allowed the determination of possible colouring agents that had been added into the composition of these tablets, and in this study, mainly with sunset yellow dye. To conclude, forensic discrimination of Erimin-5 samples was successfully carried out, at least allowing for sample-to-sample comparison based on the physcial characteristics and the profiles of colouring agents. This study could benefit the law enforcement agencies through physical and chemical examination strategies for illiciti drug related investigation and intelligence in addition to the identification and quantification of controlled substance.

Keywords: benzodiazepine, Erimin-5, physical examination, TLC, colouring agent, discrimination.

INTRODUCTION

Misuse of drug continues to be a challenging issue confronting the law enforcement authorities worldwide. Benzodiazepines, mainly contain nimetazepam as the common active composition, collectively sold as Erimin-5 in the black market [1-3]. Erimin-5 is commonly in the form of tablets. These illicit drugs are sold in relatively cheap price, and they are easily accessible as compared to other conventional illicit drugs such as heroin and cocaine. It also makes Erimin-5 one of the commonly abused sedatives [3-6]. Similar use and distribution patterns were also reported in neighbouring countries, including Singapore and Indonesia [7-10]. The severity of these illicit drugs had been frequently reported in media.

Despite harsh punishment on drug distributers and traffickers, Malaysia continues to be an attractive place for drug trafficking. This is partly due to its strategic geographical location and border entrance accessibility for the sale and distribution of Erimin-5 tablets. The Bureau of International Narcotics and Law Enforcement Affairs [11] had also reported an increase of its trafficking activities, threatening the societal well-being of the population. Therefore, determination of these tablets is deserved to direct their respective sources and to subsequently link according to the different distribution networks [11].

To confirm the identity and determine the purity of illicit drug, the law enforcement authorities frequently submit the seized tablets to the

forensic chemistry laboratories for analysis. Gas chromatography-mass spectrometry (GC-MS) technique is routinely utilised for testing of these tablets due to its strong resolving power and robustness [1,12]. The routine analytical technique had provided significant contributions to defensible qualitative of benzodiazepines in both biological and narcotic samples through the utilisation of certified reference standard, as well as the generated mass spectrum in GC-MS ([1,12]. Upon determination of the identity, the amount of the target compound, specifically the nimetazepam, was quantified using high performance liquid chromatography technique [12]. However, efforts on source tracking of such illicit drug based on their similar profiles have not been prioritised thus far with only limited information could be retrieved for forensic intelligence.

The colour of the drugs could be readily useful to differentiate them from being of the same origin. As stated by Haywood and Glass, the role of colouring agent in tablet manufacturing is to improve acceptability of the users, as well as to aid identification [13]. One other function of colouring agent is to prevent counterfeiting, and therefore the manufacturers could use different chemical compounds to make the desired appearance. In the modus operandi of counterfeiting, the objective is to make the product as similar as possible from the authentic drugs, especially the colour [14]. One important characteristic of tablet, if coloured, would be the information of the colouring agents used. The tablets could be seen to have the similar hue but could have been produced by different types if colouring agents or a combination of them. The analysis of dyes, especially the pure dyes can be carried out by TLC [15]. Discrimination of Erimin-5 tablets using various techniques including both the physical and chemical means, in this case the determination of colouring agent in the tablets, are worth to be explore with aim to establish potential clustering of the seized Erimin-5 samples. 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 illicit drugs [14]. It is hoped that the classification of the illicit 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.

This study would benefit the law enforcement agencies for forensic intelligence. The current study utilised a series of physical and chemical examination strategies for analysis of the illicit Erimin-5 samples, and the information gathered from both the physical and chemical characterisation could provide clue in term of the similarities and differences among the illicit Erimin-5 samples. To certain extent, the drug profiling would allow the establishment on their possible source of production and distribution network. The output of this study could also advance the body of knowledge in drug related investigation in increasing the successful prosecution rate, and directly promote a safer society.

METHODOLOGY

Erimin-5 samples

Within 18 months between September 2018 and April 2020, one hundred and one illicit Erimin-5 tablets samples were collected. All these samples were seized by the Royal Malaysian Police and submitted to the Department of Chemistry Malaysia for forensic analysis. Since the number of samples in each case was different from one to ten tablets, 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, and not known by any personnel besides the researcher and the supervisors. For those samples with only one tablet, it was also included in this study, but only half of the tablet was used for drug profiling to preserve the samples for repeated analyses if required.

Physical examination of illicit 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. A scale was included during the photography to estimate the size of the tablets. 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. Any variation was noted and recorded. Subsequently, the tablets were measured in term of their diameter and thickness using a Vernier callipers. Note that diameter was considered due to the round shape appearance of all the tablets. Finally, the weight of each tablet was measured using analytical balance to 0.1 mg. All the physical characteristics were clearly documented and compared among the samples.

Thin layer chromatographic analysis

Preparation of standard and drug samples

Dye standards was run by TLC to determine the dye that have been added to the composition of illicit Erimin-5 tablets. In this study, the colour formed on the plate, the fluorescence property as well as the R_f values of each dye was compared to the results arisen from those drug samples. Both the standards and drugs were in the form of powdery solids, where the latter had been grounded using mortar and pestle.

Preparation of chemical solvents

A 5% acetic acid was used to acidify the samples in a solution to transfer the dye onto the knitting wool. To prepare the solution, 50 mL of glacial acetic acid was diluted with distilled water, making a 1 L solution in the volumetric flask. A 3 N ammonia solution used in the extraction procedure to strip the dye off from the knitting wool. For this solution, 225 mL of 25% ammonia was transferred into a 1 L volumetric flask and distilled water was added to the mark. A solution containing equal volume acetone and 3N ammonia solution was prepared by mixing the equal volume of these two solvents into a volumetric flask. All the solvents used were freshly prepared in this study, for both extraction procedure as well as the solvent systems for TLC analysis.

Preparation of solvent systems

To determine the most suitable solvent system for the separation of dye contained in illicit drug samples, four solvent systems were investigated, and their outcomes were evaluated. The four solvent systems used in this study are as follows:

i. Isopropanol: ammonia (S.G. 0.880) (4: 1)
ii. n-butanol: glacial acetic acid: water (10: 5: 6)
iii. Acetone: butanone: ammonia (S.G. 0.880): water (60: 140: 1: 60)
iv. Isopropanol: ammonia (S.G. 0.880): Water (7:2:1)

These solvent systems were suggested by the Department of Chemistry Malaysia for the separation of various dye separation. Through the comparison on the experimental outcomes of these four solvent systems, the solvent system allowing for good dye separation was used for subsequently TLC analysis on the extracted solution of illicit drug samples.

Preparation of standard and sample solutions prior to spotting on TLC plate

Approximately 10 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. A white colour knitting wool was preferable to observe the successfully transfer of dye. The knitting wool was also warmed with dilute ammonium hydroxide and followed by water before its usage to clean and remove any interference that could be arisen from the wool. A treated knitting wool could also allow greater absorption of dye. After addition of the wool, the mixture was warmed on a heating plate. The heating process must be handled with care to avoid overheating and spillage of the mixture. It took about 15 min 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. Successful transfer of dye colour shall be evident through the observation. The wool was washed several times with distilled water to remove the extraneous materials attached to the wool. Then, the wool was allowed at room temperature to dry before inserted into another vial. 1 ml of solution with of equal volume of acetone and 3N ammonia was added into the vial for alkaline treatment. The mixture was heated on the heating plate for

another 15 min, or until the colour of the solution turned into the colour of the dye. As performed in the previous procedure, overheating shall be prevented during the heating process. After 15 min duration of heating, 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 from the solution and to obtain a more concentrated solution for TLC analysis.

TLC analysis

A 20 cm × 20 cm aluminium silica gel plate was cut into half for TLC separation. To remove the trapped moisture that potential disturb the TLC separation, the TLC plate was heated in an oven at 98ºC. Spotted TLC plate was placed in TLC solvent tank filled with solvent system. As described in previous section, solvent system giving the best separation among the dye standard solutions was used for drug sample analysis. TLC plates was firstly illuminated under UV light to observe the presence of any fluorescent spot. Then, the number of spots and their colours were documented. R_f values of each spot were also calculated. The spots developed from the illicit drug samples were compared to the standard reference colour spots to determine the potential dye that found in the drug samples. Subsequently, any differences among the illicit drugs samples in term of the dye that have been added into the respective composition was evaluated.

RESULTS AND DISCUSSION

Physical characterisation

In forensic cases involving drug seizures, those drugs in tablet form can be encountered in either large or small packages. The former is frequently found in the dismantled clandestine laboratories or from the drug smugglers and traffickers with the intention to bring the illicit drugs from one place to another. On the other hand, the latter is often encountered from the drug abusers, found in a small number of tablets. In this study, all 101 samples were in small packaging, ranging for one tablet up to ten tablets. Amongst, five case samples where only found with one tablet in each single package. Two samples, namely S029 and S055, were submitted to the forensic laboratory with ten tablets, respectively, and they were the seizures with the greatest number of tablets. Samples tested in this study were treated as separated samples and labelled as S001 to S101.

Imprints on the tablets

Imprint on the tablets is an important indicator on the identity of a drug, both legal medicinal and illicit misused drugs. Figure 1 illustrates the representative samples of Erimin-5 tablets with logo imprints. Through visual examination, 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. Such observation was served as a specific indication of Erimin-5 tablets, and it allowed the identification of the tablets. All 101 samples showed the same observation with the existence of such imprints on both sides of the 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 illicit Erimin-5 tablet.

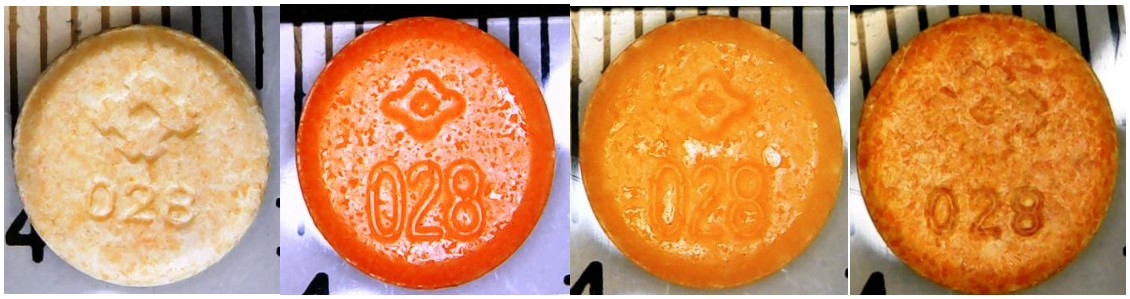

Figure 2: Variation in the logo on the illicit Erimin-5 tablets.

Additionally, as the samples tested in this study were seized samples, they could have also been exposed to certain degrees of environmental and physical insults. Atmospheric moisture level might cause rougher texture of tablets' surfaces. Some scratch marks were also evident on the surfaces of some tablets, probably made by physical insults due to storage and transport. Figure 3 illustrates Erimin-5 samples comparing the smooth and rougher textures. Figure 4 shows the presence of scratch marks on the surfaces of Erimin-5 tablets. However, the changes in texture and/or scratching did not interfere the recognition of these tablets as Erimin-5 tablets.

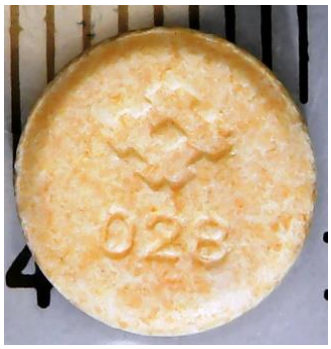

Figure 3: Changes on the texture surfaces of Erimin-5 due to environmental exposures.

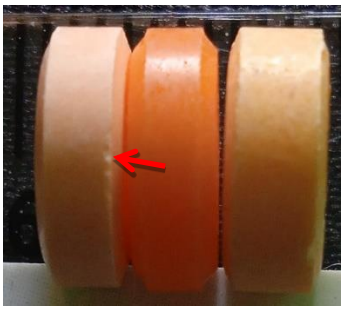

Figure 4: Presence of scratch marks on the Erimin-5 tablets.

Shape of the tablets

Tabletting machine is used to produce different types of tablets of varying shapes, size and with or without imprint. Besides the imprints, tablets' shape was decided by the tabletting machine. All the samples were found in round shape with flat face radius edge. The front, rear, and side views of Erimin-5 tablets are illustrated in Figure 5.

Figure 5: Front, rear, and side views of Erimin-5 tablets.

Colour of the tablets

Colour examination was carried out through direct and straightforward observation. In other word, no colour coding was given and documented for the samples. Through the observation, Erimin-5 tablets tested in this study were dominated by peach-like colour, as it was common colour encountered elsewhere in such illicit drug [1,12]. 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 [1,12]. Direct colour observation did not allow the determination of

dyes which can be performed through TLC in subsequently section. Colour of the tablets were very similar although slight variation had been shown. Such variations can only be objectively determined, and they could be due to the different production lines, or from the same production line but different batches. Limited information could be retrieved from the examination of the colour, except unique peach-like colour to be suggested as Erimin-5.

Dimension of the tablets

Upon compression of powdery composition into tablet form, the products would have uniform shape with specific imprint. Their dimensions were also very similar when they were produced using the same tabletting machine. With round-shaped appearance of illicit Erimin-5 tablets, the diameter and thickness of each tablet was measured using Vernier callipers. Figures 6 and 7 illustrate the distribution of tablets based on their respective diameters and thicknesses.

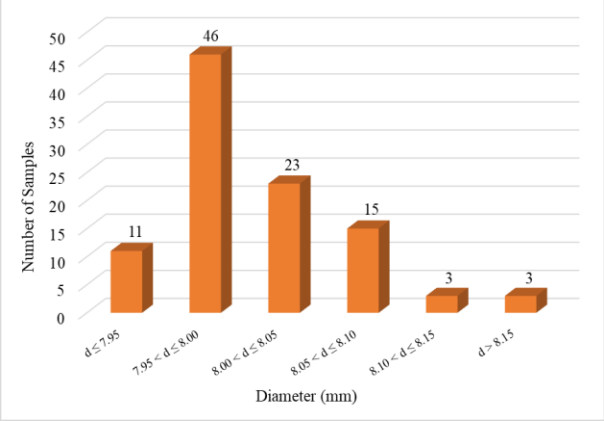

Figure 6: Diameter of illicit Erimin-5 tablets measured in this study.

In term of diameter and thickness, the measurements were determined as 8.01 ± 0.06 mm and 2.88 ± 0.12 mm, respectively. Majority of the tablets demonstrated diameters ranged between 7.95 and 8.00 mm, in which 45.5% of the samples fall into this group. The diameter variation among Erimin-5 tablets was relatively small with relative standard deviation of 0.72%. In other words, the diameter measurement was tightly clustered around the average value. On the other hand, the variation in term of the thickness was slightly greater, reporting a %RSD values of 4.3%. The thickest tablet of 3.18 mm was shown in S089, while S031 was the thinnest among the sample, varying up to 0.56 mm.

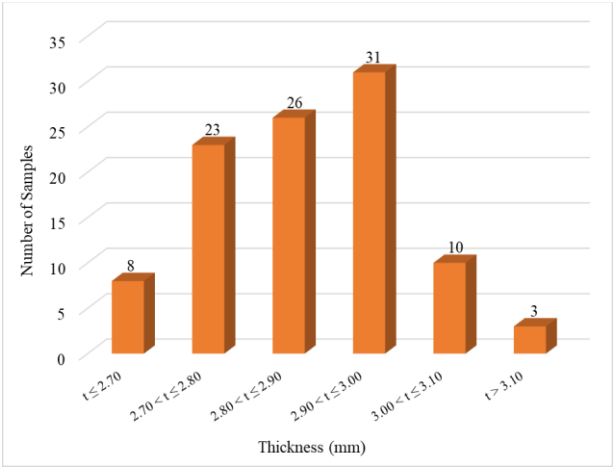

Figure 7: Thickness of illicit Erimin-5 tablets measured in this study.

Briefly, all the tablets appeared very similar to each other with slight variation. The slightly variation could be attributed to the different tabletting machine used of different illicit manufacturers. To investigate the association between the diameter and thickness of the Erimin-5 tablets, the Pearson Correlation statistical test was performed. A statistically significant linear relationship was demonstrated between the variables, namely the diameter and weight of the tablets demonstrated a (r=.214, p <0.001) where a significant level of 0.05 was considered. Based on the 101 observations, these variables are positively correlated but it was also noted with the relatively weak association. In other words, a tablet with greater diameter could have thicker characteristics in which the variables increase together.

Weight of the tablets

The seized tablets in this study were averagely weighed at 188.26 ± 6.53 mg, ranging from 174.5 mg to 207.7 mg. In general, the weight of a tablet is directly proportional to the size of the tablet. In other words, a tablet of greater size would be measured at a relatively heavier weight as compared to those smaller in size. Figure 8 demonstrates weights of illicit Erimin-5 tablets and the number of samples in each group. A %RSD of 3.47% was determined from the weight

measurement of tablet samples in this study. Sample S013 was measured the lightest at 174.6 mg, while a weight of 207.7 mg was measured in S092 as the heavier sample.

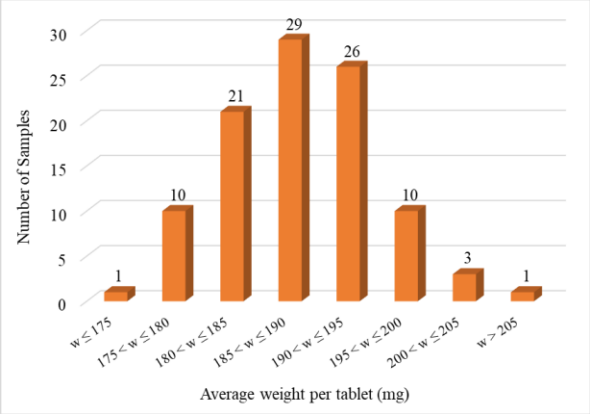

Figure 8: Weights of illicit Erimin-5 tablets measured in this study.

TLC analysis

TLC is a planar chromatography technique. It allowed qualitative, and semi-quantitative analysis in a fast and inexpensive way. Matrix components within a sample could interfere the TLC analysis. Therefore, the dye must be extracted from the illicit drug tablets to remove any sugars, fats, or other substances contained in its composition [16,17]. The extraction step prior to TLC analysis is therefore a crucial step to exclude all the possible interferences from final solutions before spotting on the TLC plate. It was reported that the existence of any sugar in the solution for TLC analysis could lead to blurring of the spots upon the chromatographic separation [15].

To extract the dye from tablets with specific formulation, a sample was suggested to be acidified followed by alkaline treatment [18]. Extraction procedure was initiated with acidification where this procedure enabled the dye to be taken up into the white knitting wool. Then, addition of alkaline ammonia solution removed the dye from the wool upon boiling [15,16]. Finally, the alkaline solution was warmed to further concentrate the content for the spotting of the dye samples onto the TLC plate. If the solution is very pale upon the sample treatment procedure, the final solution can be applied few times onto the same spot of the TLC plate to increase the concentration. TLC plate spotted with dye samples was placed in the TLC tank with solvent system for chromatographic separation. As the positive control to compare with the dye that could have added into the composition of the tablets, dye standard solution was also prepared using the same extraction procedure.

Selection of solvent system for TLC analysis of illicit Erimin-5 samples

Different solvent systems would carry different polarities depending on the composition of the solvent. In most instances, a solvent system involves a combination of different solvents, but some solvent systems contain only a single solvent. The specificity of the TLC could be highly influenced by the choice of solvent systems. With the aim to determine the best solvent system to separate the different dyes potentially found in the illicit Erimin-5 samples, four solvent systems were tested. TLC plates spotted with various dye standards upon sample extraction were also run with the four solvent systems. Note that these solvent systems were of different polarity, and they were commonly used in separating the different sources of dyes by the Department of Chemistry Malaysia. All the TLC runs included 11 dye standards obtained from the Department of Chemistry Malaysia added with two commercially available food colouring dyes, namely Egg Yellow and Apple Green. Table 1 demonstrates the calculated retention factors of each standard dye solution upon running using the different four solvent systems. It was noted that some dye standards had formed more than one spot on the TLC plate upon the analysis.

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

Standard	Isopropanol: ammonia (S.G. 0.880) (4: 1)	n-butanol: glacial acetic acid: water (10: 5: 6)	Acetone: butanone: ammonia (S.G. 0.880): water (60: 140: 1: 60)	Isopropanol: ammonia (S.G. 0.880): Water (7:2:1)
Tartrazine	0.81	0.84	1.00	0.62
Sunset Yellow	0.83	0.86	1.00	0.68
Egg Yellow	0.50	0.72	0.96	0.62
Fast Green	0.73 0.71	0.87	1.00	0.68 0.64
Green S	0.77 0.70 0.64	0.80	1.00	0.62
Brilliant Blue	0.76	0.84	1.00	0.68
Apple Green	0.73 0.71	0.86 0.84	1.00 0.99	0.68 0.62
Amaranth	0.71	0.90	1.00	0.62
Carmoisine	0.83	0.93	1.00	0.68
Erythrosine BS	(0.89)	(0.86)	(1.00)	(0.78)
Ponceau 4R	0.79	0.84	1.00	0.61
Rhodamine B	(0.86) (0.73) (0.64)	(0.90) (0.87)	(1.00) (0.87) (0.80)	(0.81) (0.78) (0.72)
Red 2G	0.84	0.86	1.00	0.70

Generally, the dye standards that produced the same colour appeared very similar physically. TLC has successfully separated various dye standards with characteristic R_f values. Some dyes fluoresced when they were visualised under UV light, and they were noted as ( ) in Table 1. 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 UV illumination with long wave. Rhodamine B showed very intense fluorescence behaviours upon illumination, as such behaviour can be easily distinguished this dye from most of the dye standards used in this study 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.

Since different dye standards had different degrees of interaction between dye component with the TLC plate and mobile phase, different patterns of separation were formed with different solvent systems. Literature suggested that isopropanol: ammonia (4:1 v/v) was a good solvent system to separate the different types of dyes, especially in the application and analysis of food dyes. This solvent system was reported in providing good separation with minimum tailing effect on the synthetic food colours [16]. It had mixed polarity characteristics, and certainly good in the separating the dyes. Majority of the dye standards were well-separated among each other, especially for those red-coloured dye standards. Each red-coloured dye had reported with different R_f values. However, it carried limited significance in separating the two yellowish dyes, namely the tartrazine and sunset yellow. Both these dyes located parallel to each other with very similar Rf values as 0.81 and 0.83, respectively. Good separation among the dye standards is crucial for more confident determination of dyes

contained in a tablet through sideway comparison. As the samples tested in this study were existed in peach-like colour, and these two dye components had been reported as the common dyes encountered in composition of Erimin-5 samples, the solvent system was found to have limited use for separation on the yellow-coloured dyes.

Another two solvent systems, namely the acetone: butanone: ammonia: water (60:140:1:60 v/v/v/v) and n-butanol: acetic acid: water (10:5:6 v/v/v) performed not as efficient as the other two solvent systems. The dye standards were not well separated but tend to wander further from the origin. The former solvent system had brought all the dye standard until reaching the end of the solvent front. Only Rhodamine B dye standard provided a R_f value lesser than 1.00 and discriminated it from the others. It could be due to the high polarity of the solvent system as it contained high proportion of water in the system. The latter solvent system could be less polar than the former system but remained too polar for the separation of target dye standard solutions in this study. It performed slightly better than the former solvent system, but nearly all the dye components appeared closely to the end of solvent front. Additionally, the R_f values reported for majority of the dye standards were too close to each other and hardly differentiated. This had limited its application especially when a coloured tablet was run by the TLC but cannot confidently determine the dye that contained in the sample. For example, if a red-coloured spot was appeared on the TLC plate upon development and reported with its specific value, ones might not be able to determine the unknown dye and potential lead to false result.

Based on the experimental results, the best separation of the various dyes was found when the standards on the TLC gel plates was run with isopropanol, ammonia, and water in a volume composition of a ratio 7:2:1. With this solvent system, all dye standards were well separated with determined Rf values, respectively. The issue encountered with solvent system consisting of isopropanol: ammonia (4:1 v/v) was solved by this solvent system. Both the dyes, namely the tartrazine and sunset yellow that hardly differentiated in the previous solvent system had been resolved. With this solvent system, these two dyes respectively reported Rf values of 0.62 and 0.68, slightly further away from each other. Note also that if the spots of the dye standards appeared very closely to each other, the accurate interpretation of the dye that contained in the real samples could be difficult, and potentially lead to wrongful determination. Based on the analytical comparison, the solvent system of isopropanol: ammonia: water (7:2:1 v/v/v) was then selected and used in the TLC analysis of illicit Erimin-5 samples in this study.

TLC analysis of illicit Erimin-5 samples

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. Upon development, their colour spot, evidence of fluorescence and R_f values were determined. The experimental outcome was also directly compared to those formed through the separation of dye standards. Figure 9 demonstrates the TLC output of illicit Erimin-5 samples in comparison to the dye standards.

This study examined 101 peach-like coloured samples. Figure 10 summarises the percentages of different combination of dyes found in the Erimin-5 samples tested in this study. 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%.

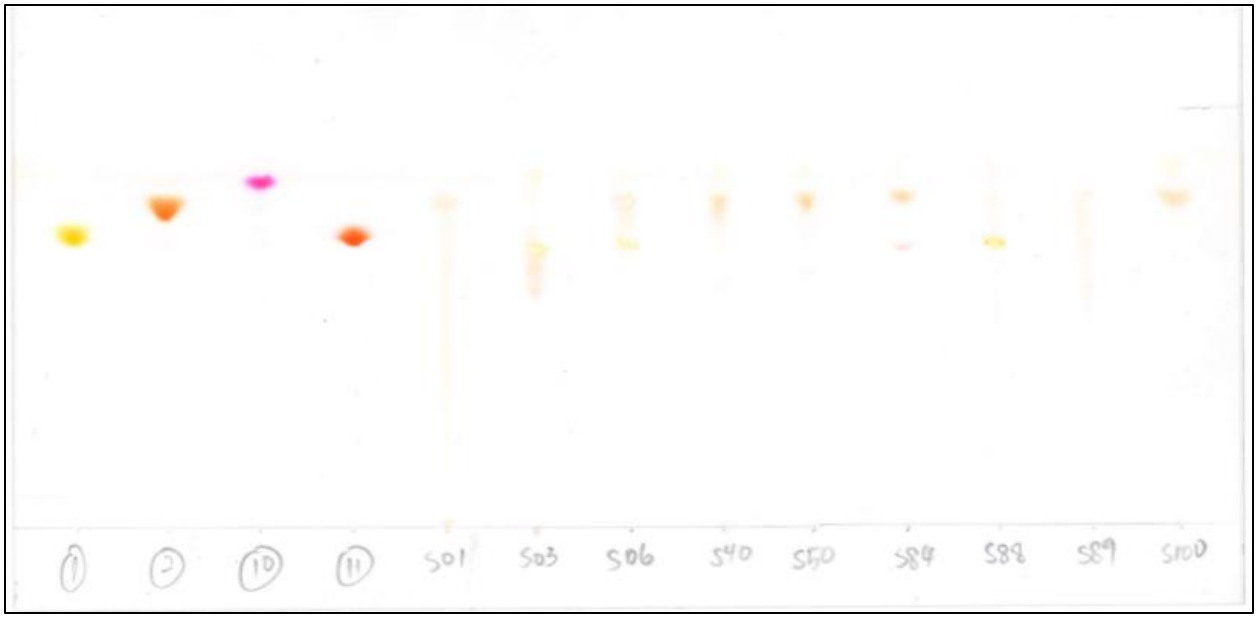

Figure 9: TLC output of illicit Erimin-5 samples in comparison to the dye standards using solvent system consisting of isopropanol: ammonia: water (7:2:1 v/v/v).

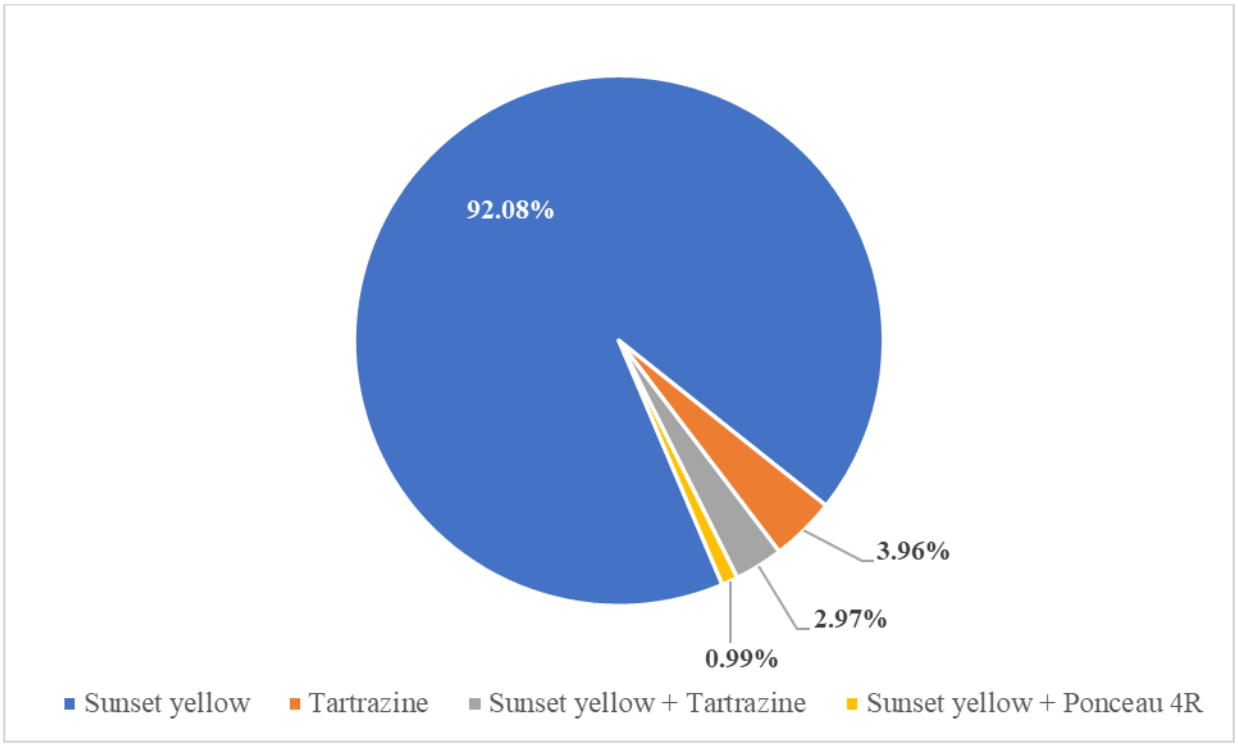

Figure 10: Percentages of dye composition in 101 Erimin-5 samples.

In these samples, only one type of dye was detected in contrary to another four samples where more than one spots indicating the presence of dyes were noticed upon the TLC analysis. Different combinations of colour dyes were detected to give their peach-like colour in their physical appearance. Three samples showed the presence of forementioned dyes in their composition where two spots were evident on the TLC plate. Additionally, one sample showed

unique dye combination where it had reported with the presence of ponceau 4R upon TLC development, as indicated by sample S84.

No fluorescence behaviour was noticed in all the samples tested under the illumination of UV light, suggesting that the dye added in the composition of Erimin-5 tablets did not fluoresce. According to a previous study [1], sunset yellow was the dye frequently encountered in Erimin-5 samples, reporting with 66.04% from a total of 53 samples tested in the study. In this study, a greater percentage of single sunset yellow dye was reported, which had supported the common of this dye to be added into Erimin-5 samples. In the previous, 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 this dye combination might no longer utilised in the Erimin-5 tablets, at least among the 101 samples tested in this study.

General Discussion

Having a unique tablet shape, colour along with the logo imprint on the surfaces of the tablets allowed the recognisability of Erimin-5. Physical examination had suggested certain degree of variations among the seized samples, but their discrimination among the samples was found very limited. In view of this, the chemical characterisation would contribute to greater opportunities in differentiating among the Erimin-5 drug tablets in accordance with their batches or manufacturers, or at least for sample-to-sample comparisons. Subsequent chemical analyses of these tablets were then performed and evaluated.

TLC enabled the determination of different dyes in a simple and quick way. To represent the identity of drug, both medicinal and illicit drugs, dye is often added for recognition purpose. All the Erimin-5 tablets encountered in this study appeared as peach-like colour through physical examination, but the separation of dyes through TLC analysis demonstrated the different dyes or combination of dyes in their composition. Utilising the solvent system consisting of a mixture of isopropanol: ammonia: water (7:2:1 v/v/v) as a mobile phase, three different combinations of dyes were found, and majority of the samples were added with sunset yellow dye. Besides, the sample extraction procedure implemented in this study also showed effective sample treatment where good separation was evident in the TLC output with minimum smearing and blurring of the spots.

TLC analysis had successfully differentiated the 101 Erimin-5 samples in this study into four groups based on their dye composition. This indicated that the dyes added during the manufacturing process were differed, probably due to the different manufacturers or production from different clandestine laboratories. The information was beneficial during the drug profiling to discriminate the seized samples, and aid in tracking the source of these illicit drugs based on their analytical profiles. Additionally, the detection of dyes in the composition of the illicit drugs could also provide the information on whether the dye added is of restricted form because certain dye might cause risk in inducing allergic reactions in some users. Such information could aid in the diagnosis of illicit drug related cases where allergic reaction was encountered.

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. Measurement of the diameter, thickness and weights of these tablets had also allowed sample-to-sample comparison. 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, allowing them discrimination from the majority groups. With a great amount of illicit 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.

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 illicit drugs, forensic profiling through physical characterisation and chemical analyses of drug substance is a strategy for intelligence purposes. This study was successfully characterised the selected Erimin-5 case samples over a period of 18 months. The comparative study could also suggested the possible linkages among the samples. The output of this study could contribute to the body of knowledge, especially in drug related investigation for tracking the source and trafficking routes of illicit drugs.

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