Forensic Drug Profiling of Erimin-5 Using TLC and GC-MS

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ABSTRACT: Sixty-four groups of Erimin-5 tablets from 23 sources were profiled based on their dye and active ingredients. Dye of the tablets was extracted using 5% acetic acid and subjected to TLC separation using isopropanol/ammonia (4:1) as the solvent system while the active ingredients were analysed using GCMS and diluents were analysed using FTIR. All tablets were of peach-like or green in colour. The dye components were identified as tartrazine, sunset yellow, erythrosine, ponceau 4R and brilliant blue. The active ingredients were identified as nimetazepam and diazepam with glucose, sorbitol, mannitol and lactose as diluents. The combination of dye information and chemical contents allowed a quick classification of these 23 sources into six different profiles. This drug profiling method can provide useful information for narcotic enforcement and intelligence purposes. Forensic drug laboratories receiving tablet-form Erimin-5 should perform forensic profiling a profile database of the drug.

Keywords: Erimin-5, nimetazepam, dye, active ingredients, forensic chemistry

Introduction

Erimin-5 a brand name of nimetazepam initially used for the treatment of short-term severe insomnia in patients. Due to worldwide abuse, nimetazepam has been listed as a controlled substance in many countries [1]. In Malaysia, both nimetazepam and flunitrazepam, the two benzodiazepines are listed as control substances under the Dangerous Drugs Act 1952. While the latter is seldom encountered, the former tablets, either in the form of Erimin-5 or its counterfeits, were frequently submitted to the forensic laboratory for analysis [2].

Erimin-5 pills were frequently reported in illicit markets in Malaysia in the mid 1980s [3]. Its wide availability, relatively low price on the local black markets and its long activity has made it one of the most widely abused sedatives today. Heroin addicts use it as a substitute and it is also increasingly used as sedative by methamphetamine abusers after binging [4]. Illicit manufacturing and/or illegal smuggling of Erimin-5 is alarming. An estimated RM9.7 million worth of Erimin-5 pills were seized by the Royal Malaysia Police [5]. During the first eight months of 2012 alone, Malaysia police have seized a total of 4,115, 694 pills worth an estimated RM80 million [5].

Drug trafficker distributed the pills through their illicit marketing network. Therefore, profiling of these tablets can provide useful information [6] to help establish links among seizures [7] or to source of origins. Dye used in the tablets could provide useful information for forensic comparison between pills or between pills and dyes found in clandestine laboratories [8] or to a particular syndicate operation while active ingredients of the tablets help establish the chemical contents and legal status of a seizure. Therefore, this study aims to determine the dye components and active ingredients of Erimin-5 tablets obtained from 23 sources using routine Thin Layer Chromatography (TLC), GCMS (Gas Chromatography Mass Spectroscopy) and FTIR (Fourier Transform Infrared) analyses. Establishing a profile of these tablets may facilitate the law enforcement operations.

Materials and methods

All chemicals used were of HPLC grade. Acetic acid (5%), ammonia solution (3N) and iso-proponal:ammnia (4:1) were freshly prepared. The 64 groups of Erimin-5 tablets were obtained from seized samples from 2011-2012. Food colour standards were obtained from The Department of Chemistry, Malaysia.

Dye extraction and identification

Erimin-5 pills tablets were powdered using the mortar and pestle, acidified with 5% acetic acid in a beaker. Two knotted pieces of wool were submerged in the mixture which was warmed on a boiling water bath until the dye was transferred to the wool. The wool was then removed and washed thoroughly with water to remove other extraneous materials. The dye on the wool was then re-extracted by warming the wool with 5 mL of acetone and 3N ammonia mixture (1:1) on a water bath for approximately 5 min. The wool was then discarded and the dye extract was

allowed to evaporate until dry before being reconstituted in methanol. Dye standards were dissolved in methanol prior to TLC.

TLC separation was performed on 200 mm x 200 mm plates coated with 0.10 mm layer of silica gel. Dye extracts and standards were spotted on the plate using capillary tubes and developed in freshly prepared solvent system of isopropanol/ammonia (4:1) until the solvent front approached about 1cm away from the top of the TLC plate. The plate was marked and left to dry. Rf value of each band was then calculated.

Active ingredient and diluents identification

Erimin-5 tablets from each source were pounded with a mortar and pestle into fine powder. It is subsequently suspended into methanol and mixed well. The mixture was filtered and the filtrate was analysed using GCMS (Agilent GC-MS 6890N) equipped with an Agilent HP-5 capillary column (30 m length, 250 µm i.d., 0.25 µm film thickness) with a flow rate of 1.2 mL/min with helium as the carrier gas. The temperature of the injector was set at 260ºC. The initial oven temperature was set at 250ºC for one minute and then raised to 300ºC at a rate of 5ºC/min and held for two minutes.

For infrared analysis, the fine powder samples were analysed. Infrared spectra were obtained with a Nicolet Magna-IR Spectrometer 550. The resolution was set at 4.000 cm^{-1}, with 32 scans between 400 cm^{-1} and 4000 cm^{-1}.

Results and discussion

TLC Analysis

All 64 groups of Erimin 5 tablets were visually examined and grouped based on their colour. 53 groups were of peach-like colour and 11 groups were green. Tartrazine, Sunset Yellow, Erythrosine, Ponceau 4R and Red 2G were the standard dyes analysed together with the dye extract from peach coloured tablets were. Standard dyes used for comparing the dye extracts from green coloured tablets were Fast Green, Brilliant Blue, Green S, and Tartrazine.

Among the 53 samples of peach-like coloured samples, 18 contained a combination of three dyes, namely Erythrosine, Tartrazine and Ponceau 4R to produce the hue when the resulting bands were compared to the retention factor (R_f) of dye standards. The other 35 samples produced only one band suggesting the colour from the tablets comes from Sunset yellow. For the 11 green samples, two bands were observed which corresponded to those observed in the standard dyes of Brilliant Blue and Tartrazine suggesting that the colours from all of these tablets come from mixture of the two dyes.

Examinations on the dye composition of the 64 sample-groups from 23 sources showed that all samples from common sources had the same combination of dyes. This indicates that the respective tablets could be from the same origin hence can provide useful information to link the drug syndicates and delineate a drug syndicate's illicit market coverage.

The TLC analysis was shown to be rapid and cheap and enabled simultaneous assay of several dozen of tablets with satisfactory results [9]. The wool extracting technique was effective in extracting colouring substances from the tablets consisting of various substances.

GC-MS Analysis

The GC parameter employed gave the peak of diazepam at 5.546 min (Figure 1) and nimetazepam at 6.017 min (Figure 2), with glucose at 2.455 min and mannitol/sorbitol eluted at 2.714 min. About 82.8% (53/64) sample groups contained nimetazepam as the active ingredient. The remaining 17.2% (11/64) groups contained diazepam as the active ingredient.

Note that commercial Erimin-5 tablets should contain nimetazepam as the active ingredient. From GC-MS analysis, it was observed that the fake "Erimin-5" tablets from 11 samples of three different sources contained only diazepam as the active ingredient. This information is important to law enforcement personnel because only nimetazepam and flunitrazepam are the two benzodiazepines listed under the Dangerous Drug Act 1952.

Mannitol and sorbitol are isomers and the only difference is the hydroxyl group on carbon 2. So, this difference could not be detected using the GCMS without using derivation agent, so it yields the similar peaks for both compounds.

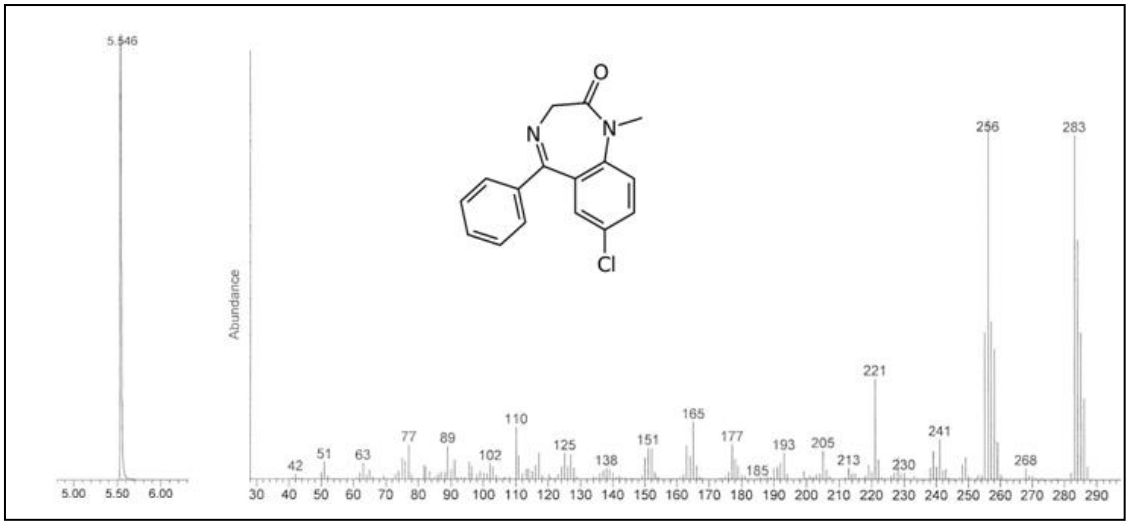

Figure 1: Gas chromatogram showing diazepam standard eluted at 5.546 min (left) and its mass spectrum (right)

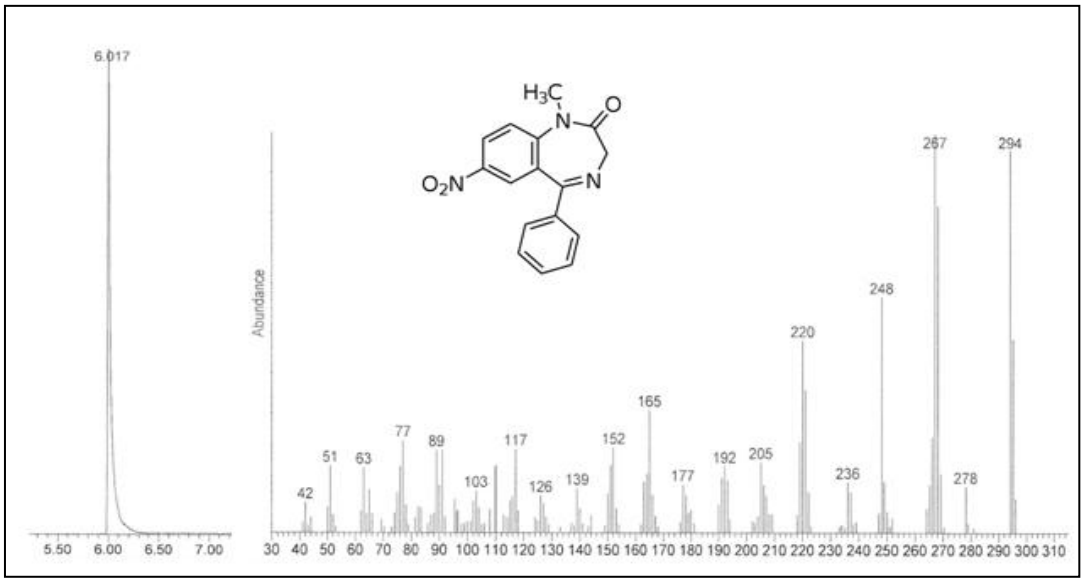

Figure 2: Gas chromatogram showing nimetazepam standard eluted at 6.017 min (left) and its mass spectrum (right)

FTIR Analysis

The FTIR analysis concluded three types of diluents in the samples that were analysed. FTIR technique has the capability to differentiate between the isomers, mannitol and sorbitol. Lactose, mannitol and glucose are the diluents identified in the samples through this technique. About 45.3% (29/64) samples contained lactose as the diluent. Seven (11.0%) samples contained glucose as the diluent and approximately 43.7% (28/64) samples contained mannitol as the diluent.

Erimin-5 profiling

Further profiling strategy were taken by combining the information of TLC, GC-MS and FTIR analyses as shown in Figure 3. It shows that the results from the three techniques enable the 64 samples for further classification into six profiles/clusters.

Tablets from six sources contain the active ingredient nimetazepam and lactose as diluent with a combination of Erythrosine, Tartrazine and Ponceau 4R to provide the hue. Tablets from nine sources contained nimetazepam, lactose and Sunset Yellow as the dye. Nimetazepam and mannitol as ingredients with Sunset Yellow as the colouring agent could be found from two sources. Only one source has sample

which contained diazepam and mannitol as ingredient and Sunset Yellow as the dye.

Tablets from three sources contained nimetazepam and mannitol with a combination of Tartrazine and Brilliant Blue to give the green colour Erimin 5. Tablets from two sources contained diazepam as active ingredient, glucose as diluent and mixture of Tartrazine and Brilliant Blue as the colouring agent.

Obviously, this simple profiling strategy could aid law enforcement operations who seized samples at different locations to link the source to its syndicate operation or source of illegal productions. Further profiling could also be made if more physical or chemical information could be generated.

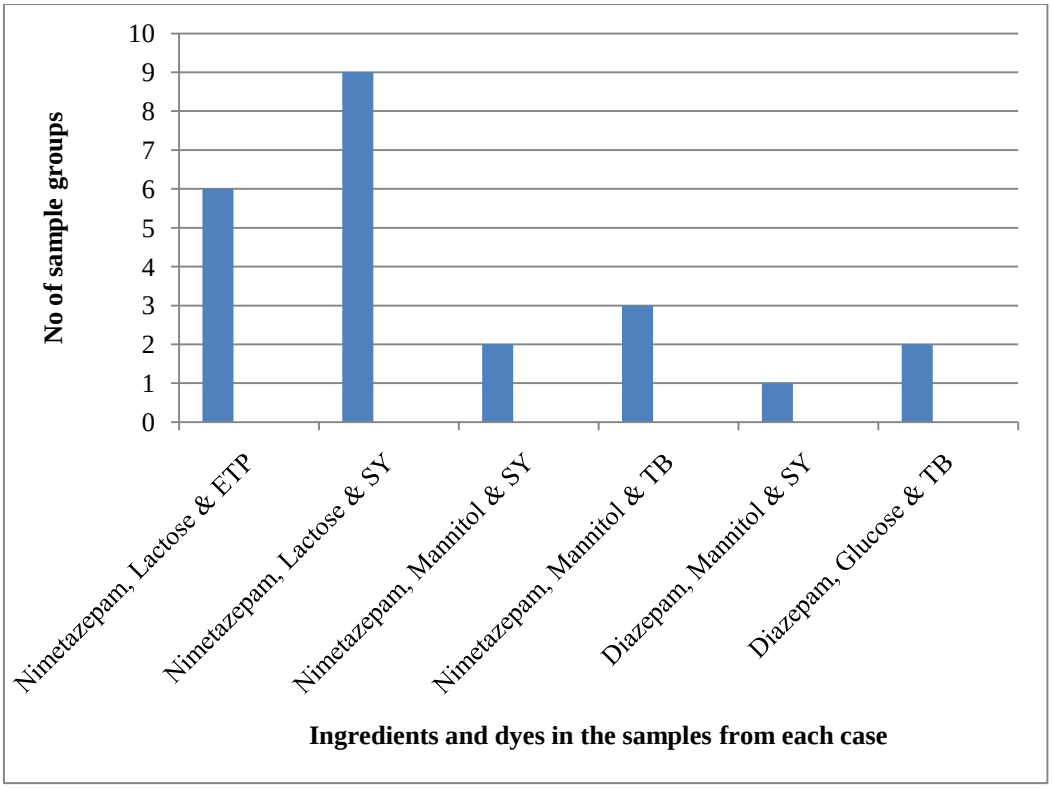

Figure 3: Bar chart shows the distribution of active ingredient, diluent and dyes according to number of sample groups (ETP = erythrosine, tartrazine and ponceau 4R, SY = sunset yellow, and TB = tartrazine and brilliant blue)

Conclusion

A framework of the Erimin-5 tablets profiling based on dye identification by TLC with active ingredient identification using GCMS and diluents identification using FTIR was developed. All the three analyses gave useful results, which were then used to compare the tablets from different sources. This rapid and cost effective drug profiling method can be easily adapted for forensic narcotic laboratories that normally receive Erimin-5 in tablet forms and could be used as routine laboratory procedure to build a drug profile database for investigative and intelligence purposes.

Acknowledgement

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