

**PROFILING OF ILLICIT ERIMIN 5 TABLET
SEIZED IN MALAYSIA**

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**A RESEARCH PROJECT REPORT SUBMITTED TO THE
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ABSTRACT

Profiling study of forty - six illicit Erimin 5 tablet samples were carried out according to their physicals and chemical characteristics. Out of 46 illicit Erimin tablet samples, 45 tablet samples were peach-orange like in colour and only one tablet sample was green coloured tablet. For physical characteristic studies, logo and color were observed whereas, others characteristics such average weight of tablet, thickness and diameter of the illicit Erimin 5 tablet samples were measured and photograph was taken. The distribution profile of average weight of tablet, diameter and thickness of the tablet samples were developed. For chemical characteristics, these illicit Erimin 5 tablet samples were characterized according to their active ingredients, adulterants, diluents and dyes. Active ingredients and adulterants were analyzed by GCMS and diluents were used ATR-FTIR. Dyes of tablets were determined by TLC using two different systems. Nimetazepam content (percentage) and the weight of nimetazepam per tablet were determined using HPLC and their distribution profiles were developed. Nimetazepam, Diazepam and Nitrazepam together with caffeine (adulterant) were detected as active ingredient with Lactose, Mannitol and Calcium phosphate as diluent. Sunset Yellow alone / combination with others dyes namely Ponceau 4R and/or Tartrazine were found in peach-orange like tablet samples and combination of Brilliant blue and Tartrazine were detected in green coloured tablet sample.

ABSTRAK

Kajian Pemprofilan ke atas empat puluh enam sampel tablet Erimin 5 telah dijalankan berdasarkan ciri - ciri fizikal dan kimia masing - masing. Dari 46 sampel tablet Erimin 5, sebanyak 45 sampel tablet adalah berwarna peach-oren dan hanya satu sampel tablet berwarna hijau. Untuk kajian ciri-ciri fizikal, logo dan warna diperhatikan manakala, ciri – ciri fizikal lain seperti purata berat tablet, diameter dan ketebalan sampel tablet Erimin 5 telah diukur dan gambar setiap sampel tablet telah diambil. Profil corak taburan bagi purata berat tablet, diameter dan ketebalan sampel tablet telah dibangunkan. Untuk ciri-ciri kimia, sampel - sampel tablet Erimin 5 telah dikategorikan mengikut bahan aktif, bahan pengubahsuai, bahan pencampur (diluent) dan bahan pewarna. Bahan aktif dan bahan pengubahsuai telah dianalisis dengan menggunakan GCMS dan ATR-FTIR telah digunakan untuk menganalisis bahan pencampur (diluent). Pewarna tablet telah ditentukan oleh TLC dengan menggunakan dua sistem yang berbeza. Kandungan Nimetazepam (peratusan) dan berat nimetazepam per tablet ditentukan dengan menggunakan HPLC dan profil corak taburannya telah dibangunkan. Bahan aktif seperti Nimetazepam, Diazepam dan Nitrazepam dengan caffein (bahan pengubahsuai) telah dikesan bersama – sama dengan Laktosa, Mannitol dan Kalsium fosfat sebagai bahan pencampur (diluent). Sunset Yellow sahaja / gabungan dengan pewarna lain seperti Ponceau 4R dan / atau Tartrazin dijumpai dalam sampel tablet berwarna peach-oren dan kombinasi Brilliant Blue dan tartrazin dikesan dalam sampel tablet berwarna hijau.

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TABLE OF CONTENTS

Abstract.....	i
Abstrak.....	ii
Acknowledgement.....	iii
Table of content.....	iv
List of Figures.....	ix
List of Graphs.....	xii
List of Tables.....	xiii
List of Abbreviations.....	xiv

CHAPTER ONE: INTRODUCTION

1.1	Patterns and emerging trends of the illicit Erimin 5 tablets.....	1
1.1.1	Patterns and trends of the illicit Erimin 5 tablets seizures in Malaysia compared to others drugs.....	2
1.1.2	Patterns and trends of the illicit Erimin 5 tablets retail prices in Malaysia compared others drugs.....	4
1.2	Erimin 5 tablets	
1.2.1	What is Erimin 5?.....	5
1.2.2	Illicit Erimin 5 tablets.....	8
1.3	Active ingredients in illicit Erimin 5 tablets	
1.3.1	Physical and chemical Properties of Common Active ingredients.....	10
1.3.2	Pharmacological effect of active ingredients.....	10
1.4	Others excipients in illicit Erimin 5 tablets.....	14
1.4.1	Adulterants.....	16
1.4.2	Diluents.....	17
1.4.3	Colourants/Colouring matter.....	19

1.5	Legislation Status	
1.5.1	Legal status in Malaysia.....	20
1.5.2	Legal status in others countries.....	20
1.6	Profiling.....	21
1.7	Significance of the study.....	23
1.8	Objective of the study.....	24

CHAPTER TWO: LITERATURE REVIEW

2.1	Introduction.....	25
2.2	Profiling and sample comparison by Physical characteristic.....	26
2.3	Profiling and sample comparison by Chemical characteristics	
2.3.1	Determination of active ingredient and adulterants.....	27
2.3.2	Determination of major diluents.....	28
2.3.3	Determination of dyes.....	30
2.3.4	Profiling and sample comparison.....	31

CHAPTER THREE: METHODOLOGY

3.1	Sample Collection.....	32
3.2	Materials	
3.2.1	Reagents and Chemicals.....	32
3.2.2	Standards.....	33
3.2.3	Apparatus.....	33
3.3	Instrumentation	
3.3.1	Gas Chromatography Mass Spectrometer (GCMS).....	36
3.3.2	High Performance Liquid Chromatography (HPLC).....	37
3.3.3	Fourier Transform Infra Red (FTIR).....	38

3.3.4	Scanning Electron Microscope – Energy Dispersive Detector (SEM-EDX).....	38
3.4	Procedure	
3.4.1	Physical characteristics.....	39
3.4.2	Chemical Characteristics.....	40
3.4.3	Identification and Confirmation of Active Ingredient and other Adulterant	
3.4.3.1	Sample Preparation.....	40
3.4.3.2	Calibration of the instrument.....	41
3.4.3.3	Interpretation of the mass spectrum.....	41
3.4.4	Quantification of Nimetazepam by High Performance Liquid Chromatography (HPLC)	
3.4.4.1	Sample Preparation.....	42
3.4.4.2	Calibration standard.....	43
3.4.4.3	Calibration by external Method.....	43
3.4.4.4	Sample Injection.....	43
3.4.4.5	Calculation.....	44
3.4.4.6	Quality Control.....	44
3.4.5	Identification and Confirmation of major Diluent by Fourier Transform Infra Red (FTIR)	
3.4.5.1	Sample Preparation.....	45
3.4.5.2	Quality control of the result.....	45
3.4.5.3	Interpretation of Spectrum.....	45
3.4.5.4	Additional analysis	
3.4.5.4.1	Sample Preparation.....	46
3.4.5.4.2	Interpretation.....	46

3.4.6	Identification and Confirmation of Dyes	
3.4.6.1	Sample Preparation (dye extraction).....	47
3.4.6.2	Preparation of Developing Solvents System of TLC.....	48
3.4.6.3	TLC analysis.....	49
3.4.6.4	Interpretation of the TLC chromatogram.....	50

CHAPTER FOUR: RESULTS AND DISCUSSION

4.1	Introduction.....	51
4.2	Physical characteristics	
4.2.1	Colour.....	60
4.2.2	Logo.....	60
4.2.3	Average Weight per tablet.....	61
4.2.4	Diameter.....	62
4.2.4	Thickness.....	63
4.3	Chemical characteristic	
4.3.1	Identification and Confirmation of Active Ingredient and others Adulterant.....	64
4.3.2	Quantification of Nimetazepam (active ingredient).....	76
4.3.3	Identification and Confirmation of major Diluent.....	82
4.3.4	Identification and Confirmation of Dyes.....	91
4.4	Characterization of illicit Erimin 5 tablets samples according to their chemical characteristics.....	98
4.5	Application of the profiling of Erimin 5 tablets to the drug intelligence purpose.....	101

CHAPTER FIVE: CONCLUSION

Conclusion.....	102
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REFERENCES.....	104
------------------------	------------

APPENDICES

Appendix 1.....	A-1
-----------------	-----

Appendix 2.....	A-11
-----------------	------

Appendix 3.....	A-13
-----------------	------

Appendix 4.....	A-15
-----------------	------

LIST OF FIGURES

Figure 1.1: Erimin 5 tablets seizures worth RM 20.13 millions.....	1
Figure 1.2 (a): Front and back views of original/commercial Erimin 5 foils.....	6
Figure 1.2(b): Zoom photo of front and back view of original/commercial Erimin 5 foil.....	6
Figure 1.3: Front, back and rear views of original/commercial Erimin 5 tablet.....	6
Figure 1.4: Front and back views of illicit Erimin 5 foils (YK Chong et al., 2004).....	9
Figure 1.5: Example of illicit Erimin 5 tablets (YK Chong et al., 2004).....	9
Figure 1.6: Chemical structures of Nimetazepam, Nitrazepam and Diazepam.....	11
Figure 1.7: Chemical structures of some diluents.....	18
Figure 3.1: Examples of apparatus used in this study.....	35
Figure 3.2: GCMS Agilent.....	36
Figure 3.3: Shimadzu HPLC with PDA detector.....	37
Figure 3.4: HazMatID portable FTIR spectrometer (ATR).....	38
Figure 4.1: Example of the slight variation of logo imprint in illicit Erimin 5 tablet samples from the left : tablet ID 003, 009, 026 and 007.....	60
Figure 4.2: TIC Chromatogram of mixture reference standard of menthol, caffeine, Diazepam, Ninetazepam and Nitrazepam.....	66
Figure 4.3: Mass spectrum of the interested compounds which are (a)Menthol (b) caffeine (c)diazepam (d) Nimetazepam and (e) Nitrazepam.....	67-68

Figure 4.4: TIC GCMS chromatogram profile of group A.....	71
Figure 4.5 (a): TIC GCMS chromatogram profile of group B.....	71
Figure 4.5 (b): TIC GCMS chromatogram profile of group C.....	72
Figure 4.6 (a): Mass spectrum of Unknown peak in TIC chromatogram profile of group B.....	72
Figure 4.6 (a): Mass spectrum of Hexadecanoic acid peak in TIC chromatogram profile of group B	73
Figure 4.7: GCMS chromatogram Profile of of group D.....	73
Figure 4.8: Profile of GCMS TIC chromatogram occurred in sample seized in 2005...	75
Figure 4.9: Calibration curve of Nimetazepam.....	77
Figure 4.10 : HPLC chromatogram of Nimetazepam Reference Standard eluted at 7.723 min.(left) and Nimetazepam in sample (ID 005) eluted at 7.765 min. (right)....	78
Figure 4.11: UV spectrum of Nimetazepam obtained in standard (left) and sample (right) at retention time mention in figure 4.10.....	78
Figure 4.12: IR spectrum of sample (ID 001) which lactose as major diluent (above) and Lactose Reference Standard (below).....	85
Figure 4.13: IR spectrum of sample (ID 002) which mannitol as major diluent (above) and Mannitol Reference Standard (below).....	85
Figure 4.14: IR spectrums of four samples which show a mixture profile of major diluent and others excipients.....	86
Figure 4.15: SEM-EDX results of samples (a) ID:025 (b) ID:026 (c) ID:036 and (d) ID:037.....	87

Figure 4.16: IR spectrum of sample ID:025 (above) and Calcium Phosphate Dibasic Dihydrate Reference Standard (below).....	88
Figure 4.17: IR spectrum of Calcium Phosphate Tribasic obtained from library.....	88
Figure 4.18 (a): Example of TLC results using solvent system A which several combination of dyes were detected. Rf values for Reference Std : Sunset Yellow (Rf 0.46), Tartrazine (Rf 0.31), Ponceau 4R (Rf 0.16) and Brilliant Blue (Rf 0.44).....	94
Figure 4.18 (b): Example of TLC results using solvent System B which several combination of dyes were detected. Rf values for Reference Std: Sunset Yellow (Rf 0.80), Tartrazine (Rf 0.67), Ponceau 4R (Rf 0.60) and Brilliant Blue (Rf 0.78).....	94
Figure 4.19 (a): Example of TLC results using solvent system A which only Sunset Yellow (Rf 0.46) was detected.....	95
Figure 4.19 (b): Example of TLC results using solvent System B which only Sunset Yellow (Rf 0.78) was detected.....	95
Figure 4.20: Characterization of illicit Erimin 5 tablets samples according to their chemical characteristics.....	99

LIST OF GRAPHS

Graph 4.1: Distribution profile of average Weight per tablet in 46 illicit Erimin 5 samples.....	61
Graph 4.2: Distribution profile of tablet diameter (mm) in 46 illicit Erimin 5 tablet samples.....	62
Graph 4.3: Distribution profile of tablet thickness (mm) in 46 illicit Erimin 5 tablet samples.....	63
Graph 4.4: The characterized of illicit Erimin 5 samples according to their chromatogram's profile and active ingredient.....	70
Graph 4.5: Tabulation of the percentage of Nimetazepam (as active ingredient) in the 42 illicit Erimin 5 tablet samples.....	80
Graph 4.6: The distribution profile of weight of Nimetazepam per tablet in 42 illicit Erimin 5 tablet samples.....	81
Graph 4.7: Type of Dyes use as colouring agents in 46 illicit Erimin 5 tablet samples.....	93

LIST OF TABLES

Table 1.1: Seizures of selected drugs in Malaysia, 2006 – 2010.....	3
Table 1.2: Retail prices of selected drugs in Malaysia (USD), 2007, 2009 and 2010.....	4
Table 1.3: Description of genuine/commercial Erimin 5 tablet.....	7
Table 1.4: Physical and chemical Properties of Common Active ingredient found in illicit Erimin 5 tablets.....	12
Table 1.5: Pharmacological effect and the used of three commonly active ingredient found in illicit Erimin 5 tablets.....	13
Table 1.6: Common excipients used in tablets formulation.....	15
Table 1.7: Type of diluents and their examples.....	17
Table 1.8: Examples of food colorings/dyes.....	19
Table 3.1: The principal peaks of some common active ingredient and adulterants.....	42
Table 4.1: Photo and Physical characteristics of 46 illicit Erimin 5 tablet samples....	52-59
Table 4.2: Retention time (RT) for each compound in the mixture of reference standard.....	66
Table 4.3: The percentage of illicit Erimin 5 samples according to their chromatogram’s profile and active ingredient categorized.....	70
Table 4.4: Comparison of IR results of sample with literature and Reference Standard of calcium phosphates.....	89
Table 4.5: Characterization illicit Erimin 5 tablet samples according to their chemical characteristics.....	100

LIST OF ABBREVIATIONS

AR – Analytical Grade

ATR – Attenuated Total Reflectance

ATS - Amphetamine Type Stimulant

°C – Degree Celcius

CI – chemical ionization

CZE-DAD - Capillary Zone Electrophoresis equipped with Diode Array Detector

cm - centimetre

EI – Electron Impact /Electron Ionization

etc. – et cetera

e.g – example

ev - electrovolt

FT – Fourier Transform

FTIR - Fourier Transform Infra Red

GC – Gas Chromatography

GCFID – Gas Chromatography Flame Ionization Detector

GC-IR.- Gas Chromatography Infra Red

GCMS - Gas Chromatography Mass Spectrometry/Spetcrometer

HPLC - High Performance Liquid Chromatography

HPTLC – High Performance Thin Layer chromatography

ID - Identification

id – internal diametre

IR – Infra Red

KBr – Potassium Bromide/ Kalium Bromide

kg - Kilogram

KeV – Kiloelectronvolt

LCMSMS – Liquid chromatography Tandem Mass spectrometry

LSD - Lysergic acid diethylamide

MDMA - 3,4-Methylenedioxymethamphetamine

m - metre

mg – milligram

min – minute

mL – mililitre

mm – milimetre

MS – Mass Spectrometer/spectrometry

N - normal

nm - nanometre

NIR - Near Infrared

NPD- Nitrogen Phosphorus Detector

ODS – Octadecylsilica

pA – pico ampere

R_f - Retardation factor

RT – retention time

SEM-EDX - Scanning Electron Microscope with Energy Dispersive X-Ray

SIM- Selected ion Monitoring

TIC – Total Ion Count/Total Ion Current

TLC – Thin Layer chromatography

UNODC - United Nations Office on Drugs and Crime

UV/PDA – Ultra Violet/Photo Diode Array

UV – Ultra violet

µm - micrometre

CHAPTER 1

INTRODUCTION

1.1 Patterns and emerging trends of the illicit Erimin 5 tablets

Pattern of drug abuse around the world are constantly changing. They vary considerably on geographical basis, from country to country and from region to region in the same country (Simon Wills, 2005). Recently, it can be seen that the trend of drugs consumption in Malaysia is changing these days with the demand for Erimin 5 tablets overtaking that of syabu (methamphetamine) among drug users (*The Malay Mail*, 02 August, 2012). The demand and seizures of the Erimin 5 tablets (psycotropic pills) have been increasing over the past few year .

The same scenario of the growing presence of Nimetazepam (Erimin 5) also could be seen on the others East and South-East Asian countries where, a rise in the abuse of nimetazepam (Erimin 5 tablet) was also reported in Brunei Darussalam, Hong Kong, China, Indonesia, and Thailand. Besides that, large seizures of this substance also have been made in Indonesia (*International Narcotics Control Board Report 2010*). Figure 1.1 shows an example of illicit Erimin 5 tablets seizures.



Source: BORNEOPOST
Online, 29 October, 2011,

Figure 1.1: Erimin 5 tablets seizures worth RM 20.13 millions.

1.1.1 Patterns and trends of the illicit Erimin 5 tablets seizures in Malaysia compared to others drugs

According to Patterns and Trends of Amphetamine-Type Stimulants and Other drugs: Asia and Pacific Report 2011, illicit Erimin 5 seizures in Malaysia has been increased year by year starting from 2006 to 2009 as shown in table 1.1. However, seizures of nimetazepam (Erimin 5) had declined about 30% in 2010 compared with the previous year with totaled more than two million tablets were seized. It could be noticed that the seizures pattern of Erimin 5 (between 2006 to 2010) were parallel with the seizures of crystalline methamphetamine.

From table 1.1 also, it could be seen the decline pattern for ecstasy seizures during 2006 to 2011 whereas, the seizures of ketamine, showed a sharp decline pattern in 2011 compared to increasing pattern within four years before. The heroin seizures remain stable within year 2007 to 2010 compared to unstable seizures pattern of cannabis herb, where the number of seizures were up and down within the same period of time.

Starting by the year of 2012, it could be seen that the trend of illicit Erimin 5 seizures in Malaysia have been changed where, more illicit Erimin 5 tablets have been seized by the police and custom department. As a result, illicit Erimin 5 tablets suddenly have a sharp increased in their seizures. Royal Malaysian Police (RMP) had reported that, in the first half of the year of 2012, 4,115,694 Erimin 5 tablets worth RM 80 million were seized compared with 87,012 Erimin 5 tablets seized in 2011 (The Malay Mail , 02 August, 2012).

Table 1.1: Seizures of selected drugs in Malaysia, 2006 - 2010

Drug type	Measurement	2006	2007	2008	2009	2010
Crystalline methamphetamine	kg	145.2	69.2	357.0	1,160.0	887.3
Methamphetamine pills	pills	●	121,629	281,343	107,952	107,963
Amphetamine	kg	2.0	●	●	●	●
Ecstasy	pills	227,932	709,888**	80,778	75,515	60,713
Ketamine	kg	110.0	268.0	553.0	1,071.0	334.1
Nimetazepam (Erimin 5)	pills	49,952	171,965	1,502,233	2,909,587	2,032,183
Cannabis herb	kg	2,379.0	1,483.0	875.0	2,352.0	1,064.0
Cocaine	kg	●	●	7.1	18.6	20.6
Codeine	lt.	10,802.0	9,630.0	●	13,131.7	1,925.1
Heroin	kg	156.0	243.0	297.0	283.4	299.3
Opium (raw and prepared)	kg	0.5	7.4	14.0	10.1	4.4
Other benzodiazepines*	pills	173,003	455,407	306,611	268,888	311,123

● = Not reported. *Mostly diazepam.

**Reported in combination of pills and kg, converted at 1 pill = 300 mg.

Source: *Patterns and Trends of Amphetamine-Type Stimulants and Other drugs: Asia and Pacific Report 2011.*

1.1.2 Patterns and trends of the illicit Erimin 5 tablets retail prices in Malaysia compared to others drugs

Retail prices of one illicit Erimin 5 tablet remained largely stable at USD 6 (about RM 20) from 2007, 2009 to 2010 compared to others drugs as displays in table 1.2. The same retail price of illicit Erimin 5 tablet also had been reported in 2012 by Royal Malaysian Police (*The Malay Mail* , 02 August, 2012), which indicates that the retails price of illicit Erimin 5 has been remained stable within a long period. However, the same pattern couldn't be seen for others drug since, the upward and downward pattern of the retails price can be seen except for Ketamine which their retail price showed decreasing pattern between the year 2007, 2009 and 2010 (refer to table 1.2).

Table 1.2: Retail prices of selected drugs in Malaysia (USD), 2007, 2009 and 2010

Drug type	Measurement	2007	2009	2010
Crystalline methamphetamine	per kg	43,290	78,370	77,750
Methamphetamine pills	per pill	6	●	●
Ecstasy	per pill	14	16	16
Ecstasy powder	per kg	●	●	31,100
Ketamine	per kg	10,101	3,448	3,421
Nimetazepam (Erimin5)	per pill	6	6	6
Cannabis herb	per kg	692	752	746
Cocaine	per kg	57,680	62,696	62,200
Heroin	per kg	49,350	●	●
Heroin (No. 3)*	per kg	●	9,404	9,330
Opium (prepared)	per kg	5,772	6,270	6,220

● = Not reported. *Low purity substance processed by adulterant heroin with other substances. (Source: *Patterns and Trends of Amphetamine-Type Stimulants and Other drugs: Asia and Pacific Report 2011.*)

On the changing trend, it can be seen that the drug user preferred Erimin 5 tablets compared to syabu (crystalline methamphetamine) due to the price was more affordable. The cost of one tablet is about RM 20 compared with syabu RM 100 for a few grams. It also has been famous by the users as “Happy 5”. Erimin 5 tablets were easily available and popular among party-goers since it gives them a mild feeling compared to syabu. (*The Malay Mail* , 02 August, 2012, Thursday).

Nimetazepam is found to be manufactured illicitly in Malaysia since, the seizures of two different Nimetazepam (Erimin 5) manufacturing operation in March 2009 and 2010. (*Patterns and Trends of Amphetamine-Type Stimulants and Other drugs: Asia and Pacific Report 2011*). As for this reason, Nimetazepam (Erimin 5) tablets were might found easily among the users.

1.2 Erimin 5 tablets

1.2.1 What is Erimin 5?

Erimin 5 is a trade name of Nimetazepam which is usually encountered on the streets in the form of a tablets strip of 1 x 5 mg Erimin tablets in an aluminum foil which red colour with printed of “Erimin 5” written (front view) at one side and silver colour at the other side (back view). Thus, this tablet has been known and popular as “Erimin 5” tablet. Each aluminium foil contains 10 orange tablets. The genuine/commercial Erimin 5 tablet is orange in colour with the bear of Sumitomo logo and marking of “028” at one side and marking of “5” at the other side. Each Erimin 5 tablet contains 5 mg of Nimetazepam (active ingredient). Erimin 5 tablets originally are manufactured by Sumitomo Pharmaceutical Co, Ltd , Japan and generally prescribed for the treatment of short-term severe insomnia in patients who have difficulty falling asleep or maintaining sleep. Figure 1.2 (a) and (b) shows photo and zoom photo views

of genuine/commercial Erimin 5 foil. While, the photo of commercial/original Erimin 5 tablet is displayed in figure 1.3:-

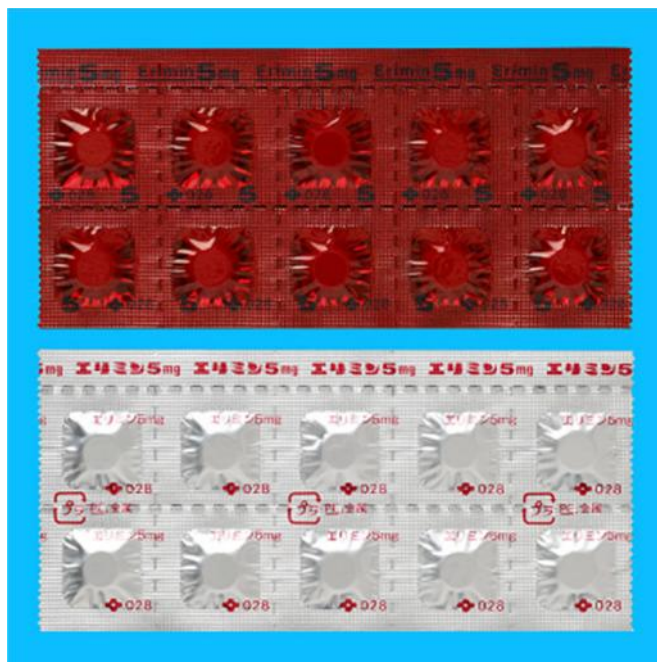


Figure 1.2 (a) :Front and back views of genuine/commercial Erimin 5 foils



Figure 1.2(b): Zoom photo of front and back view of genuine/commercial Erimin 5 foil





Figure 1.3: Front , back and rear views of genuine/commercial Erimin 5 tablet

Source of Photo: Dainippon Sumitomo Pharmacauticals website ,<https://ds-pharma.jp/product/erimin/>

The details description of the original Erimin 5 are shown in table 1.3 :-

Table 1.3 : Description of genuine/commercial Erimin 5 tablet

Brand Name	Erimin 5 mg Tablets
Storage	(It may be discolored by light) stored at room temperature, shielding
Active ingredient	5 mg Nimetazepam
Additive	Magnesium stearate, aluminum lake yellow No. 5 (tartrazine), D-mannitol, corn starch, calcium carmellose, polyvinyl alcohol (partially saponified)
Dosage form, color,	Uncoated tablets of orange color thin
Outline	
Size	A diameter of about 8 mm
Identification code	 028/5

Source of table :

Dainippon Sumitomo Pharmaceuticals website ,<https://ds-pharma.jp/product/erimin/>

1.2.2 Illicit Erimin 5 tablets

According to UNODC, the term illicit drugs is used to describe drugs which are under international control (and which may or may not have licit medical purposes) but which are produced, trafficked and/or consumed illicitly). (<http://www.unodc.org/unodc/en/illicit-drugs/definitions/index.html>)

Erimin 5 is no longer sold in most Western nations, but it is a significant drug of abuse in some Asian countries such as Japan and Malaysia. Illicit Erimin 5 was appeared in Malaysia illicit drug markets in the mid-1980's (Y.K Chong et al., 2004). It is encountered to the Asia region include Malaysia through the black market due to its scarcity and legal restriction.

The illicit Erimin 5 tablet also appear with closely resembled the commercial tablets with the foil and the physical appearance (colour, size and logo) of the tablets, were slightly same as the genuine/commercial tablets. Sometimes encountered in green and pink colour with size and logo of the tablet slightly same as the genuine/commercial tablets (Drug Net Asia, 2006). Examples of illicit erimin 5 foils and tablets are shown in figure 1.4 and 1.5:-

Most of illicit Erimin 5 found to contain Nimetazepam alone as their active ingredient. However, in some cases Nimetazepam was detected together with other active ingredient such as Diazepam and Nitrazepam. Sometimes, Diazepam or Nitrazepam had replaced Nimetazepam as active ingredient. and were detected alone/together with other adulterants such as menthol and caffeine.



Figure 1.4 : Front and back views of illicit Erimin 5 foils (YK Chong et al., 2004)



Figure 1.5 : Example of illicit Erimin 5 tablets (YK Chong et al., 2004)

This benzodiazepine (Nimetazepam) has become the most commonly abused sedative in the country (Malaysia). The popularity of Nimetazepam is due in part to its wide availability and relatively low price on the local black markets, and in part due to its long activity. More recently, nimetazepam has also been used as a sedative by methamphetamine abusers to help them sleep after bingeing. As a result, the rise in nimetazepam abuse roughly parallels with the rise in methamphetamine abuse in Malaysia. (YK Chong et al., 2004).

1.3 Active ingredients in illicit Erimin 5 tablets

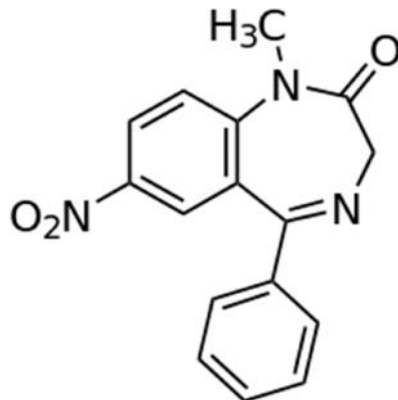
Active ingredient is the substance in a pharmaceutical drug that is biologically active. Nimetazepam is the origin active ingredient found in original/commercial Erimin 5 tablets. However, besides Nimetazepam, others active ingredients commonly found in illicit Erimin 5 tablets are Nitrazepam and Diazepam.

1.3.1 Physical and chemical Properties of Common Active ingredients

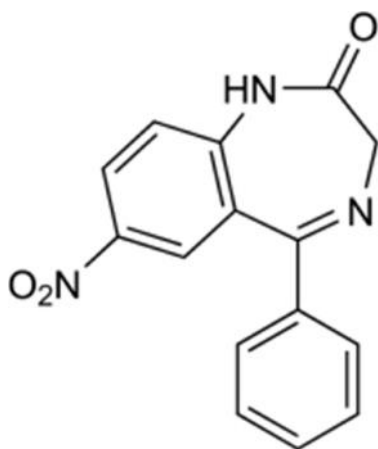
Nimetazepam, Nitrazepam and Diazepam are benzodiazepines groups of drug. A benzodiazepine is a psychoactive drug which their core chemical structure is consist of the fusion of a benzene ring and a diazepine ring. Chemical structure of Nimetazepam, Nitrazepam and Diazepam are shown in figure 1.6 and their chemical and physical properties are shown in table 1.4.

1.3.2 Pharmacological effect of active ingredients

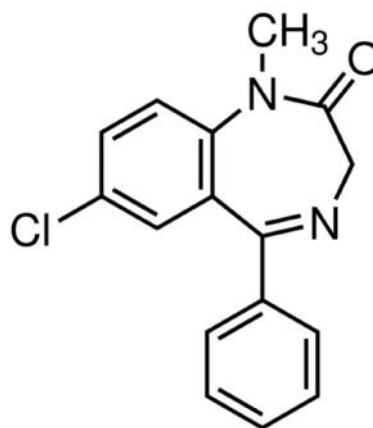
Benzodiazepines enhance the effect of the neurotransmitter gamma-aminobutyric acid (GABA-A), which gives in sedative, hypnotic (sleep-inducing), anxiolytic (anti-anxiety), anticonvulsant, and muscle relaxant properties. It could be categorized into short-, intermediate- or long-acting. Short- and intermediate-acting benzodiazepines are used for the treatment of insomnia; longer-acting benzodiazepines are preferred anxiety's treatment. Comparison of pharmacological characteristics of three Common Active ingredients (Nimetazepam , Nitrazepam and Diazepam) are displayed in table 1.5.



Nimetazepam



Nitrazepam



Diazepam

Figure 1.6 : Chemical structures of Nimetazepam, Nitrazepam and Diazepam

Table 1.4: Physical and chemical Properties of Common Active ingredient found in illicit Erimin 5 tablets

Drug Name	Nimetazepam	Nitrazepam	Diazepam
Chemical Name	1,3-dihydro-1-methyl-7-nitro-5-phenyl -2H-1,4-benzodiazepin-2-one	1,3-dihydro-7-nitro-5-phenyl-2H-1,4-benzodiazepin-2-one	7-chloro-1,3-dihydro- 1-methyl-5-phenyl-1,3-dihydro-2H-1,4-benzodiazepin-2-one
Empirical formula:	$C_{16}H_{13}N_3O_3$	$C_{15}H_{11}N_3O_3$	$C_{16}H_{13}ClN_2O$
Molecular Weight	295.3 g/mol	281.3 g/mol	284.8 g/mol
Melting Point (°C)	156.5 – 157.5 °C	224 – 226 °C	131 – 135 °C
pKa	2.63	3.2	3.3
Solubility	-Freely soluble in Chloroform -Soluble in Ethyl acetate and acetone	- Soluble in ether, acetone, ethyl acetate, and chloroform	- Soluble in ethanol, chloroform and ether.
	-Slightly soluble in alcohol -Very slightly soluble in diethyl ether	- Slightly soluble in alcohol	-
	-Almost insoluble in water	-Almost insoluble in water	- Almost insoluble in water

Table 1.5: Pharmacological effect and the used of three commonly active ingredient found in illicit Erimin 5 tablets.

Drug name	Nimetazepam	Nitrazepam	Diazepam
Action	Intermediate-acting	Intermediate- acting	Longer-acting
Time to peak (hours)	0.5-3	0.5-7	1-1.5
Elimination Half-Life (hours)	14–30 hours	15–38 hours	20–100 hours
Therapeutic use/ properties	Hypnotic, anxiolytic, sedative, anticonvulsant, skeletal muscle relaxant	hypnotic , anxiolytic, sedative and motor impairing , amnestic, anticonvulsant, and skeletal muscle relaxant	hypnotic, anxiolytic, anticonvulsant, sedative, skeletal muscle relaxant, and amnestic
Uses/ treatment	Short-term severe insomnia in patients who have difficulty falling asleep or maintaining sleep	Short-term insomnia problems namely :- -difficulty falling asleep, -frequent awakening, - early awakenings, -or a combination of each.	-anxiety, insomnia, seizures including status epilepticus, muscle spasms, restless legs syndrome, alcohol withdrawal, benzodiazepine withdrawal and Ménière's disease. -It is used before certain medical procedures to reduce tension and anxiety and in some surgical procedures to induce amnesia.

1.4 Others excipients in illicit Erimin 5 tablets

The word *excipient* is derived from the Latin *excipere*, meaning 'to except', which is simply explained as 'other than' (Alison Haywood and Beverley D Glass, 2011). The Wikipedia defines excipients as:

“An inactive substance used as a carrier for the active ingredients of a medication. In addition excipients can be used to aid the process by which a product is manufactured”.

The term of excipient is commonly used pharmaceutical field to describe the others compounds than active ingredient which make up the bulk of the tablet. The excipients includes diluents, binders, lubricants, disintegrants and colourants. However, not every excipients will necessarily to be present in any given formulation (P. J. Gomm and I. J. Humphreys, 1975). Examples of excipients and its function is shown in table 1.6.

In most illicit tablets, the percentage of active ingredient is relatively low compared to the excipients. Consequently, the major compounds of any illicit tablet will be the diluent. Those common pharmaceutical diluents such as lactose can be encountered in illicit tablets (P. J. Gomm and I. J. Humphreys, 1975). Thus, adulterants and diluents added to a batch of an illicit drugs can provide useful information regarding batches and groups (Suzanne Bell, 2009).

In recent years excipients have proved to be anything but inert. Many excipients have more than one use, which can be an advantage since it reduces the number of excipients needed and minimises the risk of interactions between them. (Alison Haywood and Beverley D Glass, 2011) .

Table 1.6: Common excipients used in tablets formulation

Excipient	Function	Examples
Diluents/ fillers	Provide bulk of the tablet with adequate weight and size.	Sugar , sugar alcohol and inorganic compounds
Binders/ Granulating agents	Bind the tablet ingredients together giving form and mechanical strength	Mainly natural (e.g mannitol, starch) or synthetic polymers (eg. Cellulose derivatives)
Disintegrants	Aid dispersion of the tablet in the gastrointestinal tract, releasing the active ingredient and increasing the surface area for dissolution	Compounds which swell or dissolve in water (e.g. starch, cellulose derivatives and alginates, Croscopolvidone)
Lubricants	To reduce the friction during tablet formulation in a and also during ejection from die cavity.	Stearic acid and its salts (e.g. magnesium stearate)
Colouring agents	Aid identification and prevent counterfeiting. Increase stability of light sensitive drugs.	Mainly synthetic dyes, lakes and natural colours.

Source : Alison Haywood and Beverley D Glass, 2011 .

As example, amphetamine tablet formulation with an amphetamine content of 2.5% in the total tablet weight of approximately 200 mg (P. J. Gomm and I. J. Humphreys, 1975) is given below:-

Amphetamine : 5 mg
Diluent : 170 mg
Binder : 5 mg
Lubricant : 2 mg
Disintegrator : 20 mg
Dye : 0.03 mg

1.4.1 Adulterants

In illicit drugs includes illicit tablets production, commonly others pharmacologically active substance which also give effects grossly similar to the drug (active ingredient) has been added into the bulk material (Suzanne Bell, 2009). This substance is called adulterant.

Adulterants is defined as pharmacologically active ingredients added to give either synergistic or antagonistic effects (Cole C et al., 2010). Adulterant are often used to enhance the biological effect of active ingredients (Sara Castiglioni et al., 2011). In the case of illicit Erimin 5 tablets, caffeine and menthol are commonly added into the bulk materials as adulterant.

Caffeine is preferred as adulterant due to cheap, legal and more readily available than illicit drugs (Cole C et al., 2010). It acts as a central nervous system stimulant by raising brain activity, warding off drowsiness temporarily and restoring alertness. Menthol is added into the illicit drug such illicit Erimn 5 tablets to provide cooling sensation effect while taking the drugs. However, the use of some adulterants cannot be explained in terms of their pharmacological effect why they had been added. Some adulterants appear on the market for only a short period of time as example menthol.

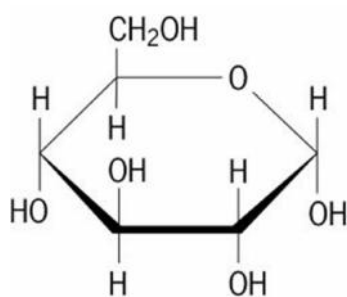
1.4.2 Diluents

Diluents refers to the inert substances added to illicit drugs to bulk out the drug and therefore decrease the amount of active ingredient (Cole C et al., 2010).

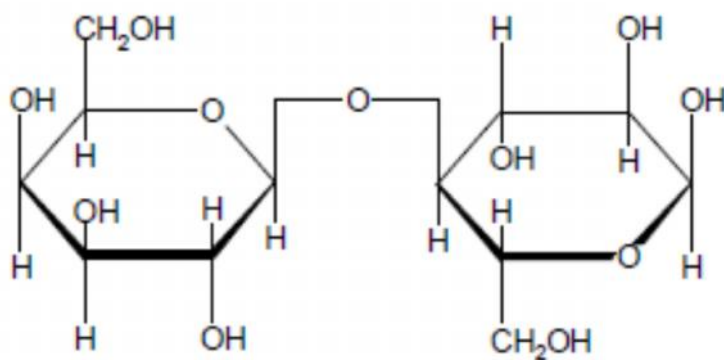
There are 3 groups of diluents commonly found in illicit tablets, which are sugar/carbohydrate group, sugar/poly alcohol groups and inorganic compound. Usually the range of diluents may vary from 5-80%. Examples of diluents are shown in table 1.7 and chemical structures of some diluents are illustrated in figure 1.7

Table 1.7: Type of diluents and their examples.

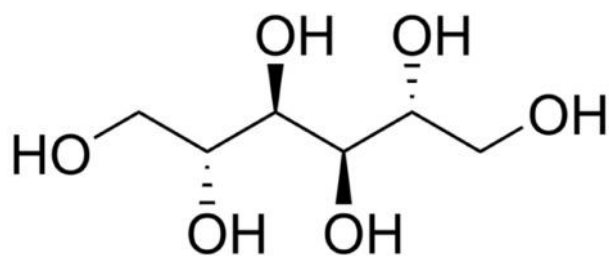
No.	Diluents	Examples
1.	Sugar/carbohydrate Monosaccharides Disaccharides Polysaccharides	Glucose, Fructose, Galactose,etc. Sucrose, Maltose, Lactose,etc. Cellulose /Modified cellulose , Starch (corn starch, potato starch, wheat, rice starch) etc.
2.	Polyalcohols / Sugar alcohols	Sorbitol, Inositol, Mannitol, Xylitol,etc.
3.	Inorganic compounds	Calcium Phosphates (Dibasic calcium phosphate and tribasic Calcium phosphates), Calcium carbonate, calcium sulphates, talc (magnesium silicates),etc.



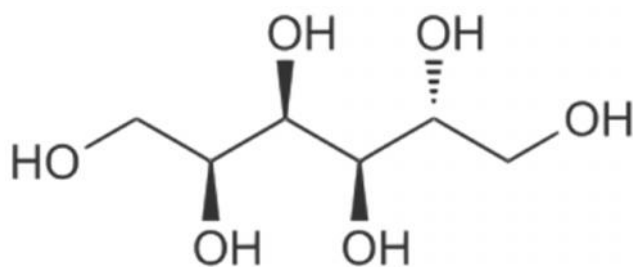
Glucose (Monosaccharide)



Lactose (Disaccharide)



Mannitol (sugar alcohol)



Sorbitol (sugar alcohol)

Figure 1.7: Chemical structures of some diluents

1.4.3 Colourants/Colouring matter

Dyes are widely used as colourant/colouring matter in manufactured of illicit tablets as well as in pharmaceutical manufactured which gives an attractive colouring appearance of the tablet. It has been used as visual appeal as well as mimic of the original pharmaceutical/commercial tablet.

The easiest and preferably dyes uses in illicit tablet manufactured are food colouring materials which easily available either direct purchasing at most retail shops/supermarket or through online order. These food colourings are easily introduced into the preparation. Dyes are usually cheaper in cost (Krishna V. A. and Gannu P.K., 2011). Examples of dyes are listed in table 1.8 .

Table 1.8 : Examples of food colorings/dyes

Colour of Dye	Examples of Dyes
Red	Ponceau 4R, Erythrosine, Carmoisine, Amarath ,etc.
Oranges	Sunset Yellow, Orange G, Orange RN,etc.
Yellows	Tartrazine, Quinoline Yellow , Naphthol Yellow etc.
Greens/ Blues/violets	Brilliant Blue, Green S, Indigo Carmine,Patent Blue V,etc.
Brown	Brown FK, Chocolate Brown, etc.

1.5 Legislation Status

Nimetazepam and others benzodiazepines such as Diazepam and Nitrazepam are currently a Schedule IV drug under the International Convention on Psychotropic Substances of 1971.

1.5.1 Legal status in Malaysia

In Malaysia, only two benzodiazepines which are Nimetazepam and Flunitrazepam are listed under first schedule of the Dangerous Drugs Act 1952 since May 2001. However, compare to Flunitrazepam, Nimetazepam is commonly found in illicit Erimin 5 tablets samples besides caffeine and others benzodiazepines groups such as Diazepam and Nitrazepam. Caffeine and others benzodiazepines (derives from 1,4-benzodiazepines structures) are controlled under Poison Act 1952. However, the use of Caffeine in baverages and foods are allowed (legal). Other adulterants such as menthol and diluents as examples sugars/carbohydrates groups (sucrose, glucose, lactose, maltose, starch etc.) sugar alcohols groups (e.g mannitol and sorbitol) or inorganic diluents groups (e.g calcium phophates, calcium sulphates and etc) are not controlled substances.

1.5.2 Legal status in others countries

Besides Malaysia, Nimetazepam is illegal and has been controlled in certain countries such as Singapore, Hong Kong and Victoria, Australia. Diazepam and Nitrazepam are regulated in most countries as a prescription drug. Whereas, Caffeine is legal in most of countries since in easily available in food and baverages.

1.6 Profiling

Drug profiling is defined as the extraction of chosen physical and/or chemical characteristics from the samples and their use as intelligence in the fight against illegal drug trafficking (Quentin M. et al., 2009). Different information can be obtained, depending on the chosen set of characteristics (i.e. profile). It is generally assumed that seized tablets having corresponding characteristics come from the same production batch, while tablets showing different characteristics come from different batches (Esseiva P. et al., 2007).

Physical characteristics such colour, diameter, thickness and average weight of tablets provides a means for tablet comparison. These information can be used as a general ideas of the pattern and trends of physical characteristic of illicit Erimin 5 tablets (average weight, diameter, thickness, colour) would be. A link between samples based on physical characteristics does not mean that the same press was used. It may be another press with the same settings in respect to diameter, thickness, and weight (Marquis R. et al., 2008). However, physical characteristics are generally not considered sufficient enough to provide evidence of a link between seizures (Quentin M. et al., 2009). A link found between samples should therefore be confronted to other information on the samples (such as contextual information or other characteristics) in order to allow a more precise understanding of the traffic (Marquis R. et al., 2008).

Illicit tablets production implies three steps: synthesis of active compound, addition of excipients (e.g adulterants, diluents, and dyes) and compression of tablets. Each step provide useful data which is delimiting the goals of an investigations (Till Goldmann et al., 2004). The first step provide organic impurities information of the

tablets, whereas the second step provide chemical characteristics (chemical profiles) information of the tablets and the last step provide physical characteristics information of the tablets.

However, in the case of illicit Erimin 5 tablets production, the second step are very important. The valuable information of chemical characteristic such as adulterants, diluents and dyes added in illicit Erimin tablets production can be used as a tool for comparison between illicit Erimin 5 tablet samples. The same profile of chemical characteristics between illicit Erimin 5 tablets can be used as indication these tablets might come from the same source of origin/batches. Besides that, chemical characteristics profile of illicit Erimin in 5 tablets also can be used to compare with raw materials found in clandestine laboratories whether they have a link or not.

Compare to chemical characteristics, physical characteristics are difficult to make a judgement whether the tablet may come from the same sources/origin/batch although they have the same physical characteristics such as diameter, thickness and average weight of tablet. This is due to the possibility of different tableting machines may yield similar features (Quentin M. et al., 2009). Sometimes, small differences in mean/average tablet weight from the same batch are often attributed to resetting of the machine. It might also due to a change in the major excipients used. Moreover, poor manufacturing expertise also contribute to variation in size and distribution of the bulk (P.J. Gomm and I.J. Humhreys, 1976).

Others physical characteristics such colour of tablet surface, may vary considerably according to age and storage condition due to some of dyes are sensitive to the light (P.J Gomm et al., 1976). Thus, colour of the tablet is not accurate measurement of the colour since, it also depends on perception and judgement of the observer.

However, in some cases the unique of tablet tone colour able to provide information whether these tablets come from the same batch/ source. As example, several tablet samples which seized in different occasions but shows similarity in poor distribution tone of orange-red colour of tablet may indicates they come from the same batch/source. To confirm, whether they come from the same source, further analysis which involves chemical characteristics such active ingredient, diluents and dyes should be done in order to compare their profiles. By combination both characteristics, the evidence whether they came from the same source/origin would be stronger.

1.7 **Significance of the study**

The trend of Erimin 5 tablets abused and their seizures in Malaysia could be seen increasing over the past few year as well as encountered a few of illicit manufacturing operation scale lab of this tablet.

By studying their physical and chemical characteristics, perhaps these can help law enforcements agencies such Royal Malaysian Police and Royal Customs Department to establish links among seizures and their illicit marketing networking of illicit Erimin 5 tablets. These information also can be used as court purposes in order to strengthen the evidence of illicit Erimin 5 tablets trafficking .

Beside that, physical and chemical characteristics data also can be used as database to compare the distribution pattern of that characteristics (physical and chemical characteristics) among others lab especially from Branch lab which provide information for Malaysian distribution as well as from others Asia region lab/others countries which provide region/international characteristics distribution of illicit Erimin 5 tablets.

1.8 Objective of the study

The objective of this project are :-

To study of physical characteristics (colour, diameter, thickness and average weight per tablet) of illicit Erimin 5 tablets and their distribution profile.

To study of chemical characteristics includes active ingredient, adulterant, major diluent and dyes of illicit Erimin 5 tablets and their distribution profile.

To quantitate Nimetazepam content in illicit Erimin 5 tablets and to study their distribution profile.

To characterized illicit Erimin 5 tablets according to their active ingredients, adulterants, major diluents and dyes for intelligence purpose.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

The study of physical characteristic and chemical characteristic include active ingredients, adulterants, diluents and dyes of illicit tablets have been done by many researchers. However, some chemical characteristic of illicit tablets such as dyes had been reported long time ago and not much further reseach was done on it. As a result, studies on dyes in illicit tablet was rarely reported and thus, studies of dyes in others field such as in food had been used as reference.

Since, general formulation used in the production of illicit tablets is similar to that used for tablet manufacture in the pharmaceutical industry (P. J. Gomm and I. J. Humphreys, 1975), any studies on tablet formulation on pharmaceutical field have been used as guide in analysis of illicit Erimin 5 tablets.

The studies on profiling of illicit tablets such ecstasy tablet and yaba tablets had been widely applied include physical, chemical (general profile), combination of physical and chemical, impurities, organic profiling of these illicit tablets. However, profiling of illicit Erimin 5 tablets are rarely done/reported due to this drug (Erimin 5-nimetazepam) has not been widely encountered in some countries or this type of drug is not their preferable since, it is legal in other countries such Japan. Thus, the profiling studies of others illicit tablet could be used as a guide for profiling of illicit Erimin 5 tablets.

2.2 Profiling and sample comparison by Physical characteristic

The studies of physical characteristics of illicit tablets had been done by several researchers. As example, P.J. Gomm and I.J. Humhreys (1976) which described physical methods of examination in comparison of illicit amphetamine tablets, in order to establish common sources of manufacturer. In this study, amphetamine and LSD tablets were compared based on the dimensions of the tablets and a microscopic examination of the surface.

Yukiko M. et al. (2003) had investigated physical characteristics of 100 illicit amphetamine type stimulant (ATS) seized in Japan. About 15 parameters of physical characteristics were studied included logo, vertical view, horizontal view, outside and inside colour of the tablet, diameter, weight, thickness, smell, toughness, colour pattern, capping ,appearance of the logo, cleavage and coat in order to obtain the nature of tablet abused in Japan.

Marquis R. et al. (2008) had studied physical characteristics of 560 of illicit MDMA tablet samples (test samples and street samples tablets) and found physical characteristic such as diameter, thickness , weight were found reliable for comparison in illicit MDMA tablets. It also was demonstrated these physical characteristics were adequate features in order to discriminate between samples coming from different seizures and also can be used in drug intelligence purpose.

J. Camargo et al. (2012) proposed a tablet profiling method based on visual appearance of tablets (such as color, texture and shape) and a tablet monitoring strategy that combined a discriminative and clustering model based on pill visual similarity functions. In this new approach, pictures of ecstasy pills were used. This powerful tool

might help finding links between illicit drugs seizures by exploring the collection of image to decipher the structure of criminal organizations behind this traffic.

2.3 Profiling and sample comparison by Chemical characteristics

Chemical characteristics have been studied to identify chemical components such as active ingredient, adulterants, diluents and dyes present in the illicit tablet preparation and their tabulation as well as to compare these chemical characteristic in order to establish of links between seizures or between a seizure and a laboratory. Since, chemical composition of tablet are hardly ever changes (eventually decomposition or very slight changes in quantities) once excipients are added to the active substance and the tablet is pressed. Therefore, a seized tablet presents the same composition than that at the initial production (Ines Baer & Pierre Margot, 2007a).

2.3.1 Determination of active ingredient and adulterants

UNODC have recommended several methods on analysis of Nimetazepam and others benzodiazepines using various chromatographic methods such as GC/FID and HPLC with UV/PDA detector either normal phase or reverse phase.

Besides that, several researchers also had reported in analyzing of benzodiazepines included Nimetazepam using various method such as thermal desorption GC (Minemasa Hida et al., 1999) GC/MS using Selected ion Monitoring (SIM) mode (Hiroyuki Inoue et al., 2000), HPLC with reverse phase (Lim WJL et al., 2004 and Chong Y.K. et al., 2004), Capillary Electrochromatography - CEC (Kiyokatsu Jinno et al., 2000).

Some of the researchers had studied various method as example Margaret et al. (1988) had compiled and described analytical data for detection and identification of Nimetazepam and others benzodiazepines using chromatographic (GC, HPLC, TLC), spectroscopic (IR and UV) and mass spectrometric methods. Some of them compared performance of Nimetazepam and others benzodiazepines between two different GC detectors as example Shimano M. et al. (1995) found sensitivity of most studied psychotropic drugs (included Nimetazepam) and other components (included caffeine) using NPD were thirty times than the average height of the peak high in FID.

2.3.2 Determination of major diluents

Common method used in determination of diluents in illicit drugs such in illicit tablets which have been described in many literatures is Infrared spectroscopy. P.J Gomm et al. (1975) described a method for the comparison and identification of the major excipients encountered in illicit tablets by infrared spectroscopy using KBr disc method without any need for its separation from the other tablet component. The result showed it is possible to obtain a rapid identification of the major excipient by IR spectroscopy directly on the powdered tablet materials due to its high percentage (often greater than 80%) in illicit tablet formulations.

Fourier Transform Infra Red (FTIR) is the most modern IR spectrophotometer technique which has been replaced the use of IR. It has been widely used in determination of active ingredient and excipients in pharmaceutical application as well as in analysis of illicit drugs. Besides that, a lot of new development in FTIR technique such as Attenuated Total Reflectance (ATR) technique, Near Infrared (NIR) technique and FTIR imaging using microscope have been extensively established.

As example, Near Infrared (NIR) technique using Near Infrared reflectance spectroscopy had been applied in analysis of cellulose and lactose (excipients) in illicit ecstasy tablets. It has capabilities in determination of different chemical forms of these two substances, as well as the differentiation of their origin (producer) (Ines Baer et al., 2007b). Others example, ATR imaging and mapping had been described widely in Instrument's Supplier application notes such as Perkin Elmer Application Note (2007) in determination of active ingredient and excipient in pharmaceutical tablets. The imaging system technique provides a visible image of a tablet surface which able to show how individual ingredient (such as starch) distribution pattern as well as a composite image from major components.

Another technique which also has been reported is Raman spectroscopy as well as Raman mapping technique. Raman spectra can be processed to give unambiguous identification of both drug and excipients (Steven E.J. Bell et al., 2000a). As Raman mapping technique provide the same application as mapping in FTIR, it able to show the spatial distribution of the compounds and also allows identifying another ingredient (Horiba Application Note). However, the application of FT Raman has limitation, in some cases pharmaceutical excipient as well as illicit tablets exhibit a weak Raman response due to exhibit large amount of fluorescence signal originating from the same target compound (Mark R. Witkowski, 2005).

2.3.3 Determination of dyes

Many analytical methods have been used in determination of dyes. The earliest method used is Paper chromatography which recommended by Association of Public Analyst (APA) in 1960. However, these have been superseded by the use of column chromatography, TLC and HPLC (Harold Egan et al., 1981). TLC procedure had been described by GJ Dickes (1965) for the identification of twenty-eight permitted and twelve non permitted colouring matter using two solvents system. It was found TLC gives more rapid and precise results than separation on paper.

Joyce J. R. et al. (1979) described a three step analytical process for identification of synthetic food dyes used in illicit drug preparation. The procedure involved TLC, visible spectrometry and reverse phase ion pair HPLC which had been successfully applied to the analysis of several dyes of illicitly produced tablets. This studied had been extended to the analysis of insoluble food dyes (Joyce J. R, 1980a) commonly used in illicit tablets using the same procedure as mention before.

Another method that had been reported in analyzing of dyes is capillary zone electrophoresis equipped with diode array detector (CZE-DAD). Till Goldmann et al., (2004) had developed method for confirmation of the identification and quantitation for 14 hydro soluble, acidic, synthetic food dyes in illicit tablets by CZE-DAD.

The newest of TLC technique, High Performance Thin layer chromatography (HPTLC) method had been reported in literature regarding on analysis of dyes. Florin et al. (2008) had proposed HPTLC method combined with image processing of scanned chromatograms for the determination of food dyes (tartrazine, azorubine and Sunset yellow) in different product. This method is relatively more cheaper than other instrument, more simple and easy to handle and acquire.

2.3.4 Profiling and sample comparison

Several studies on profiling and sample comparison according to chemical characteristics had been reported before. For example, Steven E.J. Bell et al. (2000b) applied Raman spectroscopy to profile the composition of illicit Ecstasy tablets from a seizure of 50,000 tablets in eight different bags. Despite significant variation within each bag, the contents of each were classified based on the diluent present. The samples which contained the same diluent were differentiated according to the degree of hydration of the illicit substance and the ratio of illicit substance to diluent.

Steven E. J. Bell et al. (2003) did a study on composition profiling of approximately 1500 “ecstasy” tablets from different seizures in North Ireland by Raman spectroscopy. In this study, each of the batches of tablets was classified according to active drug content, excipient used, drug/excipient ratio and degree of hydration. It is clear that, when used in combination, these data give a very powerful method for distinguishing between seized samples with similar but not identical compositions.

Till Goldmann et al., (2004) demonstrated that by comparing of dyes found in tablets with dyes found in illicit laboratory could provide strategic intelligence information. While, comparison of chemical profiles between tablets samples such as dyes and excipient could provide tactical drug information whether they came from same batch or not.

Profiling studies based on combination of physical and chemical characteristics had been done by Q. Milliet et al. (2009), where the correlation of profiling information contained in organic composition and physical characteristics of MDMA tablets had been investigated and yet found that organic impurities confirmed the links highlighted by characteristics physical characteristics of the tablets.

CHAPTER 3

METHODOLOGY

3.1 Sample Collection

46 illicit Erimin 5 tablets samples were collected as research materials in this study. These samples were selected among the Erimin 5 cases seized between January to June 2012 which had been submitted by the Royal Malaysian Police, RMP to the Narcotics Section, Chemistry Department of Malaysia. Since the amounts of the tablets samples were limited, at least 3 tablets were collected for each sample depends on the seized materials. In this study, the tablets samples were identified according to identification (ID) number due to the actual laboratory number (unique number) are private and confidential for the public interest exposed.

3.2 Materials

3.2.1 Reagents and Chemicals

- i. Chloroform HPLC grade or other suitable grade
- ii. Methanol HPLC grade or other suitable grade
- iii. Acetic Acid AR grade
- iv. Ammonia 25% AR grade
- v. 2-Propanol (iso-propanol) AR grade
- vi. Phosphoric Acid AR
- vii. Distilled water

3.2.2 Standards

- i. Nimetazepam Reference Standard- Pure Standard from Sumitomo or certified standard from other sources.
- ii. Diazepam Reference Standard from UNODC
- iii. Nitrazepam Reference Standard from UNODC
- iv. Caffeine Reference Standard obtained from Merck.
- v. Menthol Reference Standard obtained from Sigma-Aldrich.
- vi. D-Lactose Monohydrate and D-Mannitol Reference Standard obtained from Sigma-Aldrich.
- viii. Dibasic Calcium Phosphate Dihydrate Reference Standard from Merck.
- ix. Dyes Reference Standards (Food Coloring) of Sunset yellow, Ponceau 4R, Tartrazine, Erythrosine, Green S, Brilliant Blue, Q-yellow and N-Yellow obtained from Sanei Chemical Industrials.

3.2.3 Apparatus

Digital camera branded Canon Ixus 130 with 14.1 megapixels

Analytical electronic balance branded Sartorius (four-decimal)

Digital Calipers (Avenger Products)

Ruler

Ultrasonic bath

Water bath

Mortar and pestle

Domestic food blender (brand sumeet)

Volumetric flasks (10 mL, 25mL, 200 mL, 250mL, 1000mL)

Beaker

Spatula

Measuring cylinder

Pipettes

Glass rods

GC Vials

Test Tubes

Evaporating Dish

White knitting wool (100% wool)

20 mm x 10 mm plates pre-coated with 0.10 mm layers of Silica gel

TLC Tank

TLC Spotting Capillary tube

Spot plates



White knitting wool



Digital Calipers



Mortar and pestle

Figure 3.1: Examples of apparatus used in this study

3.3 Instrumentation

3.3.1 Gas Chromatography Mass Spectrometer (GCMS)

GCMS was used for identification and confirmation of the active ingredients and adulterants in illicit Erimin 5 tablets samples. GCMS analysis was performed on an Agilent 6890N GC equipped with 5975B inert mass spectrometer, an autosampler and a data management system. The following condition was applied:-

Operating Conditions

HP-5 MS capillary column (30m x 0.25 mm id x 0.25 μ m) was chosen in this study. Helium was used as carrier gas at flow rate of 1.0 mL/min. The GC injector port and GCMS interface temperatures were set at 250 °C and 280 °C respectively. A split mode at 50:1 was chosen. The oven temperature program was held at 80 °C for 2 min, then the temperature was ramped at 20°C/min to 270°C and held for 10.0 min. The MS was operated in an EI mode with 70 eV EI voltage. The MS scanning rate was 1 scan/sec with a scan range of 40 to 500 amu.



Figure 3.2: GCMS Agilent

3.3.2 High Performance Liquid Chromatography (HPLC)

HPLC was used for quantification of Nimetazepam in illicit Erimin 5 tablets samples. HPLC analysis was performed on an Shimadzu equipped with Photodiode Array (PDA) detector, an autosampler and a data management system. The following parameters were applied:-

Operating Conditions

Column: ODS column,C-18 (15 cm x 4.6 mm id x 5 μ m particle size)

Detector: PDA/UV at 265 nm

Mobile phase : Methanol: Water (50:65).The pH adjusted to 4.0 with orthophosphoric acid (to a mixture of 500 mL of methanol and 650 mL of water was added one drop of orthophosphoric acid)

Flow rate: 1.0 mL/min

Column temperature: 25°C (ambient temperature).



Figure 3.3: Shimadzu HPLC with PDA detector

3.3.3 Fourier Transform Infra Red (FTIR)

The diluent of the illicit Erimin 5 tablets samples will be definitely identified by HazMatID portable IR spectrometer. The system operates based on Fourier Transform InfraRed (FTIR) spectroscopy coupled with an Attenuated Total Reflectance (ATR) with the aid of machine press equipped with built – in diamond crystal. The number of scan and resolution were set at 64 and 4 respectively. Information was collected in the Absorbance mode within 4000 and 650 cm^{-1} .



Figure 3.4 : HazMatID portable FTIR spectrometer (ATR).

3.3.4 Scanning Electron Microscope – Energy Dispersive X-ray instrument (SEM-EDX)

Analysis of element using Scanning Electron Microscope – Energy Dispersive X-Ray instrument (SEM-EDX) will be proceed if there any difficulties in the interpretation of IR result occurs. This instrument's provide the element content of sample which could be used as aid in the IR interpretation.

Instrument Condition

Brand: Oxford instrument

Model: Leica S440

Accelerating voltage: 20 kv

current probe: 300 pA

Working Distance: 19 mm

Signal : Secondary Electron

3.4 Procedure

Analysis of illicit Erimin 5 tablets samples were consisted of two parts which were physical characteristic and chemical characteristics.

3.4.1 Physical characteristics

Physical characterization were done by physical examination of the collected illicit Erimin 5 tablets samples as follows:-

- i. Tablets were photographed using the digital camera to show the colour, the front, rear and side views of the tablets.
- ii. The colour and stamped logos on both sides were observed and recorded.
- iii. The nett weights of 3 tablets were weighed using the 4 - decimal analytical balance. Then, the nett weights were recorded and the average weight of one tablets were calculated. One point check with 20.0000 gram Standard Weight should be done on the analytical balance prior do the weighing and should met the stated acceptance value.
- iv. The diameter and thickness of the tablets were measured by using Digital Caliper which had been first adjusted to zero before use.

3.4.2 Chemical Characteristics

Chemical Characterization of the illicit Erimin 5 tablets samples were include identification and confirmation of the active ingredients, adulterants (both done by GCMS), the diluents (done by FTIR) and the dyes (done by TLC). Since Nimetazepam is the only drug, which is listed under Dangerous Drug Act 1952 in this study, the quantification of Nimetazepam by HPLC also included as a part of the analysis. The suitable amount of the illicit Erimin 5 tablets samples were homogenized by crushed with the mortar and pestle or Domestic food blender then placed inside a clear plastic packet prior chemical characterization analysis had been done.

3.4.3 Identification and Confirmation of Active Ingredient and other Adulterant

3.4.3.1 Sample Preparation

About 100 mg of homogenized Erimin 5 tablets samples was transferred directly to a 10 ml volumetric flask. Then 10 mL of a solution of Methanol:Chloroform (5:1) is added and the mixture is thoroughly shaken to extract the active ingredients. The insoluble material was allowed to settle by leaving for overnight. Transferred a suitable volume of the supernatant solution to GC vial. For some cases, if the solution remains turbid the solution shall be filter. Finally the samples were injected 1µl into the GCMS and the mass spectrum were obtained for the tablets samples.

3.4.3.2 Calibration of the instrument

GCMS system should be ensuring to be in tip top condition by auto tuning the Instrument prior performing the analysis. The auto tuning result should meet within the acceptance value as specified in manufacturer specification. The blank solution (methanol/chloroform) should be run to check the baseline and the presence of any contamination or carry over from the previous running. Then the known of mixed Standard were injected for several times to obtained a consistent retention time and the fingerprint of the mass spectrum profile for each peaks were identified and compared to be the match with the library spectra or literature. Hence, the instrument's found to be stable and ready for sample analysis.

3.4.3.3 Interpretation of the mass spectrum

The mass spectrum of the active ingredients and adulterants in Erimin 5 tablets samples were compared with a current mass spectrum of the appropriate reference standard obtained from the same instrument operated under the same condition. The mass spectrum of the interest compound also searched against the library spectra for identification. The principal peaks of some common active ingredient and adulterants in illicit Erimin 5 tablets is shown in table below in descending order the intensity.

Table 3.1: The principal peaks of some common active ingredient and adulterants

Common active ingredient and adulterants found in illicit Erimin 5 tablets	Peaks of descending intensity
Nimetazepam	294,91,295*,248,278,249,206
Diazepam	256,283,284*,285,257,255,258,286,221
Nitrazepam	280,253,281,206,234,252,254,264
Caffeine	194,109,55,67,82,195*,42,110
Menthol	81,95,71,41,67,55,138,123

*Denote molecular ion

3.4.4 Quantification of Nimetazepam by High Performance Liquid Chromatography (HPLC)

3.4.4.1 Sample Preparation

90 mg or more of homogenized illicit Erimin 5 tablets samples was accurately weighed directly into 25 mL volumetric flask and was made up to volume with a solution of Methanol : Chloroform (5:1) and placed in ultrasonic bath for about 5 - 10 minutes to dissolve the sample and then leave for overnight. Each of sample solution was transferred into separate injection vials for HPLC analysis. For each sample, duplicate determinations were performed.

3.4.4.2 Calibration standard

1. Preparation of Nimetazepam Stock Solution (1.0 mg/mL)

50.00 mg of Nimetazepam Standard was weighted accurately into a 50 mL volumetric flask and made up to volume with a solution of Methanol:Chloroform (5:1).

2. Preparation of Nimetazepam Working standard (0.120 mg/mL) and Control standard (0.08 mg/mL)

To each 3 mL and 2 mL of Nimetazepam stock solution (1.0 mg/mL) were pipetted accurately into two 25 mL volumetric flask respectively and the volume was made up with methanol.

3.4.4.3 Calibration by external Method

The Nimetazepam standard was chromatograph a number of times until a reproducible peak area and retention time was obtained. The HPLC was calibrated using the results of Nimetazepam standard and chromatograph the Nimetazepam Control standards solution to ascertain the accuracy of the calibration.

3.4.4.4 Sample Injection

Each sample solutions were chromatograph under the same conditions as for the standards solution. A blank solution (methanol/chloroform), the sample in methanol/chloroform should be chromatograph.

3.4.4.5 Calculation

1. For each determination the percentage of Nimetazepam (as free base) in homogenized sample was calculated as follows:

$$\text{Percentage.P} = A \times V \times 100/M;$$

Where ; A = HPLC read-out (mg/mL)

V= volume of flask used

M= Weight of homogenised sample taken (mg) for analysis

2. The mean of Nimetazepam percentage, P_{ave} for the each sample was computed and the weight of the Nimetazepam per tablet Erimin 5 (W) in mg was calculated as follows :-

$$W = P_{ave} \times W \text{ (mg)}$$

Where W = Average weight of one tablet Erimin 5

3.4.4.6 Quality Control

The concentration of the Nimetazepam standards solution and the Nimetazepam control standards solution should be obtained chromatographically within $\pm 5\%$ of the calculated value. The run was repeated and the HPLC was recalibrated if did not meet of this criteria. The accuracy of the calibration of the HPLC throughout the analysis must be checked with either the calibration standard or the quality control standard.

3.4.5 Identification and Confirmation of major Diluent by Fourier Transform Infra Red (FTIR)

3.4.5.1 Sample Preparation

In this study, HazMat ID portable FTIR spectrometer with Attenuated Total Reflectance (ATR) technique was used hence, there was no sample preparation required. A small amount of homogenized sample could be directly placing onto the ATR sampling port using a spatula and by lowering the 'Force applicator arm' onto the sample to ensure contact between the sample and the ATR port. This sampling technique is non - destructive technique. If necessary, the extraction and purification of diluents from the sample according to its solubility in various solvents could be done prior did the FTIR analysis.

3.4.5.2 Quality control of the result

The performance of the instrument was tested with the known sample (control sample) and reference standard of the interested compound to show its ability to identify accurately the compounds prior applying to the illicit Erimin 5 samples

3.4.5.3 Interpretation of Spectrum

The Infra Red (IR) spectrum of the diluent of the Erimin 5 tablets samples was searched against the library spectra of HAZMAT ID instrument/software for the identification and confirmation of the compound (diluent). The IR spectrum also was compared with a current IR spectrum of the appropriate reference standard obtained from the same instrument condition.

3.4.5.4 Additional Analysis

In the occasion where, the interpretation of IR spectrum is difficult and the diluent couldn't be separate physically according to their solubility, the elemental analysis using SEM-EDX will be run as an aid of IR spectrum interpretation. Instrument was calibrated with Carbon standard. This instrument is capable to detect any element which heavier than carbon in the Periodic Table.

3.4.5.4.1 Sample Preparation

There was no sample preparation required. A small amount of homogenized sample could be directly placing onto sample chamber.

3.4.5.4.2 Interpretation

Peak of element could be identified at their specified X-Ray energy line, K (some element has additional energy line K_α) in unit of KeV. Presence of any element revealed an idea for diluent prediction.

3.4.6 Identification and Confirmation of Dyes by Thin Layer Chromatography (TLC)

3.4.6.1 Sample Preparation (dye extraction)

Dye extraction of the Erimin 5 tablets sample was involved the extraction out the colour (dye) from the tablets to wool and followed by removal the colour from the wool. The details procedure as follows:-

1. A suitable amount of homogenized Erimin 5 tablets sample was acidified with 5% Acetic Acid in a beaker, then a piece of white knitting wool was added into it. The white knitting wool should be immersed in the acid. If the colour of the extracted dye was insufficient concentrated, the more amount of sample was added in order to concentrate the dye.
2. The mixture was warmed on a boiling water bath until the colour was transferred to the wool. The colour was extracted out very fast, usually within a few minutes.
3. Then, the wool was removed and washed thoroughly under the cold tap water to remove sugar and other extraneous materials.
4. Re-extract the dye from the wool by warming it with a suitable volume of 3N Ammonia solution on a water bath for approximately 5 minutes. The wool then was removed when the dye/colour of the wool almost had been faded.
5. Then, the dye solution was gently evaporated to dryness on a water bath.

3.4.6.2 Preparation of Developing Solvents System of TLC

The solvent System for TLC analysis of Dye should be freshly prepared. In this study, two recommended developing solvent system which were widely different polarity, system A and system B were used. The details of the solvents system are shown as below:-

Solvent System A: Iso-propanol (2-propanol): Ammonia (S.G 0.880) (4:1)

Solvent System B: Iso-propanol (2-propanol): Ammonia (S.G 0.880): water
(7:2:1)

The steps in Developing Solvents System as below:-

1. The TLC tank was filled with the developing solvents system to give a depth of approximately 1 cm. The mixing process could be done in the TLC tank. It is important that the solvent level should be below the line with the spot on it.
2. The lid should be closed tightly and allowed to equilibrate for approximately 10 minutes. Filter paper was soaked into the tank to check whether the developing system was mixed well and achieved the equilibrium.

3.4.6.3 TLC analysis

1. A Pencil line was drawn about 1.5 cm from the bottom of Silica gel TLC plate.
2. The Dye (food colouring) Reference Standard and evaporate dye samples were made into solution by adding a few drops of Methanol.
3. A small quantity of the concentrated Dye Standards and dye samples were applied as small spots on the line on TLC plate using spotting capillary tube. The distance between each spot was set at 1.0 cm. The spot was applied more if the colour of the dye was still not enough concentrated. Dye standard's spot was labelled with their commercial food dye name and each sample's spot was labelled according to their ID number. For each plate, a set of relevance dye reference standard should be spotted together with the dye samples.
4. Once the developing system was equilibrated, the TLC plate was placed in the TLC tank which containing selected Developing solvents system.
5. The progress of the solvents should be monitored. The plate must be removed from the tank as soon as the solvent reached the suitable level (development level). This development level (solvent front) was marked as soon as possible before it had a chance to evaporate.
6. The TLC plate was allowed to dry at room temperature in the fume hood.

3.4.6.4 Interpretation of the TLC chromatogram

The Retardation factor, R_f value for each spot was calculated according to formula as below:-

$$R_f = \frac{\text{Distance between start line to the spot (dye compound)}}{\text{Distance of solvent front}}$$

The R_f values of the dye spots of the Erimin 5 tablets sample was compared with the R_f values of the set of dyes reference standard on the same plate to aid in their identification. The other particulars such a colour, position and number spots of the sample dye spots in daylight also were compared with reference colour spots. Two solvent systems of widely different polarity were used to confirm identity of the dyes used in the illicit Erimin 5 tablets sample.

CHAPTER 4

RESULT AND DISCUSSION

4.1 Introduction

The scope of this study, are based on physical and chemical characteristics of illicit Erimin 5 tablet samples. This type of tablets are usually encountered on the streets in the form of a tablets strip of an aluminum foil however, the study on description of the aluminium foil could not be done due to most of aluminium foil seized had been marked by investigating officer as the evidence, thus the pictures of these foils are private and confidential. At least, three tablets were taken randomly as a sample for each Erimin 5 tablet seizure. All of the 46 Erimin 5 tablets collected samples were labelled as “001” to “046” as their Identification (ID) number.

4.2 Physical characteristics

A full - colour photograph, is essential for the physical characteristic description of Erimin 5 tablets samples in this study. Photographs provide an accurate and visible representation of the colour, imprint (logo) and the size of the entire tablet. Three representatives of the tablets from each sample were photographed from the front, side and back side. Ruler was introduced in the photograph, in order to estimate the real size of the tablet. The physical characteristics of 46 Erimin 5 tablet samples were analyzed based on their colour, imprint (logo), average weight per tablet, diameter and thickness. The photo and their physical characteristics are shown in table 4.1:-

Table 4.1: Photo and Physical characteristics of 46 illicit Erimin 5 tablet samples




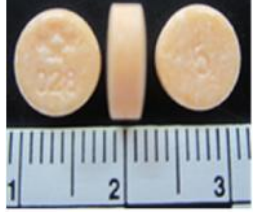

ID No.	Photo	Colour	Average Weight (mg) /tablet	Diameter (mm) ± 0.01	Thickness (mm) ± 0.01
001		Light peach (dull)	179.1	8.63	3.00
002		Peach	185.7	8.02	2.69
003		Peach	188.1	8.06	2.81
004		Peach	189.8	8.00	2.80
005		Peach	191.5	8.04	2.75
006		Peach	185.9	8.00	2.77

Table 4.1: continued





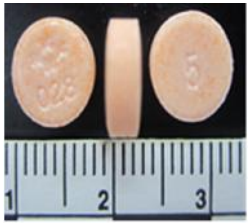

ID No.	Photo	Colour	Average Weight (mg) / tablet	Diameter (mm) \pm 0.01	Thickness (mm) \pm 0.01
007		Reddish Peach	187.8	7.93	2.80
008		Peach	190.1	8.06	2.79
009		Orange	178.2	8.07	2.79
010		Orange	203.3	8.06	2.91
011		Peach	184.0	8.06	2.55
012		Light peach	193.5	8.04	2.95

Table 4.1: Continued







ID No.	Photo	Colour	Average Weight (mg) /tablet	Diameter (mm) ± 0.01	Thickness (mm) ± 0.01
013		Orange	185.5	8.07	2.73
014		Light Peach (Dull)	193.6	8.04	2.95
015		Orange	184.6	8.06	2.64
016		Peach	191.8	8.06	2.83
017		Peach	198.5	8.06	2.87
018		Peach	183.8	8.06	2.63

Table 4.1: Continued







ID No.	Photo	Colour	Average Weight (mg) /tablet	Diameter (mm) ± 0.01	Thickness (mm) ± 0.01
019		Light Peach	194.0	8.12	2.98
020		orange	184.7	8.06	2.64
021		Peach	194.9	8.01	2.80
022		red	192.2	8.03	2.89
023		Orange	188.7	8.03	2.70
024		Peach	181.3	8.03	2.63

Table 4.1: Continued

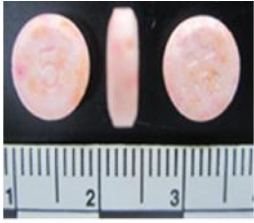





ID No.	Photo	Colour	Average Weight (mg) /tablet	Diameter (mm) ± 0.01	Thickness (mm) ± 0.01
025		Peach with red-yellow dot	183.6	8.28	2.53
026		Peach with red-yellow dot	182.0	8.28	2.44
027		Peach	189.8	8.07	2.73
028		Light Peach	192.7	8.04	2.91
029		Peach	186.5	8.01	2.47
030		Peach	187.2	8.16	2.59

Table 4.1: Continued







ID No.	Photo	Colour	Average Weight (mg) /tablet	Diameter (mm) ± 0.01	Thickness (mm) ± 0.01
031		Peach	192.3	7.91	2.81
032		Peach	192.7	8.13	2.85
033		Peach	195.6	8.10	2.85
034		Light Orange	195.7	8.20	2.80
035		Light peach	193.1	8.04	2.98
036		Peach with red-yellow dot	182.5	8.28	2.64

Table 4.1: Continued











ID No.	Photo	Colour	Average Weight (mg) /tablet	Diameter (mm) ± 0.01	Thickness (mm) ± 0.01
037		Peach with red-yellow dot	187.5	8.31	2.53
038		Orange	186.7	8.13	2.69
039		Orange	185.3	8.01	2.60
040		Peach	184.1	8.09	2.60
041		Reddish Peach	190.1	8.06	2.77
042		Reddish Peach	184.9	8.21	2.63

Table 4.1: Continue

ID No.	Photo	Colour	Average Weight (mg) /tablet	Diameter (mm) ± 0.01	Thickness (mm) ± 0.01
043		Orange	188.5	8.07	2.69
044		Peach	191.3	8.04	2.78
045		Reddish Peach	194.6	8.06	2.80
046		Green	191.2	8.09	2.78

Physical characteristics data of each illicit Erimin 5 tablet sample could be used in order to develop the distribution profile of each physical characteristic. However, among physical characteristics had been described later, only three characteristics could be used to develop distribution profile of this illicit tablets which are average weight of tablet, thickness and diameter since, measurement can be done on these characteristics. Characteristics such logo and colour are difficult to describe and measure since; there are no accurate method to evaluate this characteristics.

4.2.1 Colour

This characteristic is a simple visual examination by naked eye. As colour perception heavily depends on the human eye, an entirely free description of colours would suffer from extreme variation. From table 4.1, it can be seen that out of 46 illicit Erimin 5 tablet samples, 45 were variation of peach to orange and even some are reddish peach in colour, whereas, the remaining one illicit Erimin 5 tablet sample (ID 046) was green.

4.2.2 Logo

The imprint of “5” at one side and “028” with the Sumitomo logo (looks like a four leaved clover) at the other side of the tablet could be observed in all of the 46 illicit Erimin 5 tablet samples. However, there were slight variation in type and size of font (e.g “5”/”028”) as well as the design of Sumitomo logo and the texture of the tablet could be noticed in these illicit tablets as shown as example in figure 4.1 below.

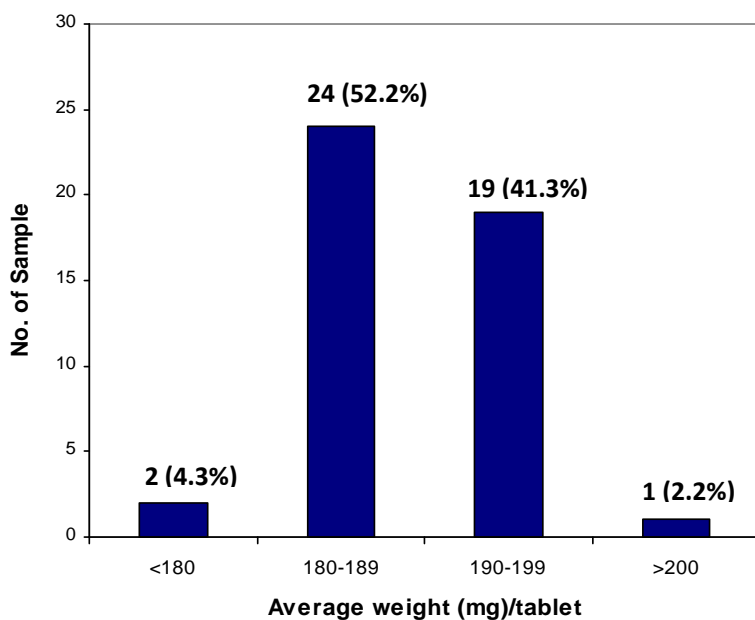


Figure 4.1: Example of the slight variation of logo imprint in illicit Erimin 5 tablet samples from the Left: tablet ID 003, 009, 026 and 007

4.2.3 Average Weight per tablet

Average weight per tablet for each illicit Erimin 5 sample was calculated and recorded in milligram. Average weight per tablet was selected instead of the weight of one tablet, due to the average weight per tablet is found to be more accurate to represent the weight of one tablet for the whole number of illicit Erimin 5 tablets in each sample. In this study, 4-decimal analytical balance within ± 0.1 mg tolerance was used.

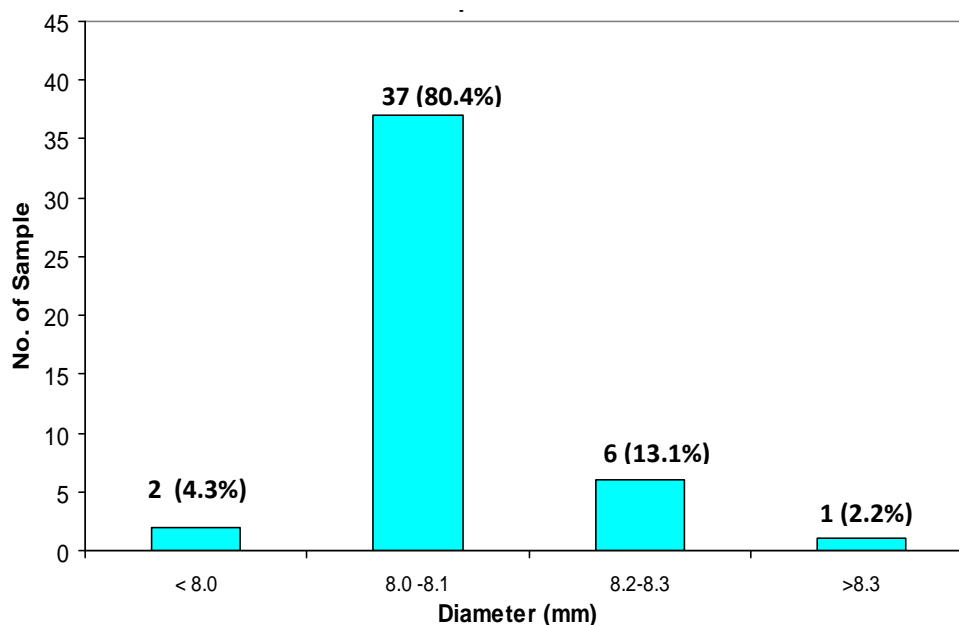
From table 4.1, the averages weight per tablet were distributed in a range of 178.2 mg (minimum) to 202.3 mg (maximum) with mean of 188.9 mg. Most of the illicit Erimin 5 tablet samples (24/46 @ 52.2 %) had the average weight per tablet between 180 to 189 mg (mode) followed by the group of average weight of tablet between 190 to 199 mg (19/46 @ 41.3 %). The tabulation of the average weight per tablet of the illicit Ermin 5 tablet samples is illustrated in graph 4.1 below: -



Graph 4.1: Distribution profile of average Weight per tablet in 46 illicit Erimin 5 samples.

4.2.4 Diameter

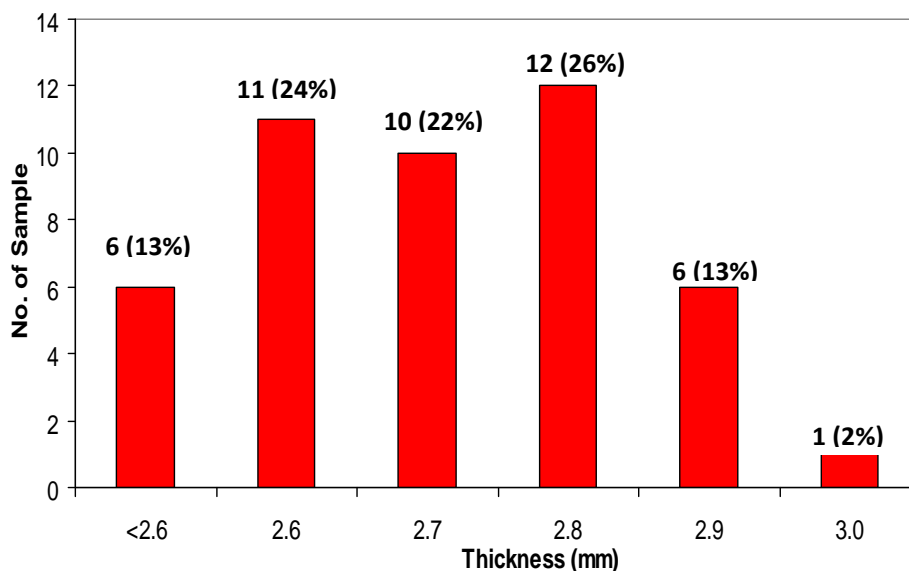
The diameters of 46 illicit Erimin 5 tablet samples were measured in millimeter (mm) using digital calipers within ± 0.1 mm tolerance. In this study, for each sample, the diameter was measured once (using only one tablet) as represent the whole tablets based on the assumption that all the tablets in the same batch sample had an uniform thickness as the same machine was used during their production. The range of the diameter of these tablets were found between 7.91 (minimum) to 8.63 mm (maximum) with the mean of 8.09 mm. Most of the illicit Erimin 5 tablets samples (37/46 @ 80.4 %) seized were noticed in the group of 8.0 - 8.1 mm diameter. The least number of illicit tablets sample were found in the group of diameter less than 8.0 mm (2/46 @ 4.3%) and diameter more than 8.3 mm (only 1 tablet sample). The following graph gives the statistical distribution profile of the diameters in 46 illicit Erimin 5 tablets samples.



Graph 4.2: Distribution profile of tablet diameter (mm) in 46 illicit Erimin 5 tablet samples

4.2.5 Thickness

In this study, only one thickness measurement (using only one tablet) was taken by digital calipers as a representative of the entire of each Erimin 5 tablet samples. It was recorded in millimeter (mm) within ± 0.1 mm tolerance. The thickness of the 46 illicit tablet samples were ranged in between of 2.44 (minimum) to 3.00 mm (maximum) with the mean of 2.75 mm. Most of these tablets samples had 2.8 mm in thickness (12/46 @ 26%), followed by 2.6 mm (11/46 @ 24%) and 2.7 mm (10/46 @ 22%). Only one tablet sample (ID 001) was observed to have the maximum of thickness value 3.00 mm. The distribution profile of illicit tablets thickness is shown in graph 4.3:-



Graph 4.3: Distribution profile of tablet thickness (mm) in 46 illicit Erimin 5 tablet samples

It could be seen that distribution of the thickness are larger compared to the distribution of diameter. The logic explanations are due to each tablet making machine has a fixed number of positions and each position has a certain diameter. The thickness of the tablets depends on the quantity of the powder and the pressure. The quantity of the powder is a variable factor. The pressure of the press is generally not changed and therefore this factor is a constant (C. Weyermann et al., 2008).

4.3 Chemical characteristic

In this study, chemical characteristic of illicit Erimin 5 tablet samples were categorized according to their active ingredients and adulterants, major diluents and dyes. The others chemical characteristic which also observed in this study were the percentage of Nimetazepam (active ingredient) and the weight of Nimetazepam per tablet. All of these chemical characteristic as mention, could provide useful information to trace the links between seizures and also connection between tablet seizures and chemicals found in clandestine laboratories (Ahmad Fahmi et al., 2012).

4.3.1 Identification and Confirmation of Active Ingredient and others Adulterant

The active ingredient and their adulterants presence in all of 46 illicit Erimin 5 tablet samples were identified and confirmed using Gas Chromatography Mass Spectrometer (GCMS) using EI mode. Gas chromatography (GC) is a powerful separation technique for volatile compounds. The combination of GC with powerful detection capabilities of Mass spectrometer provide the good separation technique with GCMS had been chosen due its versatility, ease to handle and the cost of this equipment is relatively cheaper compared to others confirmation technique such as LCMSMS and GC-IR.

Benzodiazepines group such as Nimetazepam, Diazepam, Nitrazepam and others can be conveniently identified by GCMS and analyzed directly without require of any derivatization technique from a solid free methanol extract of the drug sample (Michael D Cole, 2003).

Mixture of Methanol/Chloroform (5:1) was used as the solvents in this analysis. Methanol was chosen as the most fraction solvent in this study since, most of benzodiazepines groups and adulterants such as caffeine and menthol dissolve in methanol. The chloroform was added to into methanol to better solubilize the tablet materials and had no adverse effects on the chromatography (YK Chong et al., 2004). Most of the major tablet components such as lactose, starch, magnesium stearate (excipient which consists of diluents, binders and lubricants) are practically insoluble in chloroform (K.B. Chan et al., 2011). Some diluents such as sugar alcohol (sorbitol, mannitol etc,) are slightly soluble in methanol.

The temperature programming was used to obtain a good separation and the optimum resolution of the interested compounds (active ingredient and adulterants) presence in the samples. The temperature was started with 80°C (hold for 3 min.) then, ramped at 20°C/min. to 270 °C (hold for 10 min) allowed GCMS to scan from high to low volatility of the interested compound. The high volatility compounds were eluted at low temperature (e.g menthol, caffeine etc), whereas moderate to low volatility compounds were eluted later especially commonly benzodiazepines groups presence in the illicit Erimin 5 tablets for example Diazepam, Nimetazepam and Nitrazepam.

A mixture of reference standards which containing 5 active ingredients and diluents commonly found in the illicit Erimin 5 tablets was injected onto GCMS for comparison. The GCMS result showed all of compounds peaks had a good separation

and resolution using this specified method. Menthol was first eluted at RT 5.472 min followed by, caffeine, Diazepam and Nimetazepam. Nitrazepam was found to be the last elution at 18.408 min. The Total ion Count (TIC) chromatogram of these mixture reference standards is shown in figure 4.2 and the summary is tabulated in table 4.2.

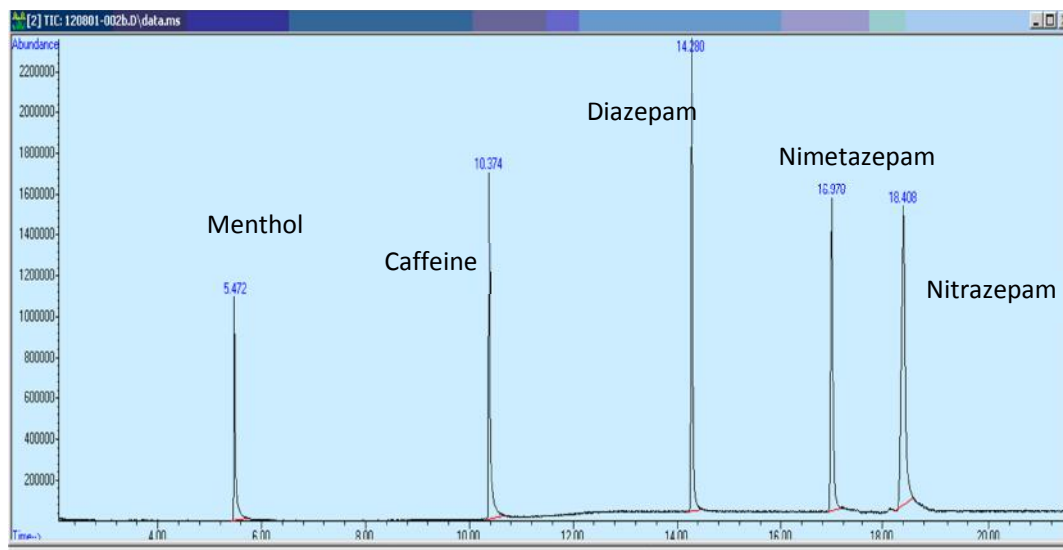


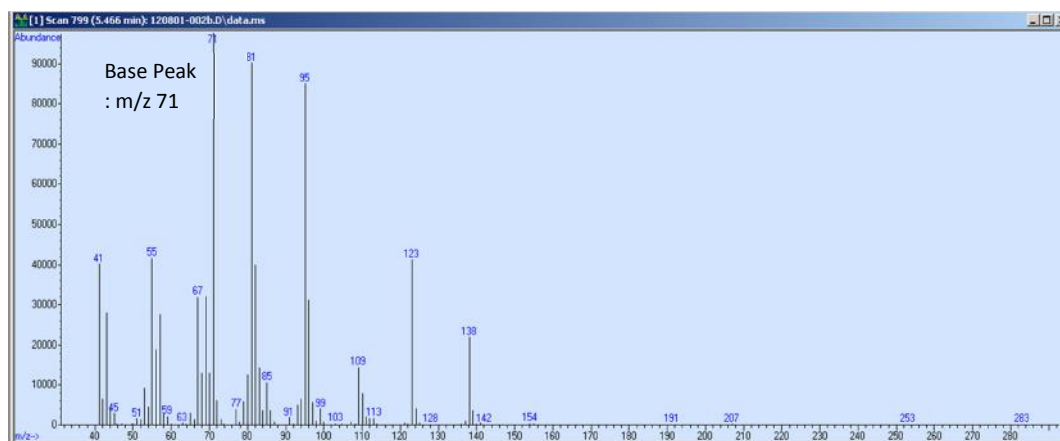
Figure 4.2: TIC Chromatogram of mixture reference standard of menthol, caffeine, Diazepam, Ninetazepam and Nitrazepam

Table 4.2: Retention time (RT) for each compound in the mixture of Reference Standard.

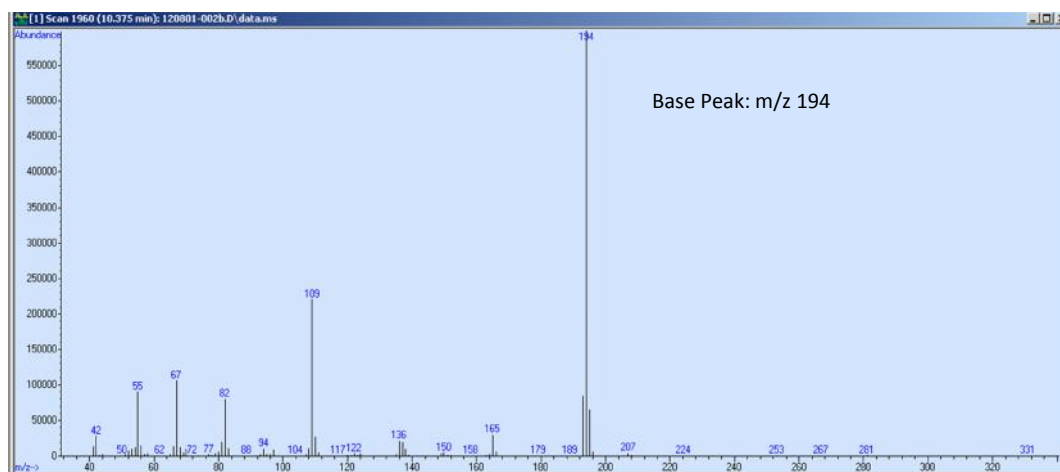
<i>Peak</i>	<i>Retention Time (minute)</i>	<i>Compound</i>
1	5.472	Menthol
2	10.374	Caffeine
3	14.280	Diazepam
4	16.978	Nimetazepam
5	18.408	Nitrazepam

Each compound has unique mass spectrum profile which can be used as a fingerprint for their confirmation. Electron Ionization (EI) mode was chosen as ionization technique since it provides more fragmentation which leads to structurally informative of the interested compound compare to others technique such as Chemical Ionization (CI) mode. Other benefit of EI mode is more commercial spectral library with full fragment of the compound have been developed as reference. Mass spectrum of each compound is introduced in figure 4.3 (a) to (e).

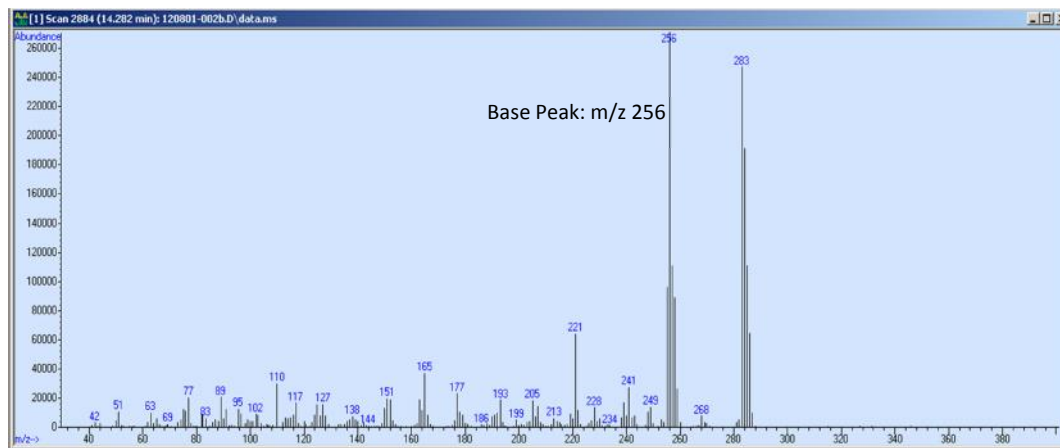
(a) Menthol RT 5.472 min.



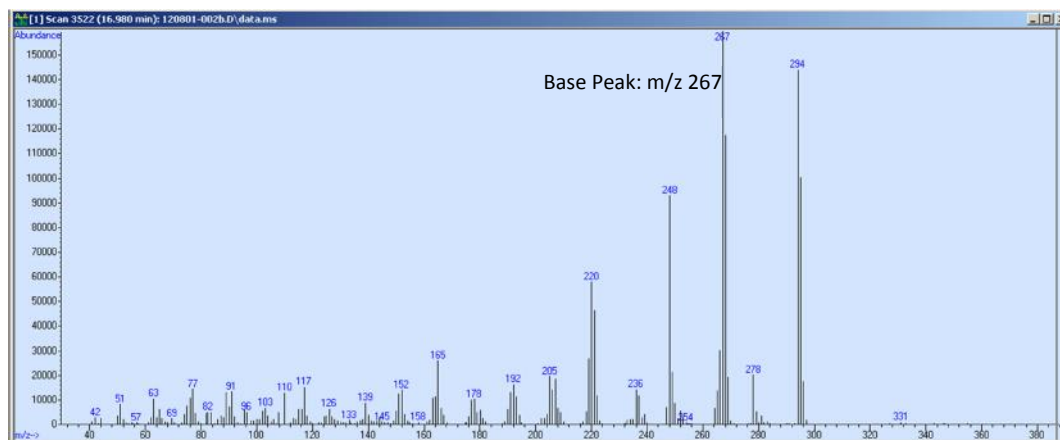
(b) Caffeine RT 10.374 Min.



(c) Diazepam RT 14.280 min.



(d) Nimetazepam RT 16.978 min.



(e) Nitrazepam (RT 18.408 min)

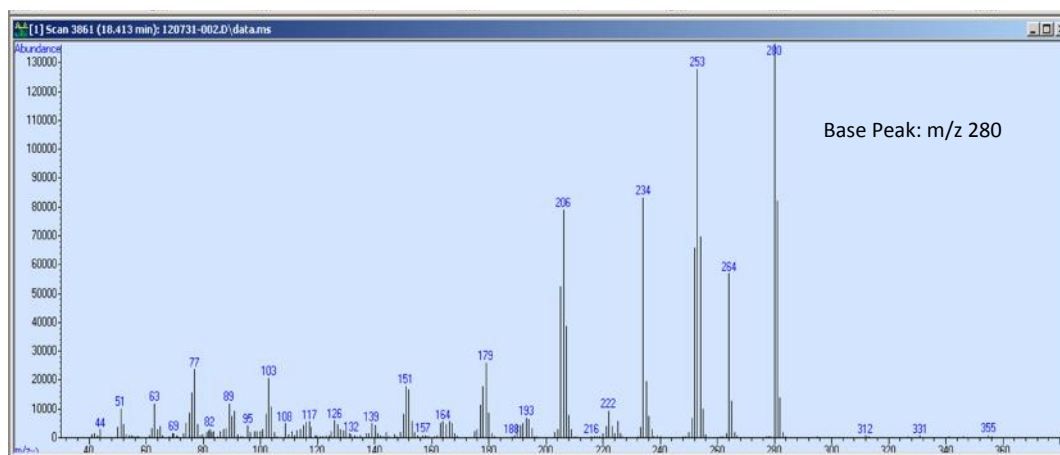


Figure 4.3: Mass spectrum of the interested compounds which are (a) Menthol (b) caffeine (c) diazepam (d) Nimetazepam and (e) Nitrazepam

The retention time of GCMS eluted peaks and the profile of mass spectrum of the each peak obtained from illicit Erimin 5 tablet samples were then compared with retention time and mass spectrum reference standards of interest (5 commonly active ingredients and adulterants found in illicit Erimin 5 tablets were chosen). Mass spectrums of the eluted peaks were also searched against the library spectra for identification and confirmation.

Generally, Nimetazepam alone as active ingredient had been detected in most of samples (42/46 @ 91%). The remaining samples contained Diazepam (1/46 @ 2%) and Nitrazepam (minor) together with Caffeine as a major compound (3/46 @ 7%).

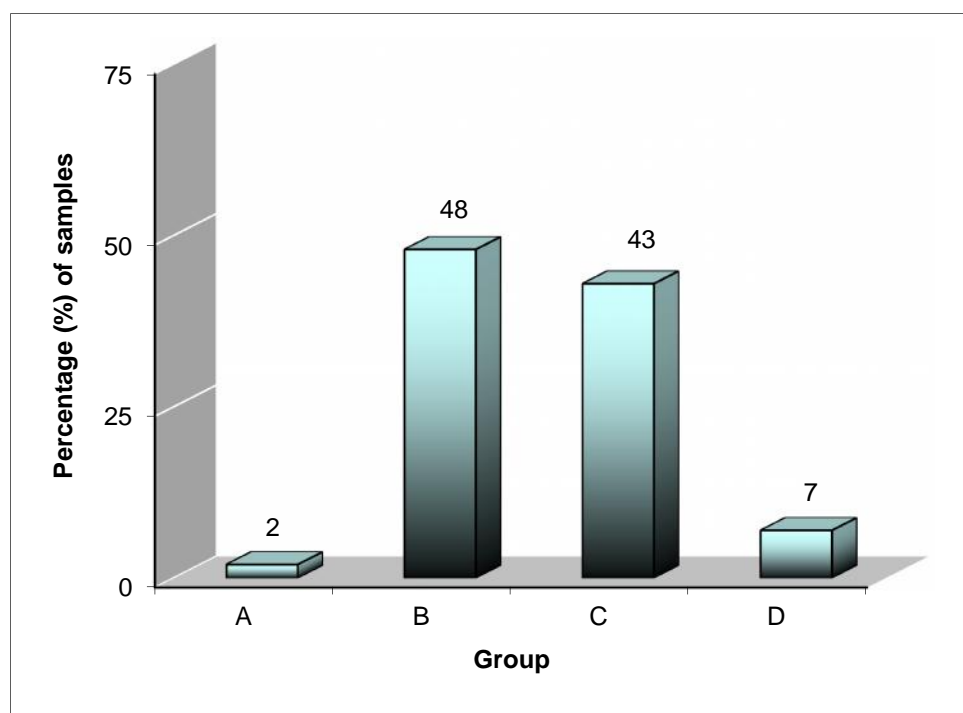
It could be noticed that some Nimetazepam's samples chromatograms profile (22/46 @ 48%) contained extra peaks at retention time around 10.4 min to 11 min. Menthol was not detected in any samples although it had been appeared in Erimin 5 tablet in the past. Fatty acids were detected at retention times of around 10.8 min (hexadecanoic acid) and 11.8 min (octadecanoic acid) in some of samples however, it couldn't be used as characterized factor, as it did not found in others samples even though, fatty acids were frequently used as lubricant in tablet production. Thus, this method was not suitable and accurate in determination of fatty acids.

Ines Baer et al. (2007a) had reported that fatty acids need to be transformed into volatile compounds by formation of fatty acid methyl ester (FAME) in order to analyze by GC. In their study, In situ transesterification method was found to be appropriate method to analyze fatty acids. Besides that, several other methods in analysis of fatty acids also had been cited in the literatures. TIC chromatogram of each tablet sample can be referred to APPENDIX 1.

As summarize, four different profiles chromatograms could be characterized from 46 illicit Erimin 5 tablet samples as shown in table 4.3 and graph 4.4.

Table 4.3: The percentage of illicit Erimin 5 samples according to their chromatogram's profile and active ingredient categorized.

Group	Compound detected	Number of sample	% Number of sample
A	Diazepam	1	2%
B	Nimetazepam with profile 1	22	48%
C	Nimetazepam with profile 2	20	43%
D	Nitrazepam (minor) and caffeine (major)	3	7%
Total		46	100%



Graph 4.4: The characterized of illicit Erimin 5 samples according to their chromatogram's profile and active ingredient/adulterant.

In group A, it was observed that diazepam was found to be active ingredient which eluted at 14.278 min in one tablet sample (ID 001). The TIC chromatogram's profile for group A is shown in figure 4.4 below:-

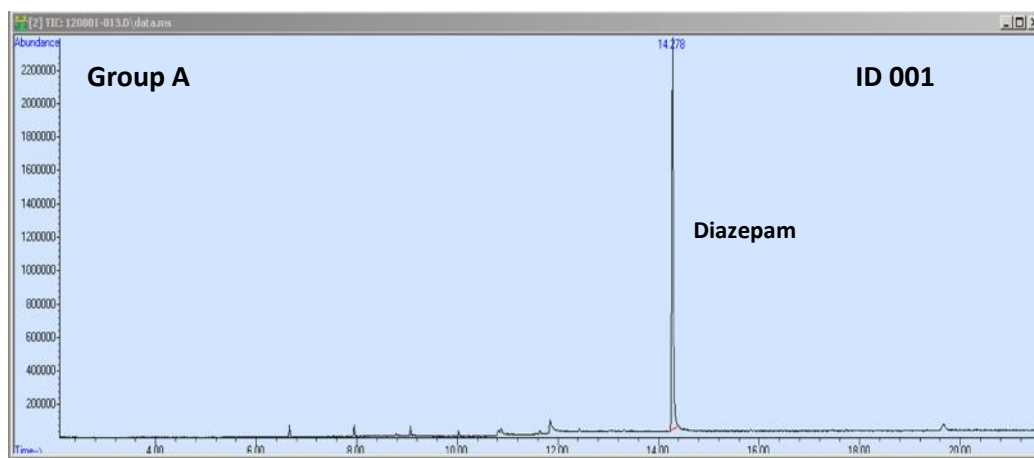


Figure 4.4: TIC GCMS chromatogram profile of group A.

Nimetazepam was detected as active ingredient in group B and C however, their TIC chromatogram's profiles were slightly different which in group B, extra peaks were observed at retention time between 10.50 min to 10.80 min. The TIC chromatogram's profile of group B and group C are shown in figure 4.5 (a) and (b) respectively.

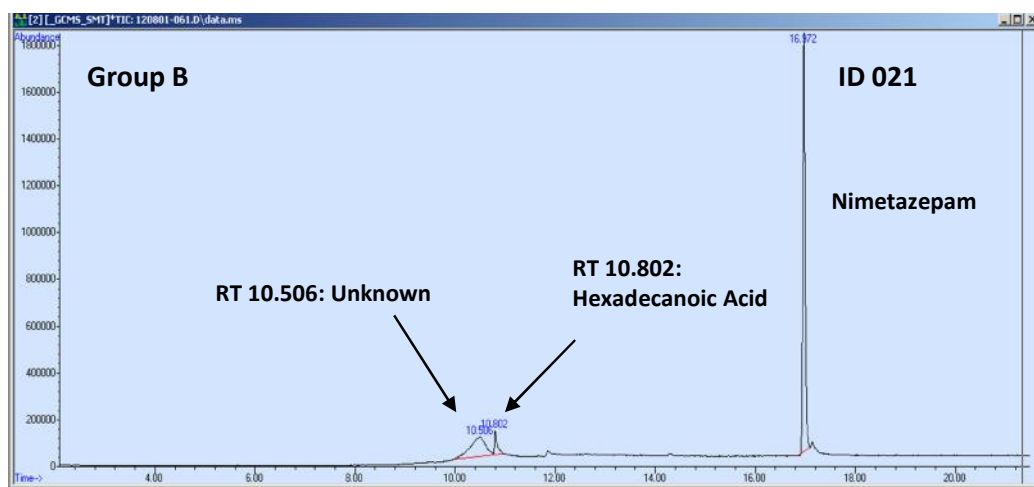


Figure 4.5 (a): TIC GCMS chromatogram profile group B.

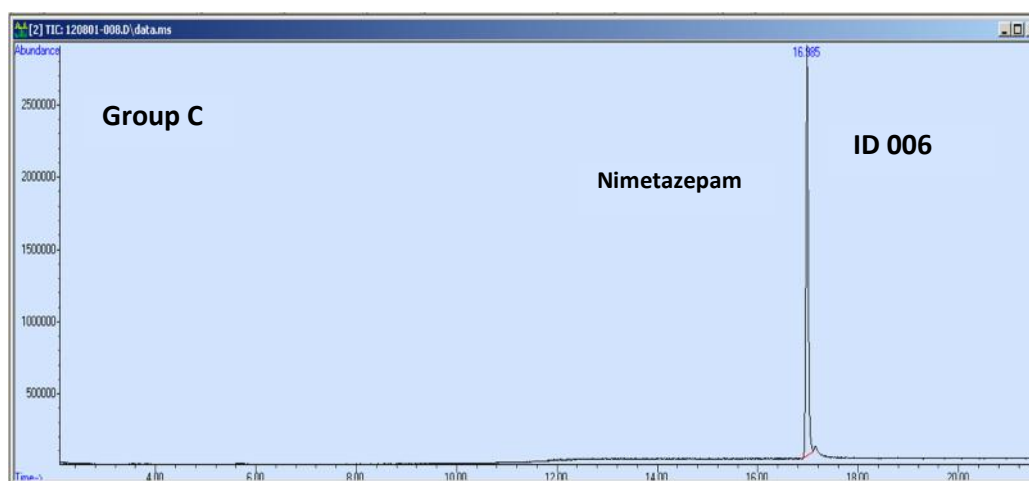


Figure 4.5 (b): TIC GCMS chromatogram profile of group C.

As we can see in example of chromatogram profile of group B (ID:021) as shown in figure 4.5 (a), the unknown peak was eluted at retention time 10.506 min and Hexadecanoic acid eluted at 10.802 min. Mass spectrum of each peak are shown in figure 4.6 (a) and (b) respectively. The unknown peak might be attributed to the interactions of hydrogen bonding between the hydroxyl groups of sugar alcohol/sugar groups (diluent) and hydroxyl groups of the methanol (solvent).

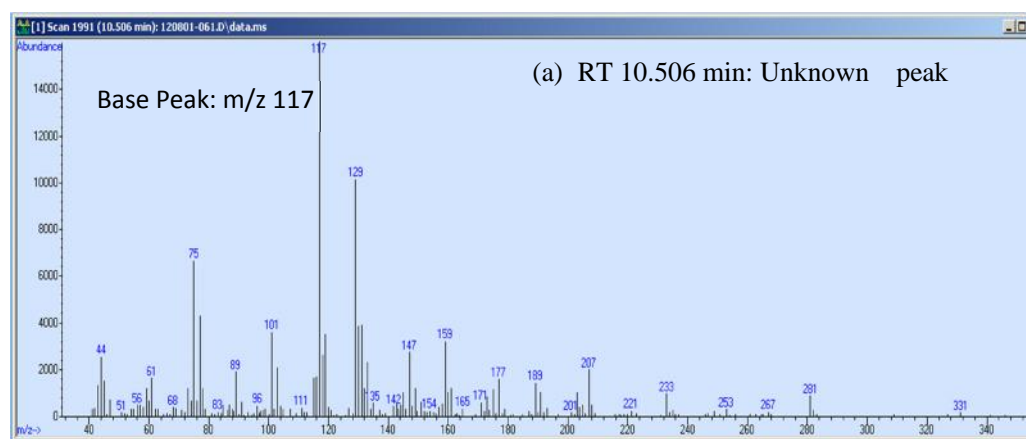


Figure 4.6(a): Mass spectrum of unknown peak in TIC chromatogram profile of group B

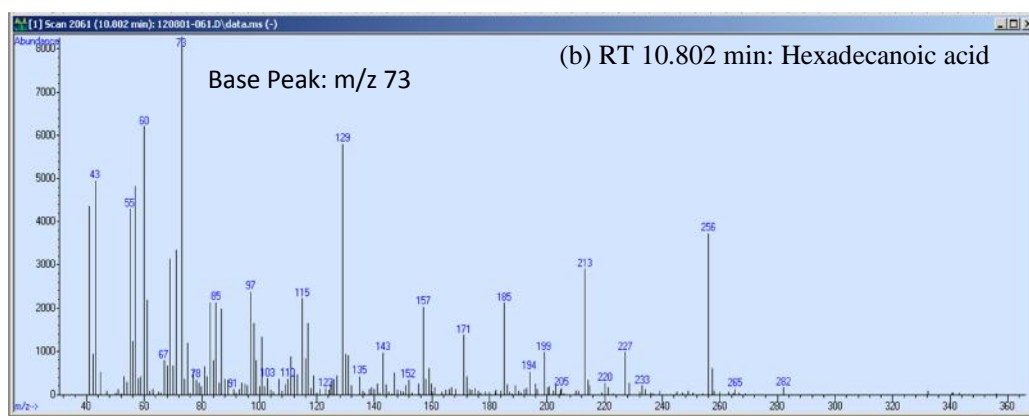


Figure 4.6(b): Mass spectrum of Hexadecanoic acid peak in TIC chromatogram profile of group B

For group D, caffeine was found as major compound together with Nitrazepam (minor). This profile could be seen in three tablets samples with ID 010, 011 and 030. However, it was noticed that fatty acids were detected in tablet samples with ID 010 and 011 while, in tablet sample ID 030 no fatty acids peak was observed. Example of TIC chromatogram profile of group D is shown in figure 4.7

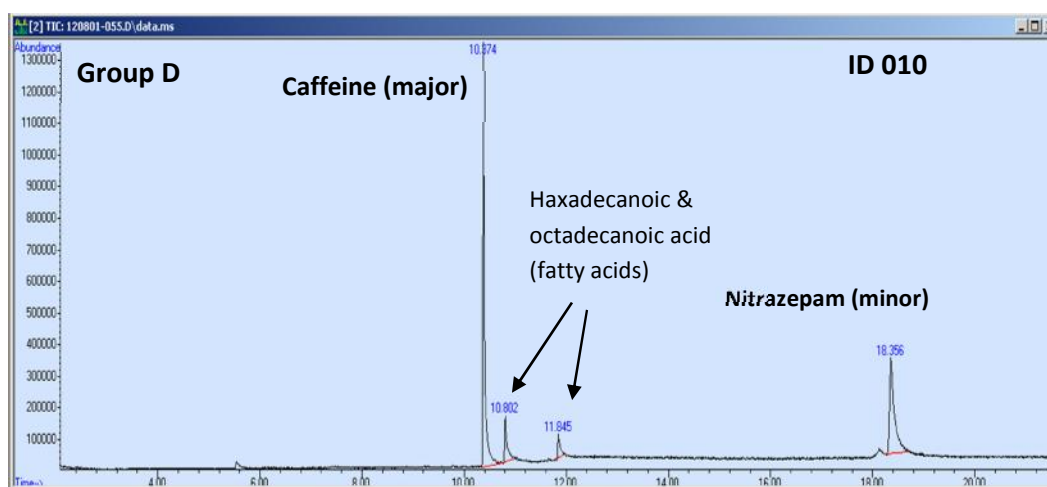


Figure 4.7: GCMS TIC chromatogram Profile of group D

The finding results in this study shows the occurrence of Nimetazepam, Diazepam, Nitrazepam and Caffeine as active ingredients and adulterants in 46 illicit Erimin 5 samples are consistent with the findings of several studies on Erimin 5 tablets had been done by other researchers.

Nimetazepam and others benzodiazepines such as Nitrazepam and Diazepam were reported presence in the illicit Erimin 5 tablets seized in Singapore. Other adulterants such as caffeine, menthol, melatonin and carbamazepine were also encountered. Meanwhile, Methamphetamine, Ephedrine and Paracetamol also were detected in one of pink Erimin5 tablets seized in Singapore in June 2006 (Drugnet Asia, 2006). Yong Kiong Chong et al. (2004) reported that few samples of Malaysian “Erimin-5” tablets had been found to contain Diazepam instead of Nimetazepam.

It can be seen that the recent profile of illicit Erimin 5 samples, do not contain much diluents such as caffeine and menthol as in the past. The same trends were also observed in 2010 and 2011 samples which almost samples contained either Nimetazepam or Nitrazepam or Diazepam alone or combination as active ingredients with absence of menthol or caffeine. It is not known whether, the same recent trend has been found in other Asia country such as Singapore, Indonesia and Brunei. Figure 4.8 below shows an example of chromatogram of illicit Erimin 5 sample which was seized in 2005 contained caffeine (diluent) as major component instead of Nimetazepam as minor components.

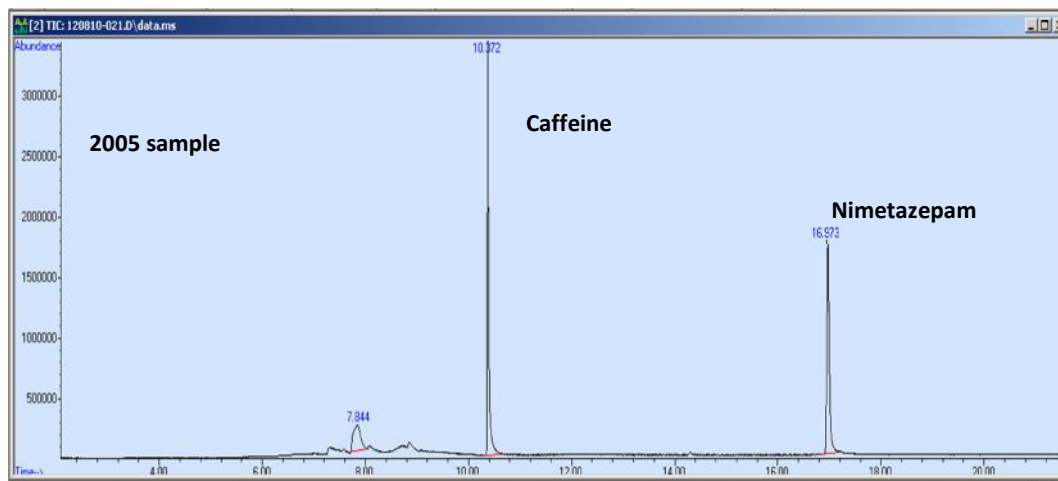


Figure 4.8: Profile of GCMS TIC chromatogram occurred in sample seized in 2005

The changing of the trends of adulterant added is may be due the market price of caffeine or menthol or other adulterant increase and not worth it to add up into the bulk. Others possibility is may be due to the strict procedure requirement in purchasing of Caffeine as it has been controlled under Malaysian Poison Act 1952 since 2006. Discontinued of caffeine and other commonly adulterant, the illicit Erimin 5 tablet manufacturer may switch to mark up with the more quantity of other diluents such as glucose, lactose, mannitol, starch, sorbitol and etc. which are relatively cheaper and easy to retrieval since they are not controlled as caffeine.

4.3.2 Quantification of Nimetazepam (active ingredient) by High Performance Liquid Chromatography (HPLC)

From GCMS results, it showed that out of 46 illicit Erimin 5 tablet sample, 42 were confirmed to contain Nimetazepam as active ingredient. All of these 42 illicit Erimin 5 tablet samples had been continued with quantitation analysis of Nimetazepam by HPLC with Photodiode Array (PDA) detector.

Only quantitation of Nimetazepam had been done since, it is the only benzodiazepine commonly encountered in the illicit Erimin 5 tablet which has been controlled under Dangerous Drug Act 1952. The Nimetazepam content (percentage) enabled the comparison of tabulation weight of Nimetazepam per tablet in illicit tablet samples with the genuine/commercial Erimin 5 tablet (contain 5 mg Nimetazepam per tablet).

In this study, HPLC with PDA detector was chosen to ensure that interference from tablet excipients (diluent, binders and lubricants) such as sugars, sugars alcohol, fatty acids did not interrupt the quantitation of Nimetazepam in the sample. HPLC provides good separation and resolution of most of benzodiazepines group such as Nimetazepam. Combination with PDA as detector instead of UV provides simultaneous measurement of absorbance at all wavelength in the UV/Visible spectrum. Hence the chromatographic peaks can be displayed at any selected wavelength after the compound has eluted from the column. The best wavelength which shows the greatest peak area/height provides the maximum sensitivity as example 265 nm was chosen in this study.

This method provides a broad linear dynamic range between 0.020 mg/mL to 0.240 mg/mL with correlation coefficient, $R^2 > 0.99$) and good precision of results (CV < 5%). Single point calibration was used with concentration of 0.12 mg/mL Nimetazepam using external method. The calibration curve of Nimetazepam analysis is shown in figure 4.9 below.

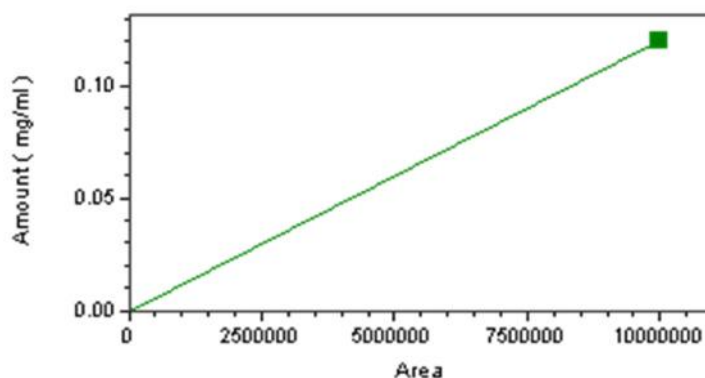


Figure 4.9: Calibration curve of Nimetazepam

In this method, it could be seen that Nimetazepam peak was eluted around 7.7 min at flow rate of 1.5mL/min. of isocratic elution of methanol:water (50:65) mobile phase. The Retention time of Nimetazepam peak in illicit tablet samples, were compared to retention time of Nimetazepam reference standard peak. Examples of HPLC chromatogram of Nimetazepam peak in Reference standard and in illicit tablet sample are given in figure 4.10:-.

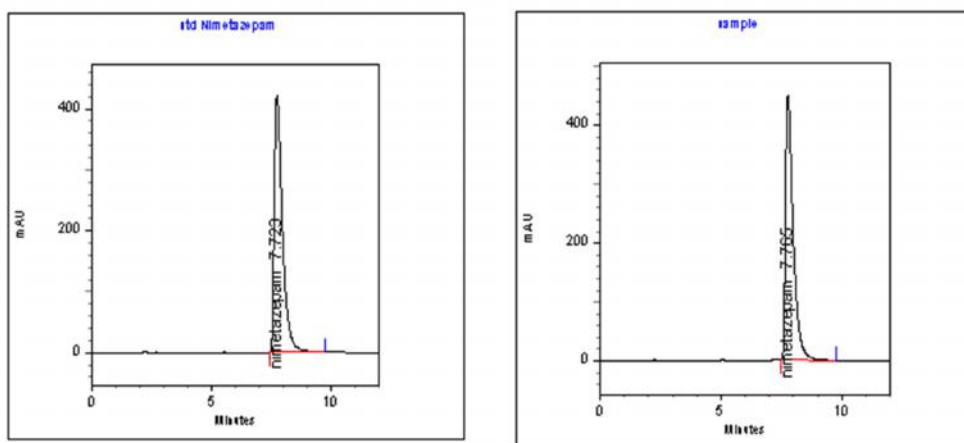


Figure 4.10 : HPLC chromatogram of Nimetazepam Reference Standard eluted at 7.723 min.(left) and Nimetazepam in sample (ID 005) eluted at 7.765 min. (right).

Besides retention time, the use of PDA as detector has its own advantage which provides the UV spectra information to each eluted peak in the time frame of analysis. This lead to useful information which permit the identification/confirmation of Nimetazepam or others eluted compound as the UV spectra is unique for each compound. Below are the example UV spectra of Nimetazepam peak in standard and sample (ID 005) as shown in figure 4.11 :-

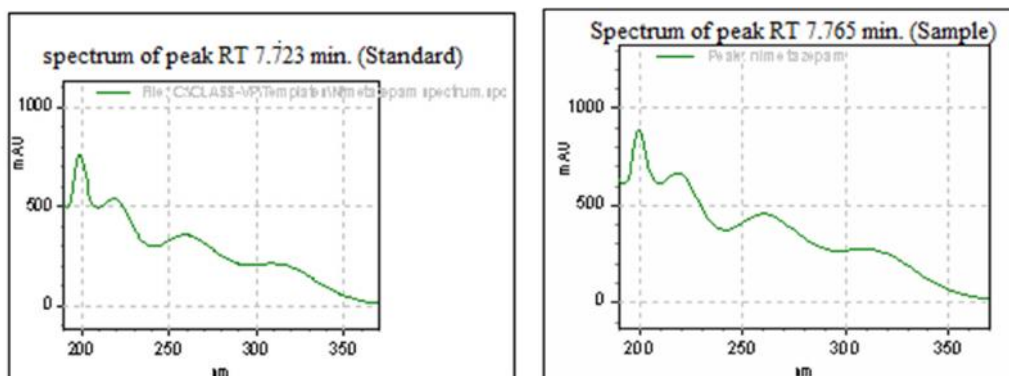


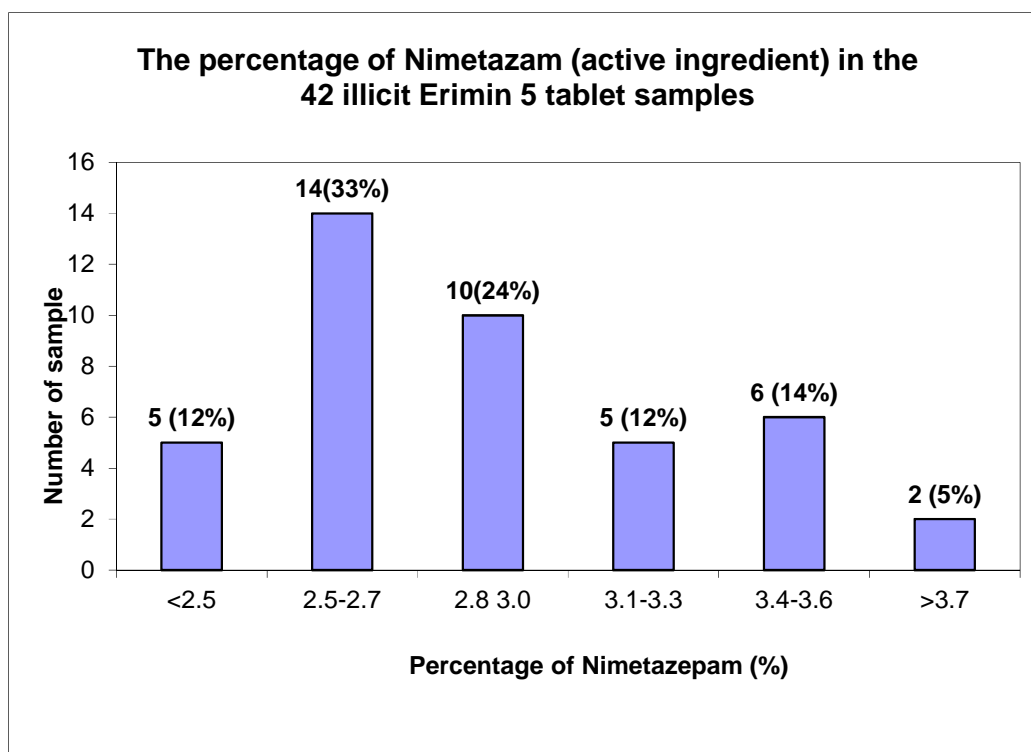
Figure 4.11: UV spectrum of Nimetazepam obtained in standard (left) and sample (right) at retention time mention in figure 4.10.

Since all of 42 illicit Erimin 5 tablet samples contained only Nimetazepam and none of other adulterant such as caffeine or extra active ingredient (for example Nitrazepam and Diazepam) were detected by GCMS, hence, their HPLC chromatograms profile were similar each other which only the appearance of Nimetazepam peak at retention time around 7.7 min .If Caffeine, Diazepam, Nitrazepam or other benzodiazepines presence in the tablet samples, it is still could be eluted within the time frame analysis of this method.

Nevertheless, adulterant such as menthol and diluents for example sugars groups (lactose, glucose, sucrose and etc.) and sugar alcohol groups (e.g mannitol,sorbitol etc.) were not detected by PDA detector. Menthol could't be analyzed by PDA detector due to the lack of chromophore in the structure of menthol, it shows very limited absorbance in the ultraviolet region. As a result, it is very difficult to analyze menthol with HPLC consisting of UV detector (KA Shaikh et al., 2010).

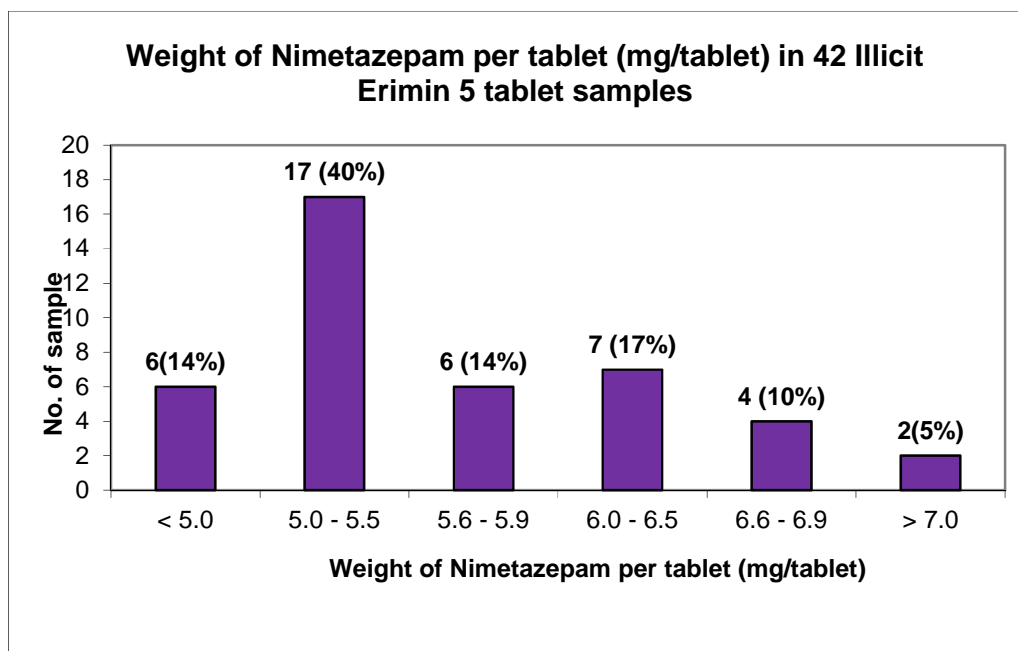
For sugar alcohol groups, due to its high polarity and lack of UV absorbing chromophore, thus require unique methods for analysis for example ion exclusion chromatography HPLC (Michael McGinley). Whereas, sugars do not absorb UV light at a wavelength longer than 200 nm (Mustafa Karkacier et al., 2003), hence, sugar do not exhibit characteristic absorbance in useful region of the UV spectrum.

Overall, the percentage of Nimetazepam found in 42 illicit Erimin 5 tablet samples were varied between 2.10% (minimum) to 4.16% (maximum) with average of 2.99%. It is noticeable that the vast majority (29/42 @ 69%) of Erimin 5 tablet samples were found to contain Nimetazepam with percentage less than 3.1%. About one third of Erimin 5 tablet samples (14/42 @ 33 %) contained a range of 2.5% – 2.7 % of Nimetazepam (mode) followed by, a group with range of 2.8% – 3.0 % of Nimetazepam which found in 10 tablet samples. The least number of samples (2/42 @ 5%) were observed in a group of percentage of Nimetazepam which more than 3.7%. The examples of HPLC chromatograms and calculation of percentage of Nimetazepam are shown in APPENDIX 2. The tabulation of the percentage of Nimetazepam found in 42 illicit Erimin 5 tablet is shown in graph 4.5.



Graph 4.5: Tabulation of the percentage of Nimetazepam (as active ingredient) in the 42 illicit Erimin 5 tablet samples.

Instead of the percentage of Nimetazepam, other chemical characteristic that could be seen from HPLC result is the weight of Nimetazepam per tablet. As mention earlier, the genuine/commercial Erimin 5 tablet contains 5 mg of Nimetazepam per tablet. This characteristic could be computed by times the percentage of Nimetazepam found in the tablet with the average weight (in mg) per tablet (in one tablet). The results show that the weights of Nimetazepam per tablet were varied from 4.1 mg per tablet (minimum) to 7.6 mg per tablet (maximum) with the mean of 5.6 mg per tablet. The majority of the illicit Erimin 5 tablets samples (17/42 @ 40%) contained 5.0 to 5.5 mg of Nimetazepam per tablet. Whereas, the minority belonged to weight Nimetazepam per tablet more than 7 mg per tablet which were only found in 2 tablet samples (2/42 @ 5%). The distribution weight of Nimetazepam per tablet is illustrated in graph 4.6. The full data of the percentage of Nimetazepam and the weight of Nimetazepam per tablet for all illicit tablet samples is shown in APPENDIX 3 as reference.



Graph 4.6: The distribution profile of weight of Nimetazepam per tablet in 42 illicit Erimin 5 tablet samples.

4.3.3 Identification and Confirmation of major Diluent

The major constituent of any illicit tablet include illicit Erimin5 tablets is diluent which their concentration may vary and up to 80% of the bulk material. Due to its high concentration thus, direct infrared examination of the powdered tablet material rapid indication of the diluent present. Common pharmaceutical diluents such as lactose, glucose, sucrose, mannitol, calcium phosphate, starch etc. could be used in the illicit tablet production. Sometimes a mixture of diluents may presence (P.J. Gomm et al., 1975). In this study, HazMat ID portable Fourier Transform Infra Red (FTIR) spectrometer with Attenuated Total Reflectance (ATR) technique was used to determine major diluent used in 46 illicit Erimin 5 tablet samples.

Absorption bands in the Infra Red (IR) spectra originate primarily from the vibration, stretching and bending modes within molecules. An IR spectrum shows the characteristic properties of a compound. It provides a fingerprint for identification and is a powerful tool for the study of molecular structure. The region between 3600 cm^{-1} to 1250 cm^{-1} provides functional group information whereas, the fingerprint region between 1200 cm^{-1} to 600 cm^{-1} shows small differences in the structure and constitution of a molecule. Consequently, a close match between two spectra (samples spectra and reference standard of interested compound spectra/library hits spectra) in the fingerprint region (as well as others) constituents almost evidence that the compounds are identical (Skoog, Holler & Crouch, 2008).

FTIR is the most modern IR spectrophotometer which operates on a different principle with the employed of interferometer compare to the use of grating monochromator (dispersive technique) or filter (non - dispersive technique) in previous IR spectrophotometer. In this technique, Fourier transform (a mathematical process)

convert the raw IR data into the actual FTIR spectrum. FTIR spectrometer instrument have several advantages such as speed of analysis, reliability, signal to noise advantage and convenience (Skoog, Holler & Crouch, 2008).

FTIR is a good technique for identification of major compound of a mixture sample as well as pure sample. However, sometimes it may difficult to interpret a complex IR spectra obtained from a mixture compound in sample as example, a mixture of diluents which their concentration slightly the same in the bulk material may produce a mixture profile spectrum of diluents. As a result, the identity of the actual diluents used is hardly to be determined. Otherwise, if possible, other physical separation technique such as solubility difference might be used before running the FTIR. However, sometimes solubility properties of the combination diluents are similar, thus this technique could n't be applied. The aid of others techniques such as Scanning Electron Microscope with Energy Dispersive X-ray (SEM-EDX) and X-Ray Diffraction (XRD) may help to identify the major diluent used.

FTIR also has the capability differentiate between two isomers such as mannitol and sorbitol (only difference at the orientation of the hydroxyl group on carbon-2) which are commonly diluents used in illicit tablets.

Attenuated Total Reflectance (ATR) technique is based on reflectance technique which provides information on the spectra absorbed by the sample surface. In this technique, IR radiation is directed into the sample which is placed closely on the surface of the prism (ATR crystal) and then, total reflected beam is collected by detector. ATR technique is a simple technique with no sample preparation requirement, quick and easy handling compared to common FTIR absorption method which need sample preparation such as KBr and Mulls for solid sample.

In this study, the result shows that just over a half (52% @ 24/46) of 46 illicit Erimin 5 tablet samples contained Lactose as their major diluent. Besides that, Mannitol as major diluent had been encountered in others 39% (18/46) illicit tablets. The remaining 9% (4/46) of illicit tablet sample contained a mixture profile spectrum of diluent and others excipient.

It could be seen that the broad absorption band was observed at the region between 3500 cm^{-1} to 3200 cm^{-1} in all illicit Erimin 5 tablet samples. This principle peak attribute to OH vibrational stretching which broad due to inter molecular hydrogen bonding. This functional group (hydroxyl group, OH) information indicates that generally either single or combination of diluents such as sugars/carbohydrates (e.g sucrose, glucose, lactose, starch, cellulose, etc.) or sugars alcohol groups (e.g mannitol, sorbitol etc.) were used. The fingerprint region between 1200 cm^{-1} to 600 cm^{-1} provides useful specifically information about the molecules skeletal structures of the diluent which lead to final identification.

Example of IR spectrum of sample which Lactose had been detected as major diluent, (tablet ID 001) is shown in figure 4.12. The region between 1300 and 1000 cm^{-1} corresponds to asymmetric C-O or C-O-C stretching vibrations (Pavia, Lampmann and Kriz, 2000). The principle peaks could be observed at wave numbers 1028, 1091, 875 and 898 cm^{-1} respectively, which are characteristics bands of lactose. These peaks accordance with the literature (Terry Mills and J. Conrad Roberson, 1987) with two additional characteristic of strong band were seen at wave numbers 1016, 1070 cm^{-1} . The two bands at wave numbers 875 and 898 cm^{-1} respectively were attributed to out of plane C-H bending (Miguel L. et al., 1995).

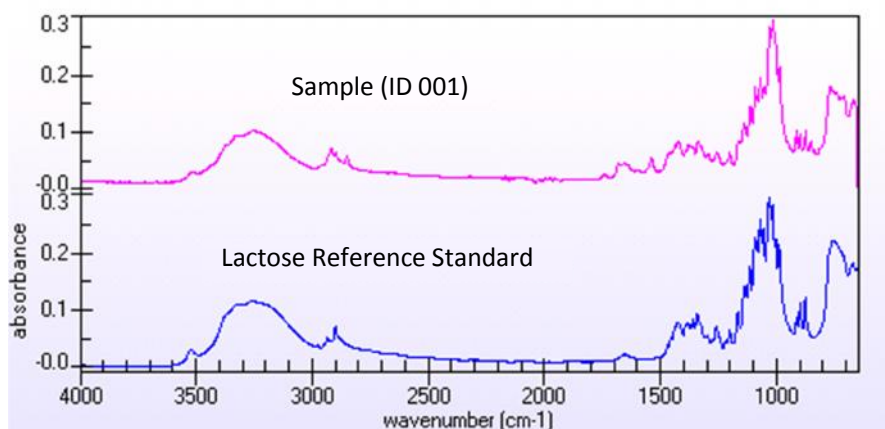


Figure 4.12: IR spectrum of sample (ID 001) which lactose as major diluent (above) and Lactose Reference Standard (below).

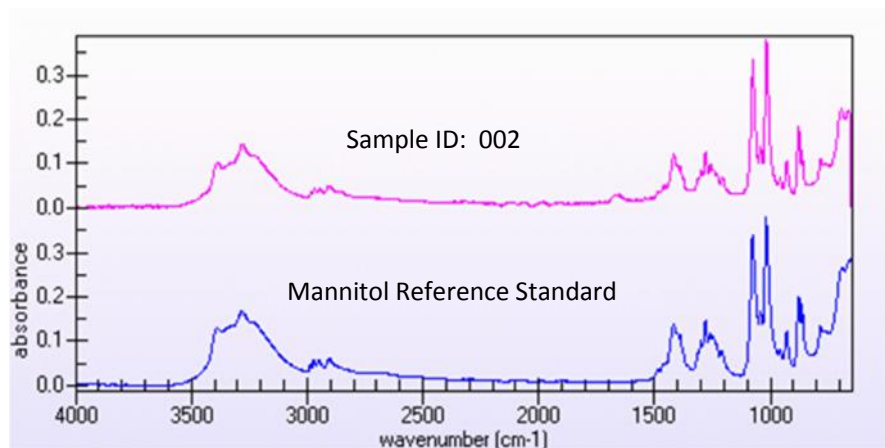


Figure 4.13: IR spectrum of sample (ID 002) which mannitol as major diluent (above) and Mannitol Reference Standard (below).

Figure 4.13 above shows example of IR spectrum of sample (tablet ID 002) which Mannitol found to be the major diluent. The principle peaks could be seen at wave numbers 1019, 1084, 1418 and 1283 cm^{-1} respectively, which are fingerprint of Mannitol. Two strong absorptions band at 1010 and 1084 cm^{-1} are attributed to asymmetric C-stretching for primary (1°) alcohol and secondary (2°) alcohol respectively in Mannitol structure. The others two peaks 1418 and 1283 cm^{-1} are corresponded to absorption band of O-H in plane bending couples with C-H wagging

vibrations. All the prominent peaks are consistent with literature (Terry Mills and J. Conrad Roberson, 1987).

IR spectrum of remaining four tablet samples did not match exactly with spectrum of any diluents provided by library. This could be due to the concentration of major diluent in this sample is either low (<80 %) or similar with others excipient in the bulk of tablet. As a result, a complex pattern of IR spectrum were produced since an attempted overlay of the spectra corresponding to the major diluents with others excipient, which lead to difficulties in the interpretation of result. The physical separation technique, unable to separate physically major diluents from other diluents/excipients since, most of them might be had the same solubility properties. However, all of four spectrum have a similar profile spectrum (Figure 4.14) which indicates its might be came from the same source of origin.

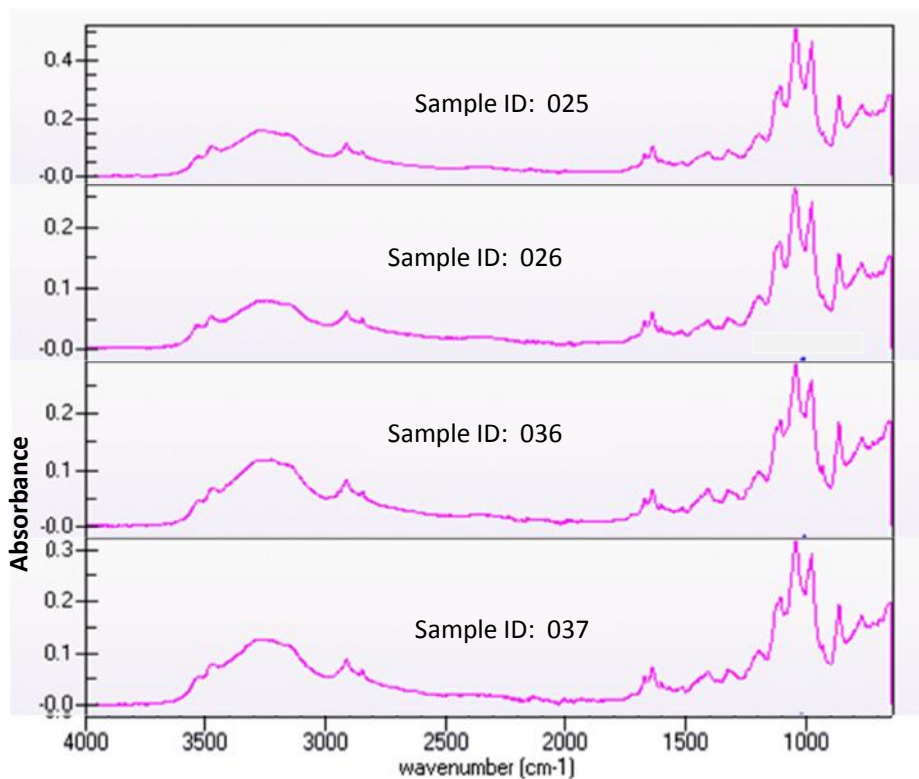


Figure 4.14: IR spectra of four samples which show a mixture profile of major diluents and others excipients.

With the aid of SEM-EDX results (Figure 4.15), three major elements of Calcium (Ca), Phosphorus (P) and Oxygen (O) are revealed to be presence in all of four tablet samples. These results give clues that calcium phosphates groups are the major diluent used in these remaining of four Erimin 5 tablet samples. However, there are two types of calcium phosphates which have been reported to use as diluents in illicit tablets and in pharmaceutical tablets which are dibasic calcium phosphate and tribasic calcium phosphate includes their dehydrate and anhydrous form.

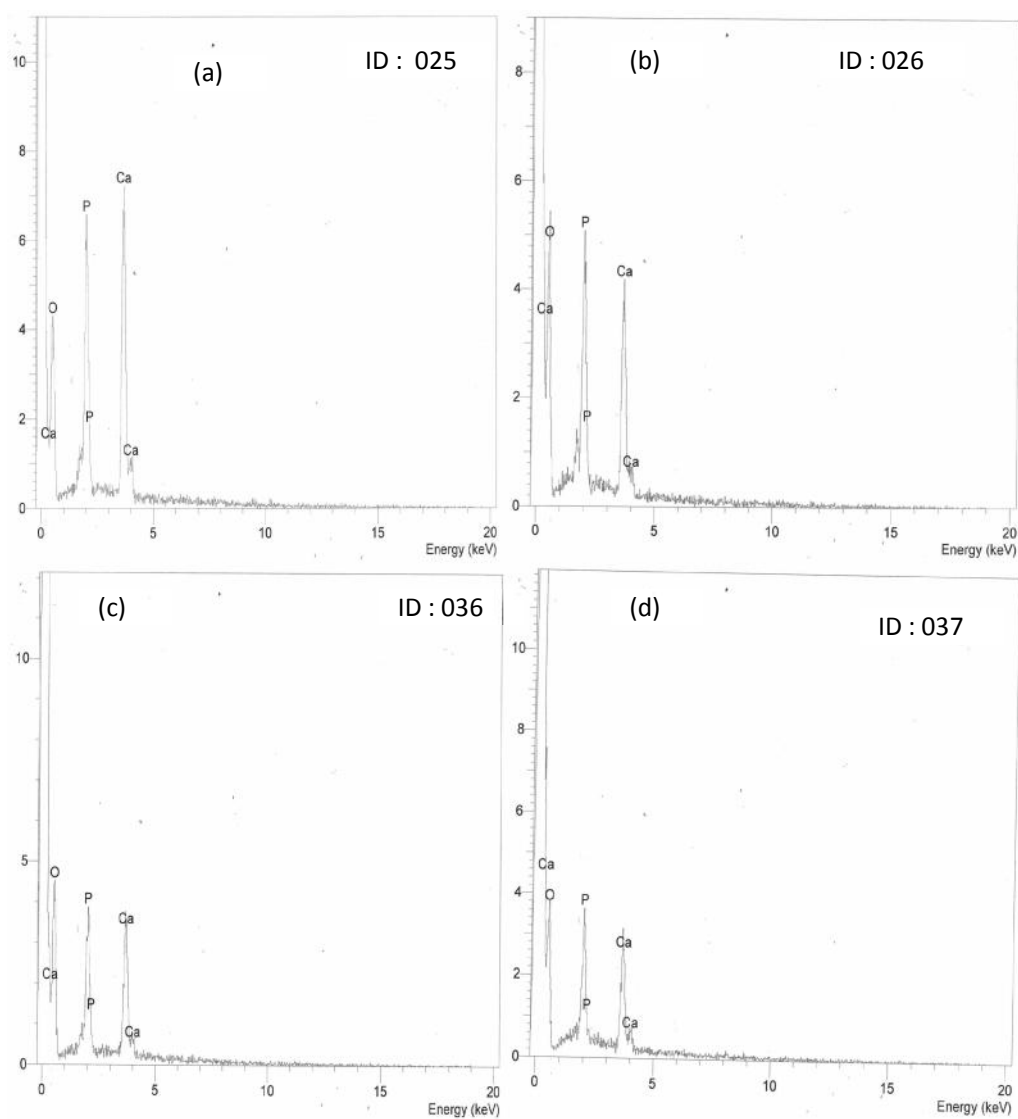


Figure 4.15: SEM-EDX results of samples (a) ID:025 (b) ID:026 (c) ID:036 (d) :037

The principle peaks of calcium phosphates IR spectrum were searched in IR spectrum of tablet samples and compared to principle peak in literature and reference Standard. The characteristics bands in calcium phosphate dibasic dehydrate and calcium phosphates tribasic spectra corresponding to the vibration modes of HPO_4^{2-} and PO_4^{3-} groups respectively. The comparison result is tabulated in table 4.4 and example of IR spectrum of one tablet sample (ID 025) is compared to IR spectra of calcium phosphates dibasic dehydrate and calcium phosphate tribasic as displays in Figure 4.16 and 4.17:-

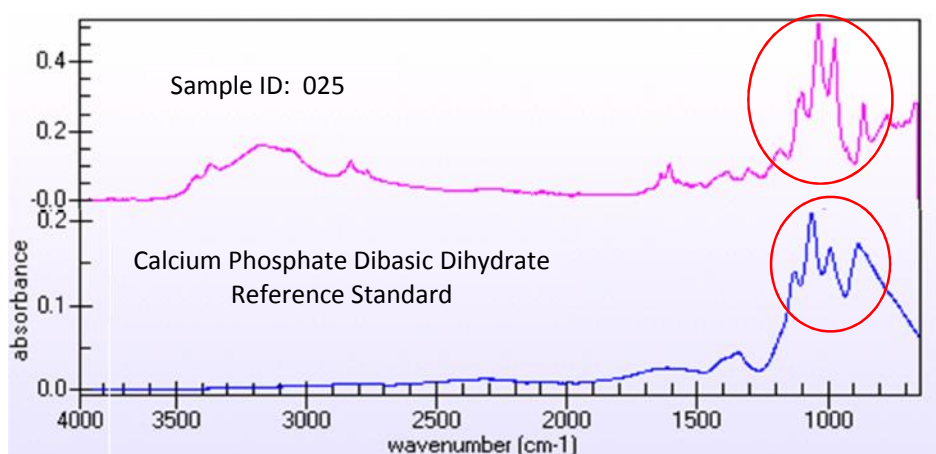


Figure 4.16: IR spectrum of sample ID:025 (above) and Calcium Phosphate Dibasic Dihydrate Reference Standard (below)

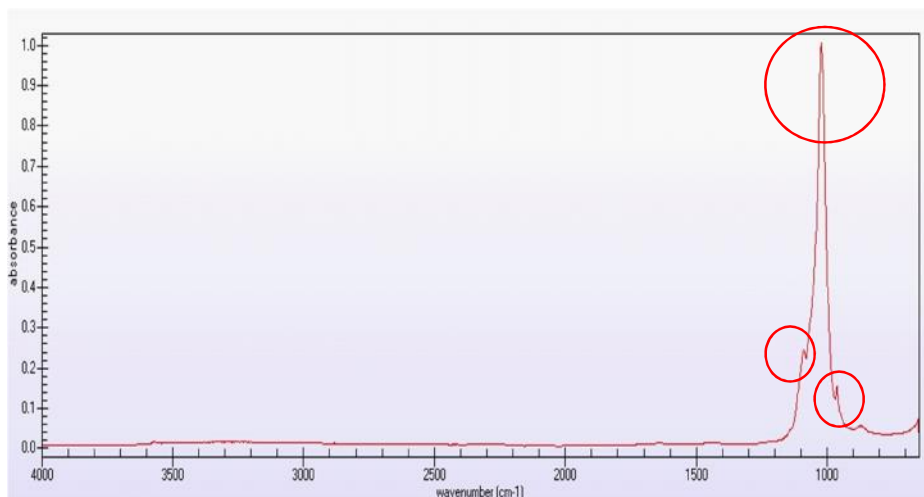


Figure 4.17: IR spectrum of Calcium Phosphate Tribasic obtained from library

Table 4.4: Comparison of IR results of sample with literature and reference Standard of calcium phosphates.

No.	Diluent	Principle peak, cm^{-1} By Literature (David et.al,1999)	Experimental result (Principle peak) , cm^{-1}					Peak Assignments
			Reference Standard	ID : 025	ID : 026	ID : 036	ID : 037	
1.	Di Calcium Phosphate dehydrate, $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$	1057	1058	1050	1054	1050	1050	Asymmetric P-O stretching
		1130	1127	1130	1132	1132	1132	Stretching of P=O
		985	991	983	983	985	985	Symmetric P-O stretching
		871	884	870	872	872	872	P-O(H) stretching
2.	Tribasic Calcium Phosphate $\text{Ca}_3(\text{PO}_4)_2$	1088	No Reference Standard	-	-	-	-	} Asymmetric P-O stretching
		1027		-	-	-	-	
		963		-	-	-	-	} symmetric P-O stretching
		891		-	-	-	-	

Based on the data as demonstrates in table 4.4 and comparison pattern of principle peaks in IR spectrum of the tablet samples with two types of calcium phosphates pattern (Figure 4.16 and 4.17), it can be strong suggested that the major diluent in the remaining four illicit Erimin 5 tablet samples (ID: 025, 026, 036 and 037) is calcium phosphate dibasic dihydrate.

Overall, all diluents findings in this study which are Lactose, Mannitol and Calcium phosphate, also have been reported by others researchers in either studies of pharmaceutical field or illicit drugs included illicit tablets. As example, Lactose and Mannitol are similar with the diluents detected in Erimin 5 tablet which had been reported by Ahmad Fahmi et al. (2012) except glucose had not been detected in this study. Both diluents also had been found in the study of illicit Heroin, Amphetamine and Cocaine samples in Denmark by Mette F. A. et al. (2009). Besides that, Lactose also had been reported as diluent in ecstasy tablets by KB Chan et al. (1996) and also in MDMA tablets by P. Gimeno et al. (2002). Mannitol has been used as diluent due to its sweet taste, cooling sensation in the mouth and also due to non- hygroscopic and water soluble properties (Mira J. et al., 2000). While, Lactose is most widely used as diluents due to inexpensive, easily available, low hygroscopic, and water soluble (Mira J. et al., 2000).

Calcium Phosphate Dibasic Dihydrate has been widely used in pharmaceutical field especially in vitamin and mineral supplement due to high calcium and phosphorous content and also due to inexpensive and desirable flow and compression characteristics (Mira J. et al., 2000). However, the use of Calcium Phosphate Dibasic Dihydrate as diluent has not been reported in any studies of illicit drugs, might be due to its percentage in the bulk tablet does not as high (<80%) as others diluent and it also

might be combined with others diluents such as microcrystalline cellulose and starch (Mira J. et al., 2000).

4.3.4 Identification and Confirmation of Dyes

Thin layer Chromatography (TLC) is one of the cheapest, quickest and most efficient separation methods for many chemical compounds. The important advantages over other chromatographic technique such as low cost of instrumentation, enabled simultaneous separation (up to 20 samples in the same time) and shortened time of analysis (Florin et al., 2008). Analysis of dyes by TLC also provides more rapid and precise results than paper chromatographic methods (GJ Dickes, 1965). It had been reported that slight amount of impurities did not give greater impact on analysis process since, assay result of several dozen samples were shown satisfactory result using TLC technique (Marta Kucharska et al., 2010). As for these reasons, TLC technique was chosen in this study, which specifically to qualitative determination of dyes identity used as colouring agent in 46 illicit Erimin 5 tablets production.

Sample preparation is the most critical step which has large impact on the interpretation results (Marta Kucharska et al., 2010). As food colorants are added to foods at low concentrations therefore, it is necessary to extract and concentrate the colorants to obtain sufficient amount for analysis (Syed Saeed-ul-Hassan et al., 2006). Besides that, dyes separation can be difficult in TLC analysis since, tablets contain other substances such as sugars, fats and fatty acids which may interfere in separation of dyes. The use of white wool in extraction technique is the most effective method in extracting of dyes/colouring matter from the tablets. For tablets, no one procedure was found to be universally acceptable (Joyce, 1980b). However, the most important is the quantity of

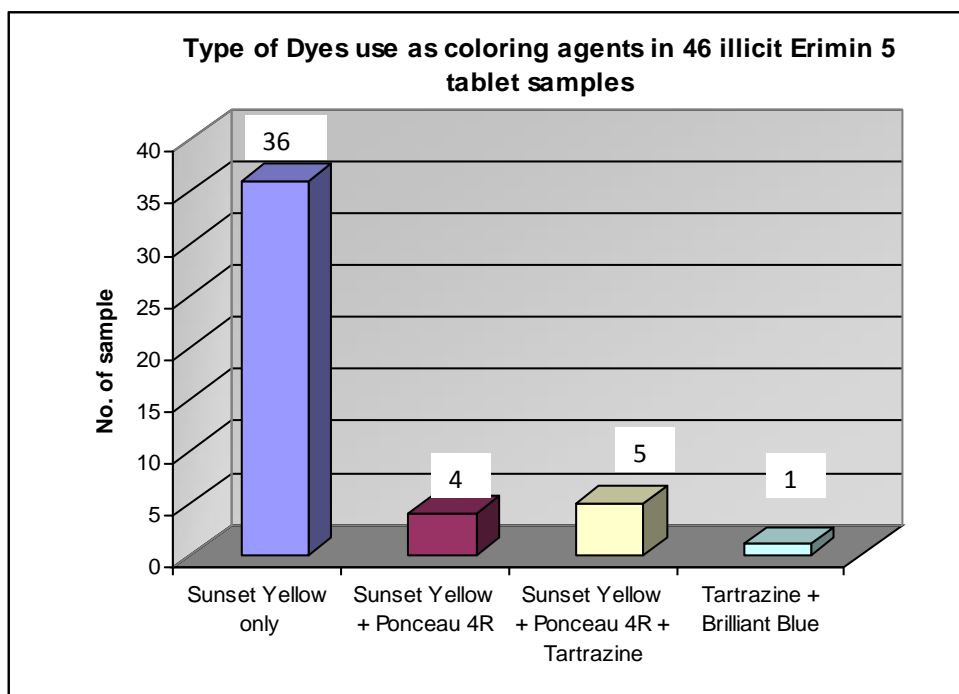
dye should be sufficient enough extracted from each of the tablets to give an accurate result.

In order, to give the desired of coloured tablet, usually either a single colouring matter/dye or a mixture of colouring matters/dyes is used. For example, a mixture of blue and yellow colouring matters/dyes gives green coloured in tablet. Others example either a mixture of red and yellow colouring matters/dyes or orange colouring matter/dye is used to give orange coloured tablet. A lot of dyes choice in the market as example, either Ponceau 4R or Amarath or Erythrosine can be used to produce red coloured tablet. Thus, in appropriate condition of TLC analysis, it is possible to separate a mixture several dyes and identify them as a result of comparison: migration coefficient R_f , spot shape and its colour compare to standards (Marta Kucharska et al., 2010).

R_f values are influenced by various factors including dye concentration, kind and quality of subsidiary dyes, composition and age of the solvent, temperature, pH value of the solution (FAO Compendium of Food Additive specifications, 2006). In order to eliminate all of these factors, therefore any unknown dye must be compared with those from a set of known dyes on the same plate and tabulated R_f values should only be employed for selecting the solvents which differentiate best between the expected spots. In addition, the solvent used should be freshly prepared prior to use.

In this study, it can be seen a mixture of dyes in 46 illicit Erimin 5 tablet samples were fairly separated on both of the TLC system, System A (Iso-Propanol/Ammonia (4:1)) and System B (Iso- Propanol/Ammonia/water (7:2:1)). However, System A provided the better separation between Tartrazine and Ponceau 4R compared to System B. Generally, among the 45 tablet samples of peach-orange like

colour, the vast majority (36/45 @ 80%) contained only Sunset Yellow as their dye/colouring matter. Whereas, three combination of dyes/coloring matters namely Sunset Yellow, Tartrazine and Ponceau 4R were used to produce the hue of peach-orange like colour to the others 5 tablet samples (5/45 @ 11%). The remaining 4 peach-orange like colour tablet samples (4/45 @ 9%), were found to contain Sunset Yellow and Ponceau 4R. For one green tablet sample (ID: 046), a mixture of the two dyes, Tartrazine and Brilliant Blue were detected. The distribution profile of types of dyes used in 46 illicit Erimin 5 tablet samples is illustrated in graph 4.7.



Graph 4.7: Type of Dyes use as colouring agents in 46 illicit Erimin 5 tablet samples

Examples of TLC results in both Solvent System A and System B are shown in figure 4.18 (a) and (b) and 4.19 (a) and (b).

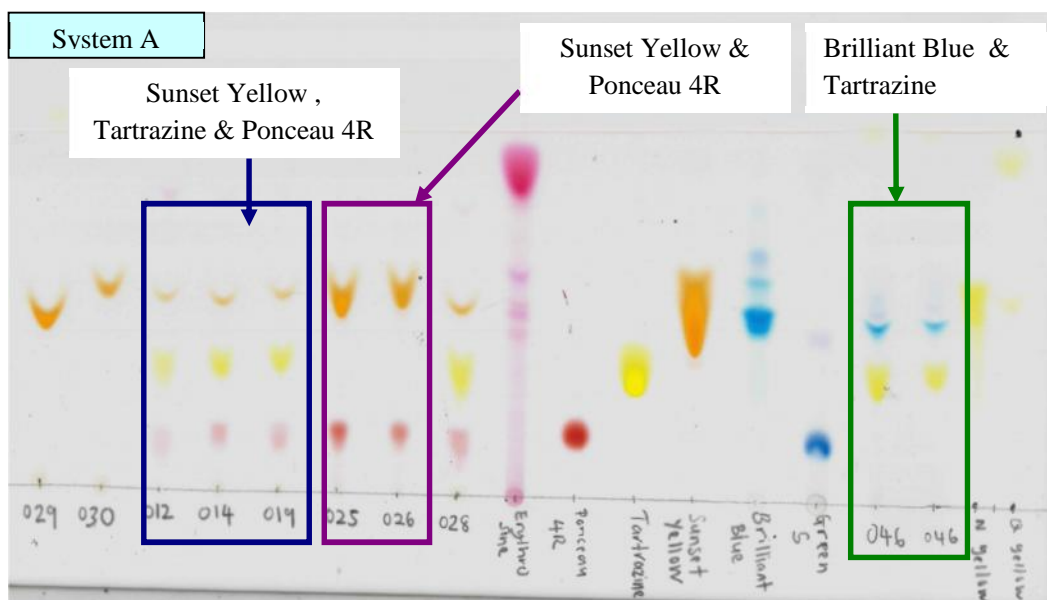


Figure 4.18 (a): Example of TLC results using solvent system A which several combination of dyes were detected. R_f values for Reference Standard : Sunset Yellow (R_f 0.46), Tartrazine (R_f 0.31), Ponceau 4R (R_f 0.16) and Brilliant Blue (R_f 0.44)

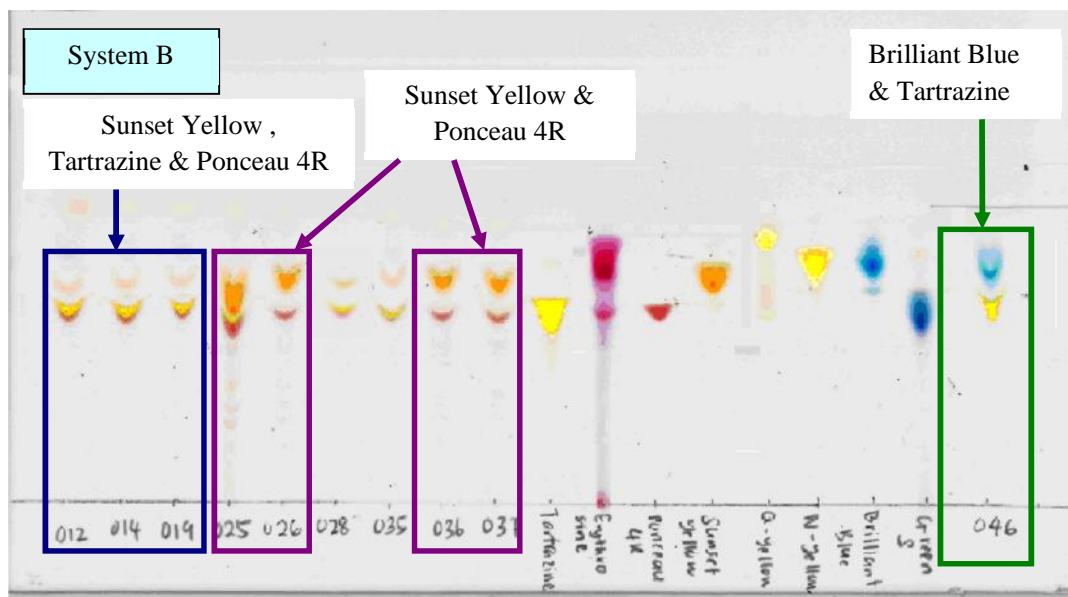


Figure 4.18 (b): Example of TLC results using solvent System B which several combination of dyes were detected. R_f values for Reference Standard: Sunset Yellow (R_f 0.80), Tartrazine (R_f 0.67), Ponceau 4R (R_f 0.60) and Brilliant Blue (R_f 0.78)

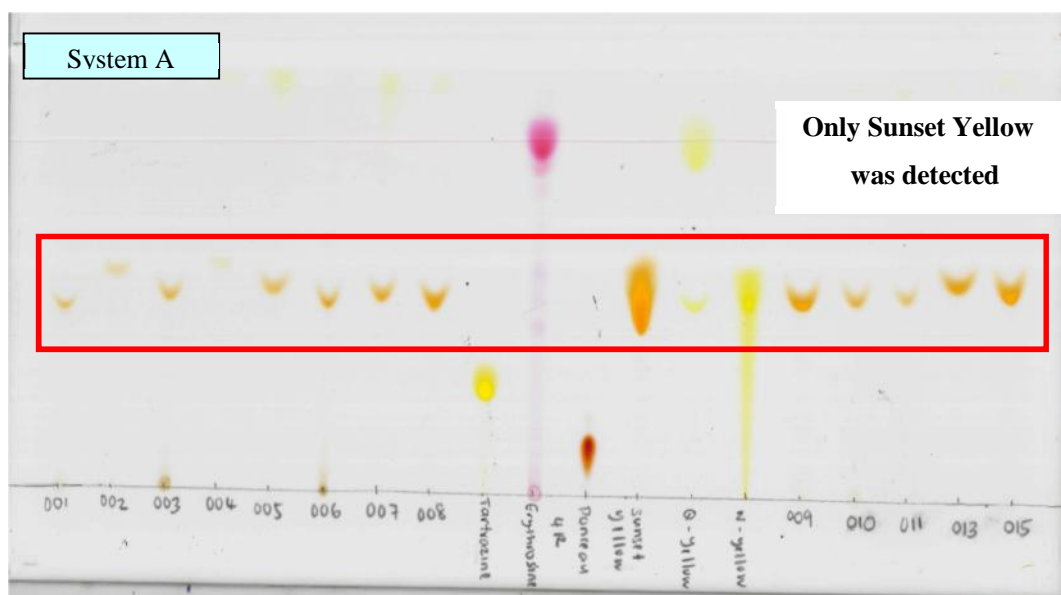


Figure 4.19 (a): Example of TLC results using solvent system A which only Sunset Yellow (R_f 0.46) was detected.

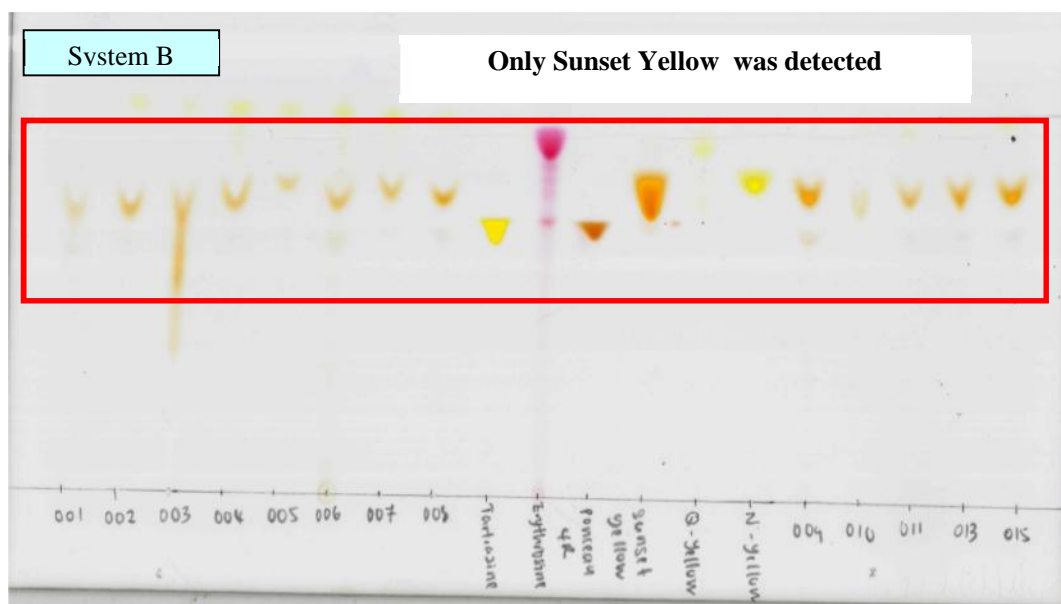


Figure 4.19 (b): Example of TLC results using solvent System B which only Sunset Yellow (R_f 0.78) was detected.

In this study, the result shows the combination of Brilliant Blue dye and Tartrazine dye were used to produce green coloured Erimin 5 tablet in illicit tablet manufactured which similar to commonly practice in hygiene and cosmetic applications (Krishna et al., 2011). The same findings also reported by Ahmad Fahmi et al. (2012) where the same combination was found in their studies of illicit Erimin 5 tablets.

It is observed that Sunset Yellow dye was detected in most of 45 peach-orange like coloured illicit Erimin 5 tablets samples, either alone or combination with other dyes. Besides Sunset Yellow alone, two combinations dyes could be seen either mixture of Sunset Yellow with Tartrazine and Ponceau 4R or Sunset Yellow with Ponceau 4R were used to give peach-orange like colour. As a result, various tone of peach-orange like colour can be seen in illicit Erimin 5 tablet samples due to difference types and quantity of dyes added into the bulk of tablets. The use of sunset yellow alone to provide orange coloured tablet also consistent with the findings of the study by Till Goldmann et al. (2004) which Sunset Yellow alone had been detected as a dye in three orange coloured illicit pills (amphetamine and derivatives).

However, other combinations of dyes could be used by illicit Erimin 5 manufacturer as their recipes in order to produce peach-orange like colour tablet as well as green tablet. As example Ahmad Fahmi et al. (2012) had reported that mixture of the three dyes namely, Erythrosine, Tartrazine and Ponceau 4R had been detected in peach-like coloured Erimin 5 tablets. Others example of combination of dyes in illicit tablets (amphetamine and derivatives) were shown in study by Till Goldmann et al. (2004) which Sunset yellow was used with Tartrazine to give orange coloured and combination of Tartrazine and Green S were used to give green tablet. It can be seen

that, any combination of dyes are possible since, a lot of food colourings choice in the market can be used in illicit tablet manufactured.

It could be assumed that those illicit tablets which have the same combination of dyes/ single dye might be come from the same source of origin. Thus, the examination of dyes composition able to provide useful information to link the drug syndicates and delineate a drug syndicate's illicit market coverage (Ahmad Fahmi et al., 2012). Dye analysis also can be used, in conjunction with other techniques, to relate tablet seized at different locations (Joyce, 1980).

The details result of active diluent, adulterant, diluent and dyes are shown in APPENDIX 4.

4.4 Characterization of illicit Erimin 5 tablets samples according to their chemical characteristics

46 illicit Erimin 5 tablet sample were then characterized according to their chemical characteristics which are active ingredient, adulterant, major diluent and dyes. As a result, 7 groups of illicit Erimin 5 tablets samples could be categorized as shown in figure 4.20. The details are shown in table 4.4

In group 1, only one orange tablet sample (ID 001) contained active ingredient Diazepam and Lactose as major diluent with Sunset Yellow as the dye. In group 2, 15 tablets samples contained Nimetazepam, Lactose and Sunset Yellow. Five tablets sample were found to contain the same chemical profile of Group 3 which active ingredient was Nimetazepam with Lactose as major diluent and combination of Sunset Yellow, Tartrazine and Ponceau 4R to produce the hue.

Nimetazepam and Mannitol together with Sunset Yellow could be found in 18 tablets samples and categorized in Group 4. For group 5, active ingredient Nimetazepam and Mannitol as major diluent were detected with combination of Brilliant Blue and Tartrazine in order, to provide green in colour of one tablet samples.

In group 6, Nimetazepam were found in 4 tablets samples which calcium phosphates is the major diluent with combination of Sunset yellow and Ponceau 4R dyes as colouring agents. Group 7, consist of three tablets samples which Nitrazepam as active ingredient and caffeine as adulterant, lactose as major diluent with Sunset yellow as the dyes.

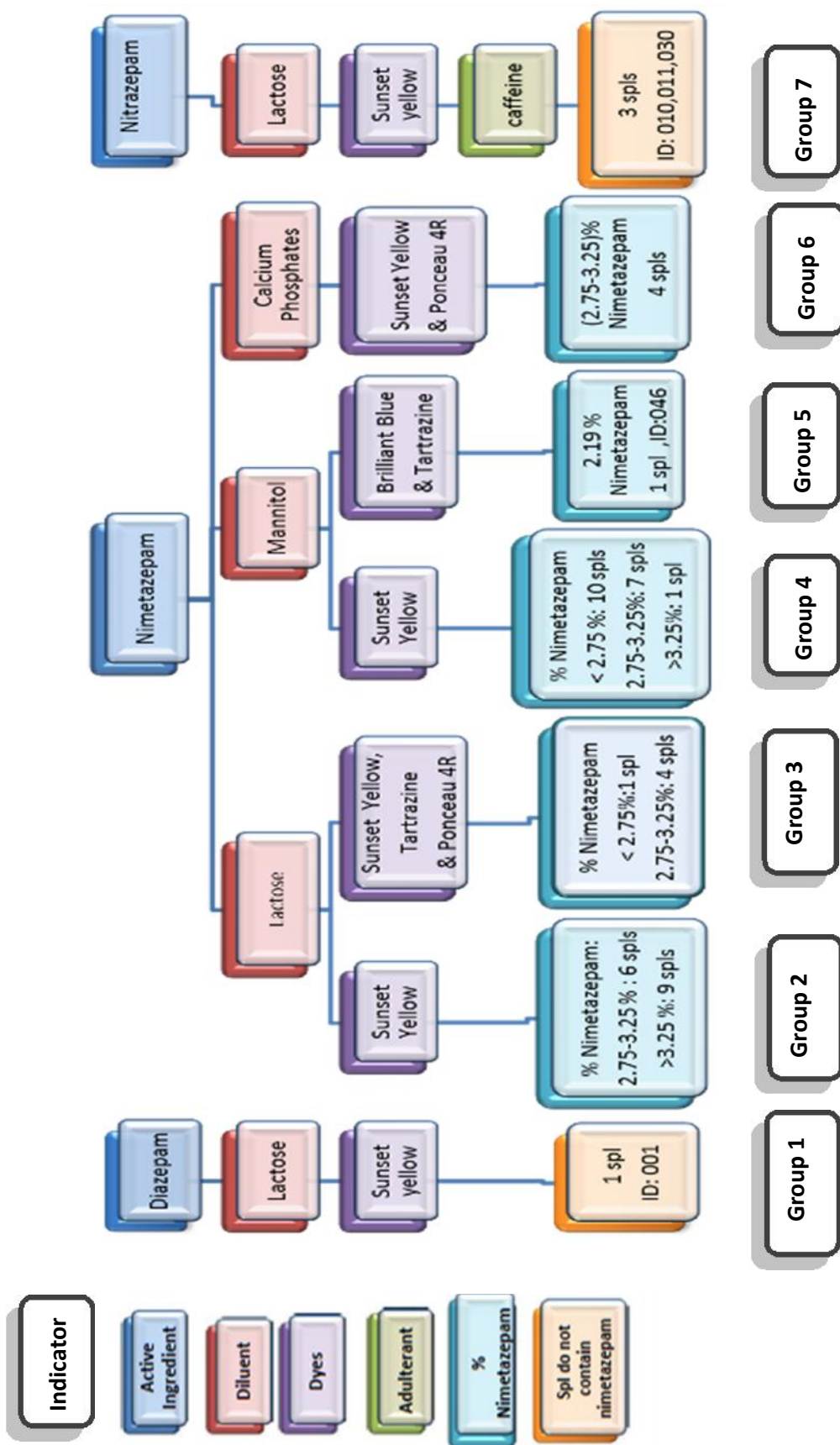


Figure 4.20: Characterization of illicit Erimin 5 tablets samples according to their chemical characteristics

Table 4.5: Characterization illicit Erimin 5 tablet samples according to their chemical characteristics

Group	GCMS result		HPLC result	IR/SEM -EDX Result	TLC	ID No.
	Active Ingredient	Adulterant	% Nimetazepam : no of spl	Major Diluent	Dyes	
1	Diazepam	-	-	Lactose	Sunset Yellow	001
2	Nimetazepam	-	2.75 - 3.25%: 6 spl	Lactose	Sunset Yellow	003, 023, 027, 038, 039, 040
		-	>3.25%: 9 spl			006, 013, 015, 018, 020, 029, 041, 043, 044
3	Nimetazepam	-	<2.75% : 2 spl		Sunset Yellow, Tartrazine & Ponceau 4R	012, 028
		-	2.75 - 3.25%: 3 spl			014, 019, 035
4	Nimetazepam	-	<2.75% : 8 spl	Mannitol	Sunset Yellow	016,017,021,022, 032,033,034,045
		-	2.75 - 3.25%: 8 spl			002, 005, 007,008, 009,024,031,042
		-	>3.25%:1 spl			004
5	Nimetazepam	-	<2.75% :1 spl		Brilliant Blue & Tartrazine	046 (2.19%) - green tablet
6	Nimetazepam	-	2.75 - 3.25%: 4 spl	Calcium phosphates	Sunset Yellow & Ponceau 4R	025, 026, 036, 037
7	Nitrazepam	Caffeine		Lactose	Sunset Yellow	010, 011, 030

4.5 Application of the profiling of Erimin 5 tablets to the drug intelligence purpose.

The abused of Erimin 5 tablets could be seen increasing for a past few years. It is a challenge for Narcotic laboratory chemists to establish the database information regarding on both characteristics, physical and chemical characteristics of the illicit Erimin 5 tablets seizures. The sharing of data between other laboratories in the countries as well as Asian region/others countries could provide intelligence purposes in order, to establish the connection between seizures.

Physical features (except the logo and colour) provided means to link tablets produced over a long period of time by tableting machines with given settings. However, different tableting machines also may produce similar features (Q. Milliet et al. 2009). Whereas, Chemical characteristics provide stronger evidence of a link between two seizures inferred to come from either the same tableting batch (Q. Milliet et al., 2009) or from the same source of origin. Therefore be very powerful intelligence tools for investigation purposes, while the added value and combination of chemical characteristics links with physical characteristics may be more adequate to refine the investigators view and to reach court requirements (Q. Milliet et al., 2009).

Obviously, this simple and quick profiling strategy could be generated easily and could aid the law enforcement agencies such as Royal Malaysian Police (RMP) and Royal Custom of Malaysia who seized illicit Erimin 5 tablet samples at different locations to check whether is there any link between of them or to link these illicit tablets samples with the raw materials found in illicit lab scale.

CHAPTER 5

CONCLUSION

The trend of Erimin 5 tablets abused have been seen arising for a last few years. Conversely, the retail price of this tablet remained stable at same price for a long period of time. The increasing of Erimin 5 tablets seizures are parallel with the increasing of methamphetamine crystal seizures. The same pattern could be observed in others Asia countries.

A framework of profiling studies based on physical characteristics and chemical characteristics of illicit Erimin 5 tablet sample was developed. For physical characteristics, overall mean of average weight per one tablet is 188.9 mg. Meanwhile, the mean of diameter and thickness of the tablets are 8.09 and 2.75 mm respectively. It could be seen that the distribution of tablet diameter among illicit Erimin 5 tablet samples is higher than thickness.

Most of illicit tablet samples contained Nimetazepam alone as active ingredient while, Diazepam was detected as active ingredient only in one tablet sample. Nitrazepam was found as minor compound together with caffeine (adulterant) as major compound in three tablet samples. The mean percentage of Nimetazepam in tablet samples was 2.99% and the mean of Nimetazepam weight per tablet was 5.6 mg per tablet.

Lactose was detected as major diluent in most of Erimin 5 tablet samples, followed by Mannitol and Calcium phosphates Dibasic Dihydrate was the least found as major diluent. Sunset Yellow was found in most of tablet samples either alone or

combination with other dyes such as Tartrazine and Ponceau 4R to give orange to peach in colour. Combination of Brilliant Blue and Tartrazine dyes were used to produce a green tablet sample.

46 illicit Erimin 5 tablet samples were categorized into 7 groups according to their active ingredient, adulterants, diluent and dyes. The similarity of chemical profile between tablet samples in each group indicates they might be come from the same source. This valuable information could be as tool in order to compare the chemical profile of illicit Erimin 5 tablets seized with other seizures at different locations as well as raw material seized at illicit manufactured lab. This data also could strengthen the evidence for enforcement and law purposes. Besides that, the database of this information enables to build the networking sharing with others countries.

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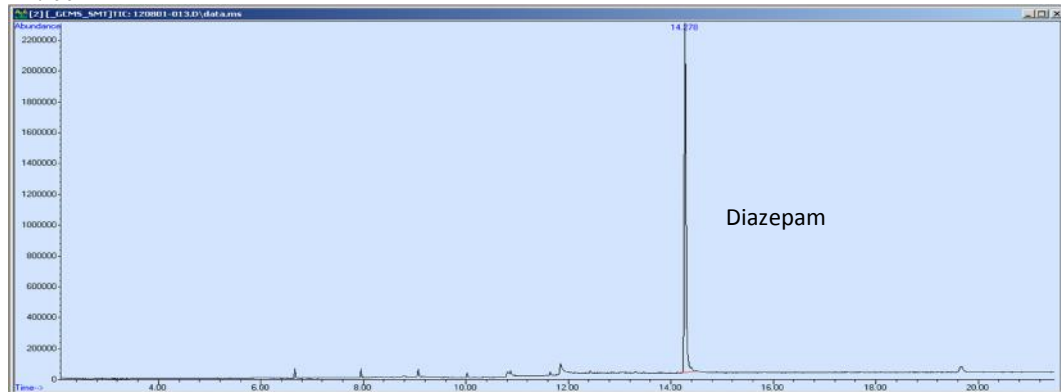
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APPENDIX 1

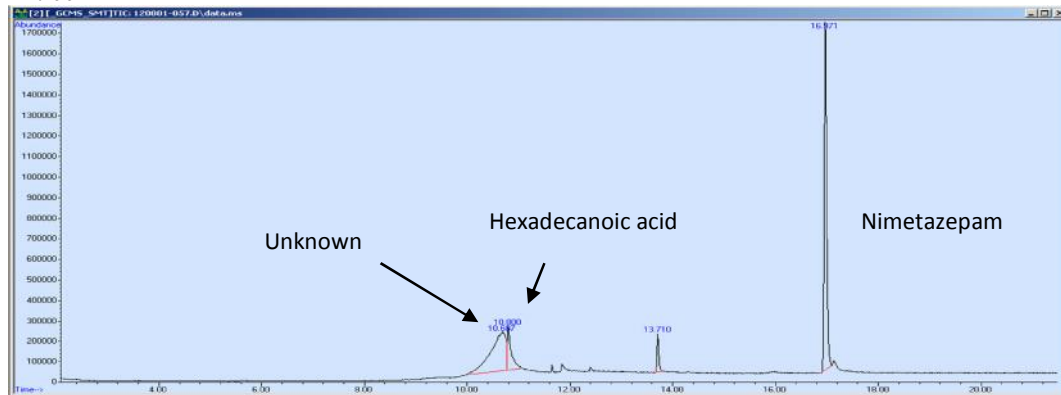
GCMS RESULT

TIC CHROMATOGRAMS OF 46 ILLICIT ERIMIN 5 TABLET SAMPLES

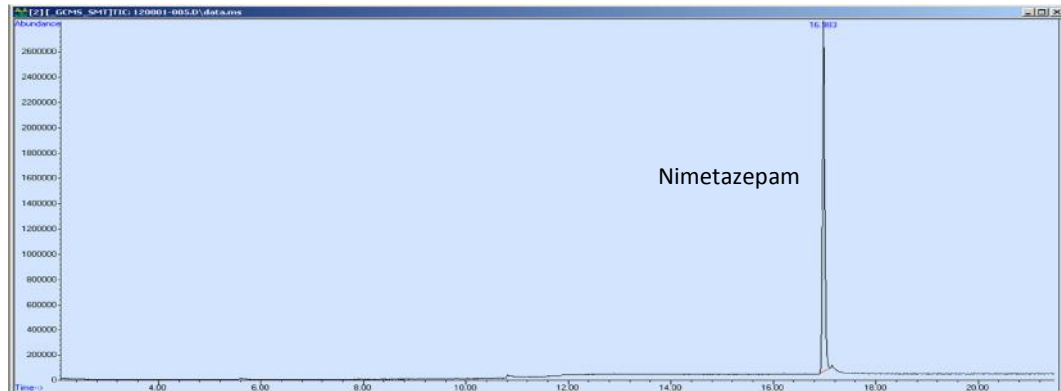
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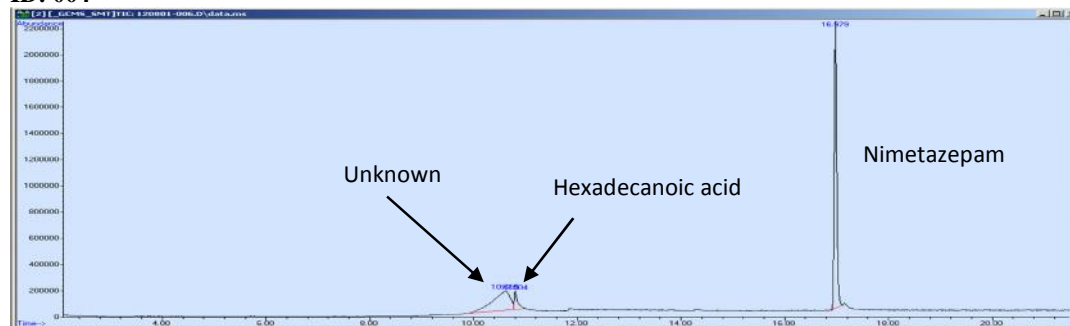
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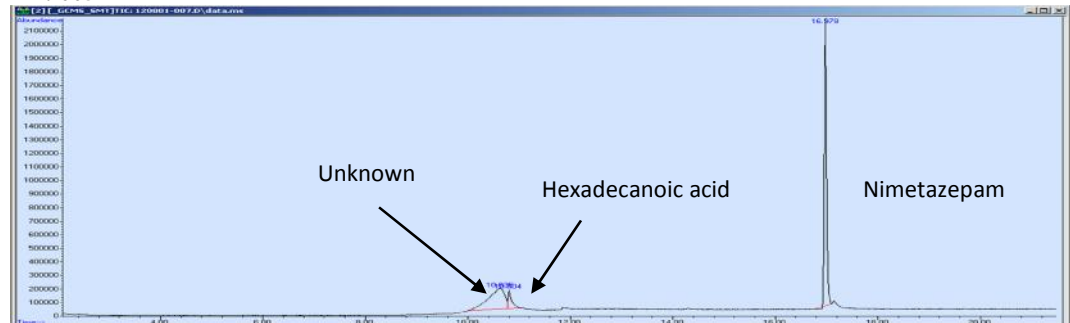
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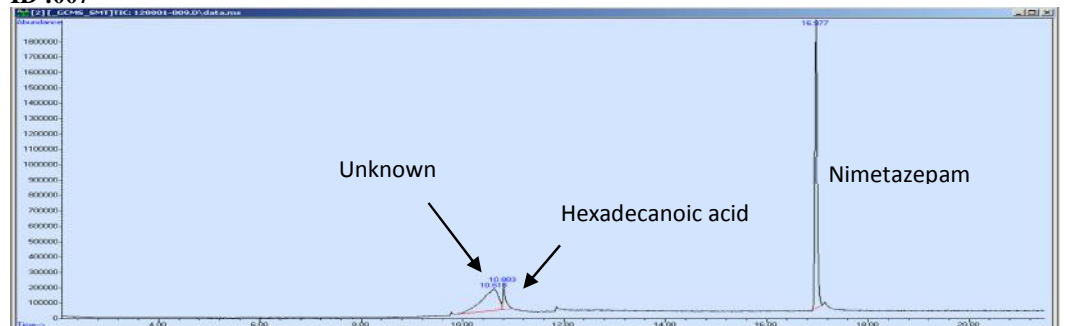
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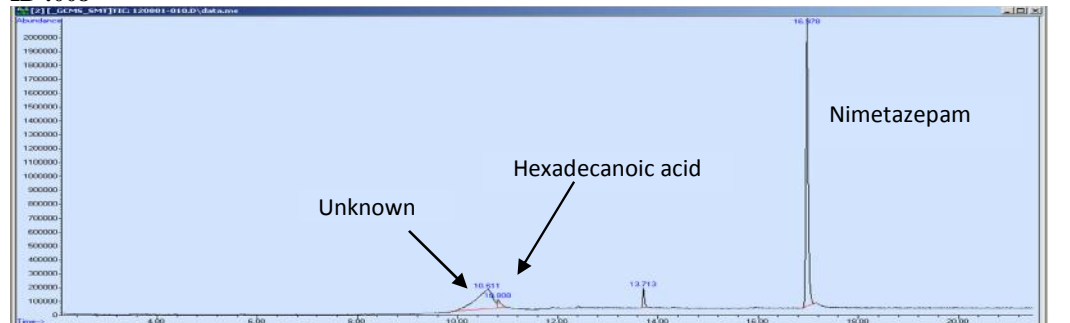
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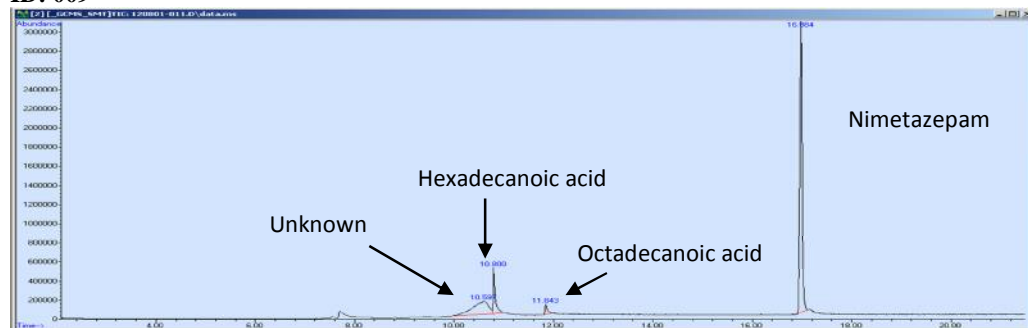
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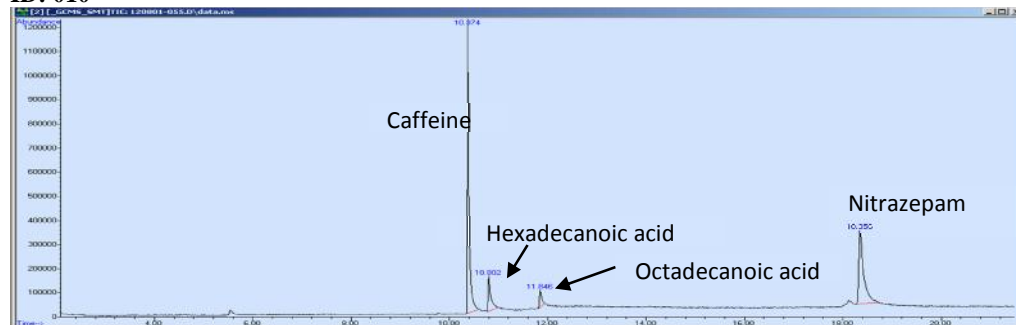
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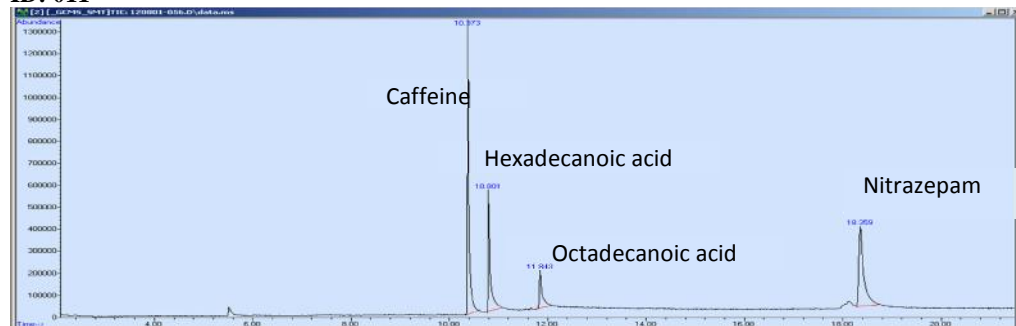
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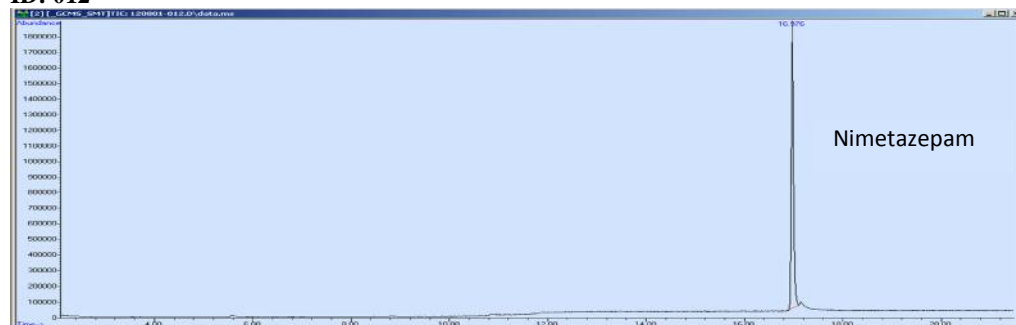
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ID: 011



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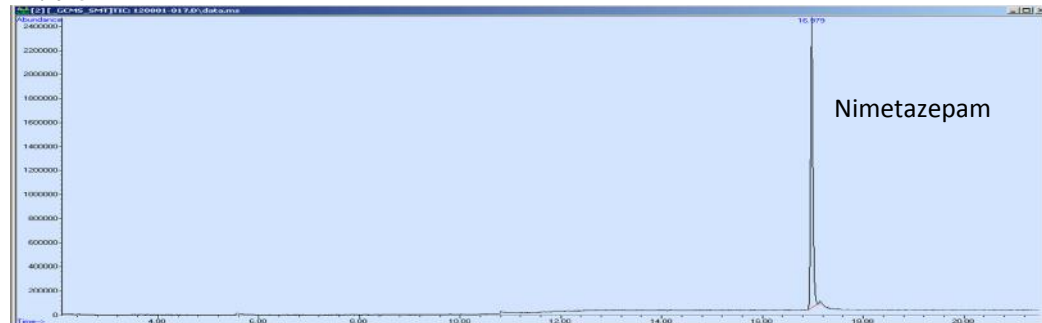
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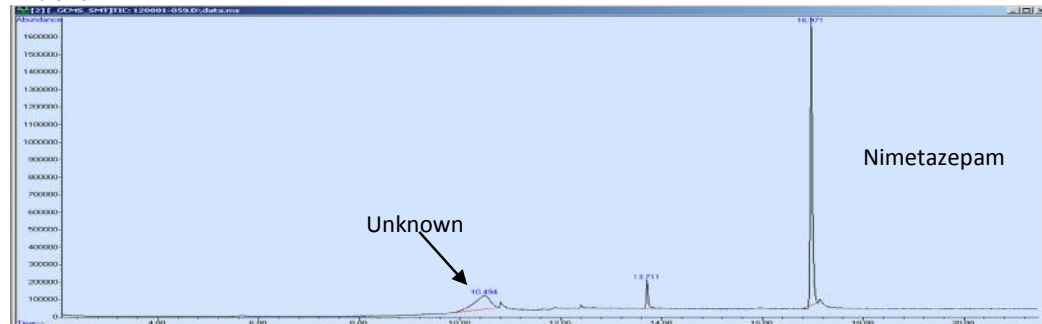
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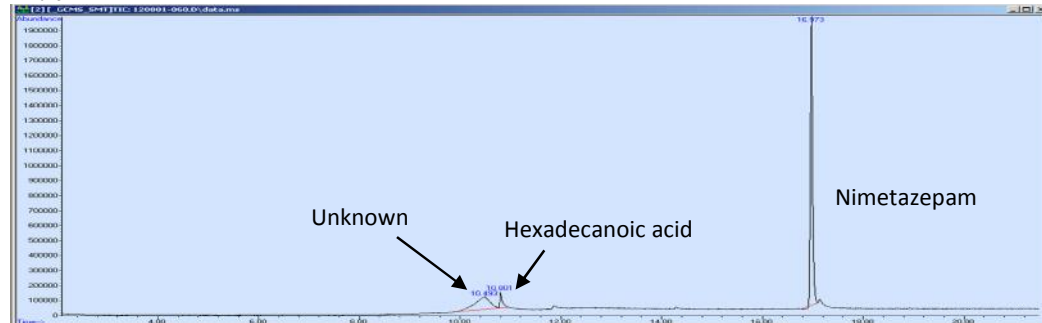
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ID: 016



ID: 017



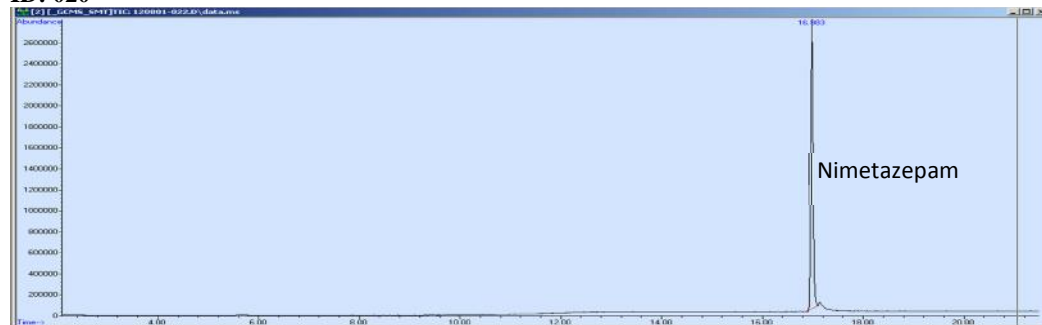
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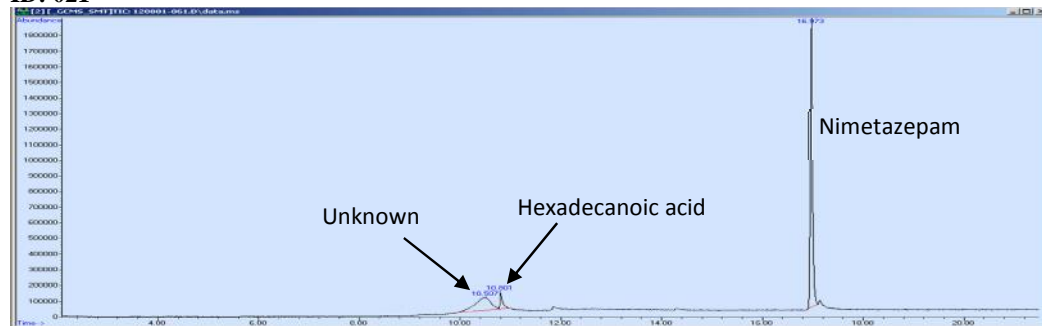
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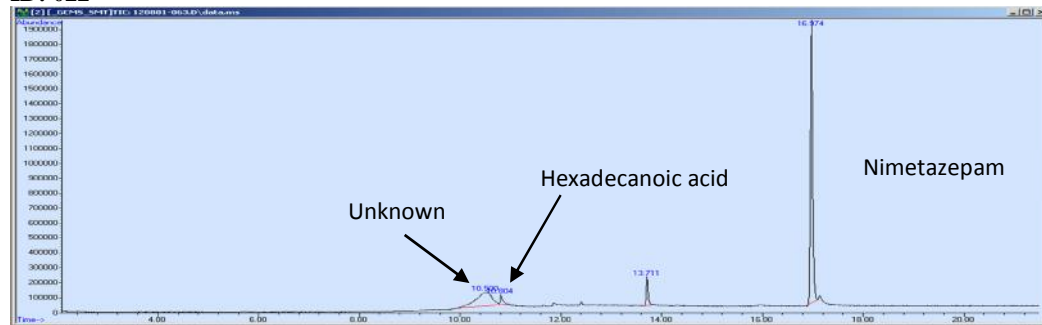
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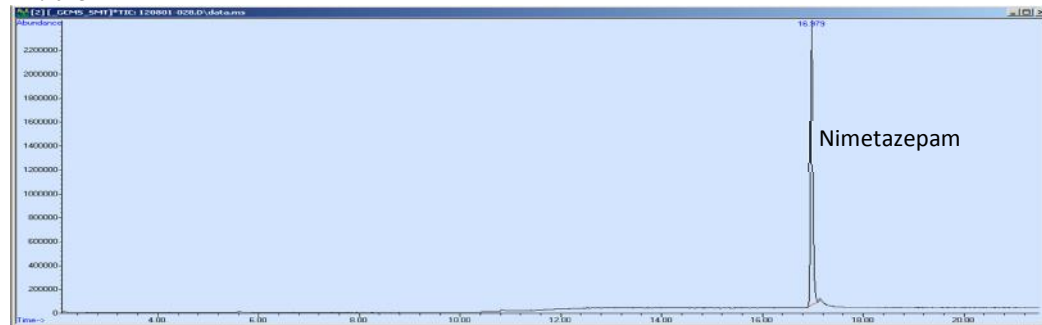
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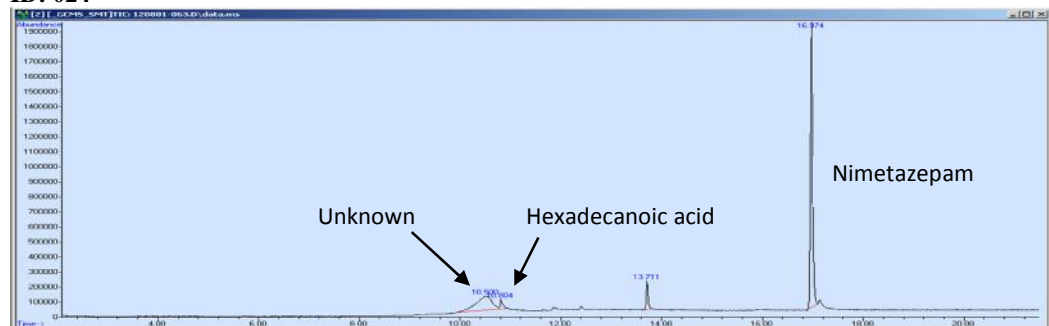
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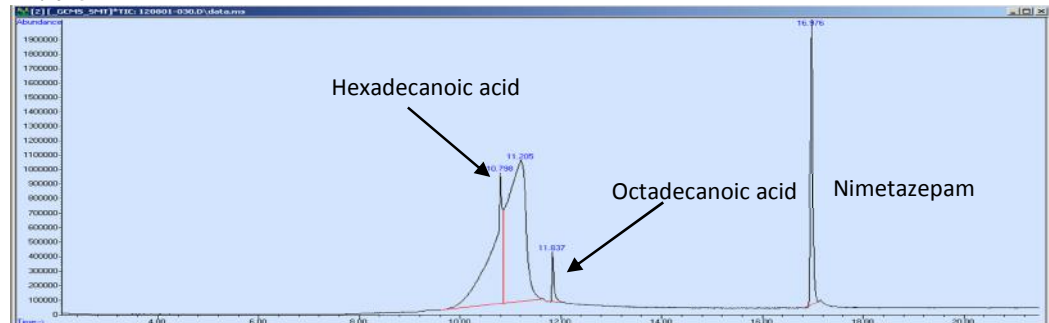
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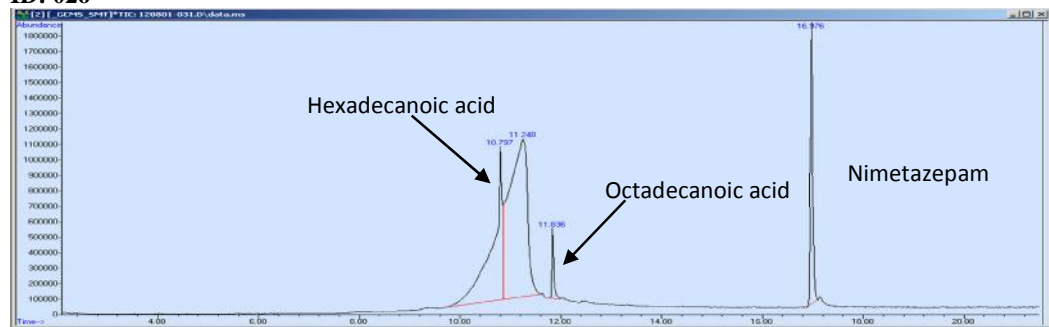
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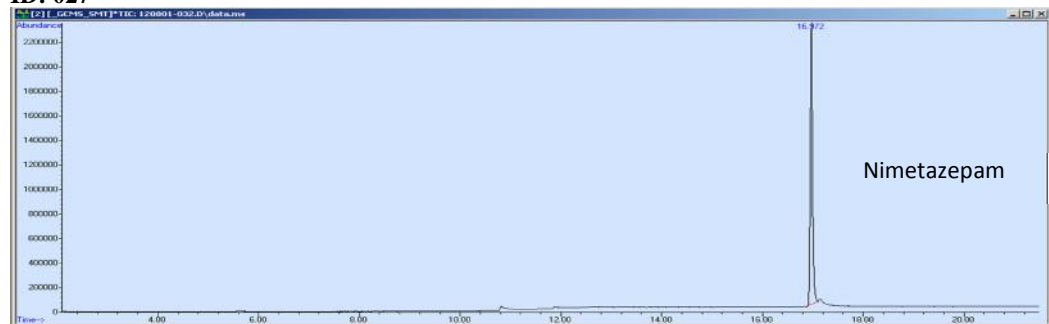
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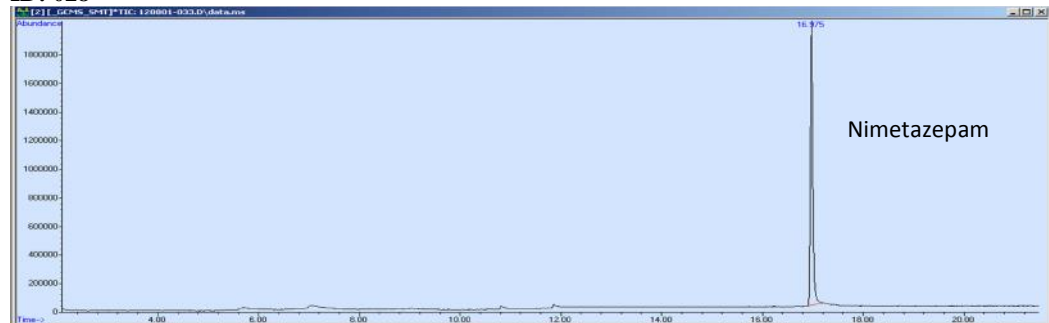
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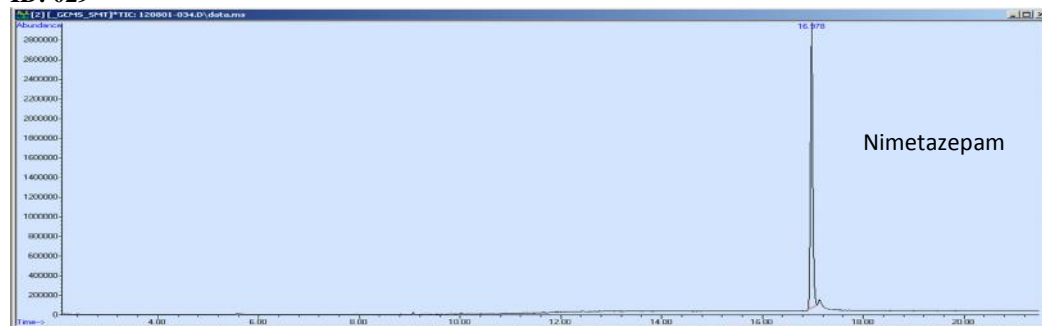
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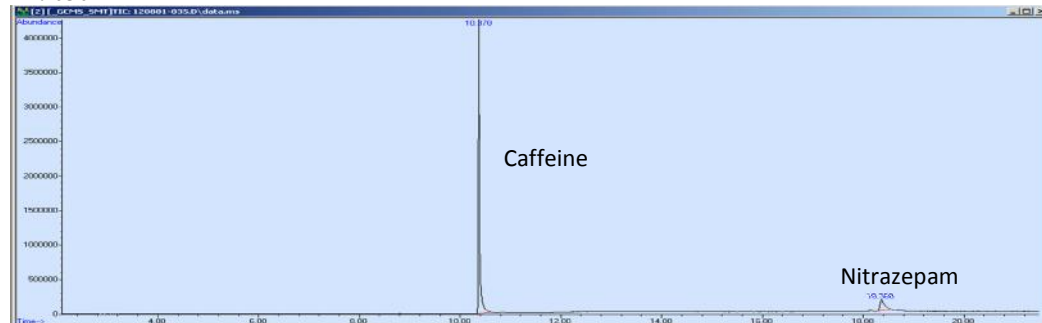
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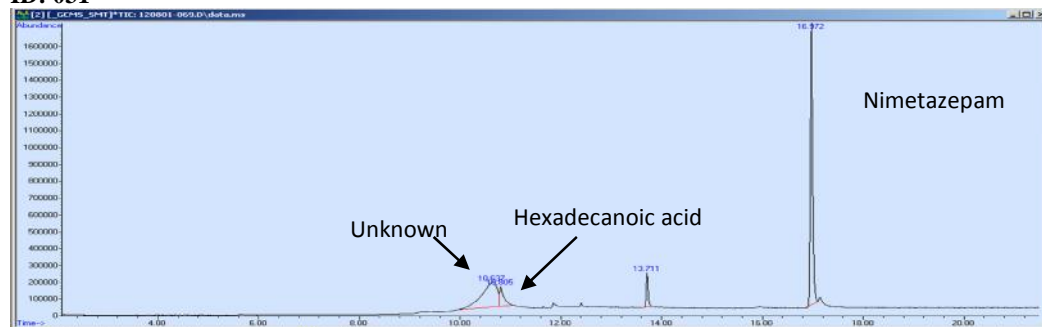
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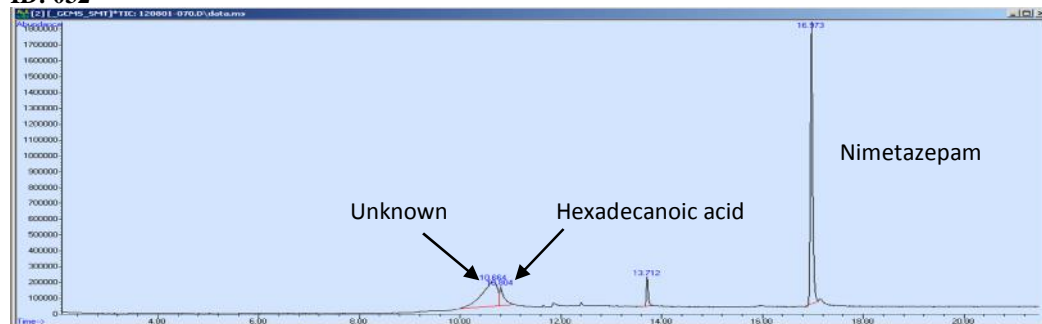
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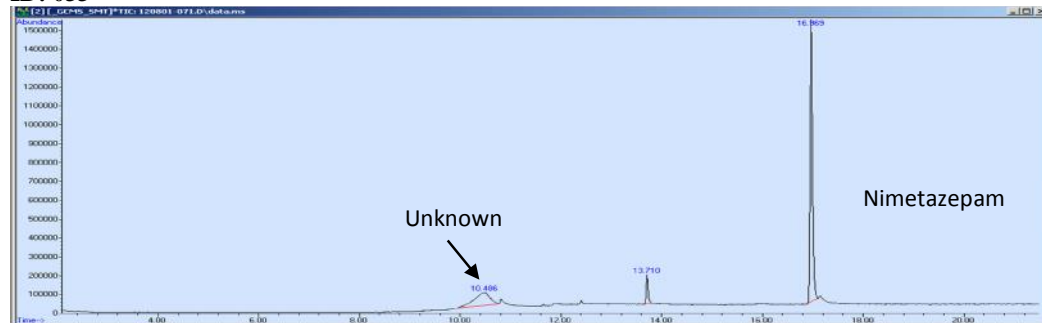
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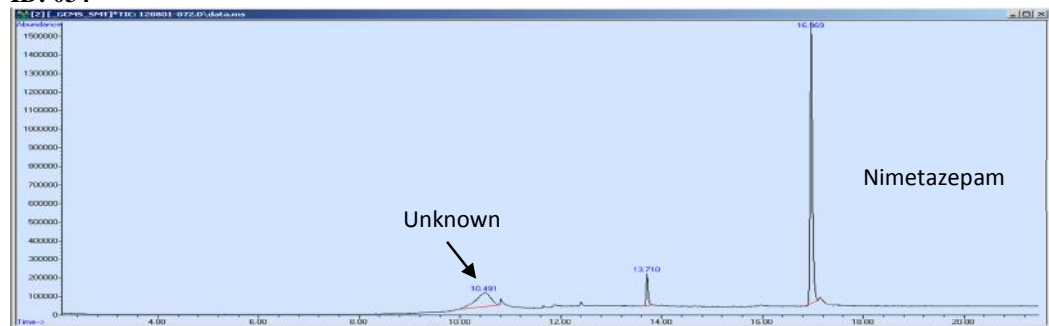
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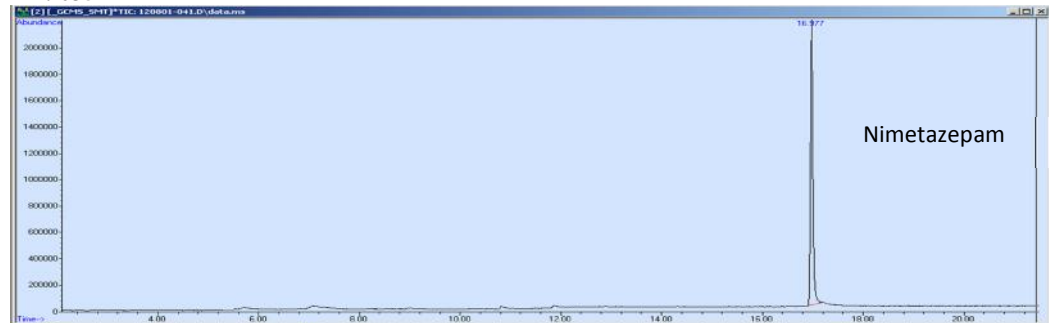
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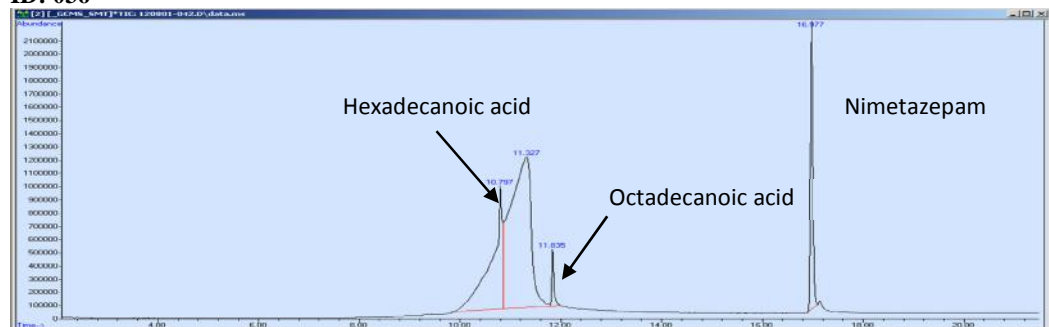
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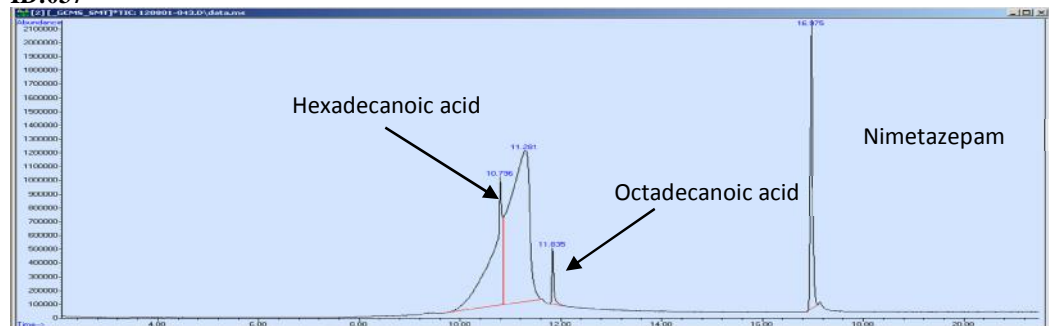
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ID: 036



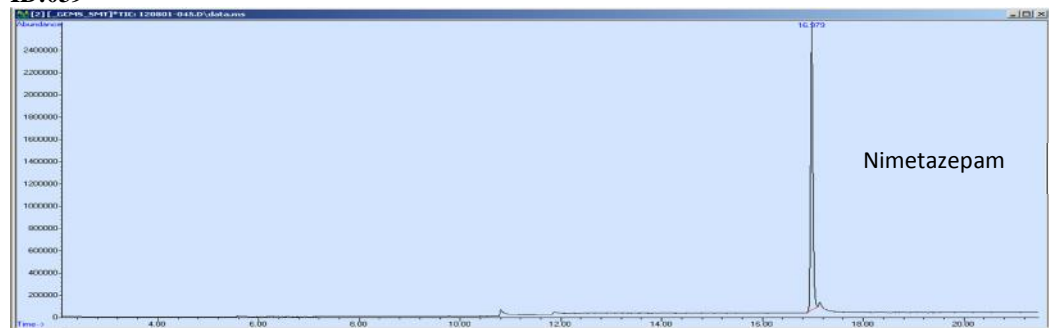
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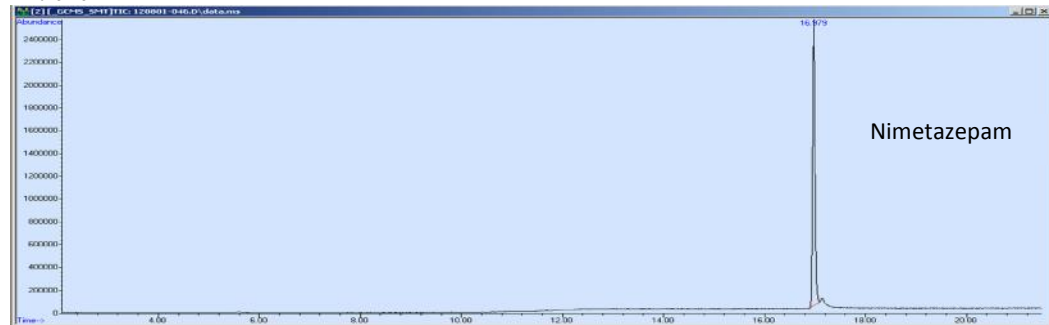
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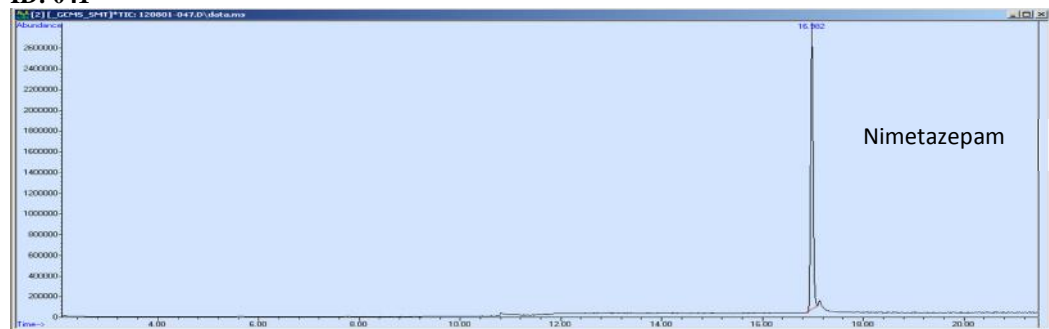
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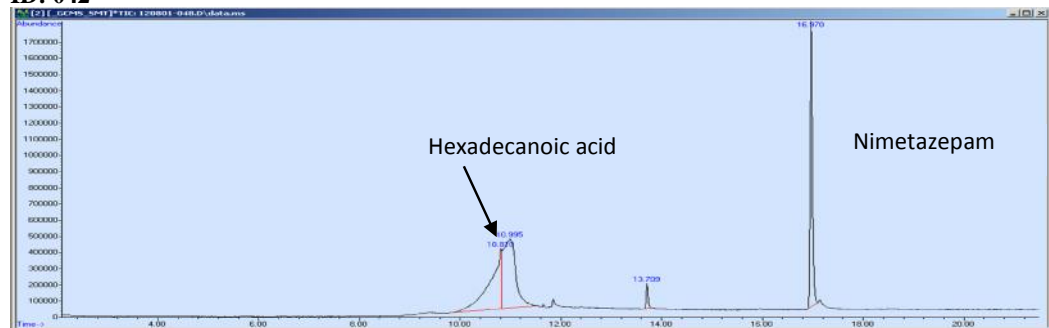
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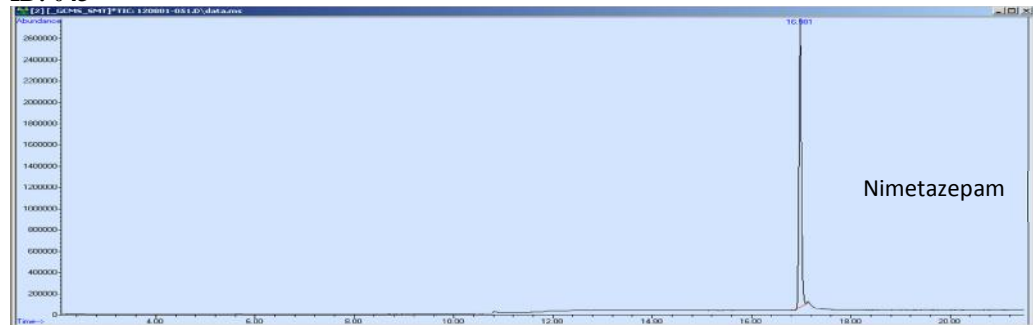
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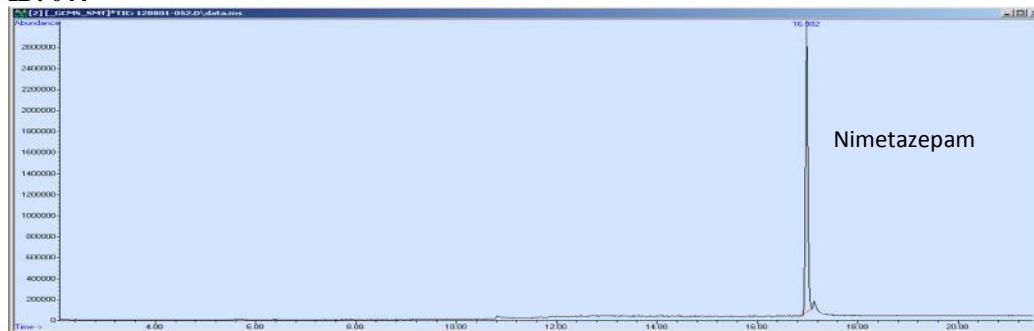
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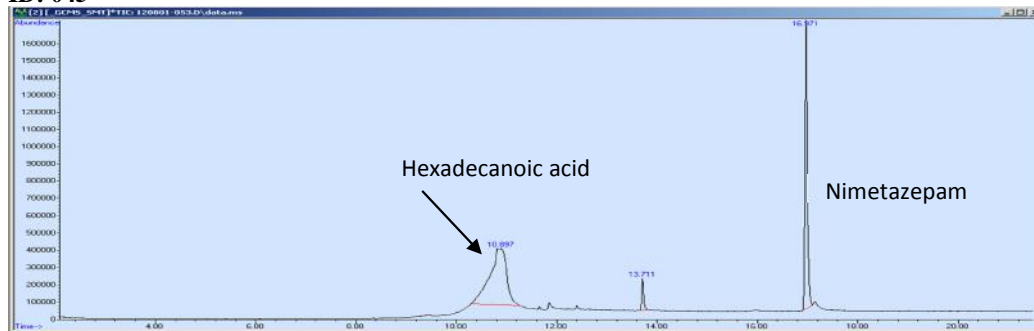
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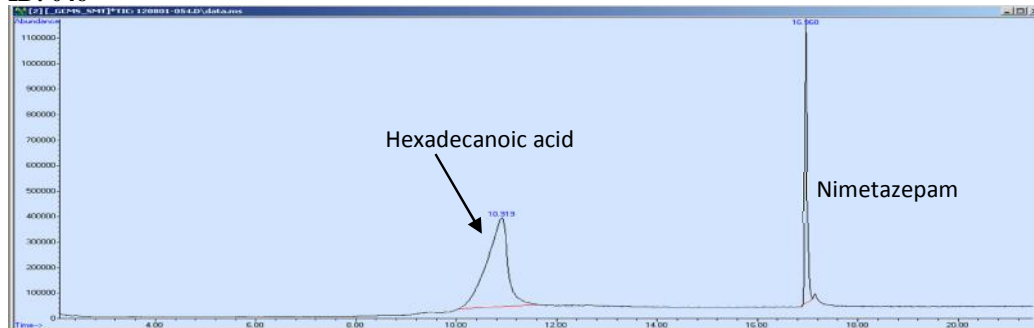
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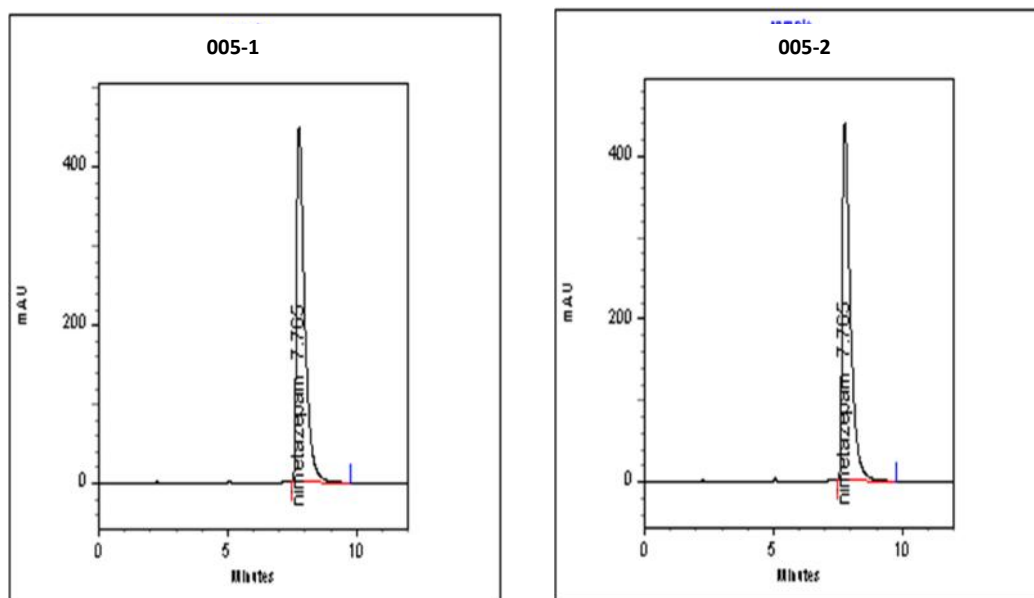
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APPENDIX 2

HPLC RESULT

SAMPLE ID: 005



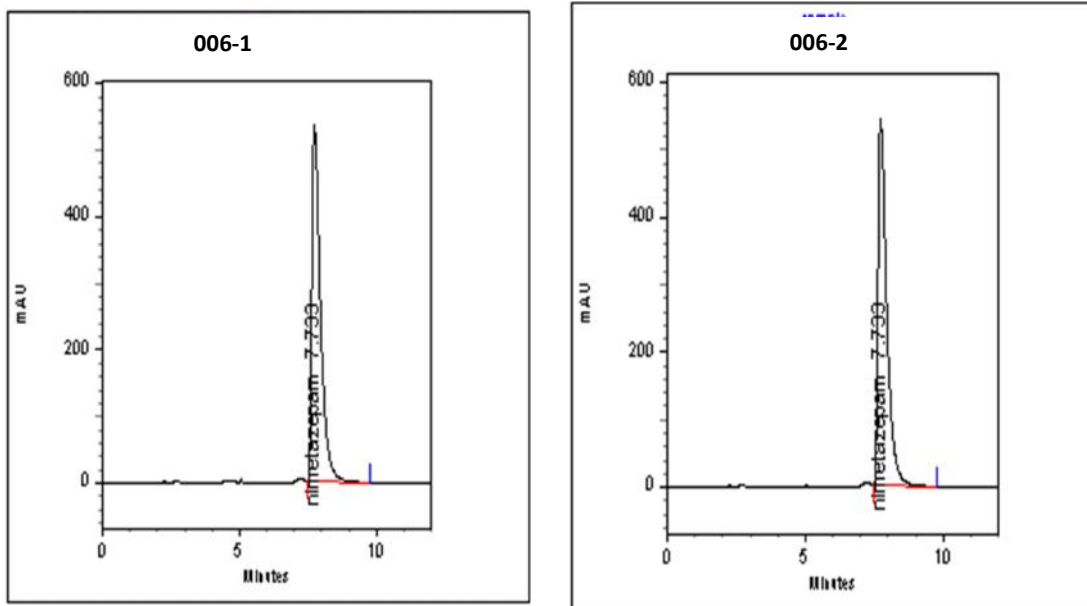
Example calculation of percentage of Nimetazepam in illicit Erimin 5 sample ID 005

Sample ID	Weight taken (mg)	HPLC reading (mg/mL)	% Nimetazepam
005-1	95.4	0.119	$= 0.119 \times 25 / 95.4 \times 100 = 3.11$
005-2	95.7	0.115	$= 0.115 \times 25 / 95.7 \times 100 = 3.00$
Mean			3.06 %

*Each sample was dissolved in 25 mL of mixture of Chloroform: Methanol (5:1)

HPLC RESULT

SAMPLE ID: 006



Example calculation of percentage of Nimetazepam in illicit Erimin 5 sample ID 006

Sample ID	Weight taken (mg)	HPLC reading (mg/mL)	% Nimetazepam
006-1	92.2	0.140	$= 0.140 \times 25 / 92.2 \times 100 = 3.79$
006-2	92.3	0.143	$= 0.143 \times 25 / 92.3 \times 100 = 3.87$
		Mean	3.83 %

*Each sample was dissolved in 25 mL of mixture Chloroform :methanol (5:1)

APPENDIX 3

HPLC ANALYSIS DATA

ID NO.	Average weight/tablet (mg)	Percentage (%) Nimetazepam	Weight Nimetazepam/tablet (mg/tablet)	Remark
001	179.1	0.00	0.00	Diazepam as active ingredient
002	185.7	3.04	5.64	-
003	188.1	3.26	6.13	-
004	189.8	3.22	6.12	-
005	191.5	3.06	5.86	-
006	185.9	3.83	7.13	-
007	187.8	2.68	5.04	-
008	190.1	2.69	5.11	-
009	178.2	3.01	5.36	-
010	203.3	0.00	0.00	Nitrazepam as active ingredient
011	184.0	0.00	0.00	Nitrazepam as active ingredient
012	193.5	2.10	4.06	-
013	185.5	3.45	6.41	-
014	193.6	2.93	5.67	-
015	184.6	3.41	6.30	-
016	191.8	2.41	4.62	-
017	198.5	2.49	4.95	-
018	183.8	4.16	7.64	-
019	194.0	3.01	5.83	-
020	184.7	3.31	6.11	-
021	194.9	2.38	4.64	-
022	192.2	2.56	4.92	-

ID NO.	Average weight/tablet (mg)	Percentage (%) Nimetazepam	Weight Nimetazepam/tablet (mg/tablet)	Remark
023	188.7	3.16	5.97	-
024	181.3	2.85	5.17	-
025	183.6	2.76	5.07	-
026	182.0	2.75	5.01	-
027	189.8	3.22	6.11	-
028	192.7	2.67	5.14	-
029	186.5	3.68	6.87	-
030	187.2	0.00	0.00	Nitrazepam as active ingredient
031	192.3	2.78	5.34	-
032	192.7	2.70	5.21	-
033	195.6	2.65	5.18	-
034	195.7	2.64	5.17	-
035	193.1	2.94	5.67	-
036	182.5	2.81	5.13	-
037	187.5	2.73	5.11	-
038	186.7	2.76	5.15	-
039	185.3	2.75	5.10	-
040	184.1	3.04	5.59	-
041	190.1	3.63	6.89	-
042	184.9	2.83	5.23	-
043	188.5	3.59	6.77	-
044	191.3	3.60	6.88	-
045	194.6	2.56	4.98	-
046	191.2	2.19	4.19	-

APPENDIX 4**ANALYSIS DATA****SUMMARY OF RESULTS**

ID NO.	Active Ingredients	Adulterant	Diluent	Dye
001	Diazepam	-	Lactose	Sunset Yellow
002	Nimetazepam	-	Mannitol	Sunset Yellow
003	Nimetazepam	-	Lactose	Sunset Yellow
004	Nimetazepam	-	Mannitol	Sunset Yellow
005	Nimetazepam	-	Mannitol	Sunset Yellow
006	Nimetazepam	-	Lactose	Sunset Yellow
007	Nimetazepam	-	Mannitol	Sunset Yellow
008	Nimetazepam	-	Mannitol	Sunset Yellow
009	Nimetazepam	-	Mannitol	Sunset Yellow
010	Nitrazepam	Caffeine	Lactose	Sunset Yellow
011	Nitrazepam	Caffeine	Lactose	Sunset Yellow
012	Nimetazepam	-	Lactose	Sunset Yellow, Tartrazine, Ponceau 4R
013	Nimetazepam	-	Lactose	Sunset Yellow
014	Nimetazepam	-	Lactose	Sunset Yellow, Tartrazine, Ponceau 4R
015	Nimetazepam	-	Lactose	Sunset Yellow
016	Nimetazepam	-	Mannitol	Sunset Yellow
017	Nimetazepam	-	Mannitol	Sunset Yellow
018	Nimetazepam	-	Lactose	Sunset Yellow
019	Nimetazepam	-	Lactose	Sunset Yellow, Tartrazine, Ponceau 4R
020	Nimetazepam	-	Lactose	Sunset Yellow
021	Nimetazepam	-	Mannitol	Sunset Yellow
022	Nimetazepam	-	Mannitol	Sunset Yellow
023	Nimetazepam	-	Lactose	Sunset Yellow
024	Nimetazepam	-	Mannitol	Sunset Yellow
025	Nimetazepam	-	Calcium Phosphate Dibasic dihydrate	Sunset Yellow, Ponceau 4R
026	Nimetazepam	-	Calcium Phosphate Dibasic dihydrate	Sunset Yellow, Ponceau 4R
027	Nimetazepam	-	Lactose	Sunset Yellow

ID NO.	Active Ingredients	Adulterant	Diluent	Dye
028	Nimetazepam	-	Lactose	Sunset Yellow, Ponceau 4R
029	Nimetazepam	-	Lactose	Sunset Yellow
030	Nitrazepam	caffeine	Lactose	Sunset Yellow
031	Nimetazepam	-	Mannitol	Sunset Yellow
032	Nimetazepam	-	Mannitol	Sunset Yellow
033	Nimetazepam	-	Mannitol	Sunset Yellow
034	Nimetazepam	-	Mannitol	Sunset Yellow
035	Nimetazepam	-	Lactose	Sunset Yellow, Tartrazine, Ponceau 4R
036	Nimetazepam	-	Calcium Phosphate Dibasic dihydrate	Sunset Yellow, Ponceau 4R
037	Nimetazepam	-	Calcium Phosphate Dibasic dihydrate	Sunset Yellow, Ponceau 4R
038	Nimetazepam	-	Lactose	Sunset Yellow
039	Nimetazepam	-	Lactose	Sunset Yellow
040	Nimetazepam	-	Lactose	Sunset Yellow
041	Nimetazepam	-	Lactose	Sunset Yellow
042	Nimetazepam	-	Mannitol	Sunset Yellow
043	Nimetazepam	-	Lactose	Sunset Yellow
044	Nimetazepam	-	Lactose	Sunset Yellow
045	Nimetazepam	-	Mannitol	Sunset Yellow
046	Nimetazepam	-	Mannitol	Tartrazine, Brilliant Blue