Preparation of a Nimetazepam Reference Standard

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ABSTRACT: Nimetazepam is very frequently submitted to the forensic laboratory for analysis and therefore a traceable nimetazepam reference standard is required. However, nimetazepam reference standard is expensive and difficult to purchase. We described how a relatively pure sample of nimetazepam (ca. 98%) was further purified, authenticated and assayed against a nimetazepam reference standard. The product fulfilled both analytical standard criteria and quality control criteria and therefore can serve as a traceable nimetazepam reference standard for forensic quantitative determination of the drug.

Keywords: Nimetazepam, reference standard, purification, analytical criteria, quality control

Introduction

In Malaysia nimetazepam and flunitrazepam are the only two benzodiazepines listed in the Dangerous Drugs Act 1952. While flunitrazepam is seldom encountered, nimetazepam in the form of "Erimin 5" tablets is widely abused and very frequently submitted to the forensic laboratory for analysis. For prosecutorial purposes a quantitative determination of the drug is required and hence the constant need of a traceable nimetazepam reference standard. Nimetazepam reference standard is expensive (most favourable offer from the suppliers is RM 9860 per gram) and difficult to purchase. In this article we describe how a relatively pure sample of nimetazepam (ca. 98%) was further purified, authenticated and assayed against a nimetazepam reference standard provided by Sumitomo Chemical Company of Japan.

In an attempt to prepare a nimetazepam reference standard for the use of our forensic drug laboratories, we aimed to determine the suitability of solvents for re-crystallisation, authenticate and assay the purified nimetazepam against a primary reference standard provided by Sumitomo Chemical Company, Japan, and determine UV absorbance of nimetazepam in 0.1N HCl and methanol.

Procedure and Results

i) Selection of solvents for recrystallisation

The solubility of nimetazepam in various solvents was tested to determine which solvent(s) was suitable for purification by re-crystallisation. As a guide the solubility of flunitrazepam and nitrazepam given in the literature [1, 2] were referred to as these compounds were most similar to nimetazepam structurally. From these solubility tests the solvent combination of chloroform/ether was chosen for re-crystallisation of the nimetazepam (**Table 1**).

Table 1: Solubility of nimetazepam in increasing order

Solvent	Solubility
Water	Practically insoluble
Diethyl ether	Very slightly soluble (1 part in 5000)
Ethanol (95%)	Slightly soluble (1 in 500)
Methanol	Slightly soluble (1 part in 100)
Ethyl acetate	Soluble (1 part in 30)
Acetone	Soluble (1 part in 25)
Chloroform	Freely soluble (1 part in 3.5)

ii) Purification of the Nimetazepam

Twenty grams of the nimetazepam powder was dissolved in about 70 mL of chloroform**.** The yellowish solution was filtered through a membrane disc filter (SUN SRI, 47 mm Nylon filter, pore size 0.45 µm, Cat. No. 24745-NN). To the clear yellow filtrate, 600 mL of diethyl ether was added to precipitate out the almost white nimetazepam crystals. The crystals were recovered by filtration after the solution had been placed in the chiller for two hours. The re-crystallisation was repeated and the final product dried in the oven at 105 °C for 3 hours. Percentage yield from each re-crystallisation was about 80%, therefore with starting weight of 20 g the yield was 12.8 g (0.8 x 0.8 x 20 g) of a pale yellow crystalline powder.

iii) Authentication

Nimetazepam was definitively identified by GC-MS and infra red spectrophotometry [3, 4, 5] with the instrumental conditions given in the Annexe.

vi) Melting Point

The melting point of the purified nimetazepam determined with the Buchi melting point apparatus was found to be 158-160 °C.

v) UV Absorbance [6]

The UV spectra in 0.1N HCl and methanol were measured with a Varian Cary 100Conc UV-Vis spectrophotometer using 1-cm wide cuvettes.

The absorbance of a 1% solution in methanol, $[A]^1_1$ at 260 nm and 309 nm were 526 and 328 respectively (or ε , molar absorptivity of 15532 and 9685).

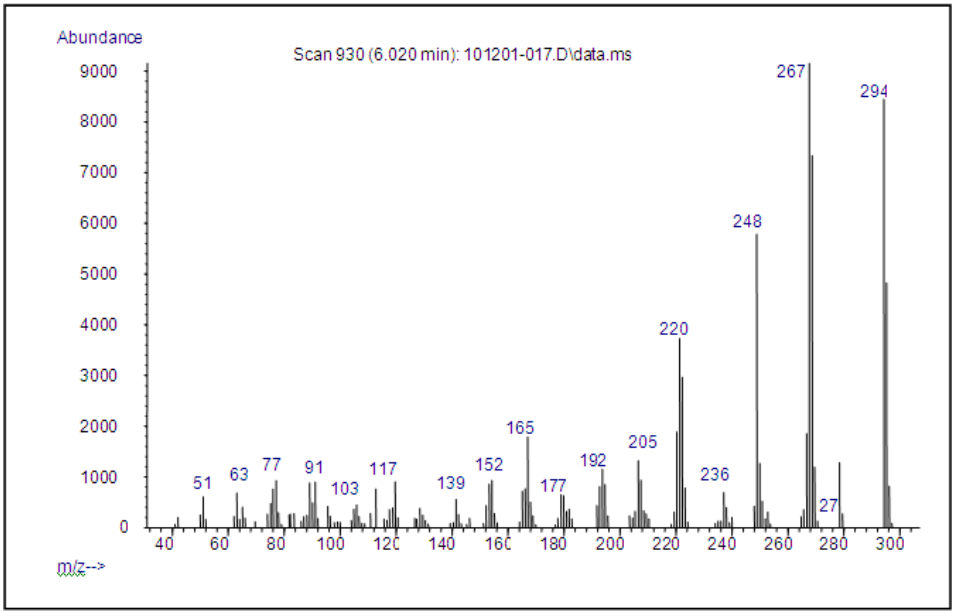

Fig. 1: Mass spectrum of the purified nimetazepam with principal ions at m/z : 267, 294, 295, 268, 248, 221, 220, 165

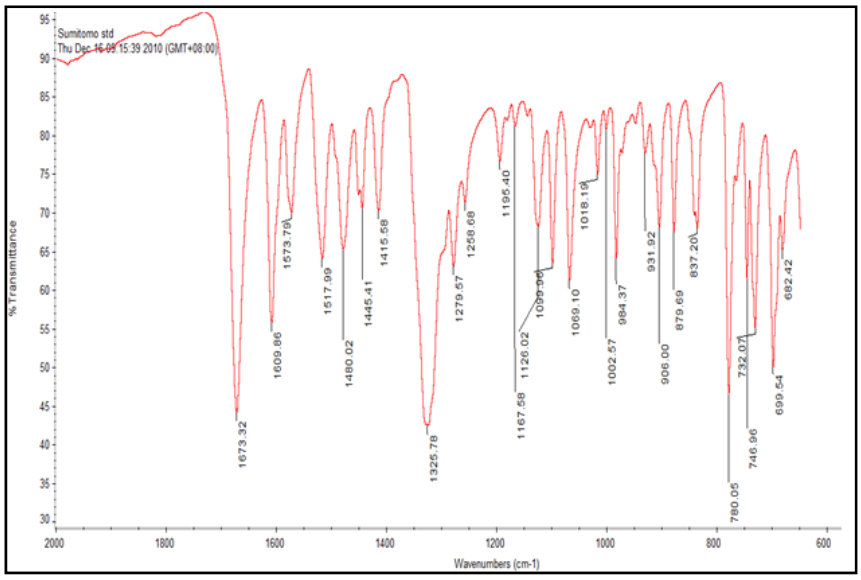

Fig.2: IR of nimetazepam reference standard from Sumitomo Chemical Company, Japan

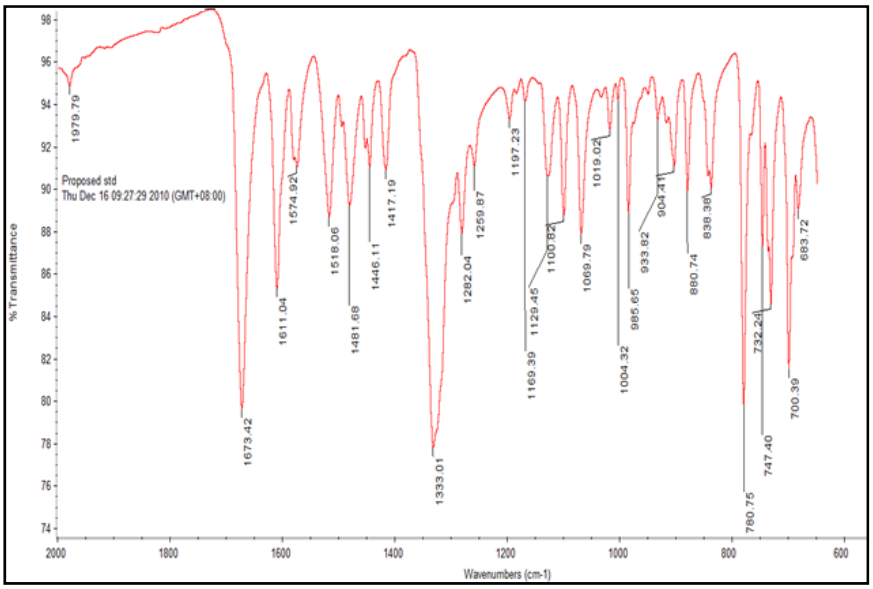

Fig. 3: IR of purified nimetazepam (the proposed reference standard)

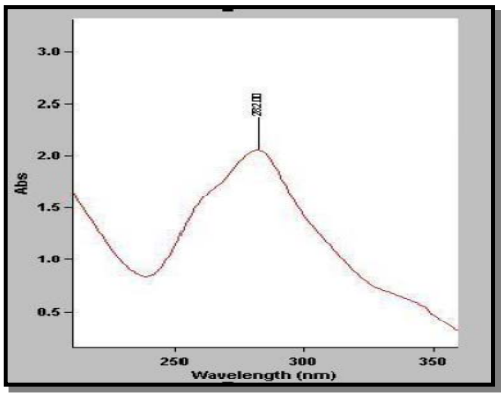

Fig. 4: λ_{max} = 282 nm (in 0.1N HCl)

vi) Assay by HPLC

The nimetazepam purified by two re-crystallisations in chloroform/ether was assayed against the Sumitomo reference standard by an HPLC external standard method [7]. To minimize instrumental drift injections of reference standard and proposed reference standard were made alternately. The % Relative standard deviation (RSD) of the five injections each for the Sumitomo reference standard and the proposed reference standard were well below the limit of 3.0% [3]. The percentage purity was found to be 99.3% (**Table 2**).

Table 2: Determination of% purity of proposed nimetazepam standard by HPLC

Run No.	Sumitomo Standard (Peak Area)	Proposed Standard (Peak Area)
1	21220979	22637279
2	21250823	22602254
3	21171821	22625576
4	21298910	22656986
5	21284413	22657026
Mean	21245389	22635824
Standard Deviation	51051	23084
% RSD	0.24	0.10
Weight, mg	52.09	55.83
Area/Weight	407859	405442
% Purity		**99.3**
% Purity of Sumitomo Standard (Lot No. 05104)		99.9

Discussion

The solubility data indicates chloroform as the most suitable solvent for the dry extraction of nimetazepam from Erimin 5 tablets when rapid identification in the field by FTIR (e.g. use of the portable HAZMAT FTIR-ATR) is required. Major tablet components such as lactose, starch, magnesium stearate used as excipients (diluents, binders, and lubricants) are practically insoluble in chloroform and therefore not extracted.

The IR spectrum of nimetazepam is almost identical with that of flunitrazepam between 1000 and 1700 cm-1. The only noticeable difference is an additional band of medium intensity at 1215 cm^{-1} in the spectrum of flunitrazepam [5]. Hence care should be taken when identification is based solely on this technique as flunitrazepam has been known to occur in Erimin 5 tablets. Both flunitrazepam and nimetazepam give a positive violet colour reaction to the Zimmermann (Janovsky) reagent.

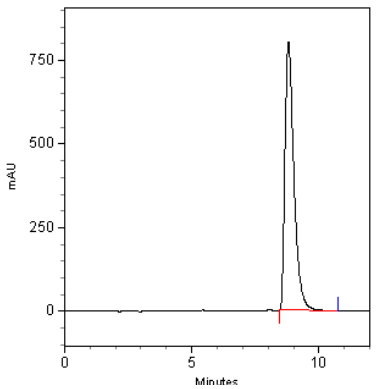

Fig. 5: HPLC trace after one recrystallisation

It was noted that most of the nimetazepam in Erimin 5 tablets analysed in this laboratory had a similar HPLC profile with regard to an unidentified impurity at about 8.0 minutes. The other peaks at 2.2 and 5.5 minutes are attributed to the solvent (**Fig. 5**).

Conclusion

A traceable nimetazepam reference standard which fulfilled analytical standard criteria and quality control criteria was prepared from purification of a relatively pure seized sample. A summary of the analysis is given in the Annexe (Certificate of Analysis). Nimetazepam has a λ_{max} at 282 nm in 0.1N HCl and the absorbance of a 1% solution in methanol (in 1-cm wide cuvettes) was found to be 328 and 526 at the wavelengths 309 nm and 260 nm respectively.

Acknowledgement

The provision of the nimetazepam reference standard by Sumitomo Chemical Company Ltd., Tokyo is greatly appreciated.

References

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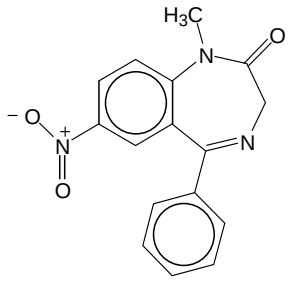
ANNEXE: Certificate of Analysis

DEPARTMENT OF CHEMISTRY MALAYSIA

Certificate of Analysis

Compound Name: **Nimetazepam**
CAS # : 2011-67-8
Description: Pale yellow crystalline powder Date prepared : 17 December 2010
Chemical Formula: $C_{16}H_{13}N_3O_3$ Mol. Wt : 295.29
Structure:



Synonyms: 1-methyl-7-nitro-5-phenyl-2H-1,4-benzodiazepin-2-one, 1-methylnitrazepam

Purity: 99.0% minimum

Melting Point: 158 - 160 °C

UV Absorbance
UV_{max} (0.1 N HCl) 282 nm ;
UV_{max} (methanol) 260, 309 nm ($[A]^{1}_{1}$ = 526, 328 or ε = 15532, 9685)

GC/MS: Spectrum matches reference sample and literature spectra

Principal m/z values:	267, 294, 295, 268, 248, 221, 220, 165
Instrument:	Shimadzu GC-MS Model QP5050
Column:	DB-5MS, 30 meter, 0.25 mm id., 0.25 µm film thickness
Temp. :	220 °C (2 mins), 20 °C/min to 300 °C and hold 5 mins.
Injector Temp:	250 °C. Transfer line: 280°C.

IR Spectrum matches reference and literature spectra

HPLC Assayed against Sumitomo reference standard (Lot No. 05104):
99.3% (mean of 5 determinations; RSD = 0.10%)

Instrument	Shimadzu
Column:	C18, (5 µm, 15 cm x 4.6 mm i.d.)
Mobile Phase:	Methanol: water (65:50); adjust pH to 4.0 with phosphoric acid
Flow rate:	0.8 mL/min
Injection Volume:	20 µL
Detector:	UV at 265 nm