The Quantitation of Nimetazepam in Erimin-5 Tablets and Powders by Reverse-Phase HPLC

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ABSTRACT: The sedative-hypnotic nimetazepam in "Erimin 5" tablets and powders was quantitated by reverse phase HPLC. The selectivity, precision, and accuracy of the procedure are presented.

KEYWORDS: Nimetazepam, Erimin-5, Benzodiazepines, HPLC, Forensic Chemistry

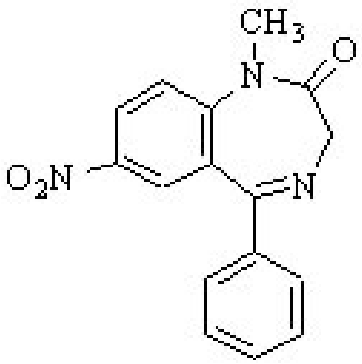


Figure 1: Structure of Nimetazepam

Introduction

Since its appearance in illicit drug markets in Malaysia in the mid-1980's, the benzodiazepine nimetazepam (Figure 1) has become the most commonly abused sedative in the country (midazolam and triazolam are the (distant) second and third most abused sedatives). 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. Most of the abusers are believed to be heroin addicts, who use it as a substitute for heroin when its availability is low. More recently, however, nimetazepam has also been used as a sedative by methamphetamine abusers to help them sleep after binging (in fact, the rise in nimetazepam abuse roughly parallels the rise in methamphetamine abuse in Malaysia). The illicit use of nimetazepam is continuing to increase, as shown by the number and size of seizures made over the past few years. For example, a seizure of 310,000 tablets was made in June 2002 at a residence near the capital city (Kuala Lumpur). Tablet submissions to the Central Laboratory have been in the hundreds of thousands for each of the three years 2002 - 2004. Similar abuse of nimetazepam has been reported in neighboring countries.

The two primary forms of nimetazepam encountered in Malaysia are a commercial product (Erimin-5 tablets in blister packs (see Photos 1 - 2)) or Erimin-5 counterfeits, and an orange colored powder that appears to be either finely crushed tablets or the tablet mixture prior to tableting. Commercially prepared tablets nominally weigh

about 170 mg and contain about 5 mg of nimetazepam each. However, as noted above, many of the Erimin-5 tablets submitted to the Narcotics Section appear to actually be counterfeit products that contain nimetazepam and/or various other benzodiazepines, notably diazepam and nitrazepam, in varying quantities.

Nimetazepam was added to the Malaysian Dangerous Drugs Act 1952 in May, 2001 and is currently the only benzodiazepine controlled in Malaysia. The analysis of nimetazepam by a variety of techniques has been previously reported (1-4), including by CE and CEC (5-7), Color Testing (8), FTIR (9), GC (10-12), HPLC and HPLC/MS (13-18), TLC (17,19), and UV/Vis (20). Herein, we report the quantitation of nimetazepam in seized tablets and powders with reverse-phase HPLC, using an external standard method.

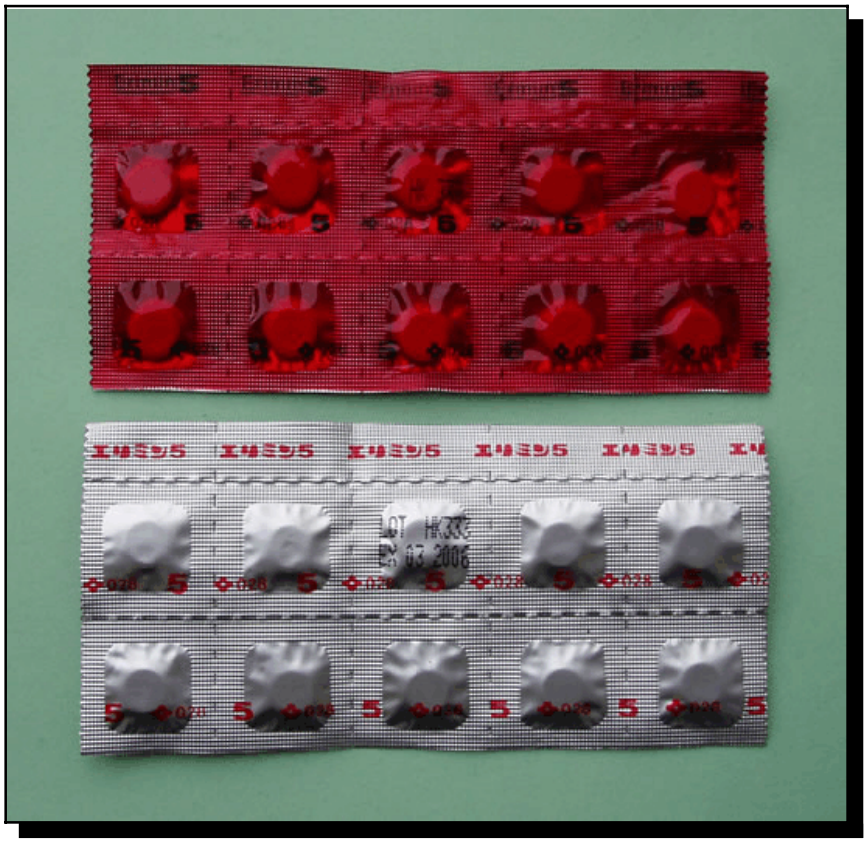


Photo 2 - Front and Back Views of a Erimin-5 Blister Pack (Note: This is a Suspected Counterfeit)

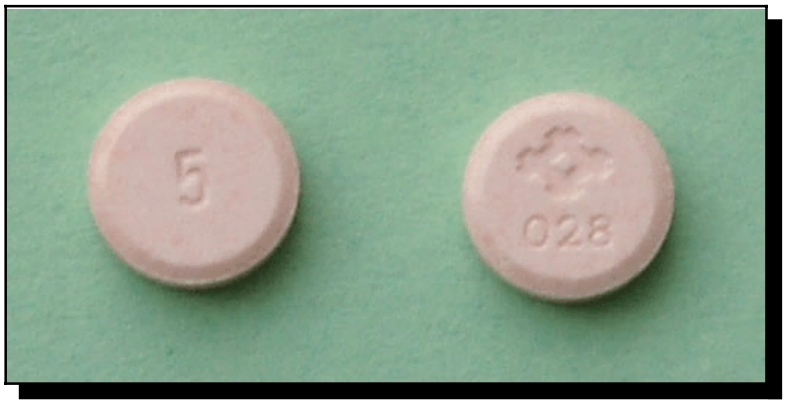


Photo 2 - Closeup of an Erimin-5 Tablet (Front and Reverse)

Experimental

Chemicals

HPLC grade methanol and chloroform were purchased from Merck, while AR grade orthophosphoric acid (84 %) was purchased from Ajax (Australia). Nimetazepam (free-base) standard of 100 % purity was kindly provided free of charge by Sumitomo Chemical Company (Tokyo, Japan). The following benzodiazepines (as free bases) were obtained from the United Nations Drug Control Programme (UNDCP) in Vienna (Austria): Nitrazepam, bromazepam, tetrazepam, flunitrazepam, oxazepam, lorazepam, clorazepate dipotassium (salt), diazepam, flurazepam, and medazepam. Unfortunately, midazolam and triazolam standards were unavailable, and so were not run.

Instrumentation

A Hewlett Packard Series 1050 HPLC was used with the following parameters:

Column :	C-18, 5 μm particle size, 15 cm x 4.6 mm i.d. (from Alltech).
Detector :	UV at 265 nm.
Mobile phase:	Methanol:Water (50:65). The pH was 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) (21).
Column temperature:	25º C (ambient temperature).
Flow rate :	1.5 mL/minute.
Average Pressure:	155 bar.
Injection:	20 μL by Rheodyne loop injector.
Attenuation:	4 (Integrator).

Standard Solutions for Linearity Study and Calibration

Standard solutions containing 0.020, 0.040, 0.080, 0.120, 0.160, 0.200 and 0.240 mg/mL of nimetazepam were prepared in a mixture of methanol/chloroform (5:1) (note that the chloroform was added to better solubulize the tablet materials, and had no adverse effects on the chromatography).

Quantitative Analysis of Samples

About 70 – 100 mg of homogenized tablet material was accurately weighed into a 25 mL volumetric flask and made up to volume with a mixture of methanol/chloroform (5:1). The sample solution was ultrasonicated for 5 minutes and filtered through a 0.45 μm filter before injected onto the column. Quantitation was by external standard and with reference to the peak area of the 0.120 mg/mL nimetazepam standard.

Procedure for Standard Addition Method

(i) 350.80 mg of tablet material was weighed into a 100 mL volumetric flask, made up to volume with methanol/chloroform (5:1), and ultrasonicated for 5 minutes. (ii) 10 mL of the solution in (i) (i.e., equivalent to 35.08 mg of tablet material) was pipetted into each of five 25 mL volumetric flasks. (iii) The following aliquots of nimetazepam standard stock solution (1.00 mg/mL) were pipetted into the solutions in (ii): 0, 1, 2, 3, and 4 mL. (iv) The solutions were made up to volume (i.e., 25 mL) with methanol/chloroform (5:1). (v) The solutions were filtered through a 0.45 μm filter and injected into the HPLC. (vi) A graph of area versus concentration of nimetazepam (mg/mL) was plotted using Excel and the native nimetazepam content calculated.

Results and Discussion

Selectivity

Identification of benzodiazepines is accomplished in this laboratory by GC/MS. However, GC and GC/MS are problematic for quantitation of nimetazepam and some related benzodiazepines due to thermal degradation at injector port temperatures, and so HPLC was selected for quantitation. Because of the wide diversity of chemical structures and solubility characteristics among the benzodiazepines, no single HPLC method will separate all of

them. The specificity of the method presented herein was defined in terms of the benzodiazepines typically found in Malaysia. The identities and retention times of these benzodiazepines using the presented methodology are presented in Table 1.

Table 1: Retention Times of Benzodiazepines (HPLC)

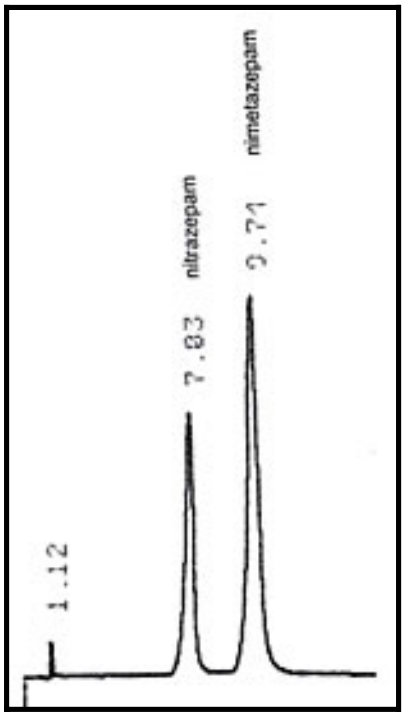
Benzodiazepine	Retention Time (min)
Nitrazepam	6.91
Bromazepam	7.62
Tetrazepam	7.80
Flunitrazepam	8.54
Oxazepam	8.55
Lorazepam	8.93
Nimetazepam	9.94
Clorazepate dipotassium	17.07
Diazepam	26.63
Flurazepam	NE
Medazepam	NE

NE: Did not elute within 30 minutes.

Of the selected benzodiazepines, flunitrazepam, oxazepam, and lorazepam elute closest to nimetazepam, and give partially overlapping peaks. Thus, the presented HPLC method is not appropriate for samples containing these compounds. Fortunately, however, experience has shown that these three benzodiazepines are very rarely present in tablets or powders containing nimetazepam. A few samples of "Erimin-5" tablets have been found to contain diazepam instead of nimetazepam; however, diazepam elutes much later than nimetazepam. A typical HPLC chromatogram of a mixture of nitrazepam and nimetazepam is displayed in Figure 2.

Figure 2: HPLC of Nitrazepam and Nimetazepam

Note: Slight variations in Retention Times between Table 1 and Figure 2 are due to natural variations over time; the order of elution was found to be consistent from run to run.



Calibration Curve and Linearity

The calibration graph (Figure 3) for the analysis was found to be linear from 0.020 mg/mL to 0.240 mg/mL. From linear regression analysis, the correlation coefficient was better than 0.99, and the percent difference between the known concentration and the predicted concentration from the regression equation was less than 5 %. In routine analyses a single point calibration was used.

Precision

The precision of the method was assessed by 10 replicate analyses of a homogenized sample of "Erimin 5" tablets. Injections were all made in triplicate and quantitation was

against the 0.120 mg/mL standard. The mean content of nimetazepam was found to be 3.1 % with a relative standard deviation of 4.4 %.

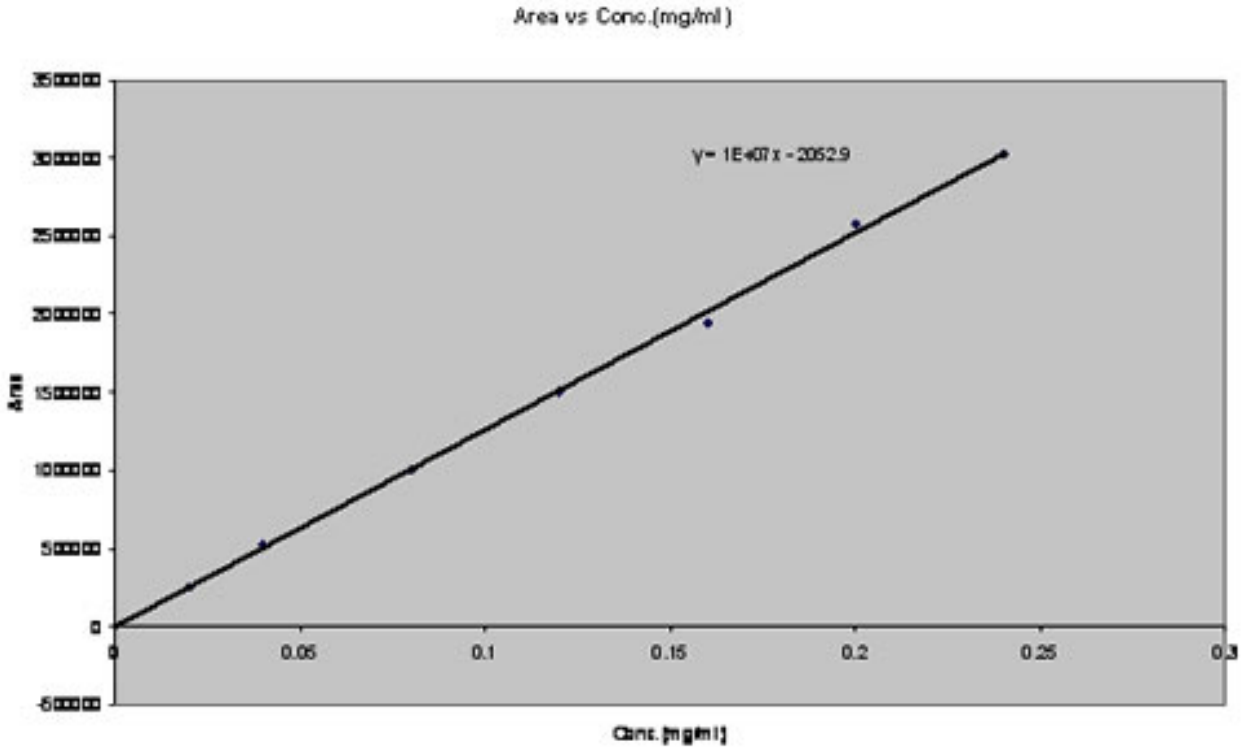


Figure 3: Calibration Curve of Nimetazepam

Accuracy

The accuracy of the method was assessed by analyzing two laboratory prepared mixtures, and re-analysis of the sample which was used for the precision study by the method of standard addition.

Analysis of Laboratory Prepared Mixtures

Owing to the limited supply of pure nimetazepam reference standards, only two synthetic mixtures were prepared, simulating 5 mg/tablet and 3 mg/tablet. Both the samples were prepared in lactose and contained 3.5 % and 1.7 % of nimetazepam, respectively. Replicate analyses (n = 7) of these two mixtures were made and the results assessed using the Student t-statistic:

$$t = | \bar{x} - \mu | \frac{\sqrt{n}}{s}$$

where $\bar{x}$ = sample mean (experimental value)
μ = true value (theoretical value)
n = no. replicate (weighings)
s = standard deviation

For both samples it was found that the t value did not exceed the critical value derived by statistical analysis, showing that there was no proven evidence of difference between the experimental value and the theoretical value at 95 % confidence level.

Standard Addition Method

The same nimetazepam tablet material which was used in the precision study was re-analyzed using the standard addition method. From the standard addition calibration graph (Figure 4) the amount of nimetazepam was found to be 3.1 %. This agreement with the precision study mean value shows that there is no interference from the tablet excipient materials, and thus to some extent shows that the method is accurate.

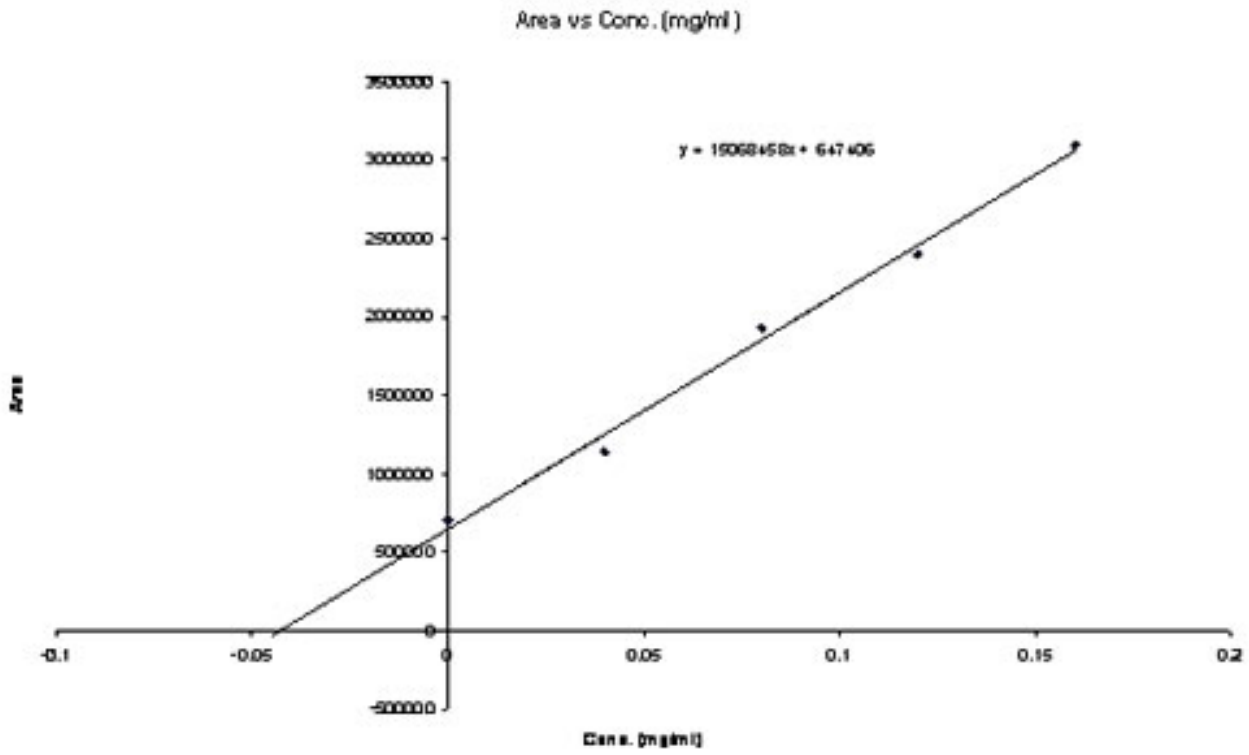


Figure 4: Standard Addition Calibration Curve

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