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STATE POLICE

DRIVING UNDER THE INFLUENCE OF DRUGS: ANALYSIS OF PHENAZEPAM IN BLOOD

FORENSICS

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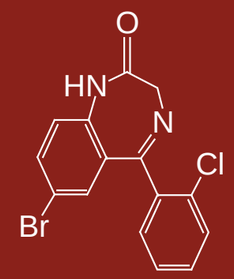

Fig. 1 The chemical structure of Phenazepam

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INTRODUCTION

Phenazepam [7-bromo- 5-(2-chlorophenyl)- 1,3-dihydro- 2H- 1,4-benzodiazepin- 2-one] (Fig. 1) is a benzodiazepine drug, which was developed in the Soviet Union and now produced in Russia and other countries. Phenazepam is used in the treatment of neurological disorders such as epilepsy, alcohol withdrawl and insomnia. It can also be used as a premedication before surgery, as it augments the effects of anesthetics and reduces anxiety. Phenazepam is available in 0.5 mg tablet. The maximum daily dosage of this drug should not exceed 10 mg.

Side effects include dizziness, loss of coordination and drowsiness, along with anterograde amnesia which can be quite pronounced at high doses. As with other benzodiazepines, in case of abrupt discontinuation following prolonged use, severe withdrawal symptoms may occur, including restlessness, anxiety, insomnia and convulsions.

Phenazepam has been determined in biological fluids by gas chromatography mass spectrometry and GC-NPD, following liquid-liquid extraction.

MATERIALS AND METHODS

Reagents

Phenazepam was purchased from Lipomed (MA, USA) , Diazepam-d5 (internal standard) from Cerilliant (TX,USA), and formic acid (99%) from Acros Organics. All other solvents were HPLC grade or better and were obtained from Fisher Scientific.

A standard stock reference solution of phenazepam (1.0 mg/L) was prepared in acetonitrile. Phenazepam standards were prepared by spiking drug free whole blood at the following concentrations: 1.0, 10, 25, and 50 µg/L. Another stock reference solution was prepared in acetonitrile (1.0 µg/L) and was used for the preparation of controls at 4.0 and 40.0 µg/L. Phenazepam concentrations in the control blood specimens were calculated from linear regression of the standard responses based on the peak-area ratio.

Extraction

Phenazepam and the internal standard (Diazepam-d5) were extracted from buffered (pH 6) blood samples (1 mL) utilizing Clean Screen® DAU SPE cartridges (UCT, Inc.). After washing the columns with DI water, acetic acid, and methanol (3 mL, 2 mL, and 3 mL, respectively), samples were eluted with CH_2Cl_2/IPA/NH_4OH (3 mL) and evaporated to dryness. To the dry eluates, 200 µL of 0.1% aqueous formic acid was added prior to the chromatographic analysis.

Instrumentation

Liquid chromatography equipment consisted of a Shimadzu Prominence (two pumps LC-20AD, autosampler SIL-20AC, and column oven CTO-20AC) using Imtakt™ C18 column (50x 2.1mm, 5 µm), at 0.5mL/min. flow gradient program. The mobile phase program (A) 0.1% aqueous formic acid / (B) acetonitrile containing 0.1% formic acid was started at 5% (B) for 0.5 min, increasing to 90% (B) over 4 min, before returning to 5% (B) and equilibrated for 1 min. The total chromatographic run time was 6 minutes including equilibration.

MS/MS analysis was conducted using an Applied Biosystems 3200 Q Trap instrument equipped with ESI source in the positive ion mode and operated with multiple reaction monitoring (MRM) under the following conditions: curtain gas 15 psi, collision gas medium, ion spray voltage 5000V, temperature 650°C, ion source gas (1 and 2) 50 psi. The following transitions were monitored (quantification ions underlined): m/z 350.8→ 206.3 and 104.1 for Phenazepam, and 290.1 → 198.3 and 154.3 for Diazepam-d5 (internal standard).

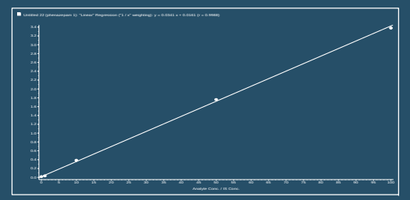

Fig. 2: Standard curve for Phenazepam SPE extraction

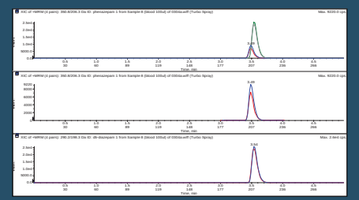

Fig. 3: Case Sample blood extracted and quantitated = 9.0 µg/L

Upper :TIC
Middle : Phenazepam
Lower : Diazepam-d5

RESULTS AND DISCUSSION

Linearity (r2 >0.99) was achieved from 0.5 to 100 µg/L (Fig 2) using a five point calibration curve (1.0, 10, 50, and 100 µg/L). The limits of detection and quantification were 0.5 and 1.0 µg/L, respectively. Recovery values for blood (target value: 4.0 µg/L) were > 90%. Intra and inter-day precision was < 5% and 8%, respectively. Ion suppression studies revealed that suppression of monitored ions was < 6%.

CONCLUSIONS

This presentation describes a method of analysis that provides a simple, sensitive, and reproducible quantitative method. It should be of great assistance to those analysts actively involved with the LC/MS/MS analysis of phenazepam in biological matrices, as well as an efficient method of extraction for phenazepam. The data from this case should add to the body of knowledge already accumulating in the literature of Forensic Toxicology .[1,2]

REFERENCES

[1.] Bailey et al J.A.T (2010) **34**: 527-32
[2.] Johnson W., Toxtalk (2010) **34**: 17-18

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