



### K35 2,4-Dinitrophenol (2,4-DNP)—An “Old New Drug”: A Gas Chromatography/Mass Spectrometry (GC/MS) Method for Screening and Quantitation of 2,4-DNP in Postmortem Blood Specimens and Three Fatal Poisoning Cases Reports

Eduardo G. de Campos, MSc\*, Center for Forensic Science Research and Education, Willow Grove, PA; Melissa Friscia, MSFS, Center for Forensic Science Research and Education, Willow Grove, PA 19090; Barry K. Logan, PhD, NMS Labs/Center for Forensic Science Research and Education, Willow Grove, PA 19090

**Learning Overview:** After attending this presentation, attendees will be able to describe a GC/MS method for the toxicological analysis of 2,4-DNP in blood and its concentration in authentic postmortem specimens.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing a novel method for the analysis of 2,4-DNP in whole blood specimens and by reporting concentrations of 2,4-DNP found in authentic postmortem blood samples for future reference.

**Introduction:** 2,4-DNP is a substance that has been used as a pesticide and for manufacturing explosives and dyes. In 1933, 2,4-DNP was initially used as a weight-loss drug, but it was prohibited for human consumption in 1938 due to its serious adverse effects, including extreme loss of weight, hyperthermia, fatigue, respiratory depression, and death. In recent years, there have been reports of 2,4-DNP reemerging as a weight-loss drug and of associated lethal intoxication induced by this compound.

**Methods:** Sample preparation was performed by Liquid-Liquid Extraction (LLE). The samples were pretreated with a clean-up basic extraction using 0.1 N NH<sub>4</sub>OH, followed by an acidic extraction using 0.1 N HCl to target the 2,4-DNP, using n-butyl-chloride as the extraction solvent. 4-nitrophenol was used as the internal standard. Samples were rotated for 15min and centrifuged for 10min at 4,600rpm. After the final acidic extraction, the organic layer was collected and dried under nitrogen flow at 35°C for 30min. The extract was reconstituted in N,O-Bis(Trimethylsilyl)Trifluoroacetamide (BSTFA) -1% Trimethylchlorosilane (TMCS) and derivatized for 30min at 80°C. Analysis was performed by GC/MS, with electron impact ionization, and using hydrogen as the carrier gas. An HP-5MS column (30m x 0.25mm x 0.25um) was used and the total run time was 8.5min. Injector, MS source, and MS quadrupole temperatures were set to 265°C, 230°C, and 150°C, respectively.

**Results/Discussion:** A method with no derivatization was first evaluated using GC/MS with hydrogen and helium as carrier gases. However, underivatized-2,4-DNP was only detected at elevated concentrations using helium as the carrier gas. This study further evaluated a BSTFA derivatization step to increase the sensitivity. A recovery of 84% for 2,4-DNP was achieved with the LLE method. The method also showed good linearity in the range of 1.0mg/L–100mg/L (R<sup>2</sup>=0.9979).

Postmortem blood samples collected during autopsies from three suspected 2,4-DNP-related cases were analyzed. Case 1 was a 21-year-old male who was admitted to the emergency room after allegedly ingesting about 3mL of wintergreen oil. He presented with shortness of breath, increased body temperature, sweating, and increased heart rate. A family member stated that he had been consuming a thermogenic supplement, and a bottle of 2,4-DNP with an Aldrich® label was found at his house. The concentration of 2,4-DNP in his blood collected at the time of autopsy was 48mg/L. Case 2 was a male who presented with a 2,4-DNP concentration in postmortem iliac blood of 13mg/L. Case 3 was a female who was found dead and had a weight-loss supplement in her purse containing 2,4-DNP. A concentration of 3.3mg/L was found for 2,4-DNP in femoral blood.

**Conclusions:** 2,4-DNP is a highly polar phenolic compound and its detection by GC is an analytical challenge because 2,4-DNP shows a strong interaction with the stationary phase. In addition, 2,4-DNP derivatization by silylation with BSTFA seems to be very sensitive to reaction medium—better results were achieved when the derivatization was promoted with no solvent in the reaction medium (as acetonitrile). In this work, a GC/MS-based method, using hydrogen as carrier gas, for 2,4-DNP detection and quantitation was successfully developed, presenting a high potential to be easily implemented and performed in routine toxicological analysis. As the 2,4-DNP seems to be reemerging as a weight-loss drug, the method can detect and quantify 2,4-DNP in authentic human whole blood samples.

#### 2,4-Dinitrophenol, Gas Chromatography, Postmortem Toxicology