2,4-Dinitrophenol: A dietary supplement that "blows" you away

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BACKGROUND

Introduction

2,4-Dinitrophenol (DNP) has traditionally been utilised as a fungicide, dye, herbicide and as a secondary explosive.[1] Despite the dangers demonstrated during World War I when workers became ill after exposure to the chemical during production of explosives; DNP became popular as an anti-obesity agent in the late 1930s.[2]

DNP causes weight loss via increased lipid metabolism initiated by an uncoupling of mitochondrial oxidative phosphorylation.[1,3] The toxicity of DNP has become more widely appreciated in recent years [3,4,5] but this has unfortunately been accompanied by a reported increase in it's use as a dietary supplement for weight loss.

Investigative work carried out during the 1930s indicated that DNP [2] had an extremely narrow therapeutic index, caused cataracts and even death.[4,6] It has more recently been linked to polyneuritis, heart disease, renal failure and depletion of white corpuscles of the blood.[1] The 1938 Food, Drug and Cosmetic Act paved the way for the illegalisation of DNP,[2] but it is now illegally sold around the world and is widely available on the internet.[1]

DNP is most commonly found as a yellow powder.[1] Clinical signs of toxicity have been reported as nausea, myalgia and headache. During increased and/or prolonged exposure patients present with vomiting, hyperthermia, sweating, increased oxygen consumption and elevated heart and respiration rates.[1] Sweating that stained the skin yellow, pupil constriction and convulsions have also been reported.[3]

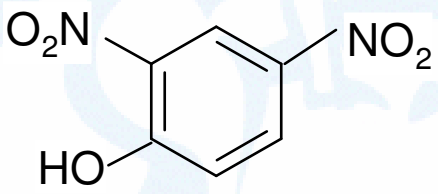

Figure 1. The structure of 2,4-Dinitrophenol

Case History

In 2007, a 36 year old female with a history of previous DNP abuse was admitted to hospital. She presented with 100% oxygen saturation, confusion, agitation, elevated heart rate and temperature, and on further examinations was found to have raised blood pressure and pinpoint pupils.

Guy's and St Thomas's Poisons Unit, London, was contacted for advice on the management of the patient. Treatment of fluids and intravenous Lorazepam was provided. The patient's condition continued to deteriorate and less than 12 hours after being admitted to hospital was eventually pronounced life extinct after a cardiac arrest.

The post-mortem report showed no obvious signs of cause of death. However, the pathologist observed a yellow colour of the stomach contents, although no tablet remnants were visible. In excess of 1kg of a yellow powdery substance was collected from the deceased's home. Both the powder and blood specimens were submitted to the Forensic Toxicology Service for analysis.

Figure 2. Yellow powder pertaining to the case, collected from the deceased's home.

EXPERIMENTAL

Materials

A pure reference standard of 2,4-Dinitrophenol was obtained from Sigma (Poole, Dorset, England) and used to prepare methanolic working standards. HiPerSolv grade methanol, acetonitrile, ethyl acetate, and sulphuric acid were obtained from BDH (Poole, Dorset, England). BSTFA N,O-bis(trimethylsilyl)trifluoroacetamide) containing 1% TMCS (trimethylchlorosilyl) was also obtained from Sigma.

Initial analysis

Due to the receipt of an excessive volume of the powder the counter terrorism explosives police attended and identified the substance as 2,4-Dinitrophenol by portable infra-red spectroscopy. Confirmation of this analysis was achieved using gas chromatography-mass spectrometry (GC-MS).

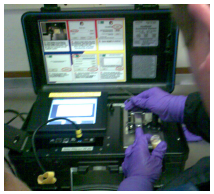

Figure 3. Analysis of 2,4-Dinitrophenol by the explosives officers.

Sample preparation

The blood calibrators (range: 50 to 250mg/L) and ante-mortem blood sample were prepared using liquid-liquid extraction. The calibrators, blank and sample (150µL) were adjusted to acidic pH with 0.025M sulphuric acid (100µL) and extracted using ethyl acetate (1mL). After centrifugation, the organic layer was evaporated to dryness. The dry residue was reconstituted in acetonitrile (30µL) and derivatised by the addition of BSFTA (70µL). The derivatisation reaction was allowed to proceed for 1 hour at 80°C, prior to sample injection onto the GC-MS system. The calibrators and samples were analysed in duplicate, and the analysis repeated over three consecutive days.

Gas chromatography-mass spectrometry

GC-MS analysis was performed using an Agilent GC-MS system (HP 5890 GC coupled to an HP 5973 MS) that utilises positive electron impact ionisation (EI). An HP-5 MS (30m x 0.2mm, 0.5 µm; (5%-Phenyl)-methyl polysiloxane) analytical column (Agilent, Palo Alto, California) was used for chromatographic separation. Helium was used as the carrier gas at a flow rate of 1mL per minute.

Samples were injected (1.0µL, splitless) onto the column, held at 80°C for 4 minutes. The temperature was then ramped by 20°C a minute up to 280°C and held for 1 minute, giving a total run time of 15 minutes. Data was collected using single ion monitoring; DNP was identified using it's principle ions of 241, 195 and 137.

RESULTS

The blood sample was negative for illicit drugs including; amfetamines, cocaine, opiates, methadone and benzodiazepines. However, DNP was detected in the blood sample at a mean concentration of 144mg/L (n=6). Previously reported fatalities have cited blood levels of 36.1mg/L, 28mg/L [1] and 48.4mg/L.[3]

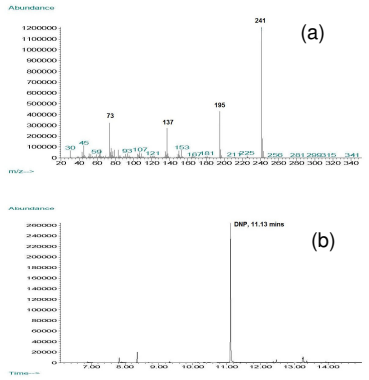

Figure 4. The EI mass spectra (a) and total ion chromatogram (b) for a 150mg/L extracted DNP.

CONCLUSIONS

A method for the quantitative determination of DNP in blood, by GC-MS, has been presented. With the increasing demand for anti-obesity agents it is important that toxicology laboratories are aware of this compound.

References:

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