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Analysis of 2,4-dinitrophenol and its phase I and phase II metabolites in a case of fatal poisoning using a 4000 QTrap LC-MS-MS system

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BACKGROUND: 2,4-dinitrophenol (DNP) has been historically used as herbicide, fungicide, and weight reduction drug, and discontinued in the 1930s owing to a variety of adverse effects; its use is still reported in the synthesis of dyes, wood preservatives, photographic developers, explosives, and insecticides. Due to its weight-reducing action, DNP has been recently shown to be illegally sold by mail/Internet as a dietary supplement for body builders. Here is presented the case of a 30-year old male found dead in his bedroom, where several medications, including stimulants, anabolic steroids, and ß2-agonists were found. Investigations revealed that probably the decedent had recently used DNP.

AIM: To assess DNP role in the death and to investigate the metabolic profile of DNP in different biofluids by LC-MS-MS.

METHODS: Blood, urine, gastric content, and bile taken at autopsy were submitted to LC-MS-MS analysis (4000 QTrap, Applied Biosystems/MDS SCIEX) after dilution in 0.1% v/v formic acid (1:10 for urine and 1: 40 for gastric content) or after deproteinisation (acetonitrile 1:1) and 1:10 dilution in 0.1% formic acid (blood and bile). Chromatographic separation was performed in gradient mode from 100% formic acid (0.1%) to 80% acetonitrile in 15 min (0.2 ml/min) on a reverse phase column (Chrompack Inertsil ODS-3, 100 × 3 mm × 3 µm). Identification of DNP and its known phase I metabolites 2-amino-4-nitrophenol (2A4NP) and 2-nitro-4-aminophenol (2N4AP) was performed in MRM mode with D3-DNP as internal standard. Another reported DNP metabolite, 2,4-diaminophenol, was not detected in any of the analysed samples. Quantification of the analytes in urine and blood samples was carried out in triplicate using a 3-point calibration curve. The limits of detection were 0.12 mg/l for DNP, 0.15 mg/l for 2A4NP and 2N4AP, and 0.35 mg/l for 2,4-diaminophenol in both urine and blood.
Phase II metabolites were tentatively identified by IDA experiments with enhanced mass scan (EMS) as survey scan, and two enhanced product ion (EPI) experiments as dependent scan.
GC-MS routine systematic analysis and EMIT assay for drugs of abuse, benzodiazepines and barbiturates were run on blood and urine samples, respectively.

RESULTS: Blood concentrations were 48.4 mg/l, 1.2 mg/l, and below the LOD for DNP, 2A4NP and 2N4AP, respectively. In urine the three analytes were found at the concentrations of 14.3, 6.0, and 0.5 mg/l, respectively. Three conjugated metabolites (DNP glucuronide, DNP sulphate, and 2A4NP glucuronide) were tentatively identified in urine on the basis of their pseudomolecular ion, isotopic and fragmentation pattern, and retention characteristics. DNP, 2A4NP and their glucuronide conjugated metabolites were found in bile, whereas DNP and 2A4NP were detected in gastric content.
The negligibility of ion suppression phenomena was ascertained both by comparison of the analytes response in spiked blank samples (urine and blood) and water, and by post-column infusion experiments (urine).

GC-MS screening and quantification in blood revealed the presence of citalopram and its desmethylated metabolite at toxic concentrations (0.58 and 0.40 mg/l, respectively), and therapeutic or lower than therapeutic levels of olanzapine (0.04 mg/l), desalkylflurazepam (0.02 mg/l), and nordazepam (0.01 mg/l). EMIT assay tested negative for all classes except benzodiazepines.

CONCLUSIONS: Based on LC-MS-MS results and on available literature data on DNP poisonings, it was concluded that DNP had a contributing role, together with citalopram, in the death.

KEYWORDS: *DNP, Poisoning, LC-MS, Metabolite identification*

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