Chemical and/or thermal conversion of analytes in a sample and/or GC-injector can mislead identification in a toxicological screening. In the past ephedrine has already been reported to cause false positive results in the identification of phenmetrazine in urine samples after carbethoxyhexafluorobutyryl chloride derivatization and high-temperature injection (1). Phenmetrazine, a central nervous system stimulant, is currently abused as a replacement for cocaine and currently is a controlled substance described in the UN Convention on Psychotropic Substances 1971 (4), while ephedrine is an over-the-counter vasoconstrictor. Recently though, the FDA prohibited the sale of dietary supplements containing ephedrine alkaloids due to the high health risk.

**Objectives**
- Identification of the causes of the erroneous identification of phenmetrazine in an ephedrine containing sample.
- Finding solutions to overcome this problem.

**Introduction**

**Experimental**

**Cold-on-column MSD configuration**

**HC-conditions**
- HP 6890 GC-5973 MSD and HP 7683 cold-on-column auto injector (Agilent technologies, Avondale, PA, USA).
- Column: 5m x 0.32 intermediate-polarity deactivated guard column (Interscience, Louvain-la-Neuve, Belgium), followed by a 30m x 0.25mm i.d. x 0.25 µm Varian factor Four VF-5ms column (Varian, Middelburg, The Netherlands). The retention gap and column were combined using a glass universal press-fit connector (Altech, Lokeren, Belgium).
- Injection temperature: 250°C.
- Injection volume: 1 µl.
- Constant helium flow = 1.2 ml/min.
- Temperature programme: 60°C (1min), 120°C at 20°C/min, 280°C (3min) at 10°C/2min.

**MS-conditions**
- Transferline: 300°C.
- Quadrupole: 150°C.
- Measurement range: 45-650amu.
- Electron voltage: 70 eV.

**Split ITMSD configuration**

**GC-conditions**
- Varian 3400GC-Finnigan Magnum ion-trap MSD and split injector (Varian, Middelburg, The Netherlands).
- Column: CP-Sil 5CB 30m x 0.25mm i.d x 0.5µm (Varian, Middelburg, The Netherlands).
- Injection temperature: 250°C.
- Injection volume: 1 µl.
- Split: 1:100.
- Constant helium pressure= 13 psi.
- Temperature programme: 80°C (2min), 280°C (2min) at 9°C/2min.

**Chemicals**
- Ephedrine base (Flandria, Gent, Belgium).
- DMPO (donation Laboratory of Toxicology Catholic University Leuven, Belgium).
- Methanol (analytical grade).
- Formaldehyde (Merck, Darmstadt, Germany).
- Herbal capsules containing ephedrine (according to the manufacturer) were contaminated by the Federal Police and transmitted for analysis.

**Results and discussion**

**Problem**
- CI-MS/MS analysis of a methanolic extract of a herbal capsule containing ephedrine (according to the manufacture) resulted in a peak at almost the same retention time as phenmetrazine, identified as phenmetrazine after library search. A 6 month old methanolic ephedrine solution gave the same spectrum in split injection mode.
- The NIST-library search for compounds with a molecular ion m/z 177 in combination with ion m/z 71 gave phenmetrazine as best hit.
- The peaks showed a 78% match between the spectrum of the artefact and phenmetrazine, while the phenmetrazine standard gave a 91% match.

**Formaldehyde contamination in solvents as possible cause?**
- Literature search (5,6) revealed that a formaldehyde contamination in solvents such as methanol can result in conversion of ephedrine-like compounds. This was described by Lewis et al. with pseudo-ephedrine being converted to 3, 4-dimethyl-5-phenyl-1, 3-oxazoline (DHMO) (see structure below).
- An ephedrine standard was analysed using the COC-MSD configuration with injection temperatures varying from 60°C until 300°C. The temperature programme of the Split-ITMSD configuration was used for the injection of a standard of ephedrine dissolved in methanol-formaldehyde (1:1, by vol.).
- Fragmentation patterns of DMPO and phenmetrazine are almost similar because both chemical structures differ only in the position of one carbon unit.
- Reaction mechanism:

**Options to counter the reaction**
- Derivatization of ephedrine leaves no functions to participate in the reaction, but can also cause problems as described by Wu et al. (1).
- Use of pure solvents, not contaminated with formaldehyde.
- Using as low as possible injection temperatures.
- Injection of a phenmetrazine standard reveals a difference in retention time.

**Conclusion**
- The combination of ephedrine, formaldehyde contamination in analytical reagents and high injection temperature can lead to an erroneous identification of phenmetrazine.
- Library search alone can cause erroneous conclusions. In the newer PMW_tox3 library the spectrum of the artefact is included (5).
- Derivatization, use of pure solvents, use of low injection temperatures and injection of a phenmetrazine standard can counter the problem.

**References**

Fig. 1: Spectrum obtained after injection of (A) a "fresh" methanolic ephedrine solution, (B) an ephedrine standard solution in methanol-formaldehyde (1:1, by vol.) (ephedrine artefact), (C) a methanolic phenmetrazine solution, using the split-ion-trap configuration.

Fig. 2: Influence of the injection temperature. Chromatogram of ephedrine (methanolic solution) using cold-on-column injector 60°C (top), cold-on-column injector 250°C (middle), cold-on-column injector 300°C (bottom).