

# Detection and characterization of 4-Methyl-Hexanamine, a stimulant analogue

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## I - INTRODUCTION

Tuaminoheptane is a volatile sympathomimetic agent with topical decongestant activity. It is primarily used for the relief of nasal congestion and may be found in over-the-counter pharmaceutical preparation such as Rinofluimucil®. Due to its central nervous system stimulating properties and potential for abuse in competition, it was introduced in 2007 in the Prohibited List of the World Anti-Doping Agency.

In our Laboratory, Tuaminoheptane was included in the LC-MS<sup>2</sup> screening for unconjugated compounds (diuretics, stimulants...). Samples are extracted at basic and acidic pH (carbonate and phosphate buffer) with 4 ml of ethyl acetate. After evaporation, the residue is dissolved with 125 µl of pH 4 Ammonium Formiate buffer 10 mM / Acetonitrile (80/20).

During routine analysis of in-competition samples, an intense peak was detected in the Tuaminoheptane window of a sample. Retention time (RT) of the unknown compound did not match exactly the Tuaminoheptane RT, but the MS/MS spectra showed strange similarity as shown in Figure 1.

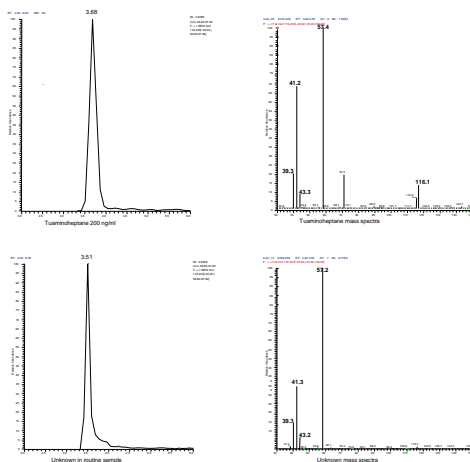


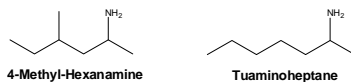
Figure 1 : Chromatogram and mass spectra of Tuaminoheptane and unknown compound

## II - CHARACTERIZATION

Accurate mass measurement was performed on the unknown compound with a Quadrupole-Time of Flight Mass Spectrometer (Waters Q-TOF LCT Premier).

With an accurate mass measurement of 116.1432 Da for the unknown compound, the only possible elemental composition (candidate compositions encompassing C<sub>9-10</sub>, H<sub>3-74</sub>, O<sub>0-4</sub> and N<sub>0-4</sub>) providing unambiguous result was C<sub>7</sub>H<sub>18</sub>N<sup>+</sup> (experimental error: 6.0 ppm). Since the experiment was conducted in the Electro Spray Ionisation positive mode, the neutral elemental composition was of C<sub>7</sub>H<sub>17</sub>N.

A research in the Merck Index gave only two compounds possibly matching this elemental formula, Tuaminoheptane and 4-Methyl-2-hexanamine, both listed as adrenergic.



## III – CONFIRMATION (GC-MS)

Standard Reference material of 4-Methyl-Hexanamine was provided by Sigma-Aldrich Library of Rare Chemicals (Milwaukee, USA).

Sample and reference material were treated by Tert-Butyl-Methyl Ether (TBME) basic extraction followed by derivatization with cyclohexanone <sup>(1)</sup>. Diphenylamine was used as an Internal Standard. Sample and controls were analysed by GC-MS in Scan mode (HP6890N / 5973N, Column ZB-5, 0.25 µm, ID 0.25 mm, 30m. Injection volume : 1 µl, splitless).

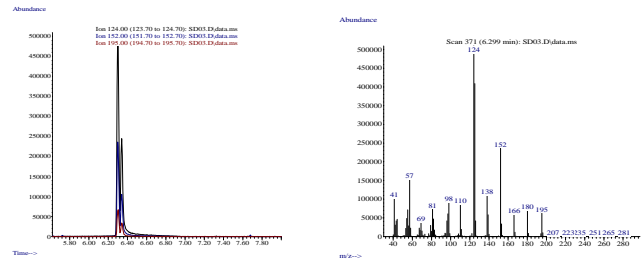


Figure 3 : Chromatogram and mass spectra of compound in routine sample

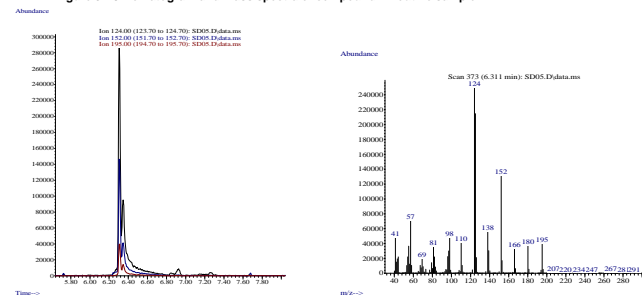


Figure 4 : Chromatogram and mass spectra of 4-Methyl-Hexanamine Reference Standard

Cyclohexanone derivatization improves the resolution of the diastereomers of 4-Methyl-Hexanamine and provides a rich mass spectra showing unambiguously the presence of the analogue in the routine sample.

## IV – QUANTIFICATION BY LC-MS<sup>2</sup>

We developed an analytical method to quantify the 4-Methyl-Hexanamine in urine by LC-MS<sup>2</sup> (Thermo TSQ Quantum Access). Samples are extracted at basic pH (carbonate buffer) with 3-fold 3 ml of ethyl acetate. After evaporation, the residue is dissolved with 125 µl of pH 4 Ammonium Formiate buffer 10 mM / Acetonitrile 90/10. Internal Standard is Tuaminoheptane.

The pre-validation parameters shows LOD < 50 ng/ml, Linearity (R<sup>2</sup> 0.988) between 50 ng/ml and 1000 ng/ml, precision 9.6% (CV) and recovery between 99 to 120%.

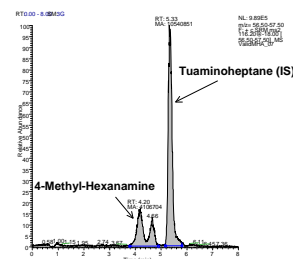


Figure 5: LC-MS<sup>2</sup> Chromatogram of 4-Methyl-Hexanamine Reference Standard (50 ng/ml)

## V – CONCLUSION

The class of stimulants has a long history of new or analogue compounds being abused (Bromantan, Carphedon, Mesocarb...). Although an extensive screening of all stimulants analogues seems unpractical <sup>(2)</sup>, the combination of the various analytical techniques available nowadays in the anti-doping Laboratories makes this task an interesting challenge.

## VI – REFERENCES

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- (2) R.K. Mueller, J. Grosse, D. Thieme, R. Preiss, Banned agents and related compounds – how many?. In Schänzer W., Geyer H., Gotzmann A., Marek U., *Recent Advances in Doping Analysis 1999 (7)*, p.13-19