In: Donike, H. Geyer, A. Gotzmann, U. Mareck-Engelke, S. Rauth (eds.)
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Old doping agent, old method: pemoline TMS-derivative[1][2]

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As a central stimulant, pemoline (Cylert®, Tradon®, Stimul®) is considered to be a doping agent by the IOC and several methods were developped for its detection in plasma and urine samples. Due to the well described difficulties encountered with the extraction and the analysis of this amino oxazolinone, two different strategies were reported: oxidation to mandelic acid and benzaldehyde or acidic conversion of extracted pemoline to the corresponding oxazolinedione followed by the GC/MS analysis of the methylated or trimethylsilylated derivatives.

In this report, we wish to present the characterization of pemoline as its TMS-derivative. For the purpose of confirmation and identification, pemoline is obtained in the free fraction (3 mL of urine sample) isolated on a Sep Pak C_{18} cartridge and eluted with methanol. Extraction is carried out at pH 9 with diethyl ether. Pemoline tri-TMS derivative is obtained by treatment with MSTFA:TMSI:dithioerythritol (final volume: 50 µL). The mixture is analysed by GC/MS in the SIM and full scan mode.

The mass spectrum of pemoline tri-TMS derivative is characterized by the presence of the molecular ion at m/z 392. As reported in litterature for 5-phenyl 2-amino oxazolinone and 5-phenyl oxazolidinedione, the cleavage of C1-C2 and C4-C5 bonds with or without hydrogen transfert is the major fragmentation observed (ions at m/z 107, 106, 105). In this case, migration of the trimethylsilyl group to the benzoyl would produce ion at m/z 178 (base peak). Ion at m/z 206 would originate from the cleavage of C1-C2 and C3-C4 bonds (see mass spectra of N,N-dimethylpemoline in figure 3) while retention of the charge on the carbodiimide moiety will produce ion at m/z 171; this fragment is the base peak of the mass spectrum of the di-TMS derivative.

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As determined by the analysis of blank urine samples spiked with pemoline, the limit of detection in the SIM mode (ions at m/z 392.3, 178.2, 105.1) and in the full scan mode is respectively 10 and 20 ng/mL.

Mass spectra[3] are presented along with proposed fragmentation pathways and GC/MS analysis in figures 1 to 4.

References:

1. Gielsdorf W., J. Clin. Chem. Clin. Biochem., 20, 65 (1982) 2. Hoffman D. J., J. Pharm. Sci., 68, 445 (1979) 3. Cartoni G. P. et coll., J. Chromatogr., 202, 131 (1980) 4. Nishira K. et coll., Therapeutic Drug Monitoring, 6, 232 (1984) 5. Libeer J.C. et coll., J. Pharm. Sci., 67, 419 (1978) 6. Igwe O.J. et coll., J. Chromatogr. Sci., 19, 617 (1981) 7. Aoyama T. et coll., J Chromatogr., 430, 351 (1988) 8. Van Boven M et coll., J Chromatogr., 134, 415 (1977) 9. Cummins L.M. et coll., J. Pharm. Sci., 762 10. Pfleger K. et coll., Mass spectral and GC data of drugs, poisons, pesticides, pollutants and their metabolites. Second revised and enlarged edition, VCH (Verlagsgesellschaft) mbH D-6940, Germany 1992, part 1,2 and 3.

[3] the minor pemoline di-TMS derivative is obtained by treatment with MSTFA at room temperature for 5 min.

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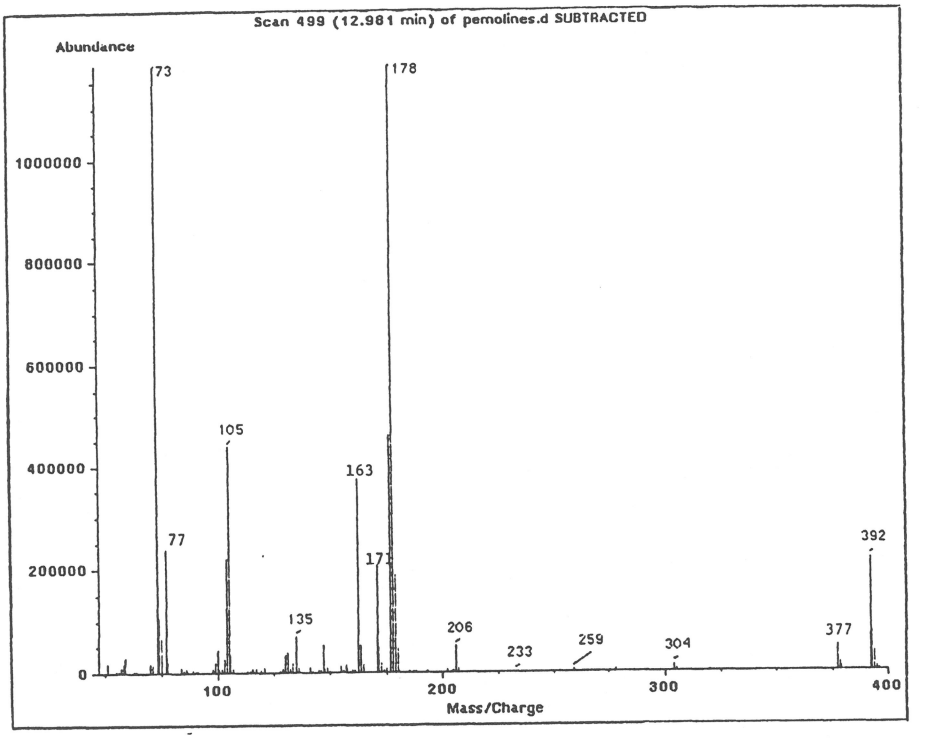

Figure 1: Mass spectrum of pemoline tri-TMS derivative (authentic standard)

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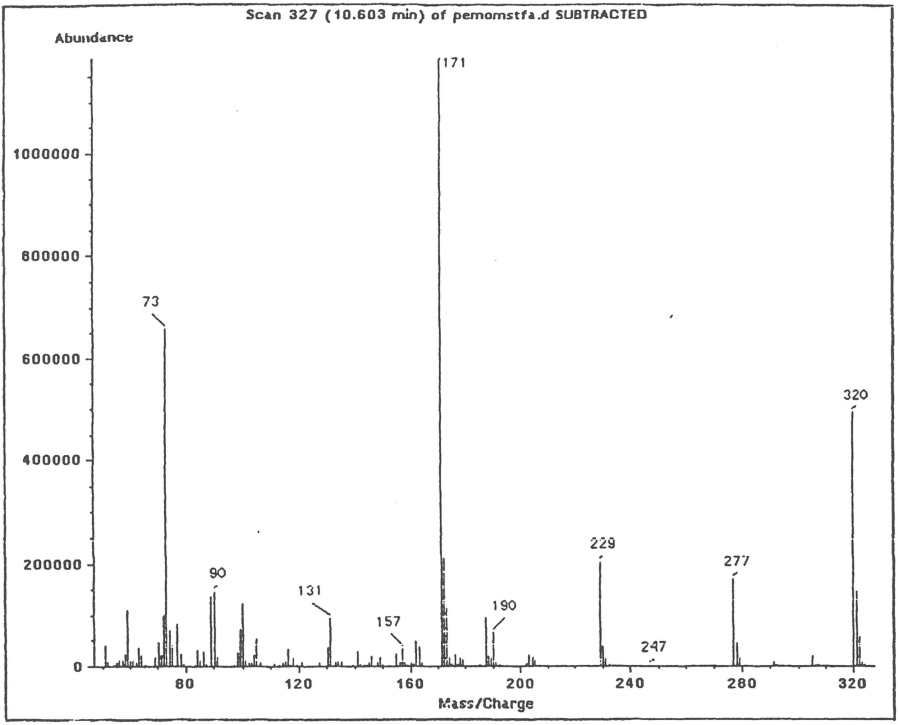

Figure 2: Mass spectrum of pemoline di-TMS derivative (authentic standard)

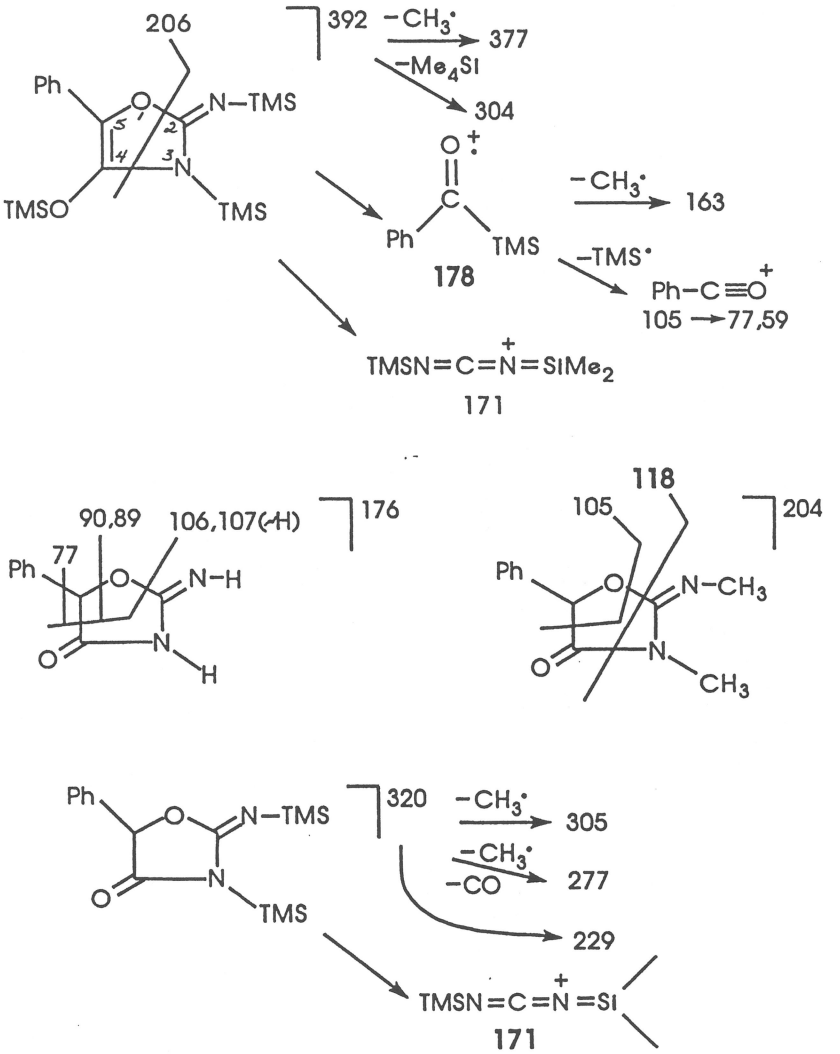

Figure 3: Proposed fragmentation pathways for pemoline derivatives

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Misc Info:
Operator : c.ayotte

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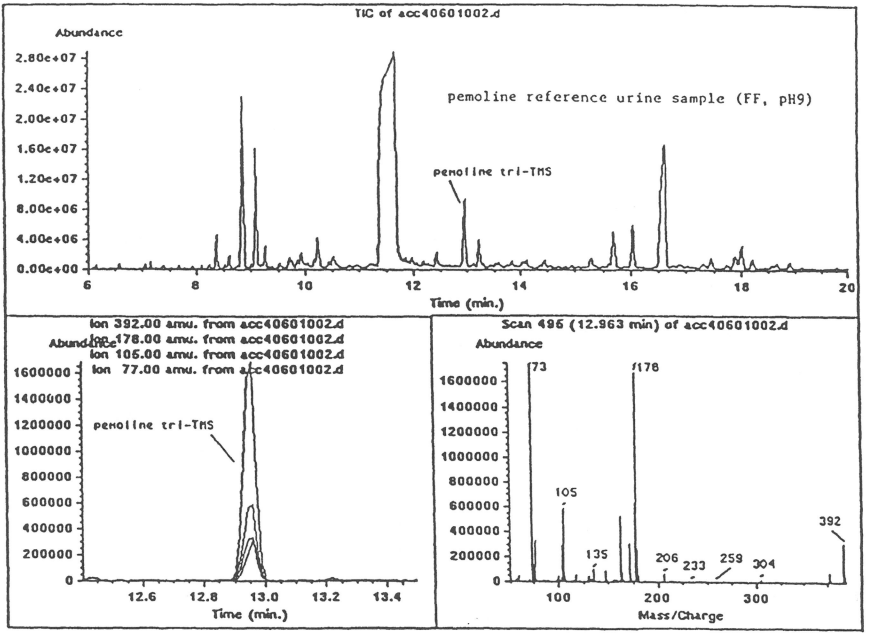

Figure 4:
GC/MS (full scan mode) analysis of the pemoline reference urine sample prepared according to confirmation procedure IV (FF, pH 9)