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Determination of methoxyphenamine in doping control analysis

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Introduction

On "The 2009 Prohibited List" of the World Anti-Doping Agency (WADA), methoxyphenamine (o-methoxy-*N*,α-dimethylphenethylamine, Orthoxine) is not expressly listed as a prohibited substance. However methoxyphenamine is related to stimulants defined in section S6, and represents a substance with a similar chemical structure and similar biological effect.[1] Methoxyphenamine was synthesised and clinically evaluated for the treatment of asthma bronchiale in the 1940s and demonstrated promising bronchodilator activity after oral administration with reduced influence on blood pressure and the central nervous system as compared to ephedrine. Studies of the metabolism resulted in three major products derived from *N*- or *O*-demethylation and ring hydroxylation at position 5, and the latter were further conjugated to glucuronic acid (Figure 2). In this study, methoxyphenamine and several of its metabolites are analysed using gas chromatography-mass spectrometry and liquid chromatography-atmospheric pressure chemical ionisation-tandem mass spectrometry.[2]

Experimental

Sample preparation for GC-MS

Common procedures for the detection of stimulants in doping controls were applied. A volume of 5 mL of urine specimens were extracted into 2 mL of *tert*.-butylmethyl ether at pH 14,[3] and 5 µL of the ether layer were injected into the GC-MS system.

Sample preparation for LC-MS/MS

Native urine was enriched with the internal standard (5 µg mL^{-1} $^2H_3$-ephedrine) and subjected to LC-MS/MS analysis without further treatment.

GC-MS analysis

GC-MS analyses were conducted using the following equipment:

- Agilent 5890/5973 GC-MSD,
- column: HP-5MS, length 24 m, i.d. 0.25 mm, film thickness 0.25 µm,
- temperature program: 85°C for 0.1 min increasing to 330°C at 28°C min^{-1}
- SCAN mode (*m/z* 40-400). Split 1:10, Carrier gas: helium

LC-MS/MS analysis

LC-MS/MS analyses were performed on an Applied Biosystems API 2000 mass spectrometer utilising atmospheric pressure chemical ionisation (APCI).

- Phenomenex Gemini C6-phenyl column (4.6x150 mm, particle size 3 µm).
- Flow rate 800 µL min^{-1}, eluents A: 5 mM ammonium acetate containing 0.1% acetic acid and B: acetonitrile.
- Isocratic at 90% A over 7 min employing a post-column split of 1:5.
- Ion source: positive mode at 400°C, methoxyphenamine was detected by means of characteristic product ions at *m/z* 149, 121, 93 and 91. Collision gas: nitrogen at 4 x 10^{-3} Pa.

Results and discussion

Methoxyphenamine was analysed with an existing validated screening procedure using a GC-MS system equipped with an additional nitrogen-phosphorus detector as analytical instrument.[3] EI-MS spectra of methoxyphenamine (a) and the metabolites *N*-demethyl methoxyphenamine (b) and *O*-demethyl methoxyphenamine (c) are illustrated in Figure 1, limit of detection (LOD) 50 ng mL^{-1}. For confirmation, a LC-MS/MS method was validated with regard to specificity, LOD (0.7 ng mL^{-1}), intraday- and interday precision (2.5-5.8% and 10.8-16.2% respectively). Its applicability was demonstrated with an authentic doping control sample, tested positive for the prohibited compound (Figure 3). A direct injection of urine aliquots was possible to analyse urine specimens for methoxyphenamine. Product ion mass spectra of metabolites obtained from a doping control sample are shown in Figure 2.

GC-MS spectra:

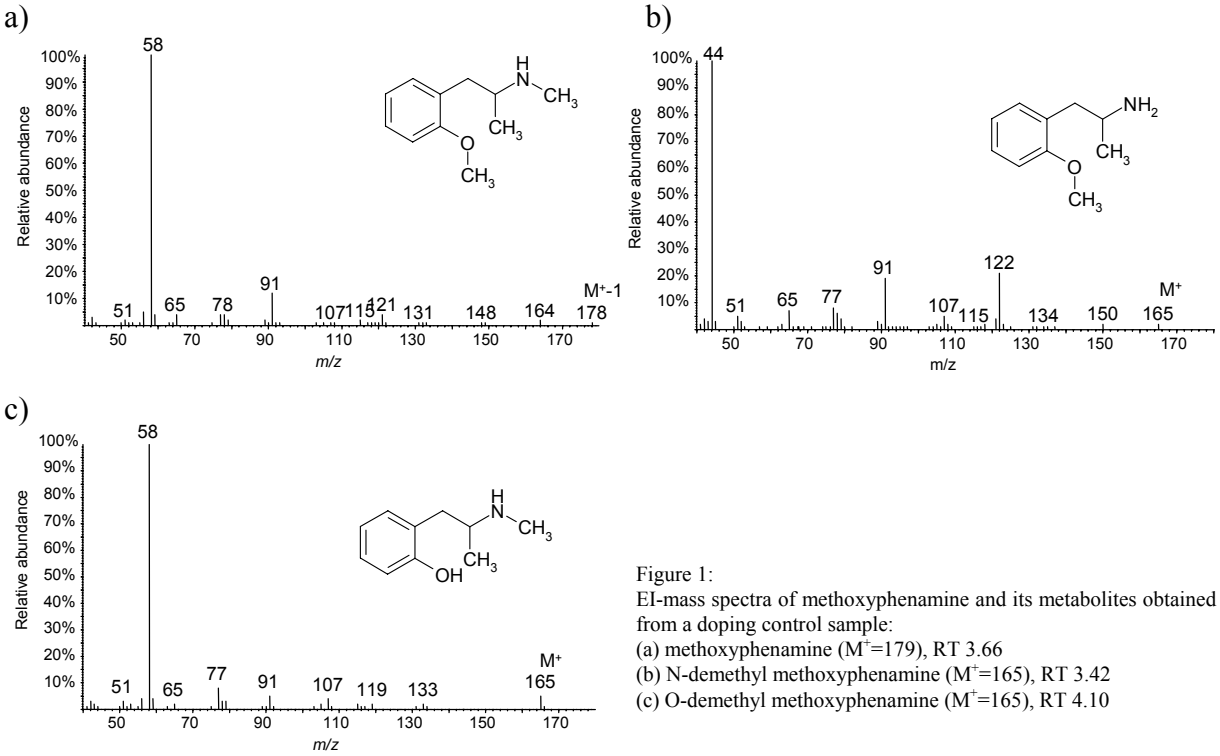
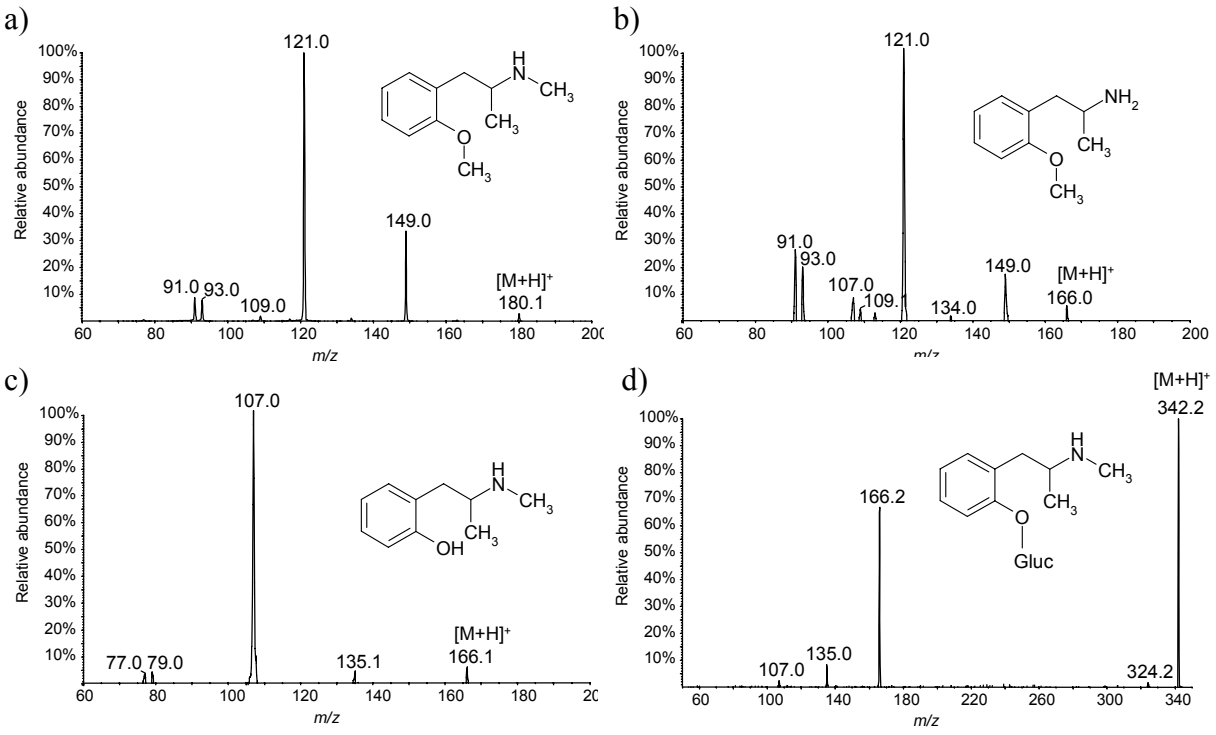


Figure 1:
EI-mass spectra of methoxyphenamine and its metabolites obtained from a doping control sample:
(a) methoxyphenamine (M^+=179), RT 3.66
(b) N-demethyl methoxyphenamine (M^+=165), RT 3.42
(c) O-demethyl methoxyphenamine (M^+=165), RT 4.10

LC-MS/MS spectra:



e)

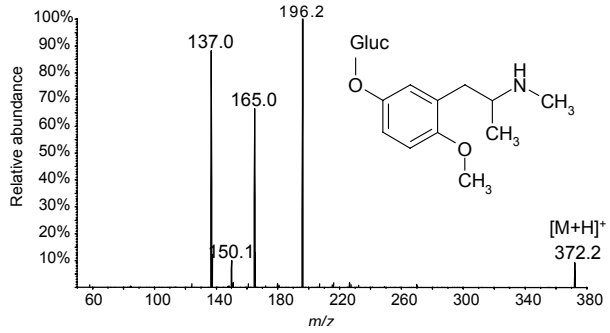


Figure 2:
Product ion mass spectra of methoxyphenamine and metabolites obtained from a doping sample:
(a) methoxyphenamine ($[M+H]^+$=180), RT 5.63
(b) N-demethyl methoxyphenamine ($[M+H]^+$=166, RT 4.65
(c) O-demethyl methoxyphenamine ($[M+H]^+$=166), RT 3.01
(d) O-demethyl methoxyphenamine glucuronide ($[M+H]^+$ = 342), RT 3.75
(e) 5-hydroxy-methoxyphenamine glucuronide ($[M+H]^+$=372), RT 3.40

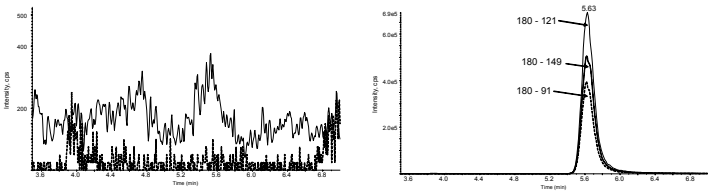


Figure 3:
Urine specimens analysed using LC-MS/MS.
Left: blank urine sample, right: adverse analytical finding for methoxyphenamine in a doping control sample.

Conclusion

The method for the detection of methoxyphenamine and its metabolites allows to confirm concentrations according to the minimum required performance limit of 500 ng mL^{-1} requested by the World Anti-Doping Agency (WADA).[4]

The identification with LC-MS/MS needs very little effort of sample preparation and complements the analytical tools commonly used in sports drug testing and facilitates the differentiation of methoxyphenamine from structurally closely related compounds and designer drug analogues.

References

1. World Anti-Doping Agency. (2009) The 2009 Prohibited list. http://www.wada-ama.org/rtecontent/document/2009_Prohibited_List_Eng_Final_20_Sept_08.pdf, (access date 02.01.2009)

2. Thevis M., Sigmund G., Koch A., Guddat S., Maurer H.H. and Schänzer W. (2008) Doping control analysis of methoxyphenamine using liquid chromatography-tandem mass spectrometry. *Eur J Mass Spectrom.* **14**, 145-152.

3. Donike M., Jaenicke L., Stratmann D. and Hollmann W. (1970) Gas Chromatographic detection of Nitrogen-Containing Drugs in Aqueous Solutions by Means of the Nitrogen Detector. *J Chromatogr.* **52**,237.

4. World Anti-Doping Agency. (2008) Minimum Required Performance Limits for Detection of prohibited Substances. http://www.wada-ama.org/rtecontent/document/MINIMUM_ REQUIRED_PERFORMANCE_ LEVELS _TD_v1_0January_2009.pdf, (access date 24.11.2008)