K. Deventer, O.J. Pozo, W. Van Thuyne, P. Van Eenoo, F.T. Delbeke

Screening and confirmation of ritalinic acid in urine by LC-MS^n

Doping Control Laboratory (DoCoLab), Ghent University-UGent, Technologiepark 30B, B-9052 Zwijnaarde, Belgium

Introduction

Methylphenidate (MPH) is therapeutically used for the treatment of attention-deficit/hyperactivity disorder (ADHD). In sports it can be abused to overcome fatigue or to increase the tolerance to exhaustion. Less than 1% of the administered dose is excreted unchanged in urine whereas 80% is excreted as ritalinic acid (RA) [1]. Moreover RA can be detected much longer in urine than MPH. Hence RA is the preferred target compound to detect MPH administration [1]. In our laboratory stimulants are screened for by GC/MS using MSTFA as derivatisating agent after liquid/liquid-extraction at pH 9.2. Unfortunately RA can not be detected by this procedure because MBTFA is necessary for its derivatisation. Moreover, even when MBTFA is used our liquid-liquid extraction at pH 9.2 does not allow to detect RA at the MRPL of 500 ng/ml. Therefore it was decided to include this compound into the LC-MS screening method for diuretics [2].

MATERIALS AND METHODS

Extraction

Internal standard solution (50 µl mefruside, 20 µg/ml) was added to 2 ml urine, followed by addition of 1 ml sodium acetate buffer (pH 5.2). Liquid-liquid extraction was performed by rolling for 20 min with 4 ml ethyl acetate. After centrifugation the organic layer was transferred into a new tube and evaporated until dry under oxygen free nitrogen at 40 ºC. The remaining residue was dissolved in 200 µl mobile phase. 50 µl were injected.

Chromatography

The chromatographic equipment consisted of a Thermo Separation Products (TSP) Model P4000 quaternary pump equipped with a TSP Model AS 3000 autosampler. A Nucleosil C18 column 3 mm x 100 mm, 5 μm, protected with a guard column (10 x 2 mm), was used for chromatographic separations (Varian, Sint-Katelijne-Waver, Belgium). The mobile phase was 1% acetic acid (solution A) and acetonitrile. Gradient elution at a flow rate of 0.3 ml/min was as follows: 85% A for 2 min, linear to 45% in 10 min, linear to 35% in 8 min followed by an equilibration step of 8 min at 85% A. Total run time: 28 min.

Mass spectrometry

Detection was performed on a Finnigan LCQ-Deca® mass spectrometer.

Ionization of analytes was carried out using Electrospray, positive ionisation (+ESI). The capillary temperature was maintained at 300 ºC; the ion source voltage was set at 5000 V and the nebulizer gas was set at 80 units. The capillary voltage was set at 44 V. for the screening full scan MS^2 was performed on the protonated molecular ion $[M+H]^+$ of ritalinic acid (m/z 220) at a collision energy (CE) of 35, activation Q (Qz): 0.25 and an activation time (AT) of 30 ms.

Full scan MS^3 was performed on the MS^2 product ion m/z 84 (CE: 37, Qz: 0.5, AT: 30 ms).

RESULTS AND DISCUSSION

Screening

RA contains both an amine-function and carboxy-group. Hence protonated as well as deprotonated molecular ions where observed in positive and negative ionization, respectively. Ionization in positive mode was much more abundant and was prefered. Full scan MS^2 generates a single product ion m/z 84 which results from the loss of the benzyl-carboxyl moiety (Figure 1). This ion is monitored in the screening procedure.

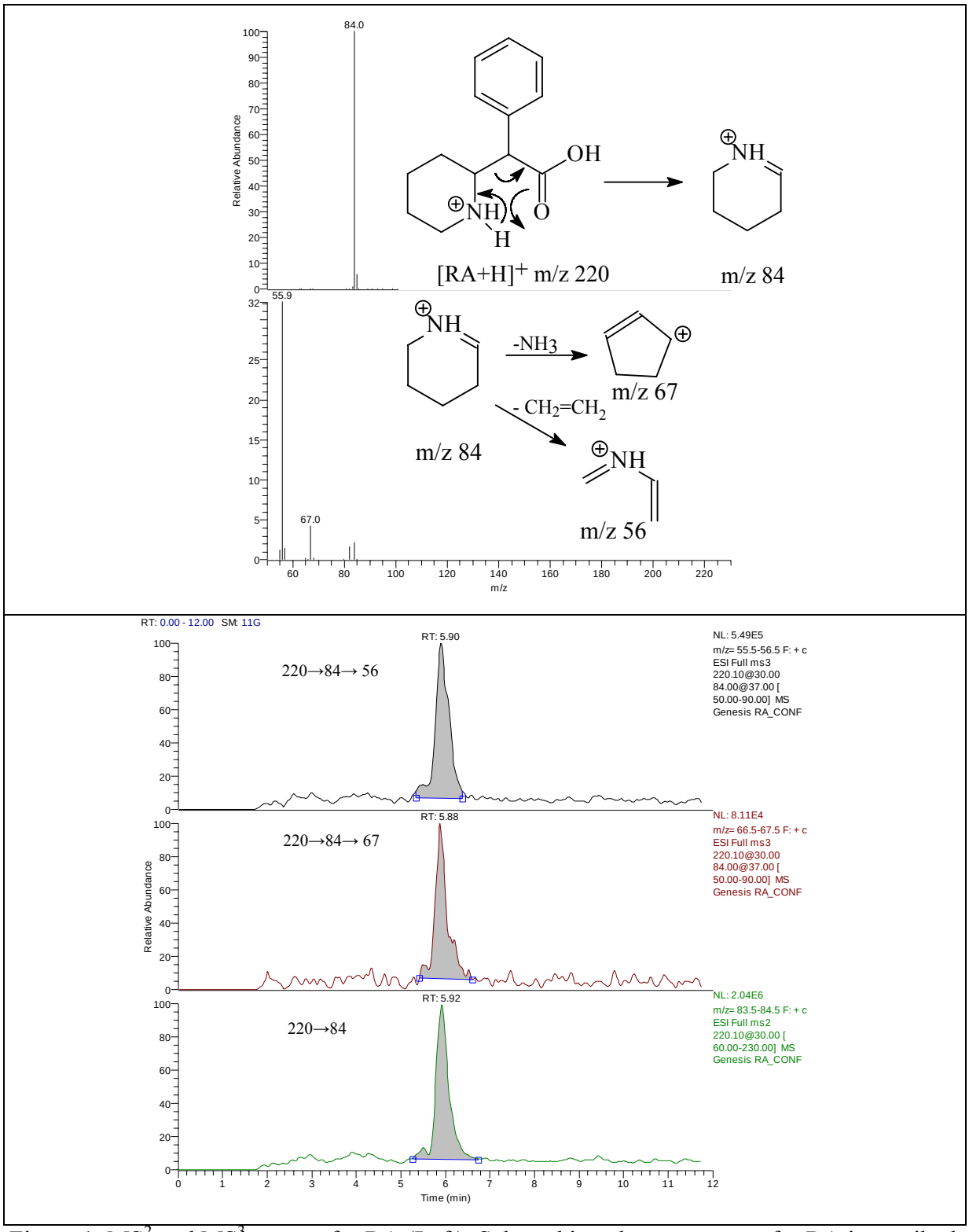

Figure 1: MS^2 and MS^3 spectra for RA (Left). Selected ion chromatograms for RA in a spiked urine sample (500ng/ml) (Right).

Confirmation

For the confirmation 2 additional ions are necessary to fullfill the WADA-criteria concerning reporting an adverse analytical finding [3]. Therefore MS^3 was applied. With the default instrument setting of 0.25 for the Qz-value, the precursor ion m/z 84 could be successfully isolated. Unfortunately no product ions could be detected when CE was applied. When the Qz- value was increased to 0.5 two product ions were detected in MS^3, m/z 56 and m/z 67 (Figure 1).

According to theoretical principles of ion trap mass spectrometry these product ions should be detectable with the default Qz-value of 0.25 [4]. However several papers [2,5] report the need to adjust the Qz-value to detect low molecular weight fragment ions (mass < m/z 100) as was also required here.

CONCLUSION

Using this LC-MS^2 screening method, the detection of RA can be guaranteed at the MRPL. For confirmation purposes, the ion trap parameter Qz-value had to be adjusted.

The method has been used routinely since one year and 3 samples were reported as an adverse analytical finding, containing RA. No MPH was detected in these samples.

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