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Urinary metabolism of ibutamoren, a small molecule growth hormone secretagogue

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Abstract

We performed an *in vitro* study with ibutamoren (non-peptidyl growth hormone secretagogue) isolated from a commercial preparation and *in vivo* experiment after oral administration of 10 mg ibutamoren to a healthy male volunteer. Applying liquid chromatography – electrospray ionization mass spectrometry it was found that the detection of parent compound is enough to reveal the abuse of ibutamoren, though the other biotransformation products such as monohydroxy metabolites, dihydroxy metabolite and desbenzyl metabolite could also support analytical finding.

Introduction

Ibutamoren (MK-677, (*R*)-1'-(2-methylalanyl-*O*-benzyl-*D*-seryl)-1-(methylsulfonyl)-1,2-dihydrospiro- [indole-3,4'-piperidine]) is a potent orally active growth hormone (GH) secretagogue discovered and developed by Merck [1]. This compound, which is studied as a drug candidate to treat somatopause or other GH-deficient conditions, has not yet passed clinical trials but is advertised and sold via the Internet [2] as a product that possesses the stimulating action of the endogenous hormone ghrelin. It has been shown that administration of 25 mg ibutamoren a day resulted in a 60% increase in serum IGF-1 levels. Therefore, it cannot be excluded that ibutamoren may be used by athletes to have the advantage like increased stamina or faster recovery after the intense training. Incorporation of this compound into routine screening procedures could be beneficial to reveal possible abuse.

Experimental

In vitro metabolism: pooled mixed gender human liver microsomes (HLM) from BD Gentest (Woburn, MA, USA); incubations according to manufacturer's protocol.
Instrument: triple quadrupole mass spectrometer TSQ Vantage (ThermoFisher Scientific, San Jose, CA, USA) coupled to a liquid chromatograph Acquity (Waters, Milford, MA, USA).
Separation: Acquity BEH C18 column (100 mm × 2.1 mm, particle size 1.7 µm) maintained at 60°C and protected by a Vanguard column (20 mm × 2.1 mm). The mobile phase flow rate 0.35 ml/min. Elution program: 0.5-min isocratic step at 95% of 0.1% formic acid in water (A) and 5% of 0.1% formic acid in methanol (B), linear increase to 95% of B within 4.5 min, hold at 95% of B for 2.5 min and then re-equilibration until the end of analysis (10min).
Detection: heated electrospray ion source (HESI II); positive ions. The collision gas pressure 1.5 mTorr (argon 99.9995%). The vaporizer and capillary temperature 370 and 300°C, respectively, spray voltage 4000 V.

Results and Discussion

We have performed an *in vitro* study with ibutamoren isolated from a commercial preparation (solution in polyethylene glycol, as evidenced by chromatographic and mass spectrometric pattern – data not shown) and an *in vivo* experiment after

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oral administration of 10 mg ibutamoren to a healthy male volunteer (urine was collected for 2 weeks after administration; ethical approval was granted by Russian Institute of Sport). In general, there was good agreement between an *in vitro* and *in vivo* metabolism, resulting in almost the same profile of metabolites. The excretion study was necessary to understand what is the optimal target for detection of use of ibutamoren. We have identified two monohydroxy metabolites (**M1**, **M2**), one dihydroxy compound (**M3**), one desbenzyl metabolite (**M4**), two desbenzyl hydroxy metabolites (**M5** major, **M6** in trace amounts), and desbenzyl des(2-methyl-2-aminopropionyl) compounds (**M7**, **M8**). Only traces of **M5** and **M6** were found *in vitro*, while **M7** and **M8** were not detected at all under these conditions (Fig. 1).

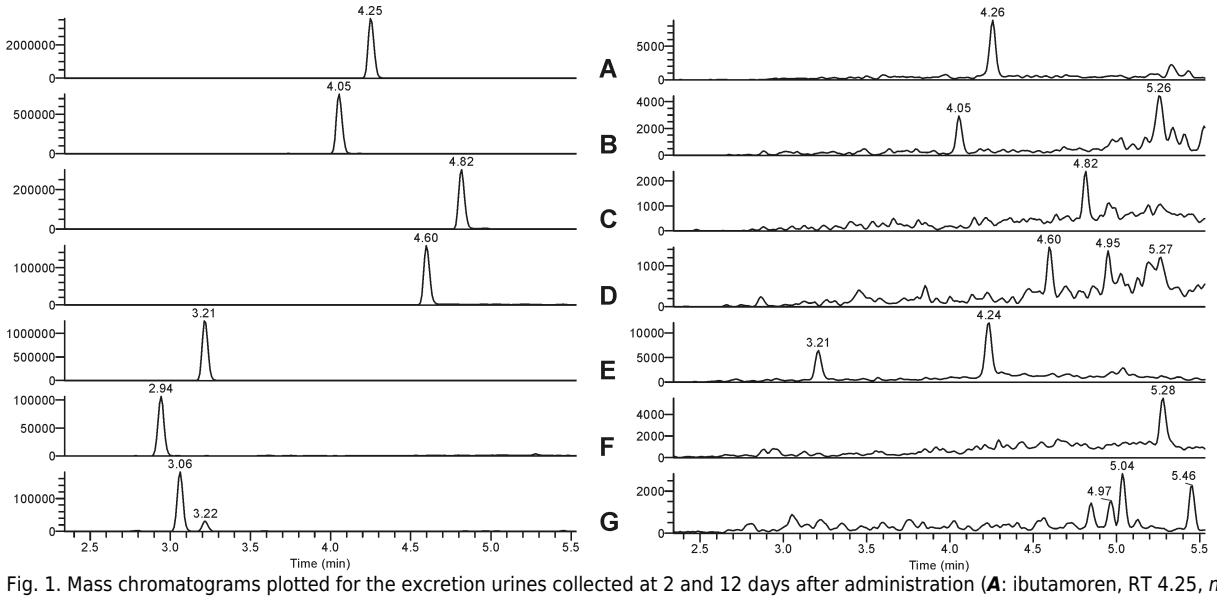

Fig. 1. Mass chromatograms plotted for the excretion urines collected at 2 and 12 days after administration (***A***: ibutamoren, RT 4.25, *m/z* 529; ***B***: **M1**, RT 4.05, *m/z* 545; ***C***: **M2**, RT 4.82, *m/z* 545; ***D***: **M3**, RT 4.60, *m/z* 561; ***E***: **M4**, RT 3.21, *m/z* 439; ***F***: **M5**, RT 2.94, m/z 455; ***G***: **M7**, **M8,** RT 3.06, 3.22, *m/z* 354)

It is important to note that hydroxylation sites of ibutamoren can only be tentatively identified based on their fragmentation data, as shown below. It was found that the detection of parent compound is enough to reveal the abuse of ibutamoren, though the other biotransformation products such as monohydroxy metabolites, dihydroxy metabolite and desbenzyl metabolite could also support the analytical finding. The fact that hydroxy metabolite **M2** elutes after intact ibutamoren is not well understood; same in case of dihydroxy metabolite **M3**.

Importantly, ibutamoren and most of metabolites are excreted unconjugated except dihydroxy metabolite **M3**, which is partly conjugated (*ca.* 30%) with glucuronic acid. Sulfate conjugates were not evaluated in this study.

Product ion positive ESI mass spectra of ibutamoren parent and the most important metabolites are given in Fig. 2 and 3, respective SRM transitions are presented in the Table 1.

Compound	SRM (CE)
ibutamoren	529 > 267 (25) 529 > 188 (40)
hydroxy ibutamoren (**M1**)	545 > 283 (23) 545 > 204 (40)
hydroxy ibutamoren (**M2**)	545 > 267 (25) 545 > 188 (40)
dihydroxy ibutamoren (**M3**)	561 > 283 (22) 561 > 204 (40)
desbenzyl ibutamoren (**M4**)	439 > 267 (25) 439 > 188 (35)
desbenzyl hydroxy ibutamoren (**M5**)	455 > 283 (21) 455 > 147 (50)

Table 1. The SRM transitions for ibutamoren and its valuable metabolites for LC-MS/MS detection

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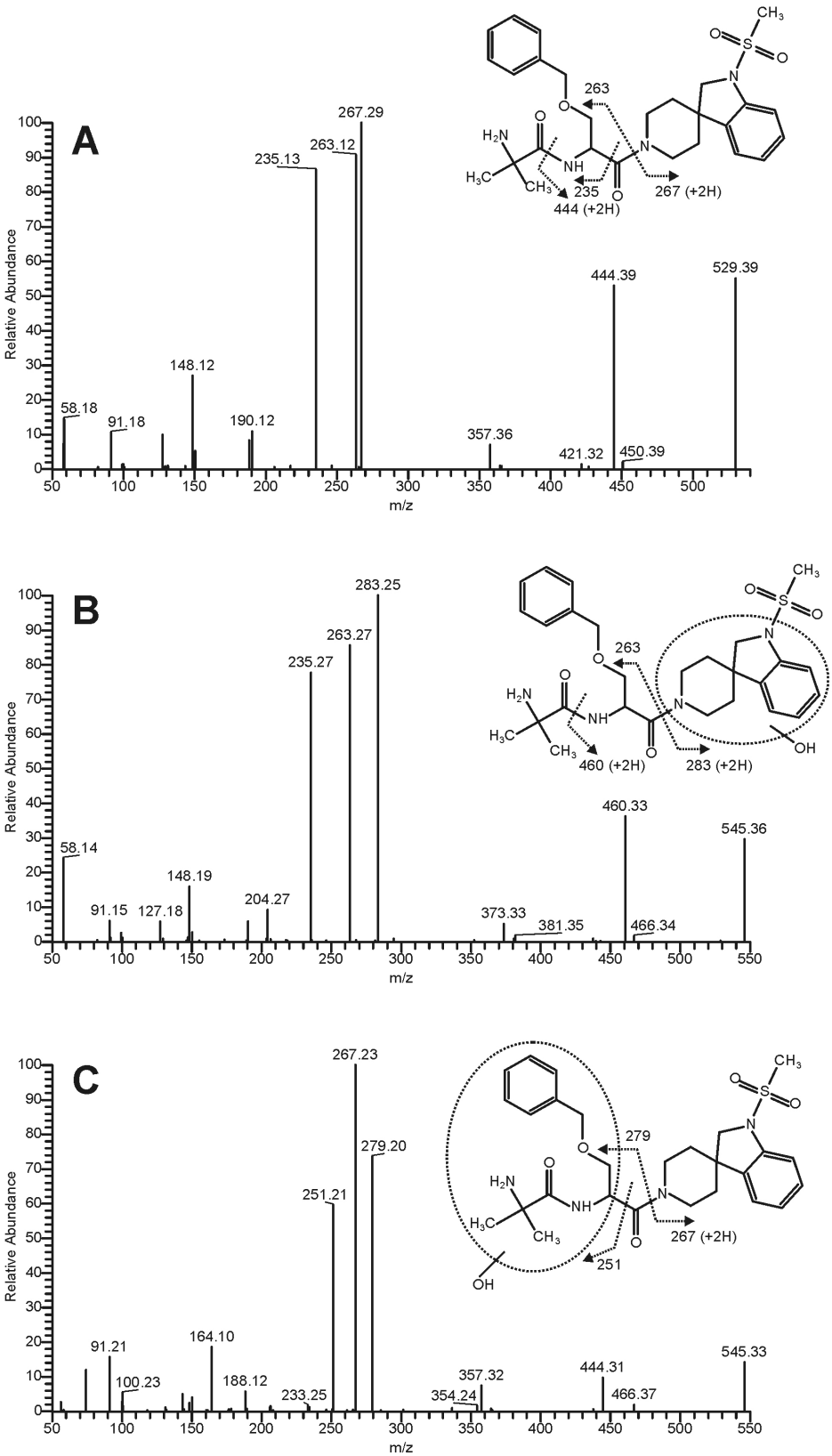

Fig. 2. ESI product ion mass spectra for ibutamoren parent (A), and its hydroxy metabolites **M1** (B) and **M2** (C).

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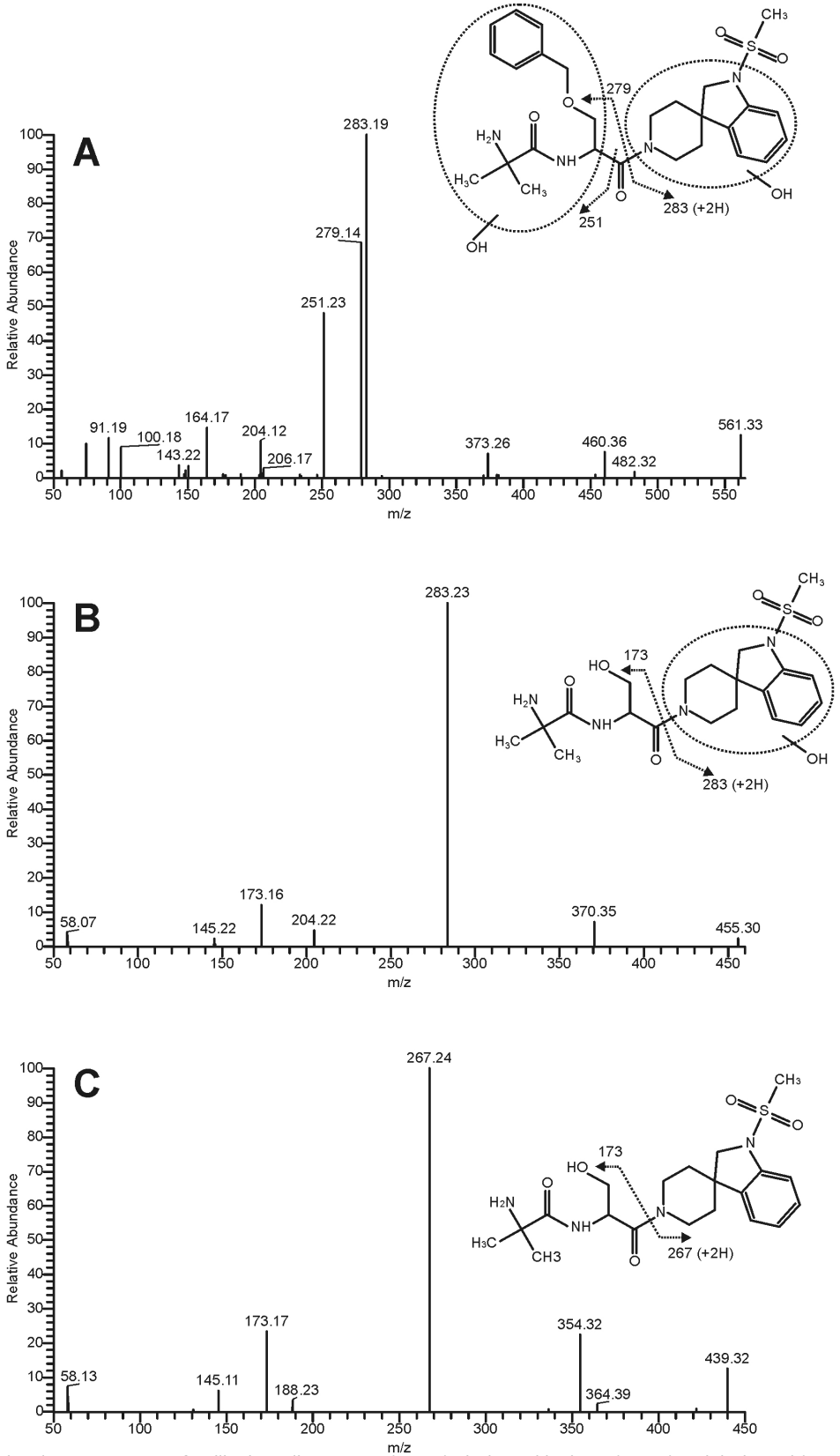

Fig. 3. ESI product ion mass spectra for dihydroxy ibutamoren (**M3**, A), desbenzyl hydroxy (**M5**, B) and desbenzyl (**M4**, C) metabolites.

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Conclusions

Analytical data necessary for detection of ibutamoren (MK-677), a low molecular weight GH secretagogue, have been presented. It was found that it is extensively metabolized to a variety of metabolites, which were tentatively identified. Parent drug and the metabolites are excreted in urine unconjugated to a large extent. Based on the elimination kinetics (which however is restricted to a single excretion study) one may suggest that the detection of intact ibutamoren is sufficient for screening purposes.

References

[1] Patchett AA, Nargund RP, Tata JR, Chen MH, Barakat KJ, Johnston DB, Cheng K, Chan WW, Butler B, Hickey G, et al. Design and biological activities of L-163,191 (MK-0677): a potent, orally active growth hormone secretagogue. Proc Natl Acad Sci USA. 1995, 18, 92 (15), 7001–7005
[2] http://researchsarms.co.uk/products/mk-677

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