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Mass spectral characterization of NBI-31772, a nonpeptide IGF-binding protein inhibitor

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Introduction

It is well known that endogenous insulin-like growth factor-1 (IGF-1) is circulated in bodily fluids bound to a family of six IGF-binding proteins (IGFBPs). They play an important role in modulating the physiological effects of IGF-1, either enhancing or attenuating them. During the last decade a nonpeptide compound that inhibits the binding of IGF-1 to all IGFBPs has been discovered by a biopharmaceutical company Neurocrine Biosciences, termed as NBI-31772 [1].

This compound was shown to elevate free IGF-1 levels both *in vitro* and *in vivo* leading to various physiological effects, such as reduction of ischemic brain damage [2], restoration of proteoglycan synthesis by chondrocytes [3] and improvement of muscle fiber regeneration [4] in mice. Though never being tested in humans, its abuse in sports cannot be excluded. Here we present mass spectrometric data on NBI-31772 needed to facilitate its detection by doping control laboratories.

Materials and Methods

NBI-31772 was obtained from Sigma (St. Louis, MO, USA).

Blank and spiked urine samples were analyzed in this study. In case of LC-MS analysis 100 µl of urine were diluted with 900 µl 3% methanol in water, centrifuged at 15 000 *g* and transferred into a vial. When GC-MS analysis was carried out an aliquot of the NBI-31772 standard solution was initially derivatized with 50 µl of MSTFA/NH_4I/dithiotreitol (1000/2/1.5 v/w/w) at 70°C for 30 min. The spiked urine samples were prepared according to the generic procedure used for analysis of the total fraction by GC-MS in our laboratory [5] except that the different extraction solvents were tested in addition to diethyl ether.

LC-MS/MS experiments were performed on TSQ Vantage AM triple quadrupole mass

spectrometer (ThermoFisher Scientific, USA) connected to an Accela liquid chromatograph (ThermoFisher Scientific, USA). Water Acquity BEH C18 column (100 mm × 2.1 mm, particle size 1.7 µm) maintained at 50°C was used for the separation. Injection volume was 10 µl. The mobile phase flow rate was set to 0.35 ml/min with the gradient elution starting at 80% of 0.1% formic acid in water (***A***) and 20% of 0.1% formic acid in methanol (***B***) (0.5 min hold) followed by a linear increase to 70% ***B*** in 3 min, linear increase to 95% ***B*** (1.5 min hold) in 1 min and then re-equilibration until the end of analysis (10 min).

Positive ESI was used for ionization. Argon was admitted into a Q2 at the pressure of 1.5×10^{-3} Torr. Sheath gas pressure (nitrogen 99.9%) was set at 55 arbitrary units corresponding to nitrogen consumption of *ca*. 17 liters per min. The vaporizer and capillary temperatures were set at 350°C and 300°C, respectively, the spray voltage was +3 kV.

GC-MS/MS system comprised a Trace GC Ultra gas chromatograph (Thermo Scientific, Italy) coupled to a TSQ Quantum GC triple quadrupole mass spectrometer (ThermoFisher Scientific, USA). The separation was done on HP-Ultra 1 column, 17 m × 0.2 mm × 0.11 µm (Agilent J&W, USA) with the temperature programming as follows: 177°C to 233°C at 4°C/min, then to 310°C at 20°C/min (held 4.15 min). One µl injections were done at 250°C in the split mode (1:20) with a carrier gas flow rate set to 0.6 ml/min (helium 99.9999%). Transfer line temperature was 300°C, the ion source was held at 250°C.

Results and Discussion

We have found that NBI-31772 is amenable to both LC-MS ESI and GC-MS EI (after trimethylsilylation) analysis. The positive ion electrospray ionization results in a structurally rich spectrum of product ions (**Fig. 1**).

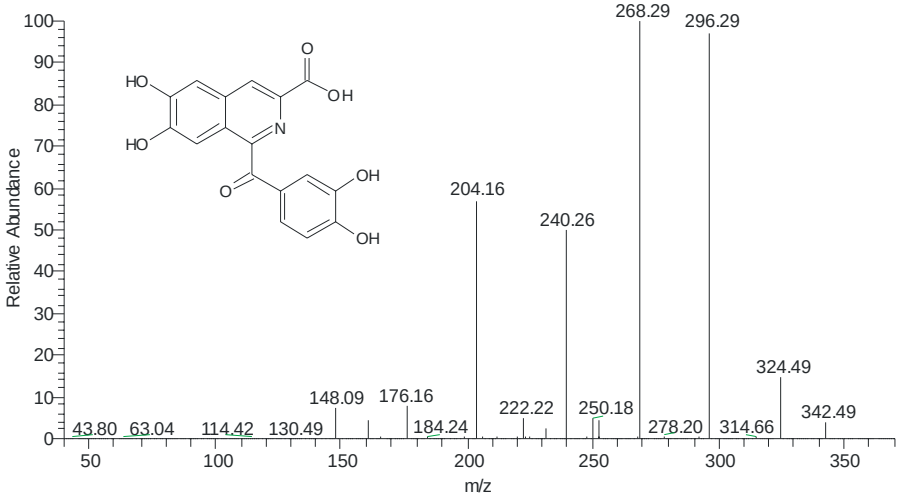

Fig. 1. ESI (+) product ion mass spectrum, *m/z* 342 @ 25 eV.

The negative ion ionization was also attempted as the structure of this compound assumes efficient deprotonation. NBI-31772 was shown to ionize reasonably well in ESI(–) (data not presented); however, at the moment of this study ESI(+) was preferred to keep the compatibility with older instruments not capable of fast polarity switching.

Our initial experiments with NBI-31772 demonstrated that its TMS derivative has good gas chromatographic properties, though it elutes at the very end of chromatogram. The electron ionization mass spectrum of the pertrimethylsilylated derivative of NBI-31772 is given in **Fig. 2**.

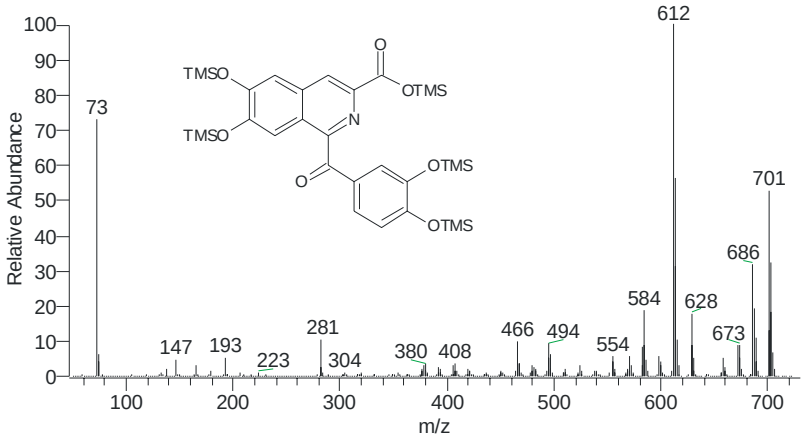

Fig. 2. EI mass spectrum of TMS derivative of NBI-31772 (MW 701).

It was found however that NBI-31772 is very difficult (if at all possible) to extract from urine using liquid-liquid extraction with ethyl acetate, diethyl ether or diethyl ether/isopropanol mix. Therefore, we attempted LC-MS/MS analysis of urine after 10-fold dilution (**Fig. 3**).

Our experiments have shown that this approach is suitable for the determination of NBI-31772 in urine with the limit of detection of *ca.* 20 ng/ml in the positive ESI mode. Application of the negative ESI could further decrease the LOD due to less background noise.

Conclusion

The procedure that enables to detect NBI-31772, a low molecular weight IGF-1 binding proteins inhibitor, is reported. This compound is very difficult to extract using liquid-liquid extraction (under typical conditions used in other screening procedures), so direct urine analysis by LC-MS/MS is preferred after urine dilution. While nothing is known about the pharmacokinetics of this compound, one may anticipate that due to its high polarity NBI-31772 will mostly excrete as parent compound.

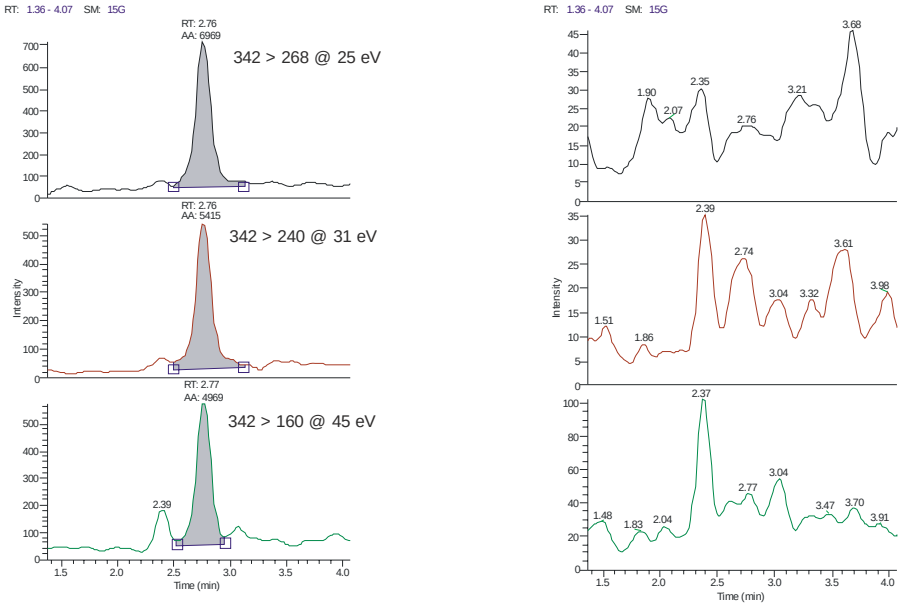

Fig. 3. Urine spiked to 100 ng/ml NBI-31772 (left) and blank urine (right).

References

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