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Analysis of small interfering (si)-RNA by means of LC-HRMS/MS for doping controls

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Abstract

Small (19 to 21 base pairs) interfering RNAs have emerged as effective therapeutic oligonucleotides with an immense potential to influence the gene expression of any gene by inducing degradation of mRNA. They are commonly designed as double strand oligonucleotides with various modifications in order to enhance the bioavailability and stability after application. Possible modifications are: phosphothioates, methylated (5`and 2`-), fluorinated or locked nucleic acids and conjugation to cholesterol. According to the actual WADA list substances on the basis of si-RNA is prohibited under category M3 gene doping.

In the present study we have designed a potential si-RNA to “knock-down” the myostatin gene with two different model compounds (2 x 21 nt as double strand, approx. MW: 7000 Da). These model compounds comprise all common modifications and were applied (i.v.) to female WISTAR rats with subsequent collection of plasma and urine specimens.

Within this study we have developed reliable assays to determine the RNA in urine and plasma by means of LC-MS or SDS-PAGE after isolation with a

microRNA purification kit. A completely unmodified oligonucleotide (20 nt single strand) was used as ISTD. The analytes were characterized by LC-HRMS as intact molecules as well as after hydrolysis (in solution and in-gel). Hereby several metabolites in the urinary samples were identified.

The analysis of the samples showed that the si-RNA metabolites were mainly excreted into urine within the first 24 h after application.

Main parts of the method (LC and Gel analysis) were validated in human urine with focus on qualitative result interpretation considering recovery, precision, specificity and linearity.

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