

## Quantitative and qualitative analysis of a siRNA and its degradation products in plasma by LC-MS/MS

Tomonori Takami; Yukiko Nishida; Shohei Shioyama; Rieko Goto

*JCL Bioassay Corporation, Nishiwaki-Shi, JAPAN*

### Introduction

Recently, following on from small molecule drugs and protein drugs, nucleic acid drugs have increased. Nucleic acid drugs have the advantages that they can be chemically synthesized and possess high specificity toward their target. However, they also have disadvantages, in that they are easily degraded in vivo and have some problems regarding their stability and delivery. Therefore, it is considered that analysis of a nucleic acid drug (parent compound) and its degradation products is necessary for nucleic acid drug development. In the present study, we investigated the possibility of using LC-MS/MS for quantitative and qualitative analysis of a siRNA, which is a type of nucleic acid drug.

### Methods

[Absolute quantification of the parent compound]

PCS-C2 siRNA was extracted from human blank plasma containing a known added concentration of PCS-C2 siRNA solution by phenol/chloroform treatment and solid-phase extraction. The extract was measured with a UPLC-Triple Quad 5500, and the possibility of creating calibration curves was investigated. The post-preparative stability, recovery, and intra-assay precision and accuracy were determined.

[Qualitative and relative quantitative analysis of the degradation products]

PCS-C2 siRNA was added to human blank plasma and incubated at 37°C for 0, 2, 4 or 6 hours. Subsequently, the samples were measured with an HPLC-LTQ FT, and the structures of the degradation products were deduced from accurate *m/z* values. The products were relatively quantified using a UPLC-Triple Quad 5500.

### Preliminary Data

[Absolute quantification of the parent compound]

Calibration curves for both the antisense and sense strands of PCS-C2 siRNA (parent compound) were successfully created in the range of 5–5000 ng/mL. It was confirmed that the recovery of PCS-C2 siRNA was stable, and that the intra-assay precision and accuracy and the post-preparative stability were high. Therefore, it was concluded that absolute quantification of the siRNA (parent compound) in plasma was possible by LC-MS/MS, similar to the case for small molecule drugs.

[Qualitative and relative quantitative analysis of the degradation products]

More than 10 PCS-C2 siRNA degradation products were detected, and their structures including the 5' and 3' terminal structures were deduced. It was confirmed that the degradation products could be measured quantitatively. Therefore, qualitative and relative quantitative analysis of siRNA degradation products was possible by LC-MS/MS.

### Novel Aspect

A siRNA and its degradation products were successfully analyzed quantitatively and qualitatively by LC-MS/MS.