

## Abstract Review:

### **Absolute Quantification of a siRNA and Qualitative and Relative Quantitative Analysis of Its Degradation Products by LC-MS/MS**

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#### **Introduction**

Recently, following on from small molecule drugs and protein drugs, nucleic acid drugs have increased. 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 or rat liver homogenate. They were incubated at 37°C for 0, 2, 4 or 6 hours in the case of adding an EDTA solution and the case of not adding of it. 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.

#### **Results and Discussion**

[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 satisfied the criteria described in the current FDA guidance. 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 in both human plasma and rat liver homogenate, and their structures including the 5' and 3' terminal structures were deduced. It was confirmed that the degradation products could be measured quantitatively. Furthermore, it could be decided whether the degradation products were produced by metal-ion-dependent reaction. Therefore, qualitative and relative quantitative analysis of siRNA degradation products was possible by LC-MS/MS.