nSMOL™ Antibody BA Kit

nSMOL is Shimadzu’s completely new and breakthrough technology that enables selective proteolysis of the Fab region of monoclonal antibodies. This technique facilitates method development independent of a variety of antibody drugs and achieves a paradigm shift in the bioanalysis of antibody drugs.

Furthermore, this is the only method with respect to antibody drugs that has fulfilled the criteria of "Guideline on Bioanalytical Method Validation in Pharmaceutical Development" for low MW drug compounds issued by the Japanese Ministry of Health, Labour and Welfare. Shimadzu also offers optimization methods and protocols, and nSMOL can be applied to clinical research at various institutions.

Antibody Drug Classification and Selection of Quantitation Peptides

Monoclonal antibodies are produced from the hybridoma with mouse spleen lymphocyte and myeloma cells. While mice are predominantly used as hosts, in recent years a variety of hosts are now available to produce monoclonal antibodies. Furthermore, production of the variable Fv by phage display technology and high-throughput screening of affinity sequence has become alternative standard procedure. Antibody drugs are classified into four classes according to specific structure.

The complementarity-determining region (CDR) of antibody specificity against human IgGs becomes smaller according to the "mouse" → "chimeric" → "humanized" → "fully human" antibody. More precise selection of quantitation peptides becomes particularly important in the nSMOL, which is used to perform structure specificity-indicated analysis. The nSMOL enables selective proteolysis in variable regions. This allows selection of quantitation peptides that reflect the structural characteristics of antibodies. Antibodies have three CDRs on each heavy and light chain, CDR2 is known as the region that makes first contact with an antigen. The signature peptide by nSMOL are mainly from CDR2 containing peptides.

Analysis Conditions for Bevacizumab Using the nSMOL

<Sample Processing Protocol>
With the nSMOL technique, the same sample processing protocol can be applied to all antibody drugs. For details, refer to Shimadzu Application News (Trastuzumab analysis).

<LCMS Analysis Conditions>
(LC) NexeraX2 System
Column : Shim-pack GISS C18 (50 mm × 2.1 mm)
Column oven : 50 °C
Solvent A : 0.1 % formic acid/water
Solvent B : 0.1 % formic acid/acetonitrile
Gradient : 1 %B (1.5 min)/1-35 %B (3.5 min)/
95 %B (1 min)/1 %B (1 min)
Flow rate : 0.4 mL/min
Injection : 10 μL

[MS] LCMS-8050, 8060
Ionization : ESI Positive
DL : 250 °C
Heat Block : 400 °C
Interface : 300 °C
Nebulizer gas : 3 L/min
Drying gas : 10 L/min
Heating gas : 10 L/min

Bevacizumab Quantitation Peptides

<table>
<thead>
<tr>
<th>Peptide</th>
<th>MRM transition</th>
<th>Purpose</th>
</tr>
</thead>
<tbody>
<tr>
<td>P14R</td>
<td>512.1&gt;292.3 (b3+)</td>
<td>For quantitation (IS)</td>
</tr>
<tr>
<td></td>
<td>512.1&gt;389.3 (b4+)</td>
<td>For structure confirmation</td>
</tr>
<tr>
<td></td>
<td>512.1&gt;660.4 (b6+)</td>
<td>For structure confirmation</td>
</tr>
<tr>
<td>FTFSDLTSK</td>
<td>523.3&gt;797.4 (y7+)</td>
<td>For quantitation</td>
</tr>
<tr>
<td></td>
<td>523.3&gt;898.5 (y8+)</td>
<td>For structure confirmation</td>
</tr>
<tr>
<td></td>
<td>523.3&gt;650.3 (y6+)</td>
<td>For structure confirmation</td>
</tr>
<tr>
<td>STAYLOQM</td>
<td>642.3&gt;748.4 (y6+)</td>
<td>For quantitation</td>
</tr>
<tr>
<td>SLR</td>
<td>642.3&gt;861.5 (y7+)</td>
<td>For structure confirmation</td>
</tr>
<tr>
<td></td>
<td>642.3&gt;620.3 (y5+)</td>
<td>For structure confirmation</td>
</tr>
<tr>
<td>VLYFTSSLH</td>
<td>588.3&gt;775.9 (y14++)</td>
<td>For quantitation</td>
</tr>
<tr>
<td>SGVPSR</td>
<td>588.3&gt;602.3 (y6+)</td>
<td>For structure confirmation</td>
</tr>
<tr>
<td></td>
<td>588.3&gt;939.5 (y9+)</td>
<td>For structure confirmation</td>
</tr>
</tbody>
</table>

* Quantitation range in human plasma : 0.15 to 300 μg/ml
Averaged accuracy : 101.3 %
Observations, Conclusions, and References

Although Bevacizumab quantitation peptide using nSMOL was obtained from the same region of Trastuzumab, the optimal peptide sequences for bioanalysis will depend on the interference with endogenous IgGs. With respect to multiplexed quantitation of three sequences, nSMOL bioanalysis for Bevacizumab fulfilled the full validation criteria.

The lower limit of quantitation is 0.15 μg/ml and the same assay method can be used from preclinical to clinical trials.

<References>
Iwamoto N et al., Drug Metab Pharmacokinet., 2016, DOI:10.1016/j.dmpk.2015.11.004

<Chief Scientists>
Noriko Iwamoto, Ph.D. and Takashi Shimada, Ph.D., Technology Research Laboratory

Notes: The product described in this document has not been approved or certified as a medical device under the Pharmaceutical and Medical Device Act of Japan. It cannot be used for the purpose of medical examination, treatment or related procedures.