

Under the above circumstances, the present inventors have attempted quantification of a variety of monoclonal antibodies by employing, as a pretreatment technique for mass spectrometry of monoclonal antibodies, a method of nano-surface and molecular-orientation limited proteolysis (hereafter, referred to as the "nSMOL" method) in which monoclonal antibodies are subjected to selective proteolysis through the solid phase-solid phase reaction, developed by the present inventors in the past. It has been demonstrated that the method of analysis via the nSMOL method is capable of detecting monoclonal antibodies existing in a biological sample with high sensitivity and selectivity, concerning a plurality of monoclonal antibodies.

[0012]

The present inventors discovered that two or more types of monoclonal antibodies existing in a biological sample could be concurrently and simultaneously detected and quantified by the nSMOL method. In addition, the method of the present disclosure involving the nSMOL method was found to satisfy the standards of the guideline on bioanalytical method validation in Japan, U.S.A., and Europe.

[0013]

Specifically, the present disclosure provides the following.

1. A method for detecting a peptide fragment having the amino acid sequence derived from the Fab region of a monoclonal antibody comprising bringing a porous body in which monoclonal antibodies to be measured are immobilized in pores into contact with nanoparticles on which proteases are immobilized in a liquid to perform selective proteolysis of the monoclonal antibodies via liquid chromatography-mass spectrometry (LC-MS), wherein peptide fragments of two or more types of monoclonal antibodies in the same biological sample are simultaneously quantified.
2. The method according to 1. above, wherein concentration of each of the two or more types of monoclonal antibodies in a biological sample is 0.5 to 300 $\mu\text{g/ml}$.
3. The method according to 1. or 2. above, wherein the results of simultaneous quantification of the two or more types of monoclonal antibodies exhibit accuracy of $\pm 15\%$ derivation from the results attained when the monoclonal antibodies are quantified independently of each other.

4. The method according to any of 1. to 3. above, wherein 3, 4, 5, 6, 7, 8, 9, 10, or more types of monoclonal antibodies are simultaneously quantified.
5. The method according to any of 1. to 4. above, wherein the monoclonal antibodies include an antibody-drug complex.
6. The method according to any of 1. to 5. above, wherein the monoclonal antibodies include two or more types of antibodies selected from among: human antibodies such as Panitumumab, Ofatumumab, Golimumab, Ipilimumab, Nivolumab, Ramucirumab, and Adalimumab; humanized antibodies such as Tocilizumab, Trastuzumab, Trastuzumab-DM1, Bevacizumab, Omalizumab, Mepolizumab, Gemtuzumab, Palivizumab, Ranibizumab, Certolizumab, Ocrelizumab, Mogamulizumab, and Eculizumab; chimeric antibodies such as Rituximab, Cetuximab, Infliximab, Basiliximab, Brentuximab vedotin, and Gemtuzumab ozogamicin; and an antibody-drug complex such as Trastuzumab emtansine.
7. The method according to 6. above, wherein the monoclonal antibodies are two or three types of antibodies selected from among Cetuximab, Rituximab, and Brentuximab vedotin.
8. A composition used for combined quantification of monoclonal antibodies in a biological sample via liquid chromatography-mass spectrometry (LC-MS), which comprises two or more types of peptide fragments obtained by selective proteolysis, each comprising an amino acid sequence derived from the Fab region of a monoclonal antibody.
9. The composition according to 8. above, which yields stable results of quantification at 5°C for 48 hours after selective proteolysis.
10. The composition according to 8. or 9. above, wherein the monoclonal antibodies include Cetuximab and a peptide fragment to be measured has the amino acid sequence as shown in SEQ ID NO: 3.
11. The composition according to 8. or 9. above, wherein the monoclonal antibodies include Rituximab and a peptide fragment to be measured has the amino acid sequence as shown in SEQ ID NO: 6.
12. The composition according to 8. or 9. above, wherein the monoclonal antibodies include Brentuximab vedotin and a peptide fragment to be measured has the amino acid sequence as shown in SEQ ID NO: 9.

[Effects of the Disclosure]

[0014]

The method of analysis involving the nSMOL method according to the present disclosure was found to be capable of detecting monoclonal antibodies existing alone or in combinations of two or more in a biological sample with high sensitivity and accuracy. The method of the present disclosure enables simultaneous measurement of a plurality of antibody drugs.

[0015]

According to the method of the present disclosure, a plurality of antibodies can be simultaneously analyzed without preparing antibodies that specifically bind to and detect relevant antibody drugs, and concentrations of antibodies *in vivo* can be monitored. Thus, the cost and the time required for development of analytical techniques in clinical settings can be reduced to a significant extent, and complicated pharmacokinetic information concerning antibody drugs can be readily provided.

[Brief Description of the Drawings]

[0016]

Fig. 1 schematically shows the nSMOL method.

Fig. 2 shows calibration curves prepared based on combined quantification of standard samples containing Cetuximab, Rituximab, and Brentuximab vedotin.

Fig. 3 shows the results of MRM assays of signature peptides following the pretreatment via the nSMOL method of a plasma sample containing all of Trastuzumab, Bevacizumab, Cetuximab, Rituximab, Nivolumab, Ipilimumab, Ramucirumab, Brentuximab vedotin, Infliximab, and Adalimumab at 10 µg/ml each and samples each containing one of the monoclonal antibodies at 10 µg/ml. The results of combined quantification are demonstrated in the form of an ion yield relative to the ion yield attained by independent quantification designated to be 100.

[Embodiments for Carrying out the Invention]

[0017]

In one embodiment, the present invention relates to a method for detecting a peptide fragment comprising an amino acid sequence derived from the Fab region of a monoclonal antibody, comprising bringing a porous body in which monoclonal antibodies to be measured are immobilized in pores into contact in a liquid with nanoparticles on which proteases are