The present inventors have found that, in order to detect an antibody using limiting protease digestion, it is most effective to detect a protease digestion fragment contained in particular in a CDR2 region in a variable region of a monoclonal antibody.

[0013]

Usually, when a peptide fragment after protease digestion is subjected to mass spectrometry, it is considered to use a protease having less self-digestion and high cleavage sequence selectivity. Therefore, for a person skilled in the art, when a commercially available protease is used, a protease of a mass spectrometry grade or of a sequencing (sequence) grade is used. For example, a native trypsin derived from a living body generates a pseudo trypsin exhibiting chymotrypsin-like activity by self-digestion and thus is known to have low cleavage site specificity. Therefore, a trypsin of a mass spectrometry grade having improved resistance to self-digestion by subjecting a lysine residue of the trypsin to reductive methylation is commercially available.

[0014]

However, the present inventors have found that, when a monoclonal antibody as a measurement target has been digested using the above-described so-called high quality protease, quantitative detection may not be possible using an obtained peptide fragment.

[0015]

The present inventors believe that the above result suggests that there may be interference due to presence of an endogenous antibody to the obtained peptide fragment, and have further studied for a purpose of obtaining a peptide fragment without such interference. As a result, it was surprisingly found that, by using an enzyme with lower specificity than a conventional structural analysis enzyme, the number of cutting sites is increased, peptide fragments of different sequences are generated, and, consequently, optimal peptides for quantitative detection can be obtained, and thus, the present invention is accomplished.

[0016]

That is, the present disclosure includes the following aspects.

In one aspect, the present disclosure is directed to a method for quantifying mogamulizumab, the method comprising a step of performing selective protease digestion of mogamulizumab as a measurement target by bringing a porous body in which mogamulizumab is immobilized in

pores into contact with fine particles onto which a specific protease is immobilized, the fine particles having an average particle size larger than an average pore diameter of the porous body; and a step of detecting a peptide fragment that is obtained from the digestion and has an amino acid sequence derived from a CDR2 region of a heavy chain or a light chain of mogamulizumab, wherein, as the protease, a protease having trypsin activity and chymotrypsin activity is used, and wherein the protease having trypsin activity and chymotrypsin activity is a trypsin that has not been subjected to a reductive methylation treatment. In another aspect, the present disclosure is directed to a kit for quantitative detection of mogamulizumab using liquid chromatography-mass spectrometry (LC-MS), the kit comprising a porous body having pores for immobilizing mogamulizumab as a measurement target; fine particles onto which a protease having trypsin activity and chymotrypsin activity is immobilized, the fine particles having an average particle size larger than an average pore diameter of the porous body; a reaction container for selectively digesting mogamulizumab by bringing the porous body into contact with the fine particles; and a buffer solution that is introduced into the reaction container together with the fine particles and the porous body and is for causing a digestion reaction by the protease to occur, wherein the protease having trypsin activity and chymotrypsin activity is a trypsin that has not been subjected to a reductive methylation treatment.

Also described herein is the following: (1) A method for quantifying a monoclonal antibody, the method including: a step of performing selective protease digestion of a monoclonal antibody as a measurement target by bringing a porous body in which the monoclonal antibody is immobilized in pores into contact with fine

WHAT IS CLAIMED IS:

1. A method for quantifying mogamulizumab, the method comprising:

a step of performing selective protease digestion of mogamulizumab as a measurement target by bringing a porous body in which mogamulizumab is immobilized in pores into contact with fine particles onto which a specific protease is immobilized, the fine particles having an average particle size larger than an average pore diameter of the porous body; and

a step of detecting a peptide fragment that is obtained from the digestion and has an amino acid sequence derived from a CDR2 region of a heavy chain or a light chain of mogamulizumab, wherein,

as the protease, a protease having trypsin activity and chymotrypsin activity is used, and wherein the protease having trypsin activity and chymotrypsin activity is a trypsin that has not been subjected to a reductive methylation treatment.

2. The method according to claim 1, wherein the peptide fragment is a peptide having an amino acid sequence expressed in SEQ ID NO: 10.

3. A kit for quantitative detection of mogamulizumab using liquid chromatography-mass spectrometry (LC-MS), the kit comprising:

a porous body having pores for immobilizing mogamulizumab as a measurement target;

fine particles onto which a protease having trypsin activity and chymotrypsin activity is immobilized, the fine particles having an average particle size larger than an average pore diameter of the porous body;

a reaction container for selectively digesting mogamulizumab by bringing the porous body into contact with the fine particles; and

a buffer solution that is introduced into the reaction container together with the fine particles and the porous body and is for causing a digestion reaction by the protease to occur, wherein the protease having trypsin activity and chymotrypsin activity is a trypsin that has not been subjected to a reductive methylation treatment.

4. The kit according to claim 3, wherein the measurement target is mogamulizumab, and an internal standard peptide is a peptide having an amino acid sequence expressed in SEQ ID NO: 10.