

Claims

1. A method of measuring acidic species generated by degradation of a Fab or Fab' component of a Fab-PEG or a Fab'-PEG comprising the steps of:
 - a) cleaving the PEG and linker from the Fab-PEG or Fab'-PEG with an enzyme,
 - b) optionally separating the PEG and linker generated in step a) from the Fab or Fab', to provide a Fab or Fab' and
 - c) quantitatively analyzing acidic species associated with the cleaved Fab or Fab' and/or the cleaved PEG.
2. A method according to claim 1, wherein the enzyme is trypsin or chymotrypsin.
3. A method according to claim 1 or 2 wherein the cleaving in step a) is performed at a temperature in the range 25 to 40°C, such as 37°C.
4. A method according to any one of claims 1 to 3, wherein the cleavage of step a) occurs between a lysine and a threonine, in particular in the C-terminal of the Fab or Fab' heavy chain.
5. A method according to claim 4, wherein the lysine and threonine are found in the sequence SCDKTHTC~~AA~~ located at the C-terminal of a Fab'.
6. A method according to claim 5, wherein the enzyme cleaves the hinge portion of the Fab' and releases the PEG and linker from the Fab'.
7. A method according to any one of claims 1 to 6, wherein the PEG and linker generated in step a) is separated from the Fab or Fab', to provide a Fab or Fab'.
8. A method according to claim 7, wherein the separation is effected using cation exchange chromatography, cIEF, and/or size exclusion chromatography.
9. A method according to any one of claims 1 to 8, wherein the quantification of acidic species is performed employing cation exchange chromatography or imaged capillary isoelectrophoresis, such as CEX-HPLC.

10. A method according to any one of claims 1 to 8, wherein the Fab' comprising or consisting of SEQ ID NO:10.

Claims

The invention claimed is:

1. A method of quantitatively measuring acidic species generated by degradation of a Fab' component of a Fab'-PEG conjugate comprising the steps of:
 - a) cleaving the PEG attached to said Fab' via a linker from said Fab'-PEG conjugate with an enzyme that cleaves the hinge portion of the Fab' and releases the PEG and linker from the Fab'-PEG conjugate,
 - b) separating the PEG and linker generated in step a) from the Fab', to provide a Fab' and
 - c) quantitatively analyzing acidic species associated with the cleaved Fab' and/or the cleaved PEG.
2. The method according to claim 1, wherein the enzyme enzyme is trypsin or chymotrypsin.
3. The method according to claim 1, wherein the cleaving in step a) is performed at a temperature in the range 25 to 40°C.
4. The method according to claim 1, wherein the cleavage of step a) occurs between a lysine and a threonine in the C-terminal portion of the Fab' heavy chain portion of the Fab'-PEG conjugate.
5. The method according to claim 4, wherein the lysine and threonine are found in the sequence SCDKTHTCAA (SEQ ID NO: 10) located at the C-terminal portion of a Fab'.
6. The method according to claim 1, wherein the separation is effected using cation exchange chromatography, cIEF, and/or size exclusion chromatography.
7. The method according to claim 1, wherein the quantification of acidic species is performed employing cation exchange chromatography or imaged capillary isoelectrophoresis.
8. The method according to claim 7, wherein said cation exchange chromatography is cation exchange HPLC (CEX-HPLC).

9. The method according to claim 1, wherein the Fab' component of said Fab'-PEG conjugate contains SEQ ID NO: 10.
10. The method according to claim 3, wherein the cleaving step in a) is performed at a temperature in the range 25°C to 38°C.