How should the test requirements be applied in related substances tests?

Answer:

This FAQ provides guidance on how to apply the test requirements and on data interpretation in the related substances test presented in an individual monograph.

The figures given in the examples below do not apply to biological and biotechnological products, oligonucleotides, products of fermentation and semisynthetic products derived therefrom, to crude products of animal or plant origin or herbal products or excipients (see Substances for pharmaceutical use (2034)); however the principle remains valid.

As stated in chapter *2.2.46. Chromatographic separation techniques*, disregard any peak due to the solvents and reagents or arising from the mobile phase or the sample matrix.

The signal-to-noise ratio of the specified impurities is calculated on the solution used for reporting threshold / disregard limit.

QUANTITATIVE METHOD

- *Reporting threshold*: inject a solution of the substance to be examined at a concentration corresponding to the reporting threshold (e.g. 0.05% of the concentration of the test solution) and note the area of the principal peak.

  a) Report the peaks in the chromatogram obtained with the test solution with an area greater than this peak area.

  b) When an impurity requires a correction factor, report the impurity peak if its corrected area is greater than this peak area.

  NB: instead of a solution of the substance to be examined at a concentration corresponding to the reporting threshold, it is possible to use the reference solution used for the quantitation of the unspecified impurities (e.g. 0.10% of the concentration of the test solution) and to extrapolate the area of the principal peak to the reporting threshold (e.g. half the peak area obtained with the reference solution at 0.1%).

  c) For an impurity controlled using a solution of an *impurity CRS*, dilute the latter solution to the reporting threshold. Report the impurity peak in the chromatogram obtained with the test solution if its area is greater than that of the principal peak in the chromatogram obtained with the *impurity CRS* solution.

- *Specified and unspecified impurities*: calculate the content of the individual impurities from the concentration of the reference solution(s) as stated in the monograph, applying any correction factor mentioned.
- *Total*: sum up the content calculated for the individual impurities and compare the figure with the limit stated in the monograph.

AREA COMPARISON

A) NO CORRECTION FACTORS STATED FOR INDIVIDUAL IMPURITIES

1. *Area comparison with a reference solution = dilution of test solution (all impurities have a response factor of 0.8 to 1.2)*

- *Disregard limit*: inject a solution of the substance to be examined at a concentration corresponding to the disregard limit (e.g. 0.05% of the concentration of the test solution) and note the area of the principal peak. Disregard the peaks in the chromatogram obtained with the test solution with an area lower than or equal to this peak area.

NB: instead of a solution of the substance to be examined at a concentration corresponding to the disregard limit, it is possible to use the reference solution used for the quantitation of the unspecified impurities (e.g. 0.10% of the concentration of the test solution) and to extrapolate the area of the principal peak to the disregard limit (e.g. half the peak area obtained with the reference solution at 0.10%).

- *Specified and unspecified impurities*: compare the areas of the reported individual peaks with (*x* times) the area of the peak obtained with the relevant reference solution, as stated in the monograph.
- *Total*: sum up the areas of the individual peaks and compare the figure with (*y* times) the area of the peak obtained with the reference solution, as stated in the monograph.

2. *Area comparison with a reference solution = dilution of test solution (for impurities having a response factor of 0.8 to 1.2) + Area comparison with a reference solution = solution of an impurity CRS (for impurities having a response factor outside the range 0.8 to 1.2)*

- *Disregard limit*: inject a solution of the substance to be examined at a concentration corresponding to the disregard limit (e.g. 0.05% of the concentration of the test solution) and note the area of the principal peak.

  a) Disregard the peaks in the chromatogram obtained with the test solution having an area lower than or equal to this peak area.

NB: instead of a solution of the substance to be examined at a concentration corresponding to the disregard limit, it is possible to use the reference solution used for the quantitation of the unspecified impurities (e.g. 0.10% of the concentration of the test solution) and to extrapolate the area of the principal peak to the disregard limit (e.g. half the peak area obtained with the reference solution at 0.1%).

b) For an impurity controlled using a solution of an *impurity CRS*, dilute the latter solution to the disregard limit. Report the impurity peak in the chromatogram obtained with the test solution if its area is greater than that of the principal peak in the chromatogram obtained with the diluted *impurity CRS* solution.

- *Specified and unspecified impurities*: compare the areas of the reported individual peaks with (*x* times) the area of the peak obtained with the reference solution, as stated in the monograph.

  For an impurity controlled using a solution of an *impurity CRS*, compare its peak area with the area of the peak obtained with the relevant reference solution, as stated in the monograph.

- *-Total*: if a maximum value is stated, see Quantitative method.

  if an area comparison is stated, see A)1.

B) CORRECTION FACTORS STATED FOR ONE OR MORE IMPURITIES

Area comparison with a reference solution = dilution of test solution (for impurities having a response factor of 0.8 to 1.2) + correction factor for one or more impurities

- *Disregard limit*: inject a solution of the substance to be examined at a concentration corresponding to the disregard limit (e.g. 0.05% of the concentration of the test solution) and note the peak area of the principal peak.

  a) Disregard the peaks in the chromatogram obtained with the test solution with an area lower than or equal to this peak area.

  b) When an impurity requires a correction factor, report the impurity peak if its corrected area is greater than this peak area.

  NB: instead of a solution of the substance to be examined at a concentration corresponding to the disregard limit, it is possible to use the reference solution used for the quantitation of the unspecified impurities (e.g. 0.10% of the concentration of the test solution) and to extrapolate the area of the principal peak to the disregard limit (e.g. half the peak area obtained with the reference solution at 0.10%)

- *Specified and unspecified impurities*: compare the (corrected) areas of the reported individual peaks with (*x* times) the area of the peak obtained with the relevant reference solution, as stated in the monograph.
- *Total*: sum up the areas of the reported individual peaks and compare the figure with (*y* times) the area of the peak obtained with the relevant reference solution, as stated in the monograph.

NORMALISATION PROCEDURE

- *Disregard limit / reporting threshold*: inject the solution prescribed in the monograph for the disregard limit/reporting threshold. Disregard the peaks in the chromatogram obtained with the test solution having an area lower than or equal to this peak area.
- *Specified and unspecified impurities*: divide the peak area due to each impurity, in the chromatogram obtained with the test solution, by the sum of the areas of all the reported peaks, including the peak due to the substance to be examined; multiply this result by 100.
- *Total*: sum up the areas of the reported peaks and divide by the sum of the areas of all the reported peaks, including the peak due to the substance to be examined; multiply this result by 100.