MEDICAL ENGINEERING TECHNOLOGIES

The Influence of Uncertainty on Chemical Characterisation

As with any other measurement or experiment analytical chemistry has uncertainties. When you use the bathroom scale to measure yourself does the screen oscillate between two numbers or the arrow on the dial point between two numbers? So which number is correct, there is an uncertainty between your perfect weight and 5g more. Equally, when we say that there are 10µg of phthalate in your sample, depending on the accuracy of the equipment and all other factors we might actually be saying that it is somewhere between 9.5µg and 10.5µg. ISO 10993-18 compels us to consider this in any analysis.

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ISO 10993-18, Uncertainty Factor

The quantification of extractables is determined by screening methods, which need to be able to detect a large variety of possible extractables. The accuracy of the estimated concentrations can vary depending on the quantification method used. Quantification methods that use internal standards, assume that all analytes give similar responses to each other, and therefore with respect to the internal standards too. If this assumption is true, the estimated concentrations for all analytes will be very accurate. However, if this assumption is false, i.e. the response factors are not similar for all analytes, the accuracy of the estimate of the concentration will vary depending on the proportional difference in the response factor of the analyte to the response factor of the internal standard.

There are other quantification methods that provide accurate estimates for concentrations. Calibration curves can be generated for expected extractables using the same screening method, by injecting standards over a range of known concentrations. These will give very accurate quantification, if the same compound is found in the extracts. Another quantification method is a hybrid of the previous two described, where relative response factors are obtained for expected extractables. The relative response factors are the ratio of the standards over a range of known concentrations versus an internal standard, which produces another calibration curve. This calibration curve adjusts for the variation in response factors of extractables compared to internal standards.

The variation in response factors of extractables and internal standards is accounted for in the calculation of the analytical evaluation threshold (AET). The AET is the threshold used to determine whether a chemical detected in the test sample is of a high enough concentration to be reported. The AET is only applicable to screening methods such as GC-MS and HPLC-MS. The AET should not be used for methods designed to identify and quantify highly toxic extractables in a cohort of concern. The formula below from ISO 10993-18 Annex E is used to calculate the AET.

$$AET = \frac{DBT \times \frac{A}{BCD}}{UF}$$

A .....is the number of medical devices extracted to generate the extract;

B .....is the volume of the extract in ml;

C .....is the number of devices a patient would be exposed to in a day under normal clinical practice;

D .....is the concentration or dilution factor;

DBT ..is the dose-based threshold (e.g. (TTC) or (SCT)) in µg/day;

UF .....is an uncertainty factor that accounts for the analytical uncertainty of the screening methods used to estimate the concentration of extractables in an extract.

Each of the variables that make up the formula for calculating the AET are easily known, when preparing the extraction, apart from the uncertainty factor, which must be calculated or justified beforehand. As shown by the formula for the AET, the uncertainty factor and the AET are inversely proportional to each other i.e. a larger uncertainty factor will give a smaller analytical evaluation threshold and vice versa. A small uncertainty factor is desired, because it shows that the variation in response factors is low and therefore suitable for reporting data, which is the foundation of a toxicological risk assessment.

For analytical methods, where the variation in response factors of expected extractables, applied internal standards and targeted extractables using qualified methods are known to be acceptably low, an uncertainty factor of 1 can be justified. An uncertainty factor of 2 can also be justified for screening methods that use GC-FID or GC-MS, as the response factors of extractables detected by these methods are deemed to be somewhat consistent. For other screening methods, such as HPLC-MS, no guidance is given by ISO 10993 for a specific uncertainty factor. However, rather than assuming and justifying the value of the uncertainty factor to be 1, 2 or another number, the uncertainty factor can be calculated for a specific method, which gives a more accurate value of the AET, and therefore a more reliable threshold to exclude or include peaks when reporting data to be assessed in a toxicological risk assessment for that specific analytical method. ISO 10993-18 has recently had an amendment on how to determine the uncertainty factor. The UF is calculated by using the formula, below, which assumes a Gaussian distribution of response factors, which is not the case for all chromatographic detection methods.

$$UF = \frac{1}{1 - RSD}$$

Where, the RSD is the relative standard deviation of the response factors from the reference database. The reference database is an internal record of response factors specific to the analytical method that the uncertainty factor is being calculated for. These response factors are the peak areas or heights of each compound at a known concentration. One analytical method for an extractables and leachables study should have many response factors in the reference database as they are screening methods. The RSD of a response factor can be obtained from the repeatability section of a method validation. To obtain the combined RSD for all of the compounds in the reference database, the RSDs for all of the compounds should be summed in quadrature.

The size of the uncertainty factor must not be too large or too small, as this indicates that the method being used is not suitable. A large uncertainty factor e.g. greater than 10, shows that the method is inaccurate and therefore, should not be used as the basis for a toxicological risk assessment. In addition, a large uncertainty factor could give an AET that is so small, that it would not be detected by the analytical method, because it is smaller than the method's limit of detection (LOD). If this occurs, the method should be improved before it is used as the foundation of a toxicological risk assessment. When the RSD is greater than or equal to 1 (this occurs when the standard deviation is greater than or equal to the mean), the uncertainty factor will equal infinity or a negative number. An analytical method with this much variation of response factors is obviously not suitable to be used as the foundation of a toxicological risk assessment, and the method should be improved.

Screening for extractables and leachables is usually done using orthogonal and complementary analytical methods, for example, GC-MS and HPLC-MS. This use of multiple techniques can be used to decrease the response factor variation and can be considered in the determination of the uncertainty factor that is then applied to all of the complementary methods. Alternatively, a separate uncertainty factor can be calculated for each method and applied to each individual method, which gives a more accurate and specific AET than combining all of the techniques for each analytical method. Whichever is chosen, the use, value and the means of calculation of the uncertainty factor used should always be justified for each analytical method used.

Conclusion

The purpose of chemical characterisation is to ascertain if a device is likely to be toxic or have negative effects when applied to a patient, and ideally obviate the need for biological testing. The data from an analysis is frequently used by a toxicologist to ascertain this. They will need to know how accurate the data is in relation to the AET in order to form conclusions. Here we show how to quantify this as required by ISO 10993-18.

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