

Appendix A.

The validation procedure: outline and example approaches

Validation is undertaken following the completion of the method development or before a method that has not been previously used is to be introduced for routine analysis. We distinguish between initial validation of a quantitative analysis method to be applied in the laboratory for the first time and to extension of the scope of an existing validated method for new analytes and matrices.

Quantitative analysis

1. Initial full validation

Validation needs to be performed

- for all analytes within the scope of the method
- for at least 1 commodity from each of the commodity groups (as far as they are within the claimed scope of the method or as far as applicable to samples analysed in the laboratory)

Experimental:

A typical example of the experimental set up of a validation is:

Sample set (sub samples from 1 homogenised sample)

- Reagent blank
- 1 unspiked sample
- 5 spiked samples at LOQ
- 5 spiked samples at 2-10x LOQ or MRL

Instrumental sequence:

- Calibration standards in solvent at LOQ level
- Calibration standards in matrix at LOQ level
- Reagent blank
- Unspiked sample
- 5 spiked samples at LOQ
- Calibration standards in matrix at LOQ level
- 5 spiked samples at 2-10x LOQ or MRL
- Calibration standards in matrix at 2-10x LOQ or MRL

Data evaluation:

Calibrate and inject the sequence and quantify as is anticipated in the AQC document.

From the data determine at least the parameters from Table 1 and verify them against the criteria.

Table 1: Validation parameters and criteria.

Parameter	What/how	Criterion	Cross reference to AQC document
Linearity	Through calibration curve	Residuals < $\pm 20\%$	35-41
Matrix effect	Comparison of response from solvent standards and matrix-matched standards	-	44-48
LOQ	By definition: lowest level for which it has been demonstrated that criteria for accuracy and precision have been met	\leq MRL	56
Specificity	Response in reagent blank and control samples	< 30% of LOQ	63
Accuracy	Determine average recovery for both spike levels	70-120%	58
Precision (RSD _r)	Determine repeatability RSD _r , determine for both spike levels	$\leq 20\%$	58
Precision* (RSD _{wR})	Determine within-laboratory reproducibility*	$\leq 20\%$	58
Robustness	Can be derived from on-going method validation / verification through establishing average recovery and RSD _{wR} ?	See above	

* Within-lab reproducibility is to be derived from on-going QC (see below)

2. Extension of the scope of the method: new analytes

New analytes that are added to a previously validated method need to be validated using the same procedure as outlined above for initial validation.

Alternatively, the validation of new analytes can be integrated in the on-going quality control procedure. As an example: with each batch of routine samples one or more commodities from the applicable commodity category are fortified at LOQ and one higher level. Determine recovery and occurrence of any interference in the corresponding unfortified sample. When for both levels 5 recovery values have been collected, the average recovery and within-laboratory reproducibility (RSD_{wR}) can be determined and tested against the criteria from table 1.

3. Extension of the scope of the method: new matrices

A pragmatic way of validation of the applicability of the method to other matrices from the same commodity category is to do this during the on-going quality control performed concurrently with analysis of the samples. See below.

4. On going performance validation / verification

The purpose of on-going method validation is to:

- demonstrate robustness through evaluation of mean recovery and within-laboratory reproducibility (RSD_{WR})
- demonstrate that minor adjustments made to the method over time do not unacceptably affect method performance
- demonstrate applicability to other commodities from the same commodity category (see also above)
- determine acceptable limits for individual recovery results during routine analysis
- collect information for estimation of the within-laboratory measurement uncertainty

Experimental:

Typically, with each batch of samples routinely analysed, one or more samples of different commodities from the applicable commodity category are fortified with the analytes and analysed concurrently with the samples.

Data evaluation:

Determine for each analyte the recovery from the fortified sample and occurrence of any interference in the corresponding unfortified sample. Periodically (e.g. annually) determine average recovery and reproducibility (RSD_{WR}) and verify data obtained against the criteria from Table 1. These data can also be used to set or update limits for acceptability of individual recovery determinations as outlined in paragraph 65 of the AQC document and for estimation of the measurement uncertainty.