Eurachem

A focus for analytical chemistry in Europe

Blanks in Method Validation

Supplement to Eurachem Guide The Fitness for Purpose of Analytical Methods

Second Edition 2025

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Blanks in Method Validation

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Contents

Foreword to the Second Edition

Following the publication of the 3rd edition of the Eurachem guide The Fitness for Purpose of Analytical Methods – A Laboratory Guide to Method Validation and Related Topics in February 2025, this supplementary document has been reviewed and updated. The main changes are:

- all cross references updated;
- revision of the section on calibration blanks in line with the 3rd edition of the Fitness for Purpose Guide;
- the addition of blanks related to sampling.

Foreword to the First Edition

The Fitness for Purpose of Analytical Methods - A Laboratory Guide to Method Validation and Related Topics (2nd ed.) was published in 2014. Since then the Method Validation Working Group has identified areas where extra guidance would be appropriate. This extra guidance has been prepared in the form of supplementary documents. This supplementary document is not intended to be used in isolation; it should be used in conjunction with the Guide.

1 Introduction and scope

Blanks are an important tool and are used in the determination of most performance characteristics during a validation process (see section 4.4.1 in the Guide [1]). They are also often included in each analytical run during routine use of the measurement procedure. There are many different types of blanks and the analyst must consider which blanks to include during preparation of the validation plan. The aim of this document is to describe the different kinds of blanks which may be used during method validation and to provide guidance for situations where it may be difficult to obtain a suitable blank matrix. Not all blanks discussed in this document are necessary for every validation and blanks used during routine use of the method e.g. to address baseline correction, do not fall under the scope of this document. It is worth noting that certain techniques, such as chromatography, rely on detecting a peak above noise. For the determination of certain performance characteristics, limit of detection (LOD) and limit of quantification (LOQ) for example, it is necessary, therefore, to use a sample containing a low level of analyte rather than a blank. Further guidance on this is provided in the Guide in section 5.3.2. Figure 1 shows the different types of blanks classified by their general intended use (calibration blanks, procedural blanks) and by composition (reagent, solvent and sample blanks) together with their possible uses in method validation. These are discussed in the following sections.

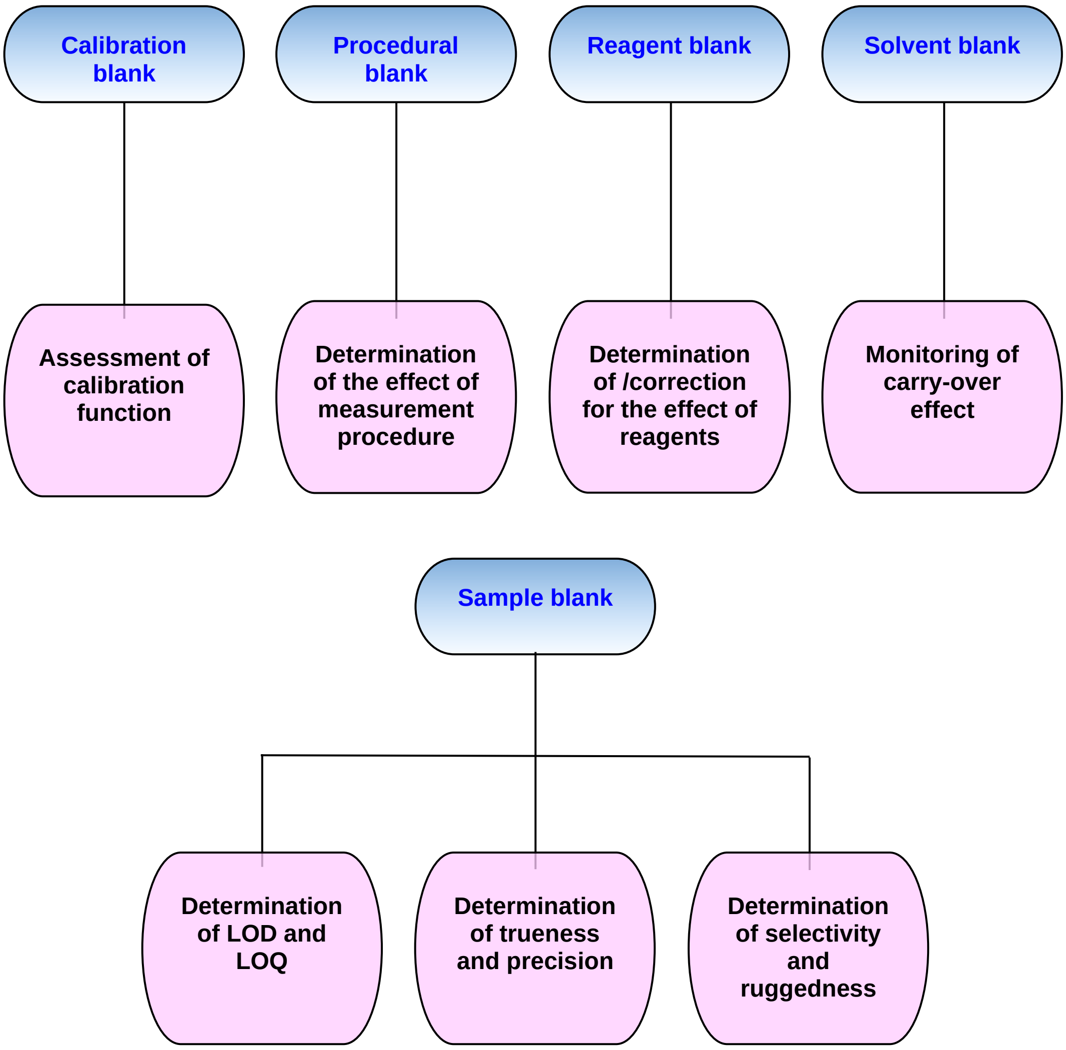

Figure 1 - Types and uses of blanks in method validation

2 Types and uses of blanks in method validation

2.1 Blanks associated with sampling

If sampling is part of the measurement procedure and therefore included in the validation plan then the plan may also need to incorporate some blanks specific to the sampling process and the transport of the samples.

2.1.1 Field Blanks

A sample of matrix known not to contain any analyte is transported to the sampling site in a clean, capped container. An empty clean, capped container is also transported to the sampling site. During the sampling procedure, the field blank is handled in exactly the same manner as the samples being collected. Sampling of water may involve a direct transfer of the blank matrix into the clean container [2] while sampling of air, for example, may involve placing a filter into the sampling apparatus but not operating the pump [3]. The container is then sealed and transported back to the laboratory for storage and analysis. This blank assesses contamination which may occur during the entire procedure from preparation of the sampling containers, through transport to the sampling site, sampling, handling, transport back to and storage in the laboratory. Field blanks should be taken over the duration of the sampling procedure and represent the entire sampling site.

2.1.2 Transport Blanks

A sample of matrix known not to contain the analyte(s) of interest is transferred to the sampling site in a clean, capped container as above. It is transported to the sampling site but is not opened. It is then transported back to the laboratory and stored until analysis. This assesses any contamination which may occur during the transport and storage of samples. This may also be referred to as a trip blank or a travel blank.

2.1.3 Equipment Blanks

To ensure there is no cross contamination of samples during the sampling procedure, sampling equipment may be cleaned after use. Following the cleaning procedure the equipment is rinsed and this rinsate is collected, stored in a clean container and transferred to the laboratory for analysis.

2.2 Calibration blank

Section 5.2 of the Guide addresses the calibration function. Although the calibration function is not a performance characteristic of a test method, ensuring that the calibration function is fit for purpose is a crucial pre-requisite to the assessment of the performance characteristics and hence to the determination of fitness for purpose of the test method. When assessing the calibration function, it is necessary to prepare and measure a calibration blank as well as the calibration standards. A calibration blank is a calibration standard that does not contain the analyte(s) of interest at a detectable level. It is necessary to determine any signal that may be produced at the detector which is not due to the presence of the analyte(s) (this signal is known as the blank indication).

2.3 Analytical procedure blank

A analytical procedure blank (referred to as “procedural blank” in the Guide and elsewhere in this document) is a sample that does not contain the matrix, and is brought through the entire analytical procedure and analysed in the same manner as a test sample [4]. When preparing procedural blanks, water is often used in place of the matrix. Procedural blanks may be used to assess any contamination or interference caused by, for example, reagents or sample tubes or introduced during any part of the measurement procedure.

2.4 Reagent blank

A reagent blank is a mixture of any solvent(s) and/or reagent(s) that would be presented to the detector for analysis of a test sample and is analysed to determine if it contributes to the measurement signal. Reagent blanks are often used with techniques such as spectrophotometry to zero the instrument before measuring test samples and other blanks. A reagent blank should also be included when a reaction (derivatization, complexation etc.) with the analyte in the test samples is required before analysis. The reagent blank can be used to determine any interferences caused by the reaction procedure and should be included in the validation process as well as during routine use of the method. A reagent blank does not contain matrix.

2.5 Solvent blank

A solvent blank is made up from the solvent(s) contained in the solution presented to the instrument. It can be used during validation to assess any interferences which may be present in the solvent. The analysis of solvent blanks carried out directly after calibration standards, reference materials or spiked sample blanks can be used to demonstrate whether there is any carryover from one sample to the next. They are often used in chromatographic methods.

2.6 Sample blank

The Guide introduces the concept of sample blanks in section 4.4.1 where it states:

These are essentially sample matrices with no analyte of interest present at detectable levels (or with very low, but well known concentrations of the analytes of interest), e.g. a human urine sample without a specific drug of abuse, or a sample of meat without hormone residues. Sample blanks may be difficult to obtain but such materials are necessary to give a realistic estimate of interferences that would be encountered in the analysis of test samples.

Sample blanks, also called matrix blanks, may be:

- included in experiments to determine the selectivity of the method. Analysis of sample blanks can be used to determine if there are matrix components that could interfere with the ability of the test method to measure the analyte of interest [5]. (Selectivity is addressed in section 5.1 of the Guide);
- included in experiments for estimating the LOD and LOQ of the method (for methods where a measurable signal is obtained for the blank e.g. atomic spectroscopy, ref: section 5.3 of the Guide);
- included in experiments for assessing the method working range (ref: section 5.4 of the Guide);
- used in the preparation of spiked samples (when reference materials are not available) for experiments to estimate the trueness, precision and ruggedness of the method (ref: sections 5.6, 5.7 and 5.9 of the Guide)

Sample blanks may also form part of the ongoing internal quality control procedures which must be in place to demonstrate the measurement procedure remains fit for purpose during routine use.

There are situations, however, when a laboratory cannot obtain a sample blank. Analysis of pesticide residues in food and feed, for example, often involves the use of multi-analyte methods used to test for the presence of hundreds of analytes. Matrix which contains no measurable quantities of all of these analytes may not be available and laboratories may have to use a matrix sample which contains low levels of some analytes. Other compounds are so widespread that they are present throughout the environment and blank matrices simply do not exist [6, 7].

Matrix components affect the detector signal in some analytical applications [8, 9, 10]. To take into account these matrix effects, the calibration curve is usually prepared in matrix blank. Difficulties arise when the matrix is variable - processed foods, for example, where the matrix components differ from sample to sample.

An alternative approach may be necessary when a sample blank does not exist.

2.7 Approaches to dealing with situations where no suitable sample blank is available

2.7.1 Blank correction

Consider the case, mentioned in section 2.6 above, of the multi-analyte method for pesticide analysis. A laboratory wishing to demonstrate selectivity of the method must do so with a sample that contains some analytes. The problem of acquiring a sample blank free of all analytes is recognised. The description given in section 2.6 above describes a sample blank as having no analyte of interest present at detectable levels or of having *very low, but well known concentrations of the analytes of interest.* If the analyte content is known, then, any measurements taken during the validation process may be corrected for the presence of analyte in the sample used in place of a blank. The laboratory must therefore determine the analyte content in the sample they propose to use in place of a blank to achieve a sample with *very low, but well known concentrations of the analytes of interest*. Options available to the laboratory include:

- repeat analysis of the sample to be used in place of a blank incorporated into the design of the experiments to demonstrate selectivity. An estimation of the analyte content in this sample may then be determined;
- determination using the method of standard addition, if appropriate;

- analysis by an alternative, validated, method (with a lower LOQ);
- analysis by a validated method (with a lower LOQ) in another laboratory.

Whichever approach is used the laboratory must ensure that it allows them to demonstrate that their method is fit for purpose.

2.7.2 Use of correction factors for calibration curves

The use of calibration curves prepared in procedural blanks, rather than matrix, followed by the application of a correction factor to the resulting calibration curve has been used in cases where sample blanks cannot be obtained [7]. Implementation of this method requires demonstration that:

- both the matrix-free and matrix-matched calibration curves (functions) are linear;
- the relationship between the matrix-matched and matrix-free calibration curves is consistent for a period of time/sequence of injections [9].

A correction factor can then be calculated and analysis carried out using calibration curves prepared in solvent rather than in matrix [11]. This correction factor must be monitored during routine application of the method to ensure it remains appropriate.

2.7.3 Simulated blank

If a sample blank cannot be obtained, then, in certain cases it may be possible to create a simulation. Matrices such as ocean water lend themselves to the production of a simulated blank by the dissolution of appropriate mineral salts in water [12, 13].

2.7.4 Alternate techniques

If the above approaches are not suitable, it may be necessary for the laboratory to revisit the type of calibration used in the method and consider an alternate technique such as that of standard addition [14].

The process of choosing a sample blank, or a suitable alternative approach, is shown in Figure 2.

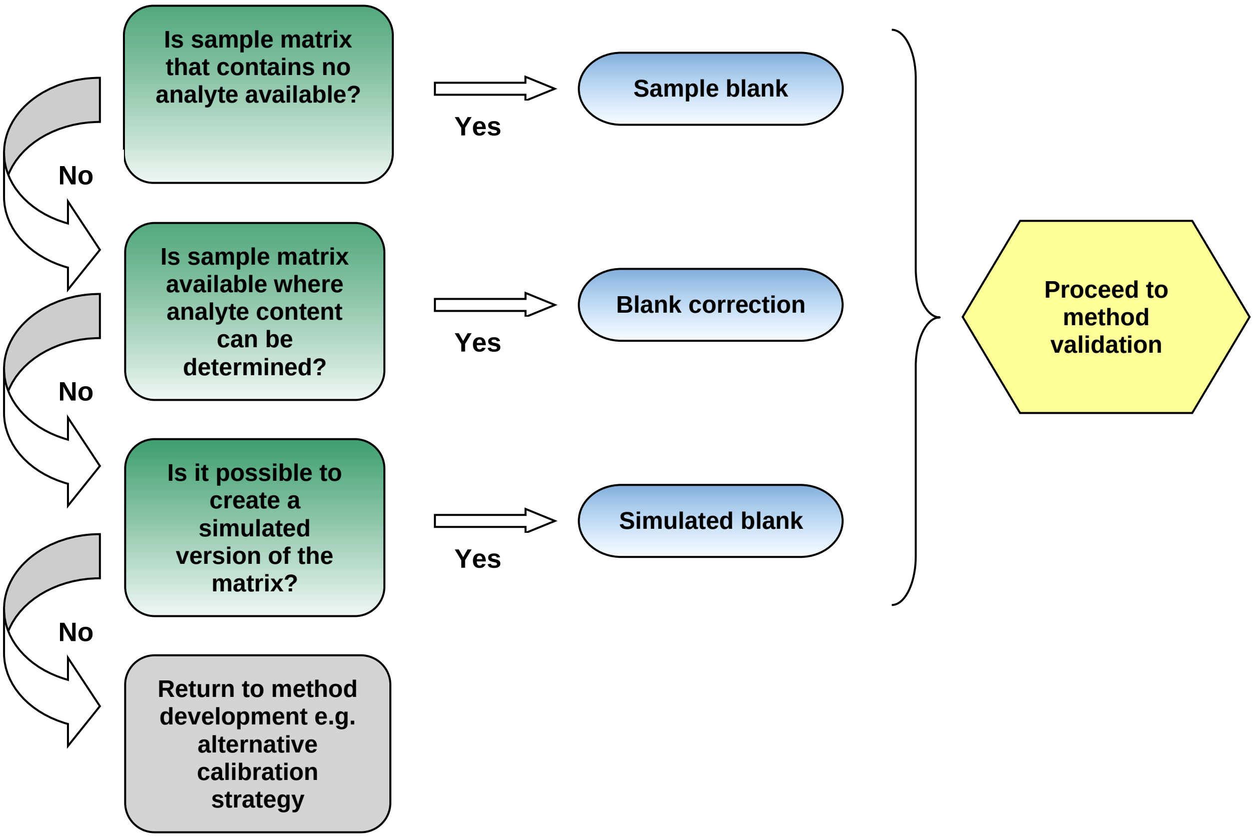

Figure 2 - Choosing a sample blank for method validation

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For a list of current references relating to quality in analytical measurement, please refer to the Eurachem *Reading List* available under the *Publications* section of the Eurachem website, *www.eurachem.org*.

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