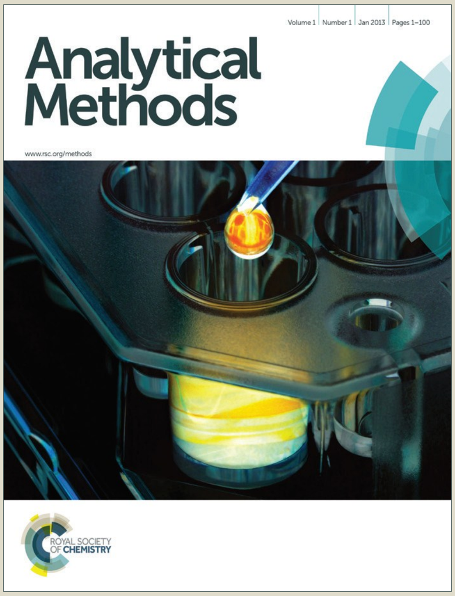
Analytical Methods

Accepted Manuscript



ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/methods

Title

In house validation for the determination of total nitrogen content in fertilisers: Developing criteria with uncertainty measurement evaluation and compliance assessment

Keywords

Nitrogen fertilizers, method validation, quality control and uncertainty measurement

Authors names and affiliations

M. João Nunes

Instituto Nacional de Investigação Agrária e Veterinária, I.P.; Unidade Estratégica de Investigação e Serviços de Sistemas Agrários e Florestais e Sanidade Vegetal

Tapada da Ajuda, Apartado 3228, 1301-903 Lisboa, Portugal, mjoao.nunes@iniav.pt

M. João de Moura

Instituto Nacional de Investigação Agrária e Veterinária, I.P.; Unidade Estratégica de Investigação e Serviços de Sistemas Agrários e Florestais e Sanidade Vegetal

Tapada da Ajuda, Apartado 3228, 1301-903 Lisboa, Portugal, mjoao.moura@iniav.pt

Corresponding author

M. João Nunes

Instituto Nacional de Investigação Agrária e Veterinária, I.P.; Unidade Estratégica de Investigação e Serviços de Sistemas Agrários e Florestais e Sanidade Vegetal

Tapada da Ajuda, Apartado 3228, 1301-903 Lisboa, Portugal, mjoao.nunes@iniav.pt

Tel: (+351) 21 361 7740

List of symbols and abbreviations

BIPEA	International Bureau for Analytical Studies
c_{H2SO4}	Concentration of the standard H_2SO_4 solution (mol L^{-1})
c_{NaOH}	Concentration of the standardized sodium hydroxide solution (mol L^{-1})
CRM	Certified reference material
E_{n}	E_{n} numbers method
k	Coverage factor
L	Limit value for compliance assessment
LOD	Limit of detection (%)
LOQ	Limit of quantification (%)
m_{N}	Total nitrogen mass in sample essay (g)
m_{NBlk}	Total nitrogen mass in blank essay (g)
m_{s}	Sample mass analyzed (g)
n	Number of samples
PT	Proficiency testing
QC	Quality control
R	Analyte Recovery
$\overline{R}$	Mean analyte recovery
RM	Reference material
s_{Exp}	Standard deviation from the average of the experimental content (%)
$s_{\mathrm{Precision}}$	Standard deviation of repeatability studies (%)
s_r	Standard deviation from duplicate samples
s_R	Standard deviation from true value
$Stdev(y_0)$	Standard deviation of bank samples concentration
t	Student t
$t_{95\%}$	Student t at a confidence level of 95%
y_0	Average of blank samples concentration (%)
y_{j1}	Replicate 1 of sample analysis j (%)
y_{j2}	Replicate 2 of sample analysis j (%)
$\% \, U$	Relative expanded uncertainty
u_c	Relative combined standard uncertainty (%)
U_{Lab}	Expanded uncertainty of the laboratory
$u_{\mathrm{Precision}}$	Standard uncertainty of the intermediate precision (%)
$u_{\overline{R}}$	Uncertainty associated with the mean analyte recovery

U_{Ref}	Expanded uncertainty of the reference laboratory
$u(w_{\mathrm{N}})$	Combined standard uncertainty (%)
$U(w_{\mathrm{N}})$	Expanded uncertainty (%)
V_{H2SO4}	Volume of the standard sulfuric acid used in the titration (mL)
V_{H2SO4Blk}	Titration volume of the standard H_2SO_4 used for the determination of the blank assay (mL)
V_{NaOH}	Volume of the standardized sodium hydroxide solution used in the titration (mL)
V_{NaOHBlk}	Volume of the standardized NaOH solution used in the back titration determination of the blank assay (mL)
X_{Lab}	Value determined by the laboratory (%)
X_{Ref}	Assigned value determined by the reference laboratory (%)
w	Nutrient content in fertilizer, expressed as a percentage by mass fraction (%)
w_{N}	Total nitrogen content in fertilizer, expressed as a percentage by mass fraction (%)
$\overline{w}_{\mathrm{Exp}}$	Mean of the average of the experimental duplicate samples of total nitrogen content in fertilizer, expressed as a percentage by mass fraction (%)

1. Introduction

Fertilisers are an integral part of the global food production chain. Effective fertilisation programmes aim to closely balance the composition and availability of the nutrients in the soil with a plant's changing requirements over its growth cycle. Targeted application maximizes plant nutrient uptake and ensures healthy and productive growth. It also minimizes nutrient losses from the soil, either to the atmosphere or waterways[1].

Nitrogen (N) is one of most important of the nutrients added to the cultivars[2] In fertilisers it may be present in several forms: ammonium, cyanamide (NH_2CN), nitrate, urea ($(CONH_2)_2$), and slow release urea-formaldehyde condensates[3].

The European fertiliser market complies with the European regulation (EC) nº 2003/2003[4]. This regulation stipulates rules by chemical fertiliser type, composition and specific analytical methods for the determination of nutrients for compliance. Annex I of regulation declares that all the categories of nitrogenous fertilisers report the total N content except for those containing only nitric or ammoniacal nitrogen. Annex IV provides accepted methods of sampling and analysis. These European standards do not give validation data for all the types and concentration ranges of N fertilisers within the scope of legislation compliance. Also, when using a standard method beyond the stated limits of operation method validation is a requirement for accreditation according to EN ISO/IEC 17025:2005[5]. Method validation entails the evaluation of several parameters of the method such as selectivity, limit of detection (LOD) and limit of quantification (LOQ), working range, trueness, precision (repeatability and reproducibility) and ruggedness. The method must be fit for the analytical purpose. Nevertheless, the method choice is usually not decided only by the analytical need. Often factors such as tradition, availability of instruments and analytical cost influence the choice of method. Also, quality assurance tools such as the use of reference materials and the participation in proficiency testing (PT) are used in the quality management of the laboratory. Comparability is an essential property of analytical results[6,7]. The adequacy of the estimated uncertainty is verified by metrological compatibility with reference materials[8] by an external experimental validation.

The Kjeldahl method is widely used for the determination of nitrogen content of organic and inorganic substances. Although the technique and apparatus have been refined and tested over the past 100 years[9], the basic principles endure today. When nitric and /or nitrous N is present it must first be reduced to ammonia/ammonium. This reducing agent used is one of the major variables in the methodologies.

In historical studies there has been a substantial focus on the stability of the reducing agents. Chromium, Cr, has been shown to be more stable than some other reducing agents. This agent is used in number of current official methods, namely ISO 5315:1984. EN 15750 also contemplates a similar method EN 15750/Method A. Other methods like this are AOAC 2.059-2.060, AOAC 2.061-2.062, AOAC 970.02. Concerns about Cr toxicity, led to the use of alternative methods. The European standards EN 15561[10] and EN 15750[11] employ reduction of nitrates and nitrites to ammonia by means of metallic iron in an acid medium in its reduction step[12].

The present work describes in house method validation for the quantification of total nitrogen according to EC 2003/2003. The performance of the two standard Kjeldahl methods with differences in nitrate reduction, digestion and titration: EN 15561[10] and EN 15750[11] was evaluated. In its scope EN 15561 gives only information for calcium cyanamide containing nitrates fertilizes and EN 15750 gives only information for UAN+S fertiliser. The fertiliser used in house validation were reference materials (RM) matrices of calcium ammonium nitrate; NPK plus MgO plus S, NPK plus micronutrients, ammonium nitrate, fluid fertiliser, NP plus S and NPK plus micronutrients which are beyond scope.

The main purpose of this work was the evaluation of in house validation parameters for an array of different fertilisers for which trueness and repeatability data are not available in standards methods, thus providing information for developing new criteria. Validation performance parameters were used for the assessment of measurement uncertainty using a top down approach[13]. The uncertainty, validation and/or traceability to standards are the first step to assess reliability of measurement results. The comparison of validation results of both methods will allow the choice of method to be used in the determination of total N in fertilisers. Also, the comparison of the analytical results from the two measurements procedures was assessed from metrological compatibility. The final aim of this work was the compliance assessment of different types of nitrogen fertilisers according European legislation.

2. Experimental

2.1. Material and chemicals

The equipment met the standards requirements: Method A[10] and method B[11]. The assays were carried out using a Shimadzu® AW 220 calibrated analytical balance for gravimetric measurements, a Gerhardt® Turbotherm TT 480 verified digestion system, a Gerhardt® Vapodest 40 verified distillation apparatus and Class A volumetric glass material for the volumetric measurements.

Analytical grade reagents and certified standard solutions were used. Certified standard solutions of H_2SO_4 and NaOH from Panreac® were used for back-titration. Blank tests allowed checking and correcting for any sources of contamination.

The analytical quality control for measurement procedure was made with standard solutions of chemical certified reference materials, certified reference materials of fertilisers (CRM) and reference materials of fertilisers (RM) also used for validation of the measurement procedure.

The standard solution for method A[10] was a mixture of potassium nitrate (KNO_3) from Fluka® and ammonium sulfate ($(NH_4)_2SO_4$) from Suprapur® Merck® and the standard solution for method B[11] was of ammonium nitrate (NH_4NO_3) from Aldrich®.

The fertiliser reference materials (RM) used for validation of the measurement procedures of methods A and B, were restricted due to availability. For this reason the in house validation procedures were conducted using the same matrices but not always the same RM. The tested matrices were for RM containing calcium ammonium nitrate, NPK plus MgO plus S, a nitrogenous compound, NPK plus micronutrients, ammonium nitrate, fluid fertilisers, NP plus S and NPK plus micronutrients. The tested RM were CRM and from inter-laboratory tests from BIPEA.

The fertiliser reference materials (RM) used for comparison of methods A and B are depicted in Table 1, were a CRM containing calcium ammonium nitrate; 178 BCR®, from Institute for Reference Materials and Measurements of European Commission, Joint Research Centre[14] and other RM used in inter-laboratory tests from BIPEA. Several fertilisers matrices containing nitrogen were used as RM: RM 18; NPK plus MgO plus S[15], RM 28; a nitrogenous compound[16], RM 27; NPK plus micronutrients[17], RM25; ammonium nitrate[18], RM 17; fluid fertiliser[19], RM 19; NP plus S[20] and RM 24; NPK plus micronutrients[21]. Other similar matrices were used for in house validation.

2.2. Measurement procedure

The measurement procedure scheme for the determination of total nitrogen content (w_N), expressed as a percentage mass fraction, by method A[10] and method B[11] is presented in Fig. 1. Samples were weighed to the nearest 0.001 g and placed in a digestion tube with the addition of the recommended amounts of iron powder and a solution of $SnCl_2$ in HCl. The reduction reaction took place for 30 minutes at room temperature. Following reduction, the digestion reagents were added and the reaction mixture was heated for 60 min in the digestion system. Fig. 2 shows the optimized digestion and distillation programmes. After the digest cooled, concentrated NaOH was added and the mixture was distilled. The ammonium distillate was collected in an Erlenmeyer flask containing an excess of standard H_2SO_4 and indicator solution. This distillate was then back-titrated with standardized NaOH solution. The procedures are similar for both methods with differences in the quantities of reduction reaction iron reagent, order of reagent addition in the digestion step and back-titration standard solution concentrations and end point indicator, as indicated in Fig. 1.

Each analytical set of samples was carried out with a blank sample, a CRM standard, duplicate RMs and duplicate samples.

2.3. Validation

Method validation entails the evaluation of various parameters of the method[22]. The in house validation studies were performed according to a plan precision study and a plan trueness study. The diagram for the scheme of measurement procedure validation strategy is depicted in Fig. 3.

The method A was evaluated in a w_N range from 2.99 to 29.42 % N. The plan precision study was conducted with 16 RM duplicate samples being 2 CRM and 14 RM and the plan trueness study was conducted with 24 RM duplicate samples being 2 CRM and 22 RM.

The method B was evaluated in a w_N range from 6.41 to 34.82 % N. The plan precision study for method B was conducted with 36 RM duplicate samples being 8

CRM and 28 RM and the plan trueness study was conducted with 47 RM duplicate samples being 7 CRM and 40 RM.

Selectivity is the degree to which the method can quantify the analyte accurately in the presence of interferences under the stated conditions of the assay for the sample matrix being studied. Since different types of nitrogen fertilisers have different properties that can be affected by the digestion process, selected matrices containing nitrogen were used for method validation.

The limits have been assessed from blank sample analysis (Fig.3).The LOD is an indication of the level at which detection becomes problematic, while the LOQ is the lowest content of the analyte that can be determined with an acceptable level of repeatability precision and trueness[23]. In this work LOD and LOQ were determined using statistical tests of blank samples following Equations (1) and (2)[24]:

$$\text{LOD} = y_0 + 3.3 \times Stdev(y_0) \qquad (1)$$

where

y_0 is the average of blank samples expressed as mass fraction

$Stdev(y_0)$ is standard deviation of bank samples expressed as mass fraction

$$\text{LOQ} = y_0 + 10 \times Stdev(y_0) \qquad (2)$$

The working range has been estimated from LOQ obtained from Equation (2) and the maximum value indicated in the standard method. The maximum value experimentally tested was the RM available and indicated in Fig. 3. The validation experimental design was delivered for a plan precision study and a plan trueness study.

Repeatability studies were performed for duplicate samples analysed over the working range with different matrices. These studies were done under intermediate precision conditions, namely, comparison with reference values through proficiency tests of different operators. The ruggedness of analytical measurement procedure was tested using different equipment of digestion systems, batches of reagents, analytical solutions, and environmental conditions such as laboratory temperature, humidity and pressure. The ruggedness was evaluated within the validation of experimental design in the assessment of precision and recovery. Regarding the repeatability assessment no differences where observed within the conditions of the study. Further details of intermediate precision and trueness studies are described within the evaluation of measurement uncertainty.

The quality control of routine tests was checked through the analysis of control standards from CRMs with known certified values. Blank samples were analysed to account for the effect of sample contaminations.

The last stage of the measurement validation procedure for the evaluation of the measurement uncertainty is described in detail in a separate section.

2.4 Quality Control

Rigorous quality control (QC) of the measurement is essential to ensure that the compliance decision is reliable[6,8,25]. Subsequent, to the measurement procedure validation, test quality control was implemented to prove that measurement performance remains fit for its intended use[23].

During the improvement and implementation of the analytical methodology, it was verified that the performance of digestion step depends upon the nitrogen fertiliser matrix. The CRM, either NH_4NO_3 or a mixture of KNO_3 and $(NH_4)_2SO_4$, are soluble in concentrated H_2SO_4 and therefore do not need to be digested. Thus following troubleshooting of the digestion equipment, it was decided to use a duplicate of RM matrix for the additional control of the digestion step.

The QC tools are:

- Blank sample, control charts
- CRM Standards, Shewhart control charts[26]
- Duplicate analysis of RMs with matrices similar to the nitrogen fertiliser samples
- Repeatability of RMs and samples, < 5 %.

3. Measurement uncertainty

3.1 Measurand

The measurand is the total nitrogen content in fertilisers, w_{N}, expressed as nitrogen as mass fraction (%). In both measurement procedures, the measurement function is defined by Equation (3):

$$w_{\mathrm{N}} = \frac{m_{\mathrm{N}} - m_{\mathrm{NBlk}}}{m_{\mathrm{s}}} \times 100 \tag{3}$$

where

m_{s} is the sample mass analysed, in g;

m_{N} is the total nitrogen mass in sample assay, in g;

m_{NBlk} is the total nitrogen mass in blank assay, in g.

3.1.1 *Standardization of sodium hydroxide titrant*

The sodium hydroxide (NaOH) solution used in the back-titration was standardized by titration against standard sulfuric acid solution (H_2SO_4) in method A[10] and method B[11]. The measurement equation is written as:

$$c_{\mathrm{NaOH}} = \frac{2 \times c_{\mathrm{H_2SO_4}} \times V_{\mathrm{H_2SO_4}}}{V_{\mathrm{NaOH}}} \tag{4}$$

where

2 is the molar equivalent

c_{H2SO4} is the concentration of the standard H_2SO_4 solution used for the standardization of NaOH solution, in mol L^{-1};

c_{NaOH} is the concentration of the standardized NaOH solution, in mol L^{-1};

V_{H2SO4} is the volume of the standard H_2SO_4 used for the standardization of NaOH solution, in mL;

V_{NaOH} is the volume of the standardized NaOH solution, in mL.

3.1.2 *Determination of total nitrogen mass*

The total nitrogen content is defined by the Equation (3) being the total nitrogen mass in sample assay, in g, defined by the following equation:

$$m_N = (2 \times c_{\mathrm{H_2SO_4}} \times V_{\mathrm{H_2SO_4}} - c_{\mathrm{NaOH}} \times V_{\mathrm{NaOH}}) \times 14.0067 \times 10^{-3} \quad (5)$$

where

2 is the molar equivalent

c_{H2SO4} is the concentration of the standard H_2SO_4 used to collect the distilled for the determination, in mol L^{-1} (Method A = 0.10 mol L^{-1} and Method B = 0.50 mol L^{-1});

V_{H2SO4} is the volume of the standard H_2SO_4 used to collect the distilled for the determination, in mL;

c_{NaOH} is the concentration of the standardized NaOH solution used in the back titration determination, in mol L^{-1} (Method A = 0.20 mol L^{-1} and Method B = 0.25 mol L^{-1});

V_{NaOH} is the volume of the standardized NaOH solution used in the back titration determination, in mL;

14.0067 is the molar mass of N, in g mol^{-1};

10^{-3} is the conversion factor for volume.

and

$$m_{\mathrm{NBlk}} = (2 \times c_{\mathrm{H_2SO_4}} \times V_{\mathrm{H_2SO_4}} - c_{\mathrm{NaOH}} \times \frac{V_{\mathrm{NaOHBlk}} \times V_{\mathrm{H_2SO_4}}}{V_{\mathrm{H_2SO_4Blk}}}) \times 14.0067 \times 10^{-3} \qquad (6)$$

where

V_{NaOHBlk} is the volume of the standardized NaOH solution used in the back titration determination of the blank assay, in mL;

V_{H2SO4Blk} is the titration volume of the standard H_2SO_4 used for the determination of the blank assay, in mL.

3.2 Identification of uncertainty sources

To identify and analyse uncertainty sources a cause and effect diagram for the analytical method was constructed. The principles of constructing a cause and effect diagram are described fully elsewhere[6,27]. In the first stage, Fig. 4(a) the sequence is mainly compiled by specification of measurand, identification of the sources of uncertainty and grouping the related effects, where possible, and quantifying the uncertainty. After this first stage, cancelling effects were removed, similar effects were combined and different instances were re-labelled. Same effects are considered for each experimental step for each branch: volume, concentration, time, temperature. Cancelling effects where considered for bias, repeatability and trueness. For our purpose, the cause and effect diagram consists in a hierarchical structure with a final outcome for the total nitrogen content. Fig. 4(b) represents a simplified cause and effect diagram for the current study. The diagram is the same for both methods. The major sources of uncertainty that are associated with the measurement procedure are indicated in the cause and effect diagram and comprise the analytical steps of sample weighing, digestion, distillation and back titration.

3.3 Top Down approach

The top down approach assesses the degree to which available information meets the requirement and thus identifies factors requiring further study[13]. This is intended to generate an estimate of overall uncertainty, not a detailed quantification of all components[22]. In this work the measurement uncertainty is assessed through validation studies of intermediate precision of repeatability and trueness evaluation using the experimental measurements of data validation indicated in Fig. 3, in the overall range of work[8,22].

The magnitude of the significant components for the uncertainty in intermediate precision conditions, $u_{\mathrm{Precision}}$, is given by the standard deviation of precision, $s_{\mathrm{Precision}}$, and is calculated following the equation for calculation of standard deviation of repeatability studies[28]:

$$u_{\mathrm{Pr}\,ecision} = s_{\mathrm{Precision}} = \sqrt{\frac{\sum_{j=1}^{n}\left(y_{j1}-y_{j2}\right)^2}{2n}} \qquad (7)$$

where

$(y_{j1}- y_{j2})$ is the difference between two replicate sample analysis;

n is the number of samples indicated in Fig. 3 and Table 1.

The analyte recovery, R, is calculated from the ratio between the average of the experimental duplicate samples and the assigned value of total nitrogen content (Table 1), w_{Exp} and w_{Ref}, respectively, in mass fraction (%),following Equation (8):

$$R = \frac{w_{\mathrm{Exp}}}{w_{\mathrm{Ref}}} \qquad (8)$$

The mean analyte recovery, $\overline{R}$, is calculated considering all the validation experimental results.

The uncertainty associated with the mean analyte recovery, $u_{\overline{R}}$, is estimated by following equation:

$$u_{\bar{R}} = \bar{R} \times \sqrt{\frac{s_{\mathrm{Exp}}^{2}}{n \times \bar{w}_{\mathrm{Exp}}^{2}}} \tag{9}$$

where

s_{Exp} is the standard deviation from the average of the experimental content;

n is the number of independent analysis carried out to estimate s_{Exp};

$\bar{w}_{\mathrm{Exp}}$ is the mean of the average of the experimental duplicate samples.

The recovery study includes a representative range of matrices and levels of analyte. The dispersion of results incorporates uncertainty contributions from the experiment, all run-to-run precision elements are included, as variation in sample preparation, in matrix and concentration effects are accounted.

The measurement trueness is evaluated through the mean analyte recovery. To verify if $\bar{R}$ is significantly different from 100 % and to confirm if it is necessary a content correction, a statistical *t*-test was performed following Equation (10):

$$t = \frac{\left|1 - \bar{R}\right|}{u_{\bar{R}}} \tag{10}$$

The content correction is necessary when t is above the critical t at a confidence level of 95 %.

The overall uncertainty is calculated from the dispersion of recoveries found in the experiment and from the repeatability studies in intermediate precision condition. The measurement uncertainty quantification is performed from these both contributory components through the uncertainty propagation law[8,23], related with the bias of the analytical method. The relative combined standard uncertainty, u_c, is then obtained following Equation (11):

$$u_c=\sqrt{(u_{\text{Precision}})^2+(u_{\bar{R}})^2} \tag{11}$$

and the combined standard uncertainty, $u(w_\text{N})$, is estimated by multiplying u_c with the total nitrogen content, w_N, expressed in Equation (12):

$$u(w_\text{N}) = w_\text{N} \times u_c \tag{12}$$

The relative expanded uncertainty, % U, is obtained by multiplying u_c by the coverage factor, k, at a confidence level of 95%, k = 2 and expressing the result as a percentage.

The expanded uncertainty, $U(w_\text{N})$, is obtained by multiplying $u(w_\text{N})$ by the coverage factor, k, at a confidence level of 95%, $k = 2$. The high number of degrees of freedom associated with the standard uncertainty of the input quantities guarantees the adequacy of the proposed coverage factor.

3.4 External Experimental Validation

The validity of the experimental research design is essential for validation studies. The metrological compatibility of measurement results assesses their validity[6]. The metrological traceability of a measurement result does not ensure that the measurement uncertainty is adequate for a given purpose or that there is an absence of mistakes.

Performance is assessed through the analysis of reference materials. The compatibility test is the criteria for this assessment for both method A[10] and method B[11]. This is carried out using data from BIPEA proficiency testing (PT) for checking the calculated uncertainty with results from PT exercises for a single laboratory[22]. This is evaluated through the E_n numbers method, a performance statistic calculated according the following equation[29]:

$$E_{\mathrm{n}} = \frac{\left|X_{\mathrm{Lab}} - X_{\mathrm{Ref}}\right|}{\sqrt{U_{\mathrm{Lab}}^{2} + U_{\mathrm{Ref}}^{2}}} \tag{13}$$

where

X_{Lab} is the value determined by the laboratory

X_{Ref} is the assigned value determined by the reference laboratory

U_{Lab} is the expanded uncertainty of X_{Lab}

U_{Ref} is the expanded uncertainty of X_{Ref}

It is common to use a critical value of 1.0 with E_{n} numbers. The criteria for statistical evaluation for scores[30] for E_{n} numbers for $|E_{\mathrm{n}}| \leq 1$ is satisfactory and for $|E_{\mathrm{n}}| \geq 1$ is unsatisfactory.

3.5 Compliance Assessment

To decide if a result indicates compliance with a specification is necessary to take into account the measurement uncertainty. The key to the assessment of compliance is the concept of "decision rules"[25]. On this basis of the Decision rules, an acceptance zone" and a "rejection zone" are determined.

Considerable care is necessary in designing the decision rule when the uncertainty is proportional to the value of measurand. In the case in hand the decision rule is based on the EC 2003/2003[4], measurement result and in the information available about the uncertainty depends on the value of the measurand. The acceptable value is deduced by applying the decision rule number 2[25]. The result will be taken as indicating non-compliance if the measured value w_{N} plus the guard band is higher than the limit value L. The guard band is obtained by $t_{95\%} \times u(w_{\mathrm{N}})$; being $t_{95\%}$ the value that could be obtained following the t-distribution at a confidence level of 95%. The L value is obtained using regulation EC 2003/2003[4].

4. Results and discussion

The evaluation of the measurement procedure parameters in house validation, according Fig. 3, is indicated in Tables 2 and 3. For both standard methodologies the LOD and LOQ have been estimated from blank assays (Equations (1) and (2)). From LOD values is shown that nitrogen in mineral fertilisers can be detected for a mass fraction of 0.80 % using method A and of 0.82 % using method B. LOQ values show that the quantification can be performed for values higher than 1.92 % if method A is used and for values higher than 1.01 % if method B is used. The smallest value that can be declared for N fertilisers according to regulation EC 2003/2003 in the annex I, is 2 %[4].

The repeatability and reproducibility limits have been set prior to validation.

The working ranges are indicated in Table 2. These ranges were estimated under the repeatability and reproducibility conditions for RMs used in the in house validation, indicated in Table 2 and Fig. 3. The RMs used in each method for measurement procedure validation were similar or equal matrices than the RMs indicated in Table 1, the use restrictions were due to the availability of RMs. The minimal values are obtained from LOQ. The maximum value for method A is indicated as 40 % in mass fraction[10]. The maximum value for method B, 47 %, was estimated considering that this method can be applied for fertilisers containing nitrogen only as nitric, ammoniacal and urea[11]. These maximum values indicated for w_N working ranges are stated in the respective standard methods[10,11]. In this work the tested maximum value for method A was 29.42 % N and for method B was 34.82 % N due to the availability of RMs, as indicated in Fig. 3.

A criterion for separately testing measurement precision and trueness was not defined considering the intended use of measurements, Fig. 3. These performance parameters are tested together in the evaluation of the magnitude of the measurement uncertainty[31]. The measurement trueness results, presented in Table 3, evaluated from the mean analyte recovery with an application for evaluation of a t statistical test (Equation (10)) show that t_{exp} is lower than t_{crit} confirming that it is not necessary to correct the analyte results due to recovery, at a confidence level of 95 % for either method A or B.

The need of replacement of a new digestion system equipment and solutions during in house validation results obtained do not present any significant variation and contribution to the evaluated validation parameters (Tables 2 and 3). Validation parameters verify the adequacy of the measurement procedure.

Uncertainty components and relative combined standard uncertainty estimated by the top down approach (Equations (7), (9) and (11)) are presented in Table 4. The precision evaluated from repeatability of duplicate measurements was used for assessing $u_{\text{Precision}}$ in intermediate precision conditions. Results show that the magnitudes of uncertainty components for method A are higher than the ones for method B, showing that sources for the uncertainty measurement for both EN measurement procedures are quite different. The major source contribution for measurement uncertainty for method A is $u_{\bar{R}}$, about two times higher than $u_{\text{Precision}}$, and conversely for method B is $u_{\text{Precision}}$, about four times higher than $u_{\bar{R}}$. It is also observed that there is no relation from these differences for both methodologies. The relative standard combined uncertainty, u_c, from the application of the propagation law for method A is two times higher than the one for method B. However, the results from the measurements procedures in house validation show that both methods can be applied for the matrices under study. The relative expanded uncertainty of the measured value for method A is 16 % and for method B is 8.8 %. The estimated expanded relative uncertainty must be multiplied by the measured quantity value (i.e. the best estimation of measurand) to obtain the expanded uncertainty. For instance, using method A, for a w_N of 16.0 %, since U is 2.6 %, the reported result would be (16.0 ± 2.6) % for a confidence level of approximately 95 % considering a coverage factor of 2. For instance, using method B, the reported result would be (16.0 ± 1.4) %.

The external experimental validation uses RMs, being the reference value reported with uncertainty estimated[14-21]. In Table 1 information about the nitrogen, phosphorus and potassium nutrient composition mass fraction for the various fertilisers types tested is given. To test the metrological compatibility for both methods E_n numbers proficiency test by Equation (13) was used. The statistical results from proficiency test by E_n numbers, in Fig. 5, show that the specified requirements are adequate for the intended use of both measurement procedures, $|E_n| \leq 1$, confirming the reliability of uncertainty measurement, thus fulfilling the requirements for verification. The use of RMs also assures metrological traceability.

It is noteworthy that the validation step does not suffice to secure the quality assessed analytical procedure. The values assumed during validation process will differ and, in effect, that is going to influence the uncertainty value. So, besides validation it is also critical to pay attention to internal quality control. The QC was checked in the course of the analysis. Shewhart control charts were constructed for CRM standards, RM from BIPEA were controlled using its reference value and reference tolerance value, blank analysis was used for the determination of nitrogen content (Equation (3)). The experimental results of the measurement value, w_N (%), for both measurements procedures with the relative uncertainty, $u(w_N)$ (%), are reported in Table 5. The Fig. 6 shows evidence that the results from both analytical methods are compatible illustrating that the measurement ranges for method A are higher than the ones for method B as estimated by the uncertainty measurement.

Compliance assessment results are indicated in Table 5. The decision rules are for:

- limit value , L, that is established by regulation EC 2003/2003[4] through tolerance for each type of fertiliser, being *L* calculated from measurement value less tolerance;

- measurement value plus guard band, using decision rule number 2 from Eurachem/CITAC Guide[25]

The tests of decision in Table 5 show evidence for the compliance of the measurement values for both methods A and B. This indicates that the goal of requirements is met and that measurement procedures are appropriate for the determination of nitrogen content in fertilisers.

5. Conclusion

In house validation of measurements performance, metrological traceability and compatibility give evidence that both measurement procedures are adequate for analysis of nitrogen content in fertilisers. The method A and B give specific information about their application for different types of fertilisers and do not address the measurement uncertainty. The methods comparison and in house validation with several types of fertilisers provide information concerning the RMs tested and assess measurement uncertainty. This comparison enables to choose the most appropriate method concerning the type of fertilisers tested in this work. Analytical range, relative to w_N for method A is from 1.92 % to 40.0 % and for method B is from 1.10 % to 47.0 % for nitrogen content. The relative expanded uncertainty measurement for method A is 16 % and for method B is 8.8 %.

The choice of measurement procedure depends of the reagents availability and nitrogen content of the fertilisers. Thus, either for fertilisers with high nitrogen content, higher than 40 % and/or with values to be declared for conformity approval close to 2 to 3 % of N the method B should be used preferentially, because presents an appropriate LOQ as well as a wider measurement range and a lower uncertainty measurement. In addition, method B should be used preferentially because it presents a relative expanded uncertainty that is a half of the one determined for method A. However, for fertilisers with content in the working range of method A (Table 1), the final choice of the method, depends on reagents availability, regarding that method B expends 10 times more iron for reduction of nitric nitrogen than method A.

The reliability of the uncertainty measurement leads to conclude that the developed metrological model for the quantification of uncertainty measurement; the top down approach is adequate. The presented examples relating measurement, measurement uncertainty, declared value and conformity approval criteria show the importance of the in house method validation with evaluation of the measurement uncertainty for the compliance assessment.

References

1 *Fertilizers Europe - 2013 Overview-Closing the loop*. Online, http://www.fertilizerseurope.com/fileadmin/user_upload/publications/communications/2013_AO_Fertilizers_Europe-VF.pdf, (accessed July 2014).

2 J. Quelhas dos Santos, *Fertilização. Fundamentos da utilização dos adubos e correctivos*, 2nd edn., Publicações Europa-América. EUROAGRO, Portugal, 1996,161-318.

3 N. T. Faithfull, *Methods in Agricultural Chemical Analysis: A Practical Handbook* CABI Publishing, New York, USA, 2002.

4 *Regulation (EC) N° 2003/2003 of the European Parliament and of the Council of 13 October 2003 relating to fertilizers*, Annex IV, method 2.3.2, 2003, Official Journal L 304, 0001-0194.

5 *Requisitos gerais para competências para laboratórios de ensaio e calibração*, CEN Committee for European Normalization, NP EN ISO/IEC 17025, Brussels, Belgium, 2005.

6 *VIM International vocabulary of metrology – basic and general concepts and associated terms*, JCGM Joint Committee for Guides in Metrology, BIPM Bureau international des poids et mesures, 2012, JCGM **200**. Online, http://www.bipm.org/en/publications/guides/vim.html, (accessed July 2014).

7 S.L.R. Ellison and V. J. Barwick, *Analyst*, 1998, **123**, 1387-1392.

8 *Evaluation of measurement data – The role of measurement uncertainty in conformity assessment*, JCGM Joint Committee for Guides in Metrology, BIPM Bureau international des poids et mesures, 2012, JCGM **106**. Online, http://www.bipm.org/utils/common/documents/jcgm/JCGM_106_2012_E.pdf, (accessed July 2014).

9. *Recommended best practice for the analysis of total nitrogen content in urea ammonium nitrate (UAN) liquid fertilizer*, IFA Working Group on the Harmonization of Fertilizer Sampling and Methods of Analysis, International Fertilizer Industry Association Paris, France, 2011. Online,

http://www.fertilizer.org/imis20/images/Library_Downloads/2011_ifa_recommended_best_practice_uan.pdf?WebsiteKey=411e9724-4bda-422f-abfc-8152ed74f306&=404%3bhttp%3a%2f%2fwww.fertilizer.org%3a80%2fen%2fimages%2fLibrary_Downloads%2f2011_ifa_recommended_best_practice_uan.pdf, (accessed November 2014).

10 *Fertilizers – Determination of total nitrogen in calcium cyanamide containing nitrates*, CEN Committee for European Normalization, EN 15561, Brussels, Belgium, 2009.

11 *Fertilizers – Determination of total nitrogen in fertilizers containing nitrogen only as nitric, ammoniacal and urea nitrogen by two different methods*, CEN Committee for European Normalization, EN 15750, Brussels, Belgium, 2009.

12 S. Choea, H. M. Liljestrandb and J. Khima, *Applied Geochemistry*, 2004, **19**, 335-342.

13 S. L. R. Ellison and V. J. Barwick, *Accred. Qual. Assur.*, 1998, **3**, 101 – 105.

14 *Certificate of analysis of Certified Reference Material BCR® - 178 of Calcium Ammonium nitrate fertilizer,* IRMM Institute for Reference Materials and Measurements, European Commission, Joint Research Centre, Belgium, 2011.

15 *Interlaboratory Comparisons Report* n.º 24/349, November 2008, BIPEA International Bureau for Analytical Studies, 2009.

16 *Interlaboratory Comparisons Report* 24/364 RCIL n.º 2010-2011-0256, BIPEA International Bureau for Analytical Studies, 2011.

17 *Interlaboratory Comparisons Report* 24/363 RCIL n.º 2010-2011-0197, BIPEA International Bureau for Analytical Studies, 2011.

18 *Interlaboratory Comparisons Report* 24/357 RCIL n.º 2009/2010-233, BIPEA International Bureau for Analytical Studies, 2010.

19 International Bureau for Analytical Studies (BIPEA), Interlaboratory Comparisons Report RCIL n.º 24/348, 2008.

20 *Interlaboratory Comparisons Report* RCIL n.º 24/350, BIPEA International Bureau for Analytical Studies,2009.

21 *Interlaboratory Comparisons Report* 24/356 RCIL n.º 2009/2010-164, BIPEA International Bureau for Analytical Studies, 2010.

22 *Quantifying uncertainty in analytical measurement*, Eurachem/CITAC Guide CG4, 3rd edn., Eurachem, UK, 2012. Online, http://www.eurachem.org/index.php/publications/guides/quam, (accessed July 2014).

23 *Evaluation of measurement data. Guide to the expression of uncertainty in measurement*, JCGM Joint Committee for Guides in Metrology, BIPM Bureau international des poids et mesures, 2008, JCGM **100**. Online, http://www.bipm.org/en/publications/guides/gum.html, (accessed July 2014).

24 *Validação de Métodos Internos de Ensaio em Análise Química*, Associação de Laboratórios Acreditados em Portugal, RELACRE, 2000, Guia RELACRE **13**.

25 *Use of uncertainty information in compliance assessment*, 1st edn., Eurachem/CITAC Guide, Eurachem, 2007. Online, http://www.eurachem.org/index.php/publications/guides/uncertcompliance, (accessed July 2014).

26 *Shewhart control charts*, ISO International Organization for Standardization, ISO 8258, Geneva, Switzerland, 1991.

27 *Total quality management. Part 2. Guidelines for quality improvement*, ISO International Organization for Standardization, ISO 9004, Geneva, Switzerland, 1993.

28 *Guia para a quantificação de incerteza em ensaios químicos*, IPAC Instituto Português de Acreditação, Portugal, 2007, OGC007.

29 *Statistical methods for use in proficiency testing by interlaboratory comparisons*, ISO International Organization for Standardization, ISO 13528, Geneva, Switzerland, 2005.

30 *Proficiency testing by interlaboratory comparisons – Part 1: Development and operation of proficiency testing schemes*, ISO International Organization for Standardization, ISO/IEC Guide 43-1, Geneva, Switzerland, 1997.

31 A.M.E. Silva, R.J. Silva and M.F.Camões, *Analytica Chimica Acta*. 2011, **699**, 161-169.

32 J.N. Miller and J.C. Miller, *Statistics and chemometrics for analytical chemistry*, 5th edn. Pearson Education Limited, UK, 2005, 256.

Table 1 Types of fertilisers, CRM and RMs, used for validation and comparison of analytical methodologies showing reference values for total nutrients (NPK), *w*, with expanded uncertainty, *U*.

Reference material	Fertiliser type	$(w_{N} \pm U)$ (%)	$(w_{P_2O_5} \pm U)$ (%)	$(w_{K_2O} \pm U)$ (%)
CRM 178[14]	Calcium ammonium nitrate	26.02 ± 0.11	-	-
RM 18[15]	NPK plus MgO plus S	6.41 ± 0.14	14.55 ± 0.08	25.29 ± 0.70
RM 28[16]	Nitrogenous compound	29.42 ± 0.28	-	-
RM 27[17]	NPK plus micronutrients	16.00 ± 0.14	5.99 ± 0.06	-
RM 25[18]	Ammonium nitrate	24.67 ± 0.26	-	-
RM 17[19]	Fluid fertilizer	9.24 ± 0.26	11.26 ± 0.06	13.42 ± 0.26
RM 19[20]	NP plus S	29.26 ± 0.20	5.71 ± 0.04	-
RM 24[21]	NPK plus micronutrients	12.40 ± 0.12	10.40 ± 0.08	18.46 ± 0.30

Table 2 In house validation of the measurement procedure for determination of w_N (%).

Parameter of the validation	Method A	Method B
LOD (%)	0.80 ($n = 48$)	0.82 ($n = 22$)
LOQ (%)	1.92 ($n = 48$)	1.01 ($n = 22$)
Working range (%)	1.92 – 40.0	1.10 – 47.0
Repeatability[10-11]	< 5 %	< 5 %
Reproducibility[10-11]	< 5 %	< 5 %

n is the number of blank samples

Table 3 Measurement trueness evaluated through the mean analyte recovery.

Parameter of the validation	Method A	Method B
n	24	47
$\overline{R}$	0.97	0.98
t_{exp}	0.29	0.37
t_{crit}[32]	2.06	2.01
Evaluation	$t_{\mathrm{exp}} < t_{\mathrm{crit}}$	$t_{\mathrm{exp}} < t_{\mathrm{crit}}$

n is the number of RM samples analysed

Table 4 Uncertainty components for the uncertainty measurement of w_N (%).

Uncertainty component	Method A (% N)	Method B (% N)
$u_{\mathrm{Precision}}$	0.06 (n = 16)	0.04 (n = 36)
$u_{\bar{R}}$	0.11 (n = 24)	0.01 (n = 47)
u_c	0.08	0.044
% U	16	8.8

Table 5 Nitrogen mass fraction content from duplicate measurements, w_N (%), and its relative uncertainty, $u(w_N)$ (%) for experimental results of in house validation of both methods. Compliance assessment of in house validation results with measurement value plus guard band, $w_N + (t_{95\%} \times u(w_N))$, limit value, L (%) and the test decision for both measurement procedures.

RM	Tolerance[11]	Method A					Method B				
		Experimental		Compliance assessment			Experimental		Compliance assessment		
		w_N (%)	$u(w_N)$ (%)	$w_N + (t_{95\%} \times u(w_N))$	L (%)	Test	w_N (%)	$u(w_N)$ (%)	$w_N + (t_{95\%} \times u(w_N))$	L (%)	Test
CRM 178	0.4	24.4	3.9	28.0	24.0	√	25.6	1.1	27.6	25.2	√
RM 18	1.1	6.5	1.0	7.4	5.4	√	5.87	0.26	6.4	4.8	√
RM 28	1.1	28.0	4.5	32.1	26.9	√	28.7	1.3	31.0	27.6	√
RM 27	1.1	16.7	2.5	18.1	14.6	√	15.5	0.68	16.8	14.4	√
RM 25	0.8	25.0	4.0	28.8	24.2	√	24.3	1.1	26.3	23.5	√
RM 17	0.6	9.2	1.5	10.6	8.6	√	9.27	0.41	10.0	8.7	√
RM 19	1.1	29.0	4.6	33.3	27.9	√	28.6	1.3	31.0	27.5	√
RM 24	1.1	12.3	2.0	14.1	11.2	√	12.4	0.55	13.4	11.3	√

Fig. 1 Measurement procedure for the determination of total nitrogen content.

Fig. 2 Optimized digestion and distillation programmes.

Fig. 3 Scheme of measurement procedure validation strategy.

Fig. 4 Detailed (a) and simplified (b) cause and effect diagram for the uncertainty evaluation for the total nitrogen mass fraction.

Fig. 5 Evaluation of analytical methodologies performance by *En* numbers test.

Fig. 6 Comparison of total nitrogen mass fraction determined by method A and method B.

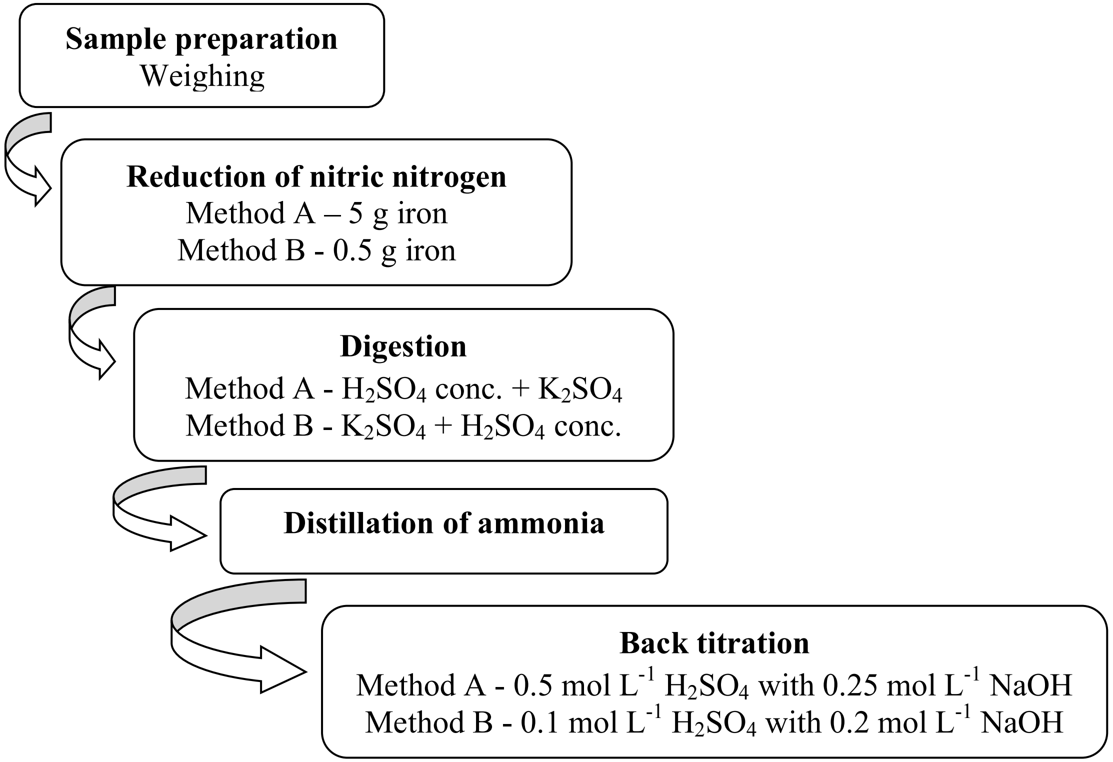
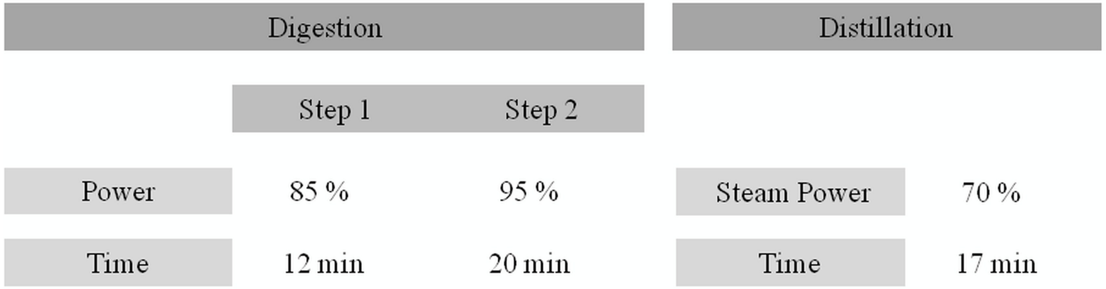

Sample preparation
Weighing
Reduction of nitric nitrogen
Method A – 5 g iron
Method B - 0.5 g iron
Digestion
Method A - H_2SO_4 conc. + K_2SO_4
Method B - K_2SO_4 + H_2SO_4 conc.
Distillation of ammonia
Back titration
Method A - 0.5 mol L^{-1} H_2SO_4 with 0.25 mol L^{-1} NaOH
Method B - 0.1 mol L^{-1} H_2SO_4 with 0.2 mol L^{-1} NaOH

Fig. 1



Digestion			Distillation	
	Step 1	Step 2		
Power	85 %	95 %	Steam Power	70 %
Time	12 min	20 min	Time	17 min

Fig. 2

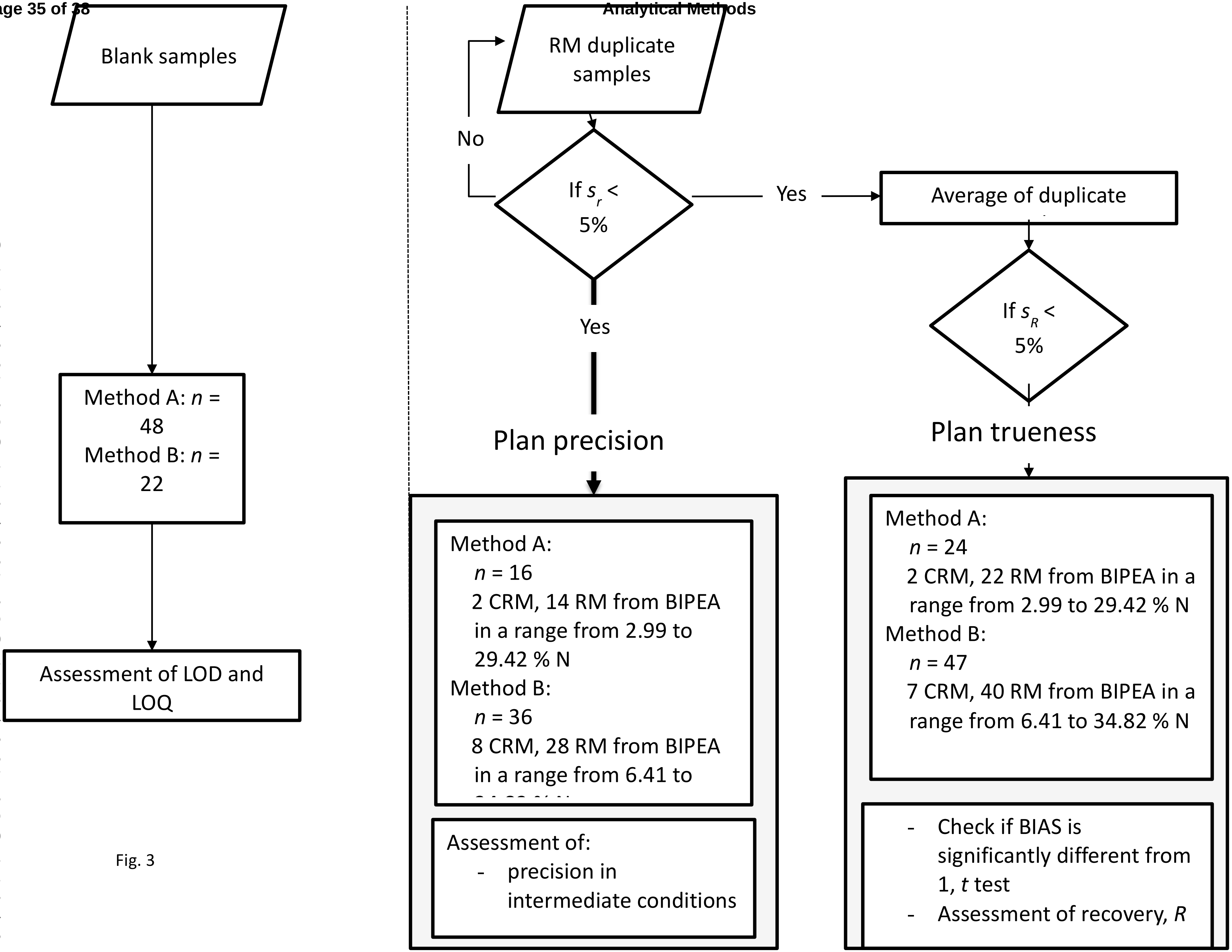

Blank samples
Method A: n = 48
Method B: n = 22
Assessment of LOD and LOQ
RM duplicate samples
No
If s_r < 5%
Yes
Average of duplicate
If s_R < 5%
Yes
Plan precision
Method A:
n = 16
2 CRM, 14 RM from BIPEA in a range from 2.99 to 29.42 % N
Method B:
n = 36
8 CRM, 28 RM from BIPEA in a range from 6.41 to
Assessment of:
- precision in intermediate conditions
Plan trueness
Method A:
n = 24
2 CRM, 22 RM from BIPEA in a range from 2.99 to 29.42 % N
Method B:
n = 47
7 CRM, 40 RM from BIPEA in a range from 6.41 to 34.82 % N
- Check if BIAS is significantly different from 1, t test
- Assessment of recovery, R

Fig. 3

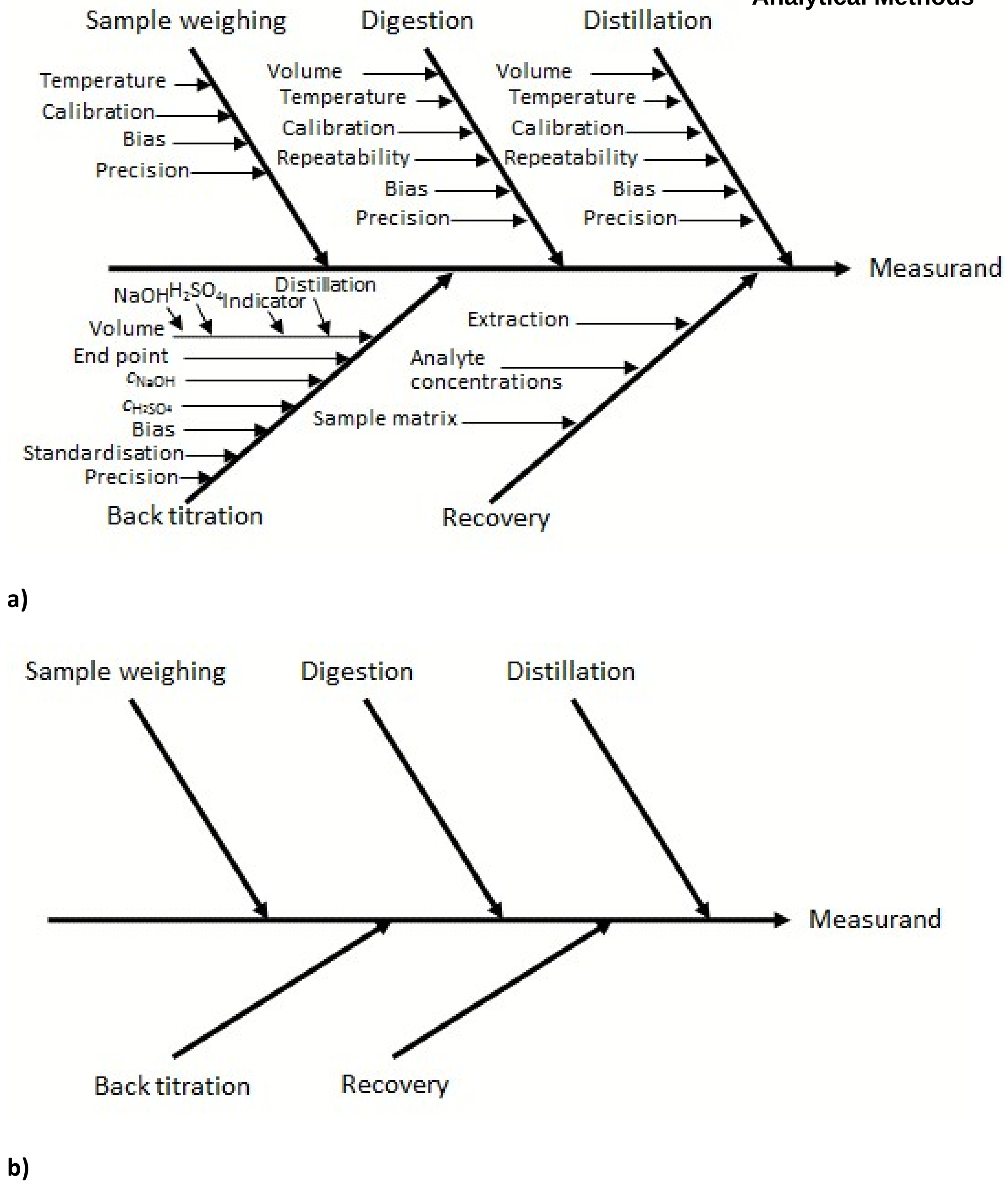

Sample weighing
Digestion
Distillation
Temperature
Calibration
Bias
Precision
Volume
Temperature
Calibration
Repeatability
Bias
Precision
Volume
Temperature
Calibration
Repeatability
Bias
Precision
Measurand
NaOH
H_2SO_4
Indicator
Distillation
Volume
End point
c_{NaOH}
$c_{H_2SO_4}$
Bias
Standardisation
Precision
Extraction
Analyte concentrations
Sample matrix
Back titration
Recovery
a)
Sample weighing
Digestion
Distillation
Measurand
Back titration
Recovery
b)

Fig- 4

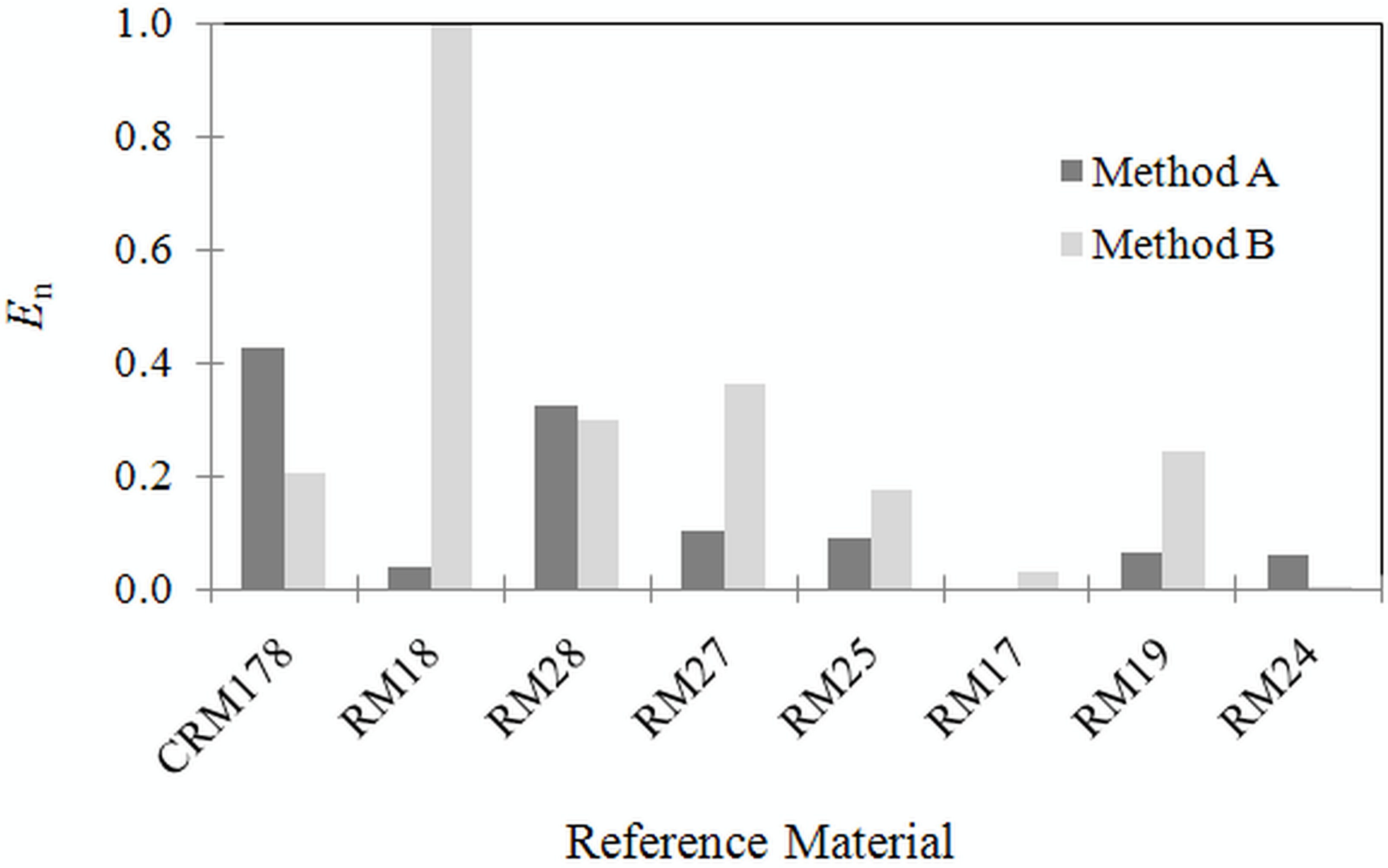

1.0
0.8
0.6
0.4
0.2
0.0
E_n
Method A
Method B
CRM178
RM18
RM28
RM27
RM25
RM17
RM19
RM24
Reference Material

Fig- 5

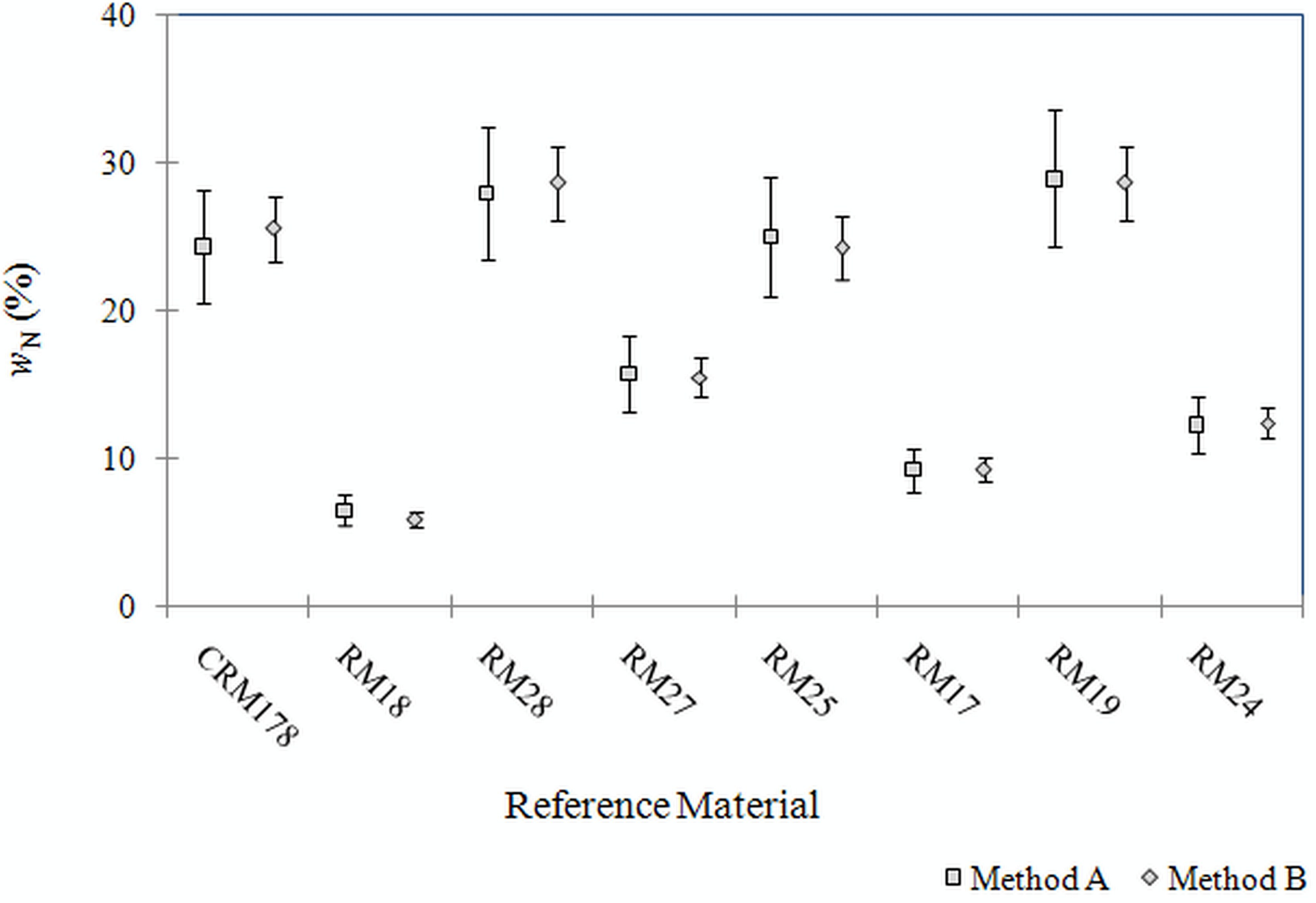

40
30
20
10
0
w_N (%)
CRM178
RM18
RM28
RM27
RM25
RM17
RM19
RM24
Reference Material
□ Method A
◇ Method B

Fig- 6