INTERNATIONAL STANDARD

ISO 22241-2

Second edition
2019-02

Diesel engines — NOx reduction agent AUS 32 —

Part 2: Test methods

Moteurs diesel — Agent AUS 32 de réduction des NOx —

Partie 2: Méthodes d'essai

Reference number
ISO 22241-2:2019(E)

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Published in Switzerland

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Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights. Details of any patent rights identified during the development of the document will be in the Introduction and/or on the ISO list of patent declarations received (see www.iso.org/patents).

Any trade name used in this document is information given for the convenience of users and does not constitute an endorsement.

For an explanation on the voluntary nature of standards, the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT) see www.iso.org/iso/foreword.html.

This document was prepared by Technical Committee ISO/TC 22, *Road vehicles*, Subcommittee SC 34, *Propulsion, powertrain and powertrain fluids*.

This second edition cancels and replaces the first edition (ISO 22241-2:2006), which has been technically revised. It also incorporates the Technical Corrigendum ISO 22241-2:2006/Cor. 1:2008. The main changes compared to the previous edition are as follows:

— Major revisions to test methods of Annex C and Annex I,

— Precision values for all test methods were revised,

— Annex K was updated.

A list of all parts in the ISO 22241 series can be found on the ISO website.

Any feedback or questions on this document should be directed to the user's national standards body. A complete listing of these bodies can be found at www.iso.org/members.html.

Diesel engines — NOx reduction agent AUS 32 —

Part 2: Test methods

WARNING — The use of this document can involve hazardous materials, operations and equipment. This document does not purport to address all the safety issues associated with its use. It is the responsibility of users of this document to respond appropriately to ensure the safety and health of personnel prior to application of the document.

1 Scope

This document specifies test methods required for determination of the quality and chemical characteristics of NOx reduction agent AUS 32 (aqueous urea solution) as specified in ISO 22241-1.

2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 3696, *Water for analytical laboratory use — Specification and test methods*

ISO 4259 (all parts), *Petroleum and related products — Precision of measurement methods and results*

ISO 12185, *Crude petroleum and petroleum products — Determination of density — Oscillating U-tube method*

ISO 17034, *General requirements for the competence of reference material producers*

ISO 22241-1, *Diesel engines — NOx reduction agent AUS 32 — Part 1: Quality requirements*

3 Terms and definitions

For the purposes of this document, the terms and definitions given in ISO 22241-1 and the following apply.

ISO and IEC maintain terminological databases for use in standardization at the following addresses:

— ISO Online browsing platform: available at https://www.iso.org/obp

— IEC Electropedia: available at http://www.electropedia.org/

3.1
certified reference material
CRM
substance or material used to check the quality and metrological traceability of products, to validate analytical measurement methods or for the calibration of instruments

4 Specifications

Conformance with the limits specified in ISO 22241-1 shall be determined by the test methods specified in Annexes B through I of this document. If necessary, the identity of the product can be determined as specified in Annex J.

5 Sampling

Samples shall be taken in accordance with Annex A.

6 Precision and dispute

Each of the test methods specified in Annex B through Annex I include a precision statement using guidance from ISO 4259 (all parts). In cases of dispute, the procedures described in ISO 4259 (all parts) shall be used for resolving the dispute, and interpretation of the results based on the test method precision shall be used.

For the convenience of the user, the respective precision data are summarized in Table K.1.

Annex A
(normative)

Sampling

A.1 General

The sampling method specified in this annex is valid for each sampling of AUS 32 throughout the supply chain after the shipment from the manufacturer's site to the AUS 32 containers of the vehicles.

A.2 Principle

The limits for the quality characteristics of AUS 32, which are specified in ISO 22241-1, are the representative analytical results that can only be obtained when the sample is protected from any contamination before the analysis.

Therefore, suitable bottles shall be used for sampling, which do not contaminate the sample, especially regarding the trace elements.

NOTE The sampling method specified in this annex is based on ISO 5667-3.

A.3 Possible contaminants

During the sampling process, foreign matter can lead to contamination of the sample. Under realistic conditions, the following sources of contamination will pose a major hazard:

— residues of process aids used for the production of the sampling bottles;

— contaminants which have been deposited in the empty bottles during the time they are stored empty;

— contaminants from the air, i.e. dust or any foreign matter from the surrounding, during the sampling;

— residues of cleaning agents, which have been used for cleaning the sampling equipment and the bottles as well;

— fuel.

A.4 Apparatus

A.4.1 Sampling bottles.

1 000-ml wide neck bottles shall be used. Suited materials for these bottles are high density polyethylene (HDPE), high density polypropylene (HDPP), polyvinylidene fluoride (PVDF) and perfluoroalkoxy alkane (PFA).

Prior to the first use with AUS 32, the bottles shall be cleaned and finally rinsed with deionized water followed by AUS 32.

A.4.2 Labels.

Each bottle shall be labelled using labels of approximately 10 cm by 5 cm. The labels and the writing on these labels shall be resistant to water and to AUS 32.

A.5 Sampling

The locked wide-neck bottle shall be opened and the cap shall be placed on a clean surface with the opening turned downward. After flushing the sampling pipe, the bottle shall be filled completely with AUS 32 from the container. The first filling shall be discarded, and the bottle shall immediately be re-filled with AUS 32 and closed tightly. The label shall be attached to the bottle (see A.4.2). During the filling of the sample, maximum care shall be taken that neither dust nor liquid pollutants get into the bottle.

The filled bottle should reach the laboratory as soon as possible. During transportation and storage, the sample should be kept at the lowest possible temperature, preferably between 0 °C and 15 °C.

It is recommended to conduct the analysis within three weeks in order to take into account possible changes in the ammonia content.

A.6 Sample quantity

The minimum quantity of sample material depends on the type of analysis conducted. Whenever possible, make sure that a sufficient volume of sample material is available (recommendation: 1 litre), and at least double that which is required for complete verification of AUS 32 specifications. In case of dispute, a sufficient number of samples shall be taken according to ISO 4259 (all parts).

A.7 Labelling

The label should contain the following information:

— product name;

— name of the company which owns the sample product[1)];

— address where the sample was taken from[1)];

— manufacturer of the sample product[1)];

— batch or lot number;

— container from which the sample was taken[1)];

— part of the container where the sample was taken from (sampling point)[1)];

— date and time of sampling[1)];

— sample shipment date[1)];

— name and signature of the person who took the sample[1)].

1) Mandatory only in cases of dispute.

Annex B
(normative)

Determination of urea content by total nitrogen

B.1 General

This annex specifies the procedure for determining the urea content of AUS 32.

The method is applicable for the determination of the urea content in the range of 30 to 35 % (mass fraction).

B.2 Principle

The sample is combusted at high temperatures in a stream of oxygen. Following the reduction of formed nitrogen oxides to elemental nitrogen and removal of any interfering products of combustion, nitrogen is measured with a thermal-conductivity detector. The urea content is calculated from the determined total nitrogen minus the nitrogen content of biuret.

B.3 Apparatus

B.3.1 Automatic nitrogen analyser, based on combustion methods.

B.3.2 Analytical balance.

The accuracy of the balance is a function of the analyser used and the required weighed portions. Resolution should be 0,1 % of the weighed portion or better.

B.3.3 Auxiliary devices for sample preparation, for example:

— tweezers with a blunt tip;

— micro-spatula with a flattened tip;

— pipette.

The pipette is recommended for weighing in and thus does not need to be calibrated. It is important, however, to obtain a good droplet size (small droplets). Fixed-volume pipettes or pipettes with an adjustable volume in the range from 10 µl to 1 000 µl or single-trip Pasteur pipettes with a fine tip may also be used.

B.3.4 Customary chemically resistant glass.

B.4 Chemicals

B.4.1 Distilled or deionized water, conductivity less than 0,1 mS/m, according to ISO 3696 grade 2.

B.4.2 Auxiliary combustion agent and other equipment, appropriate for use with the selected nitrogen analyser.

The following materials are merely examples. Other or similar materials may be used as required, depending on the system that is available:

— tin capsule or similar sample containers;

— auxiliary combustion agent, non-nitrogenous, such as saccharose, cellulose;

— absorbing agent for liquids, non-nitrogenous, such as magnesium oxide.

B.4.3 Standard substances for nitrogen determination, preferably with certified nitrogen content.

EXAMPLE Suitable standard substances include ethylenediamine tetraacetic acid (EDTA), nicotinic acid amide.

Low-biuret urea of adequate purity (for example crystalline ultra pure or analytical) or other such standard substances recommended by and available from the equipment manufacturer may also be used. Certified standard substances should be preferred.

NOTE Liquid standard substances (e.g. urea solutions) are not suited for calibration purposes.

B.4.4 Oxygen, min. 99,995 % O_2.

B.4.5 Other ultrapure gases, if required to operate the nitrogen analyser, such as helium, min. 99,996 % He.

B.4.6 Other reagents or auxiliary agents, as required by the equipment.

B.5 Procedure

B.5.1 General

The sample should be fully dissolved and free from urea crystals. It may be heated to max. 40 °C as required prior to further processing.

NOTE Different types of apparatus are available on the market. The resulting various resources and modes of operation are not an object of this document. Rather, operation is based on the respective operation manuals.

B.5.2 Reference curve

Perform calibration as required for the specific type of analyser and according to the respective operation manuals (for example, after replacement of the combustion tube, reagent or similar) by performing measurements as described in B.5.4. Weigh in an appropriate amount of standard substances repeatedly as appropriate for the respective types of apparatus to obtain a reference curve.

B.5.3 Inspecting the apparatus for good working order and the reference curve

Use an appropriate standard substance to review the good working order of the apparatus and the reference curve. Preferably, a certified urea standard solution should be used.

Frequency of inspection is a function of the analyser used.

B.5.4 Measurement

Weigh a portion of the sample in a suitable holder (such as a tin capsule) as specified for the type of nitrogen analyser used. The amount should be such that the absolute amount of nitrogen is in the middle range of the reference curve.

Use approximately the three-fold amount of combustion agent (for example, non-nitrogenous cellulose), and additional binders (for example, magnesium oxide) as required.

When using liquid feeder systems, the volume used should be no less than 100 µl. The sample mass density shall be determined according to ISO 12185.

Enter the required data (weighed portion, sample identification) into the analyser or a control computer, depending on the type of apparatus. Feed the weighed-in sample to the analyser and start combustion.

Perform at least three (3) single determinations.

B.6 Results

B.6.1 Calculation

Prior to calculating the reference curve, drift of the baseline or samples, determine the blank reading value by means of blank samples and use this value to correct the respective analytical sequences.

Use the apparatus-specific programme to calculate the reference curve or the drift correction for the samples.

Calculate the mean value for the samples. If there is a strong dispersion of single values (relative standard deviation RSD > 1,0 %), repeat the affected sample. After that, determine the mean value for this sample from all single values.

Determine the urea content from the mean value from at least three nitrogen determinations:

$$w_U = 2{,}143\,8 \times \left(w_N - F \times w_{Bi}\right)$$

where

w_U is the urea content (mass fraction, in %);

w_N is the mean value of the nitrogen content (mass fraction, in %) to the nearest 0,01 %;

w_{Bi} is the mean value of the biuret content (mass fraction, in %), determined according to Annex E;

F is the factor for converting the biuret content to nitrogen (0,407 6).

B.6.2 Expression of results

The result is the arithmetic mean value from at least three (3) single determinations (nitrogen determinations).

Round off the result of the urea content calculation to the nearest 0,1 %.

B.7 Precision

B.7.1 General

The precision evaluation programme with a matrix of only four samples of AUS 32 solutions with urea content in the range 31,09 % (mass fraction) to 35,12 % (mass fraction) did not conform to the requirements of ISO 4259 (all parts), and thus only an estimate of precision based upon inter-laboratory test results is given in B.7.2, B.7.3 and Table B.1.

B.7.2 Repeatability, *r*

The difference between two test results, obtained by the same operator with the same apparatus under constant operating conditions on identical test material would, in the long run, in the normal and correct operation of the test method, exceed the following in only one case in twenty.

r = 0,466 % (mass fraction)

B.7.3 Reproducibility, *R*

The difference between two single and independent results, obtained by different operators working in different laboratories on identical test material would, in the long run, in the normal and correct operation of the test method, exceed the following value in only one case in twenty.

R = 1,053 % (mass fraction)

Table B.1 — Precision (estimated)

Urea content w_U % (mass fraction)	Repeatability r % (mass fraction)	Reproducibility R % (mass fraction)
31,09 to 35,12	0,466	1,053

B.8 Test report

The test report shall contain at least the following information:

a) type and description of tested product;

b) reference to this document, i.e. ISO 22241-2:2019;

c) sampling method used;

d) test result (see B.6);

e) deviations from the specified mode of operation;

f) any unusual features observed; and

g) test date.

Annex C
(normative)

Refractive index and determination of urea content by refractive index

C.1 General

This annex specifies the procedure for the determination of the refractive index, relative to air, for AUS 32 at 20 °C, and at a reference wavelength at 589,3 nm ± 5 nm.

Based on the measurement of refractive index, the method shall be used for determining the concentration of urea in the range of a mass fraction of 30 % to 35 % using existing data.

NOTE The method specified in this annex is based on ISO 5661.

C.2 Principle

Measurement is based on the dependence of refractive index on the concentration of urea in an aqueous solution at a definite temperature and wavelength.

The content is determined by comparison to the agreed upon mathematical relationship between AUS concentration and refraction index.

C.3 Apparatus

C.3.1 Refractometer, capable of measuring refractive index at a reference wavelength of 589,3 nm, with a measuring range of at least 1,330 00 to 1,390 00, and a resolution of 0,000 01, with means of controlling temperature to (20 ± 0,1) °C.

NOTE Different types of apparatus are available on the market. The resulting various resources and modes of operation are not an object of this document. Rather, operation is based on the respective operation manuals.

C.4 Chemicals

C.4.1 Distilled or deionized water, conductivity less than 0,5 mS/m according to ISO 3696 grade 3.

C.4.2 Certified reference material, fluid of known refractive index, prepared in accordance with ISO 17034 and traceable through an unbroken chain of calibrations to a national measurement institute (NMI). For purposes of this standard, CRMs should have an uncertainty of ±0,000 05 nD or better.

C.5 Procedure

C.5.1 General

Each test sample should be fully dissolved and free from urea crystals. They may be heated to <40 °C as required prior to further processing. Care should be taken to avoid heating solutions above 40 °C for any time longer than is required to bring the crystals into solution.

C.5.2 Refractometer calibration and verification

Daily, the refractometer shall be zero-set to distilled or deionized water at (20 ± 0,1) °C in accordance with the instructions provided by the refractometer manufacturer.

Weekly, the refractometer shall be verified using distilled or deionized water and at least one CRM with a value greater than or equal to 1,382 7 nD20. If the refractive index of any CRM, as read on the refractometer, deviates by more than 0,000 05 nD20 from the certified value, then the refractometer shall be recalibrated according to the manufacturer's instructions, using a series of CRMs.

Monthly, the refractometer calibration shall be verified using distilled or deionized water and a series of CRMs (a minimum of three) spanning beyond the measuring range (1,381 4 to 1,384 3 nD20). If the refractive index of any CRM, as read on the refractometer, deviates by more than 0,000 05 nD20 from the certified value, then the refractometer shall be recalibrated according to the manufacturer's instructions, using a series of CRMs.

After completion of each verification/calibration, the refractometer shall be marked or tagged with the date and type of that verification/calibration.

If, after the calibration, the certified value for any CRM cannot be verified within ±0,000 05 nD20, then the instrument shall be tagged as "Out of service" until instrument calibration can be properly verified.

C.5.3 Sample preparation and measuring

If using a circulating water bath to maintain a constant temperature in the refractometer, adjust the thermostat to the desired temperature, reading this temperature on the refractometer thermometer on the discharge side. Maintain the flow of water so that the desired temperature is maintained at (20 ± 0,1) °C.

If the refractometer is equipped with a solid-state Peltier temperature control device, adjust the controls so that the refractometer is controlled to a temperature of (20 ± 0,1) °C.

Measure the sample refractive index three times and then determine the arithmetic mean of the three refractive index values. If any two of the three measurements deviate by more than 0,000 05 nD20, the measurements shall be repeated.

C.6 Calculation and expression of results

C.6.1 Calculation

Urea content, w_U, shall be calculated from the refractive index determined in C.5.3 using the following formula, which has a correlation of R2 = 1,00, less the Biuret content as determined according to Annex E.

$$w_U = (-742,747\ 88 \times (nD20)^2 + 2\ 669,653\ 61 \times (nD20) - 2\ 238,799\ 1) - B$$

where

nD20 represents the refractive index of the sample at 20 °C as determined in C.5.3;

B is the Biuret mass fraction (%) according to Annex E.

NOTE Biuret has the same refractive index per unit of mass as urea.

C.6.2 Expression of results

The result is defined as the arithmetic mean of the three refractive index measurements rounded to the nearest 0,000 1 nD20. For the urea content, the result shall be rounded to the nearest 0,1 % (mass fraction).

C.7 Precision

C.7.1 General

The precision, as determined by statistical examination in accordance with ISO 4259 (all parts) of inter-laboratory study results on AUS 32 blends with test results in the range 31,76 % (mass fraction) to 34,75 % (mass fraction) (and refractive index nD20 from 1,379 to 1,385), is given in C.7.2, C.7.3 and Table C.1.

C.7.2 Repeatability, *r*

The difference between two test results, obtained by the same operator with the same apparatus under constant operating conditions on identical test material would, in the long run, in the normal and correct operation of the test method, exceed the value given in Table C.1 in only one case in twenty.

r (Urea content) = 0,154 % (mass fraction)

r (refractive index nD20) = 0,000 25

C.7.3 Reproducibility, *R*

The difference between two single and independent results, obtained by different operators working in different laboratories on identical test material would, in the long run, in the normal and correct operation of the test method, exceed the value given in Table C.1 only in one case in twenty.

R (Urea content) = 0,211 % (mass fraction)

R (refractive index nD20) = 0,000 33

Table C.1 — Precision

Property	Repeatability *r*	Reproducibility *R*
Urea content (mass fraction, in %)	0,154	0,211
Refractive index nD20	0,000 25	0,000 33

C.8 Test report

The test report shall contain at least the following information:

a) type and description of tested product;

b) reference to this document, i.e. ISO 22241-2:2019;

c) sampling method used;

d) test result (see C.6);

e) deviations from the specified mode of operation;

f) any unusual features observed; and

g) test date.

Annex D
(normative)

Determination of alkalinity

D.1 General

This annex specifies the procedure for the determination of the alkalinity of AUS 32, calculated as ammonia, in the range of 0,1 % to 0,5 %.

D.2 Principle

The measurement is based on potentiometric titration of free ammonia of a test portion with a standard volumetric hydrochloric acid solution to the endpoint at pH = 5,7.

D.3 Apparatus

D.3.1 Analytical balance, resolution 0,1 mg or better.

D.3.2 Automatic burette.

D.3.3 Potentiometer, capable of measuring with a precision of 0,01 pH units, equipped with glass combined pH-electrode.

D.3.4 Magnetic stirrer.

D.3.5 Beaker, 150 ml, tall shaped.

D.3.6 Measuring cylinder, 100 ml.

D.4 Chemicals

D.4.1 General.

During the analysis, use only reagents of recognized analytical grade and only distilled or deionized water of an electric conductivity lower than 0,5 mS/m, according to ISO 3696 grade 3.

D.4.2 Hydrochloric acid, 0,01 mol/l standard solution.

D.4.3 Buffer solutions.

The following standard buffer solutions shall be used for the determination of alkalinity:

— standard buffer solution, pH = 4,008;

— standard buffer solution, pH = 9,184;

— standard buffer solution, pH = 8,00 or 6,86.

NOTE Such solutions are commercially available.

D.5 Procedure

D.5.1 Interferences

The samples of AUS 32 taken shall be stored and shipped at a temperature not higher than 25 °C in order to avoid ammonia formation.

The containers shall be closed tightly and the analysis time shall not be protracted by interruption to avoid evaporation of ammonia.

D.5.2 Check of potentiometric system

The correct function of the potentiometric system shall be checked by use of the standard buffer solutions at pH = 4,008 and pH = 9,180.

The standard buffer solution at pH = 8,00 or 6,86 shall be used for daily check of the potentiometric system.

D.5.3 Preliminary test

Weigh about 1 g of the homogenous sample to 0,05 g (sample mass m_S) and put it into a 150 ml beaker filled with about 100 ml distilled or deionized water.

Titrate with the hydrochloric acid solution (0,01 mol/l) under stirring to the endpoint at pH = 5,7. Calculate the content of ammonia.

Depending on the content of alkalinity found, weigh the following sample portions for the determination:

— alkalinity content found by the preliminary test (%)	0,02	0,05	0,1	0,2 to 0,5
— mass of test portion for the determination (g):	10	5	2	1

— see D.6.1 for an example.

D.5.4 Determination

Weigh the mass of the homogenous sample to 0,05 g found by the preliminary test (sample mass m_S) and put it into a 150 ml beaker filled with about 100 ml distilled or deionized water.

Titrate with the hydrochloric acid solution (0,01 mol/l) under stirring at first to pH = 7,5 with normal speed, then titrate to the endpoint at pH = 5,7 with reduced speed.

Perform two measurements.

D.6 Results

D.6.1 Calculation

The alkalinity, expressed as a percentage by mass of ammonia (NH_3), is given by the formula

$$w(NH_3) = (V \times 0{,}017) / m_S$$

where

$w(NH_3)$ is the alkalinity, calculated as ammonia (mass fraction, in %);

V is the volume of the hydrochloric acid solution used for the titration (ml);

m_S is the mass of the test portion (g).

D.6.2 Expression of results

Calculate the mean value of the two measurements. Express the result to the nearest 0,01 % (mass fraction).

D.7 Precision

D.7.1 General

The precision evaluation programme with a matrix of only four samples of AUS 32 solutions with alkalinity in the range 0,084 % (mass fraction) to 0,529 % (mass fraction) did not conform to the requirements of ISO 4259 (all parts), and thus only an estimate of precision based upon inter-laboratory test results is given in D.7.2, D.7.3 and Table D.1.

D.7.2 Repeatability, *r*

The difference between two test results, obtained by the same operator with the same apparatus under constant operating conditions on identical test material would, in the long run, in the normal and correct operation of the test method, exceed the following in only one case in twenty.

r = 0,077 % (mass fraction)

D.7.3 Reproducibility, *R*

The difference between two single and independent results, obtained by different operators working in different laboratories on identical test material would, in the long run, in the normal and correct operation of the test method, exceed the following value in only one case in twenty.

R = 0,124 % (mass fraction)

Table D.1 — Precision (estimated)

Alkalinity content $w(NH_3)$ % (mass fraction)	Repeatability r % (mass fraction)	Reproducibility R % (mass fraction)
0,084 to 0,529	0,077	0,124

D.8 Test report

The test report shall contain at least the following information:

a) type and description of tested product;

b) reference to this document, i.e. ISO 22241-2:2019;

c) sampling test method;

d) test result (see D.6);

e) deviations from the specified mode of operation;

f) any unusual features observed; and

g) test date.

Annex E
(normative)

Determination of biuret content

E.1 General

This annex specifies the procedure for the determination of the biuret content of AUS 32 with contents of biuret with 0,1 % (mass fraction) to 0,5 % (mass fraction) by photometric method.

E.2 Principle

Biuret forms in alkaline solution in the presence of sodium-potassium-tartrate with bivalent copper a violet-coloured complex with an absorption maximum at 550 nm. The colour complex is read spectrophotometrically at 550 nm and the biuret concentration is determined by reference to a calibration curve prepared from standard biuret solutions.

E.3 Apparatus

E.3.1 Laboratory balance, resolution in reading 0,001 g.

E.3.2 Vacuum filtration unit, applicable for filter with 0,45 µm pore size.

E.3.3 Spectrophotometer, for use at 550 nm with 10-50-mm-cell.

E.3.4 Volumetric flasks, 1 000 ml, 250 ml, 100 ml, 50 ml.

E.3.5 Pipettes.

E.3.6 Rotary evaporator.

E.3.7 Constant-temperature bath, capable of maintaining a temperature of 30 °C ± 1 °C.

E.4 Chemicals

E.4.1 Chemicals of analytical grade.

These shall be used in all tests. The water shall be distilled or deionized, conductivity less than 0,5 mS/m according to ISO 3696 grade 3.

E.4.2 Copper sulphate-solution.

Dissolve 15 g copper sulphate ($CuSO_4 \cdot 5H_2O$) in CO_2-free water and dilute to 1 000 ml.

E.4.3 Alkaline potassium sodium tartrate-solution.

Dissolve 40 g sodium hydroxide in 500 ml water in a 1 000 mI volumetric flask. After cooling, add 50 g potassium sodium tartrate ($KNaC_4H_4O_6 \cdot 4H_2O$) and fill up the flask with water to the mark. Let the flasks stand 1 day before use.

E.4.4 Biuret-standard-solution, of 0,8 mg biuret/ml.

Dissolve 800 mg pure biuret in CO_2-free water and dilute to 1 000 ml. Dry the biuret for 3 h at 105 °C before use.

Biuret may be purified as follows:

— add 50 g biuret to 500 ml ammonia solution of 25 % (mass fraction) concentration and stir for 15 minutes;

— filter, rinse with ammonia-free water and dry the biuret;

— dissolve in ethanol (1 litre/10 g), filter, and concentrate by gentle heating to one-fourth the volume;

— cool to 5 °C and filter;

— dry the biuret in vacuum oven at 80 °C;

— check the purity by photometrical measurements according to E.5.5.

The step of re-crystallizing from ethanol shall be repeated until there is no more noticeable improvement of purity.

E.4.5 Standard acid, 0,1 N hydrochloric or sulfuric acid.

E.5 Procedure

E.5.1 Interferences

Spectrophotometric measurements are only suitable for clear solutions. If the sample is not clear, filter through a 0,45 µm filter to get a clear solution.

Ammonia forms with bivalent copper a coloured complex, which absorbs light energy at 550 nm. The method is applicable only if the ammonia-content of the sample is less than 500 mg/kg.

To eliminate ammonia content greater than 500 mg/kg, neutralize pre-weighed sample to less than 7,0 pH with standard acid.

E.5.2 Preparation of the calibration curve

Into a series of six 50 ml volumetric flasks, transfer 2 ml, 5 ml, 10 ml, 15 ml, 20 ml and 25 ml of the biuret standard solution and add water (to each of the six flasks) to a total of mixture volume of approximately 25 ml. Add, while stirring after each addition, 10 ml of the alkaline potassium sodium tartrate-solution and 10 ml of the copper sulphate-solution. Immerse the flasks in the constant-temperature bath, regulated at 30 °C ± 1 °C and leave them there for about 15 minutes.

Carry out a blank test in parallel with the determination, following the same procedure and using the same quantities of all the reagents used for the measurement (see E.5.5).

After cooling to room temperature, fill up the flasks with water to the mark and mix well. Carry out the photometric measurements with the spectrophotometer at a wavelength of about 550 nm using a 10-50 mm cell against water as the reference.

Subtract the extinction of the blank test from the extinction of the measured values and set up the calibration curve. In the concentration range, the curve shall be strictly linear.

E.5.3 Calculation of the calibration factor

Calculate the calibration factor according to the following formula:

$$F_C = \frac{\sum_{i=1}^{6} m_{Bi,i}}{\sum_{i=1}^{6}\left(E_{1,i} - E_2\right)} = \frac{61{,}6}{\sum_{i=1}^{6}\left(E_{1,i} - E_2\right)}$$

where

F_C is the calibration factor (mg);

$m_{Bi,i}$ is the mass of biuret of the i-th sample (mg);

$E_{1,i}$ is the extinction of the i-th sample;

E_2 is the extinction of the blank test.

The determination of the calibration curve and the calibration factor shall be repeated on a yearly basis and shall be documented.

E.5.4 Day-factor

The day-factor shall be determined weekly.

Perform a measurement of 10 ml of the biuret standard solution (8 mg biuret) as described in E.5.5.

Calculate as follows:

$$F_D = \frac{8}{\left(E_{1,i} - E_2\right)}$$

where

F_D is the day-factor (mg);

E_1 is the extinction of the standard solution (average from 2 measures);

E_2 is the extinction of the blank test.

The deviation of the day-factor shall be within ±5 % to the calibration factor. For measuring of samples, the day-factor shall be used.

E.5.5 Measurement

Weigh 100 g of the test sample, to the nearest 0,01 g, in a 250 ml beaker. Neutralize to below 7,0 pH with standard acid. Quantitatively transfer the sample to a 250 ml volumetric flask. Fill the flask to the mark with demineralized water and mix thoroughly.

Transfer an aliquot of 10 ml from the test solution into a 50 ml volumetric flask and add water to approximately 25 ml. Add, with stirring after each addition, 10 ml of the alkaline potassium sodium tartrate-solution and 10 ml of the copper sulphate-solution. Immerse the flask in the constant-temperature bath, regulated at 30 °C ± 1 °C and leave it there for about 15 minutes.

Carry out a blank test in parallel with the determination, following the same procedure and using the same quantities of all the reagents used for the determination.

After cooling to room temperature, fill up the flask with water to the mark and mix well. Carry out the photometric measurements with the spectrophotometer at a wavelength of about 550 nm using a 50 mm cell against water as the reference.

To determine non-specific absorptions, put another 10 ml of the test solution into a 50 ml volumetric flask, fill the flask up to the mark with water and measure the absorption in the same order.

Duplicate determinations shall be carried out.

E.6 Results

E.6.1 Calculation

The biuret content is given, as a percentage by mass, by the formula:

$$w_{Bi} = \frac{(E_S - E_B) \times F_D \times 250}{m_S \times 10 \times 1\,000} \times 100$$

where

w_{Bi} is the biuret content (mass fraction, in %);

E_S is the extinction of the sample;

E_B is the extinction of the blank test (reagent blank + sample blank);

m_S is the mass of sample used to prepare the test solution (g);

F_D is the day-factor (mg).

E.6.2 Expression of results

Express the result to the nearest 0,01 % (mass fraction).

E.7 Precision

E.7.1 General

The precision evaluation programme with a matrix of only four samples of AUS 32 solutions with biuret in the range 0,115 % (mass fraction) to 0,461 % (mass fraction) did not conform to the requirements of ISO 4259 (all parts), and thus only an estimate of precision based upon inter-laboratory test results is given in E.7.2, E.7.3 and Table E.1.

E.7.2 Repeatability, *r*

The difference between two test results, obtained by the same operator with the same apparatus under constant operating conditions on identical test material would, in the long run, in the normal and correct operation of the test method, exceed the following in only one case in twenty.

r = 0,008 % (mass fraction)

E.7.3 Reproducibility, *R*

The difference between two single and independent results, obtained by different operators working in different laboratories on identical test material would, in the long run, in the normal and correct operation of the test method, exceed the following value in only one case in twenty.

R = 0,044 % (mass fraction)

Table E.1 — Precision (estimated)

Biuret content w_{Bi} % (mass fraction)	Repeatability r % (mass fraction)	Reproducibility R % (mass fraction)
0,115 to 0,461	0,008	0,044

E.8 Test report

The test report shall contain at least the following information:

a) type and description of tested product;

b) reference to this document, i.e. ISO 22241-2:2019;

c) sampling method used;

d) test result (see E.6);

e) deviations from the specified mode of operation;

f) any unusual features observed; and

g) test date.

Annex F
(normative)

Determination of aldehyde content

F.1 General

This annex specifies the procedure for the determination of the content of free and bound aldehyde, calculated as formaldehyde, of AUS 32 with contents of aldehyde from 0,5 mg/kg to 10 mg/kg.

F.2 Principle

Formaldehyde forms in strong sulphuric acid solution with chromotropic acid a purple colour with absorption maximum at 565 nm. The colour complex is read spectrophotometrically at 565 nm and the aldehyde concentration is determined by reference to a calibration curve prepared from standard formaldehyde solutions.

NOTE The method specified in this annex is based on Reference [4].

F.3 Apparatus

F.3.1 Laboratory balance, resolution in reading 0,001 g.

F.3.2 Spectrophotometer, for use at 565 nm with 10-mm-cell.

F.3.3 Volumetric flasks.

F.3.4 Pipettes.

F.4 Chemicals

F.4.1 Chemicals of analytical grade, which shall be used in all tests.

F.4.2 Sulphuric acid, a mass fraction of 96 %.

F.4.3 Chromotropic acid (4,5-dihydroxynaphthalene-2,7-disulphonic acid sodium salt or 4,5-dihydroxy- naphthalene-2,7-disulphonic acid disodium salt dihydrate), with a mass fraction of 3 % in a mass fraction of 15 % sulphuric acid.

In order to make this solution, add 41 ml sulphuric acid to 410 ml of water while cooling the mixture, then add 15 g of chromotropic acid and mix them well.

NOTE If stored in a brown glass bottle, this solution is usable for at least 3 months.

F.4.4 Formaldehyde standard solution prepared as follows:

— put 6,5 g to 7 g of formaldehyde solution having a mass fraction of 37 % into a 500 ml volumetric flask, fill up the flask with water and mix it well;

— determine the formaldehyde content of the solution, e.g. by using the sulphite procedure in ISO 11402;

— dilute the solution to 1:1 000. Mark the exact value of the formaldehyde content to the flask (the formaldehyde content as determined in the previous step divided by 1 000).

F.5 Procedure

F.5.1 Preparation of the calibration curve

Into a series of six 50 ml volumetric flasks, transfer 0,2 ml, 0,5 ml, 1 ml, 2 ml, 5 ml and 10 ml of the formaldehyde standard solution and add water to a total mixture volume of approx. 10 ml. Add, while stirring, 1 ml of chromotropic acid solution followed by gradual addition of 20 ml sulphuric acid during the course of 5 minutes. The temperature rise during the addition of sulphuric acid shall exceed 100 °C, which is necessary for the reaction to come to completion. Let the flask stand at ambient air for 15 minutes without any further cooling.

Carry out a blank test in parallel with the determination, following the same procedure and using the same quantities of all the reagents used for the measurement (see F.5.4).

After cooling to room temperature, fill the flask with water up to the mark and mix well. Carry out the photometric measurements with the spectrophotometer at a wavelength of about 565 nm using a 10 mm cell against water as the reference.

Subtract the extinction of the blank test from the extinction of the measured values and set up the calibration curve. In the concentration range, the curve shall be strictly linear.

F.5.2 Calculation of the calibration factor

Calculate the calibration factor according to the following formula:

$$F_C = \frac{\sum_{i=1}^{6} m_{HCHO,i}}{\sum_{i=1}^{6} \left(E_{1,i} - E_2\right)}$$

where

F_C is the calibration factor (µg);

$m_{HCHO,i}$ is the mass of the formaldehyde of the i-th sample (µg);

$E_{1,i}$ is the extinction of the i-th sample;

E_2 is the extinction of the blank test.

The determination of the calibration curve and the calibration factor shall be repeated on a yearly basis and shall be documented.

F.5.3 Check of the method

Every 3 months, the method shall be checked as follows.

Into a series of three 50 ml volumetric flasks, transfer 2 ml of the formaldehyde standard solution and add water to a total volume of approx. 10 ml. Follow the procedure described in F.5.4 and calculate the aldehyde content as shown in F.6.

Compare the findings with the content of the standard solution. If the deviation is less than or equal to 2 %, the method is ready to use. If the deviation is more than 2 %, repeat the check. If the deviation is more than 2 % again, the method shall not be used unless a new calibration curve is prepared.

F.5.4 Measuring of samples

Weigh, to the nearest 0,01 g, a test sample of between 5 g and 10 g of the test sample in a 50 ml volumetric flask and dilute it with water to a total mixture volume of approximately 10 ml. Add, while stirring, 1 ml of chromotropic acid solution followed by gradual addition of 20 ml sulphuric acid during the course of 5 minutes. The temperature rise during the addition of sulphuric acid shall exceed 100 °C, which is necessary for the reaction to come to completion. Let the flask stand at ambient air for 15 minutes without any further cooling.

Carry out a blank test in parallel with the determination, following the same procedure and using the same quantities of all the reagents used for the determination.

After cooling to room temperature, fill the flask with water up to the mark and mix well. Carry out the photometric measurements with the spectrophotometer at a wavelength of about 565 nm using a 10 mm cell against water as the reference.

F.6 Results

F.6.1 Calculation

The aldehyde content is given by the formula

$$w_A = \frac{(E_S - E_B) \times F_C}{m_S}$$

where

w_A is the content of aldehyde (mg/kg);

E_S is the extinction of the sample;

E_B is the extinction of the blank test (reagent blank + sample blank);

m_S is the mass of sample used (g);

F_C is the calibration factor (µg).

F.6.2 Expression of results

Express the result to the nearest 0,1 mg/kg.

F.7 Precision

F.7.1 General

The precision evaluation programme with a matrix of only four samples of AUS 32 solutions with aldehyde content in the range 0,479 mg/kg to 4,422 mg/kg did not conform to the requirements of ISO 4259 (all parts), and thus only an estimate of precision based upon inter-laboratory test results is given in F.7.2, F.7.3 and Table F.1.

F.7.2 Repeatability, *r*

The difference between two test results, obtained by the same operator with the same apparatus under constant operating conditions on identical test material would, in the long run, in the normal and correct operation of the test method, exceed the following in only one case in twenty.

r = 0,109 mg/kg

F.7.3 Reproducibility, *R*

The difference between two single and independent results, obtained by different operators working in different laboratories on identical test material would, in the long run, in the normal and correct operation of the test method, exceed the following value in only one case in twenty.

R = 0,464 mg/kg

Table F.1 — Precision (estimated)

Content of aldehyde w_A mg/kg	Repeatability r mg/kg	Reproducibility R mg/kg
0,479 to 4,422	0,109	0,464

F.8 Test report

The test report shall contain at least the following information:

a) type and description of tested product;

b) reference to this document, i.e. ISO 22241-2:2019;

c) sampling method used;

d) test result (see F.6);

e) deviations from the specified mode of operation, if any;

f) any unusual features observed; and

g) test date.

Annex G
(normative)

Determination of insoluble matter content by gravimetric method

G.1 General

This annex specifies the procedure for determining the insoluble matter content >1 mg/kg in AUS 32.

G.2 Principle

The sample is filtered and the mass of the residue is determined by gravimetric analysis.

G.3 Apparatus

G.3.1 Filtration equipment for vacuum filtration, suitable for 47 mm or 50 mm diameter membrane filters.

G.3.2 Membrane filter, pore size 0,8 µm, cellulose mixed ester.

G.3.3 Petri dish with cover, suited to the fitting of membrane filters (e.g. 80 mm by 15 mm).

G.3.4 Flat-tipped tweezers.

G.3.5 Analytical balance, resolution 0,01 mg or better.

G.3.6 Balance, resolution 0,01 g or better.

G.3.7 Glass beaker, nominal volume 400 ml (preferably high shaped with volumetric separation).

G.3.8 Drying oven, capable of maintaining a temperature of 105 °C with a precision of ±2 °C.

G.3.9 Desiccator filled with a drying agent.

NOTE Sulphuric acid or calcium chloride are not suitable as drying agents.

G.3.10 Standard laboratory glass.

G.4 Chemicals

G.4.1 Distilled or deionized water, conductivity less than 0,1 mS/m according to ISO 3696, grade 2.

G.5 Procedure

The sample shall be completely dissolved and free from any urea crystals. If required, the sample shall be warmed before being further processed to ≤40 °C.

The filter for use in the test shall be washed with water in advance. To do this, a filter shall be moistened in approximately 100 ml water in the vacuum filtration unit and the water shall be sucked through the filter. The filter shall then be dried to a mass consistency in the drying chamber and shall be stored in a Petri dish (one filter per Petri dish) in the desiccator. The membrane filters shall be weighed to 0,01 mg precision immediately before an analysis is made.

The filters shall always stay in the Petri dish for weighing.

The sample shall be shaken thoroughly to be homogeneous. Immediately after this, about 100 ml to 150 ml of the sample shall be placed in a dry, calibrated 400 ml glass beaker, weighed to 0,01 g precision, and 200 ml of water added. The sample shall not be pipetted for weighing.

The filtration equipment shall be set up using the prepared membrane filter. The filter shall be moistened with a little water (1 ml to 2 ml) without applying vacuum. The prepared sample shall be placed in the filtration vessel and the vacuum shall be arranged so that the sample is drawn swiftly through the filter.

The glass beaker shall be rinsed with 5 portions of water each of approximately 30 ml to 50 ml. The rinsing solution shall also be passed through the filter (sample vessel of the filtration equipment shall also be rinsed). The sample shall have completely run through the filter before the start of the first rinse (allow the filter to dry briefly).

The filtering equipment shall be dismantled and the filter dried at 105 °C until constancy of the mass is achieved. After cooling to room temperature in a desiccator, the filter shall be weighed to 0,01 mg resolution.

It shall be ensured that the filter is washed completely clean of all urea remnants. If it is found that filters are still stuck to the glass floor of the Petri dish, this indicates that the washing has not been adequate. These filters shall be disposed of and the analysis shall be repeated.

G.6 Results

G.6.1 Calculation

$$w_{\text{ins}} = \frac{\left(m_{\text{FR}} - m_{\text{FL}}\right)}{m_{\text{S}}} \times 1\,000$$

where

w_{ins} is the content of insoluble matters (mg/kg);

m_{FL} is the mass of the dried empty filter (mg);

m_{FR} is the mass of dried filter with sample deposit (mg);

m_{S} is the sample mass (g).

G.6.2 Expression of results

The mean value of the calculation is valid as the result. If the difference between the separate values is more than 25 % of the higher value, the determination shall be repeated. The result shall be rounded off as follows:

— <10 mg/kg to the nearest 0,1 mg/kg;

— ≥10 mg/kg to the nearest 1 mg/kg.

G.7 Precision

G.7.1 General

The precision evaluation programme with a matrix of only three samples of AUS 32 solutions with insoluble matter content in the range 7,42 mg/kg to 33,2 mg/kg did not conform to the requirements of ISO 4259 (all parts), and thus only an estimate of precision based upon inter-laboratory test results is given in G.7.2, G.7.3 and Table G.1.

G.7.2 Repeatability, *r*

The difference between two test results, obtained by the same operator with the same apparatus under constant operating conditions on identical test material would, in the long run, in the normal and correct operation of the test method, exceed the following in only one case in twenty.

r = 4,871 mg/kg

G.7.3 Reproducibility, *R*

The difference between two single and independent results, obtained by different operators working in different laboratories on identical test material would, in the long run, in the normal and correct operation of the test method, exceed the following value in only one case in twenty.

R = 8,220 mg/kg

Table G.1 — Precision (estimated)

Insoluble matter content w_{ins} mg/kg	Repeatability R mg/kg	Reproducibility R mg/kg
7,42 to 33,2	4,871	8,220

G.8 Test report

The test report shall contain at least the following information:

a) type and distinguishing characteristics of the tested products;

b) reference to this document, i.e. ISO 22241-2:2019;

c) the sampling process applied;

d) the test result (see G.6);

e) deviation from the specified mode of operation, if any;

f) any unusual features observed; and

g) test date.

Annex H
(normative)

Determination of phosphate content by photometric method

H.1 General

This annex specifies the procedure for the determination of the content of total phosphorus as phosphate in AUS 32 with contents from 0,05 mg/kg to 10 mg/kg. Expansion of the measurement range is possible by varying the sample amount.

H.2 Principle

The sample is evaporated and incinerated with calcium carbonate to mineralize the phosphorus compounds.

After this treatment, the sample is transformed from phosphate to orthophosphate by means of hydrochloric acid.

Orthophosphate-ions react in acid solvent with molybdate and antimony-ions to an antimony-phosphomolybdate complex.

The reduction of this complex with ascorbic acid leads to an intensive coloured molybden blue-complex. The intensity of the colour indicates the concentration of orthophosphate-ions.

H.3 Apparatus

H.3.1 Analytical balance, resolution 0,01 g or better.

H.3.2 Incineration dish (platinum or quartz glass).

H.3.3 Heating plate or sand bath.

H.3.4 Muffle furnace (700 °C).

H.3.5 Spectrophotometer (for use at 800 nm with a 1 cm cuvette).

H.3.6 Cells, made of optical glass, 1 cm.

H.3.7 Graduated flasks.

H.3.8 Bulb pipettes.

H.4 Chemicals

H.4.1 Distilled or deionized water, conductivity less than 0,1 mS/m according to ISO 3696 grade 2.

H.4.2 Calcium carbonate, analytical grade.

H.4.3 Hydrochloric acid, having a concentration of 25 % (mass fraction).

H.4.4 Sulphuric acid, having a concentration of 96 % (mass fraction).

H.4.5 Ascorbic acid, analytical grade.

H.4.6 Ammonium heptamolybdate tetrahydrate, analytical grade.

H.4.7 Potassium antimony(III) oxytartrate hemihydrate; alternatively, **Bis [(+)-tartrato] diantimonate (III) dipotassium antimony (III) oxytartrate hemihydrate** may be used.

H.4.8 Ascorbic acid solution, having a concentration of 100 g/l.

Dissolve 10 g ascorbic acid (see H.4.5) in 100 ml water (see H.4.1).

NOTE The solution is durable for two weeks if it is placed in a refrigerator. The solution can be used as long as it is colourless.

H.4.9 Molybdate solution.

Dissolve 13 g ammonium heptamolybdate tetrahydrate $(NH_4)_6Mo_7O_{24}{\cdot}4\ H_2O$ (see H.4.6) in 250 ml water (see H.4.1). Put 150 ml sulphuric acid (see H.4.4) while cooling and stirring into the first solution.

After this, dissolve 0,35 g potassium antimony(III) oxytartrate hemihydrate $K(SbO)C_4H_4O_6{\cdot}½\ H_2O$ (see H.4.7) in 100 ml water (see H.4.1) and mix it into the sulphuric acid molybdate solution.

NOTE The whole solution is durable for two months if stored in a brown glass bottle.

H.4.10 Potassium hydrogen phosphate, KH_2PO_4, analytical grade, dried at 105 °C.

H.4.11 Phosphate stock solution, having a concentration of 200 mg/l.

Weigh in 286,6 mg potassium hydrogen phosphate (H.4.10), put it into a 1 000 ml graduated flask (H.3.7) and dissolve it with water (see H.4.1). Add 2 ml sulphuric acid (see H.4.4), fill the flask with water (see H.4.1) up to the calibrating mark and homogenize.

NOTE The solution is durable in a closed glass bottle for at least 3 months.

H.4.12 Phosphate stock solution, having a concentration of 2 mg/l.

From the stock solution (see H.4.11), generate a second stock solution of 2 mg/l by dilution with water (see H.4.1) to 1:100.

H.5 Procedure

H.5.1 Preparation of the calibration curve

Put 1 ml, 2 ml, 5 ml and 10 ml stock solutions (see H.4.12) (corresponding 2 µg, 4 µg, 10 µg and 20 µg phosphate) separately into a 50 ml graduated flask (see H.3.7) and dilute them with water (see H.4.1) to 40 ml. Every concentration shall be measured 10 times in accordance with H.5.7.

H.5.2 Calculation of the calibration factor

Calculate the calibration factor according to the following formula:

$$F_C = \frac{\sum_{i=1}^{4} m_{\text{phosphate},i}}{\sum_{i=1}^{4} \left(E_{1,i} - E_2\right)}$$

where

F_C is the calibration factor (µg);

$m_{\text{phosphate},i}$ is the mass of phosphate of the i-th sample (µg);

$E_{1,i}$ is the extinction of the i-th sample;

E_2 is the extinction of the blank test.

H.5.3 Process examination

H.5.3.1 Purpose

The process shall be checked as to whether the method delivers correct results.

H.5.3.2 Principle

The stock solution (see H.4.12) is determined like a routine sample. The phosphate amount of the stock solution is the measured value that shall be proved.

H.5.3.3 Execution

Pipette the phosphate stock solution of 5 ml and put it into a 50 ml graduated flask (see H.3.7), analyse it (see H.5.7) and calculate the content of phosphate (see H.6). Repeat this procedure three times.

The method is deemed valid if the measured values differ less than ±2 % related to the given phosphate amounts.

H.5.3.4 Frequency

The examination of the method should be done once every three months.

H.5.4 Examination of the calibration curve

H.5.4.1 Purpose

The calibration curve shall be checked in fixed intervals as to whether the slope of the calibration curve is correct.

H.5.4.2 Principle

Phosphate stock solutions are determined and the results are compared with the values from the calibrating curve.

H.5.4.3 Execution

Similar to H.5.1, a minimum of three concentrations in measurement range of the calibrating curve should be determined three times.

The calibration curve is deemed valid if the mean value of the calculated values differs less than ±2 % related to the given point of the calibration curve. If the difference is greater, repeat the procedure.

If the greater differences are confirmed, the analysing method for the determination of phosphate contents shall not be used until a new calibrating curve (see H.5.1) is established.

H.5.4.4 Frequency

The examination of the calibrating curve should be done minimum once every three years.

H.5.5 Sample preparation

The sample shall be completely dissolved and shall be without any urea crystals. If required, the sample may be heated to ≤ 40 °C.

H.5.6 Decomposition

Weigh in approximately 100 g (record the mass) of the prepared sample (see H.5.5) into an incineration dish (see H.3.2) and add 100 mg calcium carbonate (see H.4.2). Put the prepared sample on the heating plate and dry it slowly. Afterwards, incinerate the sample in the muffle furnace at 700 °C (see H.3.4) until the sample is completely decomposed. Cool the sample and add 1 ml hydrochloric acid (see H.4.3) and 20 ml to 30 ml water (see H.4.1) into the dish. Boil it until the residues are dissolved and the CO_2 is removed. Transfer the solution completely into a 100 ml graduated flask (see H.3.7), fill it with water (see H.4.1) to the calibration mark and homogenize it.

H.5.7 Photometric determination

Pipette (using a bulb pipette; see H.3.8) an exact volume from the solution (see H.5.6) into a 50 ml graduated flask (see H.3.7). Use a ≤ 40 ml sample solution. If less than 40 ml is used, dilute it with water (see H.4.1) until 40 ml is reached.

While stirring, add 1 ml ascorbic acid solution (H.4.8) and 2 ml molybdate solution (see H.4.9), fill the flask with water (see H.4.1) up to the calibrating mark and homogenize. The blank test shall be made in the same way but without sample solution.

After 10 to 30 minutes, determine the extinction of the sample and the blank test with the photometer (see H.3.5) at 800 nm.

H.6 Results

H.6.1 Calculation

The phosphate content is calculated by the following formula:

$$w_P = \frac{(E_S - E_B) \times F_C \times V_S \times F_1}{V \times F_2 \times m_S}$$

where

w_P is the content of phosphate (mg/kg);

E_S is the sample extinction;

E_B is the blank test extinction;

F_C is the calibration factor (µg);

V_S is the volume of the decomposed solution (ml);

F_1 is 1 000 (conversion factor from kg to g);

V is the volume used for the photometrical determination (ml);

F_2 is 1 000 (conversion factor from mg to µg);

m_S is the mass of urea solution (g).

H.6.2 Expression of results

Express the result to the nearest 0,01 mg/kg.

H.7 Precision

H.7.1 General

The precision evaluation programme with a matrix of only four samples of AUS 32 solutions with phosphate content in the range 0,218 mg/kg to 1,007 mg/kg did not conform to the requirements of ISO 4259 (all parts), and thus only an estimate of precision based upon inter-laboratory test results is given in H.7.2, H.7.3 and Table H.1.

H.7.2 Repeatability, *r*

The difference between two test results, obtained by the same operator with the same apparatus under constant operating conditions on identical test material would, in the long run, in the normal and correct operation of the test method, exceed the following in only one case in twenty.

r = 0,028 mg/kg

H.7.3 Reproducibility, *R*

The difference between two single and independent results, obtained by different operators working in different laboratories on identical test material would, in the long run, in the normal and correct operation of the test method, exceed the following value in only one case in twenty.

R = 0,075 mg/kg

Table H.1 — Precision (estimated)

Phosphate content	Repeatability	Reproducibility
w_P	r	R
mg/kg	mg/kg	mg/kg
0,218 to 1,007	0,028	0,075

H.8 Test report

The test report shall contain at least the following information:

a) type and description of tested product;

b) reference to this document, i.e. ISO 22241-2:2019;

c) sampling method used;

d) test result (see H.6);

e) deviations from the specified mode of operation, if any;

f) any unusual features observed; and

g) test date.

Annex I
(normative)

Determination of trace element content (Al, Ca, Cr, Cu, Fe, K, Mg, Na, Ni, P and Zn) by ICP-OES method

I.1 General

This annex specifies the procedure for the determination of aluminium, calcium, chromium, copper, iron, potassium, magnesium, sodium, nickel, zinc and total phosphate from phosphorus in AUS 32.

I.2 Principle

The trace element content is determined by using an **i**nductively **c**oupled **p**lasma-**o**ptical **e**mission **s**pectrometer (ICP-OES). This method requires reference curves for each element.

Sampling preparation is by direct determination, which includes dilution with water (base procedure). The sample to be examined is diluted with water and fed directly into the ICP-OES spectrometer. Depending on the plasma configuration, a dilution ratio of at least 1 + 1 (m/m) to 1 + 9 (m/m) has been proven optimal.

I.3 Apparatus

I.3.1 Analytical balance, resolution 0,1 mg or better.

I.3.2 Volumetric flask, with a nominal volume of 100 ml, class A.

Plastic flasks or volumetric flasks made from quartz glass may be used. Borosilicate glass flasks should not be used due to possible leach out of potential sodium and potassium contamination in borosilicate glass.

I.3.3 Fixed volume pipettes or variable piston pipettes.

The pipettes shall be calibrated.

I.3.4 Atomic emission spectrometer with inductively coupled plasma (ICP-OES).

A nebulizer system shall be used which can convert even high salt loads into an aerosol (cross flow, V-groove or similar). Humidification of the ICP gas (argon) is recommended.

Where auto samplers are used, the vessels, the needle and the supply hoses to the spectrometer shall be manufactured from polymer material (HDPE, PP, PTFE, etc.). Borosilicate glass flasks should not be used.

The use of a feed pump for the sample dosage to the atomiser is required. For this purpose, the spectrometer shall be able to measure interference for the wavelengths listed in Table I.1 without any malfunctions. Since the level of the spectral background can be influenced by the composition of the sample, the net intensities (background correction) shall be used.

Table I.1 provides information regarding proven wavelengths of the analysis.

Table I.1 — Recommended wavelengths

Element	Wavelength nm
Al	396,15 or 394,40 or 167,08
Ca	396,85 or 317,93 or 393,37
Cr	205,56 or 267,72
Cu	324,75 or 327,39
Fe	259,94 or 239,56
K	766,49 or 769,90
Mg	279,55 or 285,21
Na	588,99 or 589,59
Ni	352,45 or 231,60 or 227,07 or 221,65
P	178,20 or 177,50 or 213,62
Zn	213,85 or 206,20 or 202,55

Table I.2 provides information regarding proven element lines when using an internal standard.

Table I.2 — Recommended wavelengths for the measurement of the element as an internal standard

Element	Recommended wavelengths nm	Recommended wavelengths nm	Recommended wavelengths nm
Sc	361,38	256,02	227,32
Y	371,03	224,30	377,43

I.4 Reagents and materials

For the determination of elements at trace level, the reagents shall be of adequate purity. The concentration of the analyte or interfering substances in the reagents and the water should be negligible compared to the lowest concentration to be determined.

The procedures in this document shall be conducted with only one kind of acid.

I.4.1 Water, in accordance with ISO 3696 grade 1 or water with resistance of 18,2 MΩ, for all sample preparations and dilutions.

I.4.2 Urea, analytical reagent grade to mix with water to an aqueous urea solution with a mass fraction of 32,5 %.

I.4.3 Nitric acid, HNO_3, trace metal grade or purer, $c(HNO_3)$ = 15 mol/l, $w(HNO_3)$ = 65 %.

I.4.4 Hydrochloric acid, HCl, trace metal grade or purer, c(HCl) = 12 mol/l, w(HCl) = 37 %.

I.4.5 Single-element standard stock solutions.

Al, Ca, Cr, Cu, Fe, K, Mg, Na, Ni, P, and Zn ω(Element) = 1 000 mg/l

For stability of the solutions, refer to manufacturer guarantee statement.

In the case of using several mono-element standard solutions, attention shall be paid to ensure that they are free of other analyte elements.

Ready-made commercial multi-element standard solutions may be used instead of the single element standard solutions as the method is designed to avoid interferences at the wavelength specified.

I.4.6 Multi-element standard stock solutions, ω(Element) = 100 mg/l.

In general, when combining multi-element standard stock solutions, their chemical compatibility and the possible hydrolysis of the components shall be regarded. Care shall be taken to prevent chemical reactions (e.g. precipitation).

I.4.7 Internal standard solution.

To have the same amount of internal standard in all samples and calibration solutions, both the reference solutions (refer to I.5.4.1) as well as the samples are added the same amount of internal standard solution. The following is an example for preparing 1 litre of internal standard solution:

— Approximately 10 g of internal standard (Table I.2) is weighed in a 1 000 ml volumetric flask (I.3.2), added 20 ml HNO_3 (I.4.3), filled to the mark with deionized water (I.4.1) and shaken.

— The amount of the internal standard can be customized individual to the ICP spectrometer.

NOTE The addition of the internal standard via a multi-channel (peristaltic) pump cannot be recommended, since the precision can deteriorate in cases of different viscosity between the sample and the internal standards. The precision statements in I.7 were determined by manual addition of the internal standard.

I.4.8 Argon, with a purity ≥99,996.

I.5 Procedure

I.5.1 Interferences

Traces of the elements to be determined may be present on the inside of plastic vessels (sample bottles, volumetric flasks, etc.); the inside shall therefore always be cleaned with acid-(I.4.3 or I.4.4) before use.

I.5.2 Sample preparation

There are different ways to prepare the samples for axial or lateral torch configurations.

Because of more robust plasma conditions with radial plasma configuration, the samples can be prepared with higher salt content. For axial plasma configuration, the dilution can be higher because of higher sensitivity.

Two examples of preparations for radial or axial plasma configurations are given as follows:

EXAMPLE 1 Radial plasma configuration procedure:

— weigh 50 g of the sample into a 100 ml volumetric flask with a tolerance of 0,1 g;

— add some 30 ml of water (I.4.1) and 5 ml of acid (I.4.3 or I.4.4) in this sequence;

— if manually adding internal standard, add 10 ml of internal standard solution (I.4.7);

— fill up the volumetric flask with water (I.4.1) and homogenize the solution.

EXAMPLE 2 Axial plasma configuration procedure:

— weigh 20 g of the sample into a 100 ml volumetric flask with a tolerance of 0,1 g;

— add some 60 ml of water (I.4.1) and 5 ml of acid (I.4.3 or I.4.4) in this sequence;

— if manually adding internal standard, add 10 ml of internal standard solution (I.4.7);

— fill up the volumetric flask with water (I.4.1) and homogenize the solution.

I.5.3 Setting the ICP OES spectrometer

The establishment of the ICP OES spectrometer and equipment tests are to be executed in accordance with the manufacturer's recommendations.

The selection of the equipment parameters shall be executed to obtain the best signal/background ratio for analytes.

Since the magnitude of the background signal is highly dependent on the spectral structures, which are caused by the type of sample and its origin, only net intensities shall be evaluated. To keep background noise to a minimum, the signal and background shall be measured simultaneously.

The net intensity of the analysis lines shall be calculated by subtracting the measured intensity with the respective background wavelengths. Most of the devices are equipped with a software which enables an automatic correction for the background.

NOTE For distilled or long sample runs, it can be helpful to add argon around the nebulizer argon flow to prevent clogging of the injector tube.

I.5.4 Generation of reference curves

The frequency of the acquisition of the reference curve depends on the spectrometer used (in accordance with the instrument manufacturer's specifications and guidelines). To check the reference curve and correct its drift, the lowest and highest standard should be measured on each working day.

I.5.4.1 Reference solutions

The element concentrations as specified in Table I.3 for radial plasma configuration and Table I.4 for axial plasma configuration are recommended.

Table I.3 — Preparation example of reference solutions for radial plasma configuration

Reference solution ω(Element) mg/l	Parent solution (I.4.6) ω(Element) = 1,00 mg/kg ml	Acid solution ml	Internal standard solution (I.4.7) (if used) ml	Urea solution 32,5 % (I.4.2) ml	Water (I.4.1) ml
5	5	5	10	50	Filled up to 100 ml
2	2	5	10	50	Filled up to 100 ml
1	1	5	10	50	Filled up to 100 ml
0,3	0,3	5	10	50	Filled up to 100 ml
0	0	5	10	50	Filled up to 100 ml
Calibration control	0,5	5	10	50	Filled up to 100 ml