
**Determination of organonitrogen
compounds in air using liquid
chromatography and mass
spectrometry —**

Part 1:

**Isocyanates using dibutylamine
derivatives**

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*Détermination des composés organiques azotés dans l'air par
chromatographie liquide et spectrométrie de masse —*

Partie 1: Isocyanates par les dérivés de la dibutylamine



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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).

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For an explanation on the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the WTO principles in the Technical Barriers to Trade (TBT) see the following URL: Foreword - Supplementary information

The committee responsible for this document is ISO/TC 146, *Air quality*, Subcommittee SC 2, *Workplace atmospheres*.

This second edition of ISO 17734-1 cancels and replaces ISO 17734-1:2006, which has been technically revised.

ISO 17734 consists of the following parts, under the general title *Determination of organonitrogen compounds in air using liquid chromatography and mass spectrometry*:

- Part 1: *Isocyanates using dibutylamine derivatives*
- Part 2: *Amines and aminoisocyanates using dibutylamine and ethyl chloroformate derivatives*

Introduction

Isocyanates have been used in industry for about 50 years. They are commercially important chemicals mainly used for the production of polyurethane (PUR). In spite of controls to limit exposures, there are adverse health effects such as asthma, contact dermatitis, and hypersensitivity pneumonitis as consequences of exposure to isocyanates in some industrial sectors.

The analytical method for the determination of isocyanates in workplace air must be sensitive due to the high irritation and sensitization properties of isocyanates. Extremely low occupational exposure limits (OELs) exist in many countries, and concentrations well below the OEL ($< 1/100$) are often to be determined. Isocyanates are very reactive and therefore cannot be analysed directly. Derivatization during sampling is required in order to prevent interfering reactions. Hundreds of different isocyanates are used in industry, and many more are formed during thermal degradation of PUR. Therefore, high selectivity of the analytical method is required for accurate results.

The determination of isocyanates in the work environment using di-*n*-butylamine (DBA) as a reagent and liquid chromatography-mass spectrometric detection (LC-MS) has been demonstrated to be a robust method. The development of the method was initiated when difficulties using the “older” methods during sampling of isocyanates in complex atmospheres were encountered (e.g. thermal decomposition of PUR). [1][2][3] The reaction rate between DBA and isocyanates was found to be fast, and high concentrations can be used to secure instantaneous reactions and eliminate problems with interfering compounds. [4][5] Using impinger flasks containing a reagent solution and a filter in series efficiently collects and derivatizes isocyanates in both the gas and the particle phase. [6] LC-MS/MS of the isocyanate-DBA derivatives enables highly selective and precise determinations down to levels below 10^{-6} of the OEL. [7]

Solvent-free sampling can also be performed by using a tube coated with a DBA-impregnated glass fibre filter followed by an impregnated filter. An impregnation solution containing DBA together with an acid is used, and the formed ion pair reduces volatility. DBA remains on the filter even after 8 h of sampling. [8]

Monomeric isocyanates that are formed during thermal decomposition of polymers [typically PUR and phenol/formaldehyde/urea (PFU) resins], such as isocyanic acid and methyl isocyanate, can also be determined. [6][7][8][9][10] Volatile isocyanate-DBA derivatives can be determined using gas chromatography-mass spectrometric detection (GC-MS). [9] Using the DBA method and derivatization with ethyl chloroformate makes simultaneous determinations of amine, aminoisocyanates, and isocyanates possible, as described in the companion method ISO 17734-2.

For quantification, reference compounds are necessary but are only available for a few monomeric isocyanates. Most of the isocyanates that are used in industry for the production of PUR can only be obtained in technical grade mixtures. Many isocyanates that are formed during thermal degradation are not available and are not easily synthesized. In this method, a nitrogen-sensitive detector has been used for quantifying isocyanates in reference solutions. This technique has been demonstrated to be a useful tool, together with MS characterization, in greatly facilitating the production of reference solutions. [10][11][12]

For quantifying isocyanates in complex mixtures, MS detection appears to be the current best available detection technique and provides a unique possibility of identifying unknown compounds. This method has enabled assessment of new areas for which exposure to isocyanates was not known previously and has identified new kinds of isocyanates in the work environment. [6][7][8][9][10][11][12]

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Determination of organonitrogen compounds in air using liquid chromatography and mass spectrometry —

Part 1: Isocyanates using dibutylamine derivatives

1 Scope

This part of ISO 17734 gives general guidance for the sampling and analysis of airborne isocyanates in workplace air. When amines and aminoisocyanates are suspected to be emitted (e.g. from thermal degradation of PUR), it is recommended that, in addition to isocyanates, the amines and aminoisocyanates in the air are determined, using DBA and ethyl chloroformate as reagents (see ISO 17734-2).

The method is suitable for the determination of a wide range of different isocyanates in both the gas and particle phases. Typical monofunctional isocyanates that can be determined are isocyanic acid (ICA), methyl isocyanate (MIC), ethyl isocyanate (EIC), propyl isocyanate (PIC), butyl isocyanate (BIC), and phenyl isocyanate (PhI). Typical monomeric diisocyanates include 1,6-hexamethylene diisocyanate (HDI), 2,4- and 2,6-toluene diisocyanate (TDI), 4,4'-methylenediphenyl diisocyanate (MDI), 1,5-naphthyl diisocyanate (NDI), isophorone diisocyanate (IPDI) and 4,4'-dicyclohexylmethane diisocyanate (HMDI). Multifunctional isocyanates that can be determined are typically oligomers in polymeric MDI, biuret-, isocyanurate-, and allophanate-adducts, and prepolymeric forms of isocyanates.

The instrumental detection limit for aliphatic isocyanates is about 5 nmol/sample and for aromatic isocyanates, it is about 0,2 nmol/sample. For a 15 l air sample, this corresponds to 0,6 ng·m⁻³ for HDI and 0,02 ng·m⁻³ for TDI.

The useful range, for a 5 l air sample, of the method is approximately 0,001 µg·m⁻³ to 200 mg·m⁻³ for TDI.

2 Normative references

The following documents, in whole or in part, are normatively referenced in this document and are indispensable for its application. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 5725-2, *Accuracy (trueness and precision) of measurement methods and results — Part 2: Basic method for the determination of repeatability and reproducibility of a standard measurement method*

ISO 16200-1, *Workplace air quality — Sampling and analysis of volatile organic compounds by solvent desorption/gas chromatography — Part 1: Pumped sampling method*

3 Principle

Samples are collected by drawing a known volume of air through a midget impinger flask followed by a filter. The impinger contains 10 ml of 0,01 mol·l⁻¹ of DBA in toluene and the filter is a glass fibre filter with no binder.

Solvent-free sampling can also be performed by drawing air through a tube coated with a DBA-impregnated glass fibre filter followed by an impregnated filter. An impregnation solution containing DBA together with acetic acid is used, the ion pair so formed reduces the volatility and enables long-time sampling.

After sampling, deuterium-labelled isocyanate-DBA derivatives (used as internal standard) are added to the sample solutions. The excess reagent and solvent are evaporated, and the samples are dissolved

in acetonitrile. The samples are analysed using reversed-phase LC and electrospray (ESP)-MS detection, monitoring positive ions. Quantification is made by monitoring selected ions. See [Figure 1](#).

Quantification and qualitative determinations can be performed using different LC-MS or LC-MS/MS techniques. Liquid chromatography-chemiluminescent nitrogen detection (LC-CLND), or liquid chromatography-ultraviolet detection (LC-UV) for aromatic isocyanates, can be used for the determination of higher concentrations of isocyanates.

Reference materials can be characterized using LC-MS/CLND. For characterization of volatile compounds, gas chromatography-thermionic specific detector (GC-TSD) can also be used.

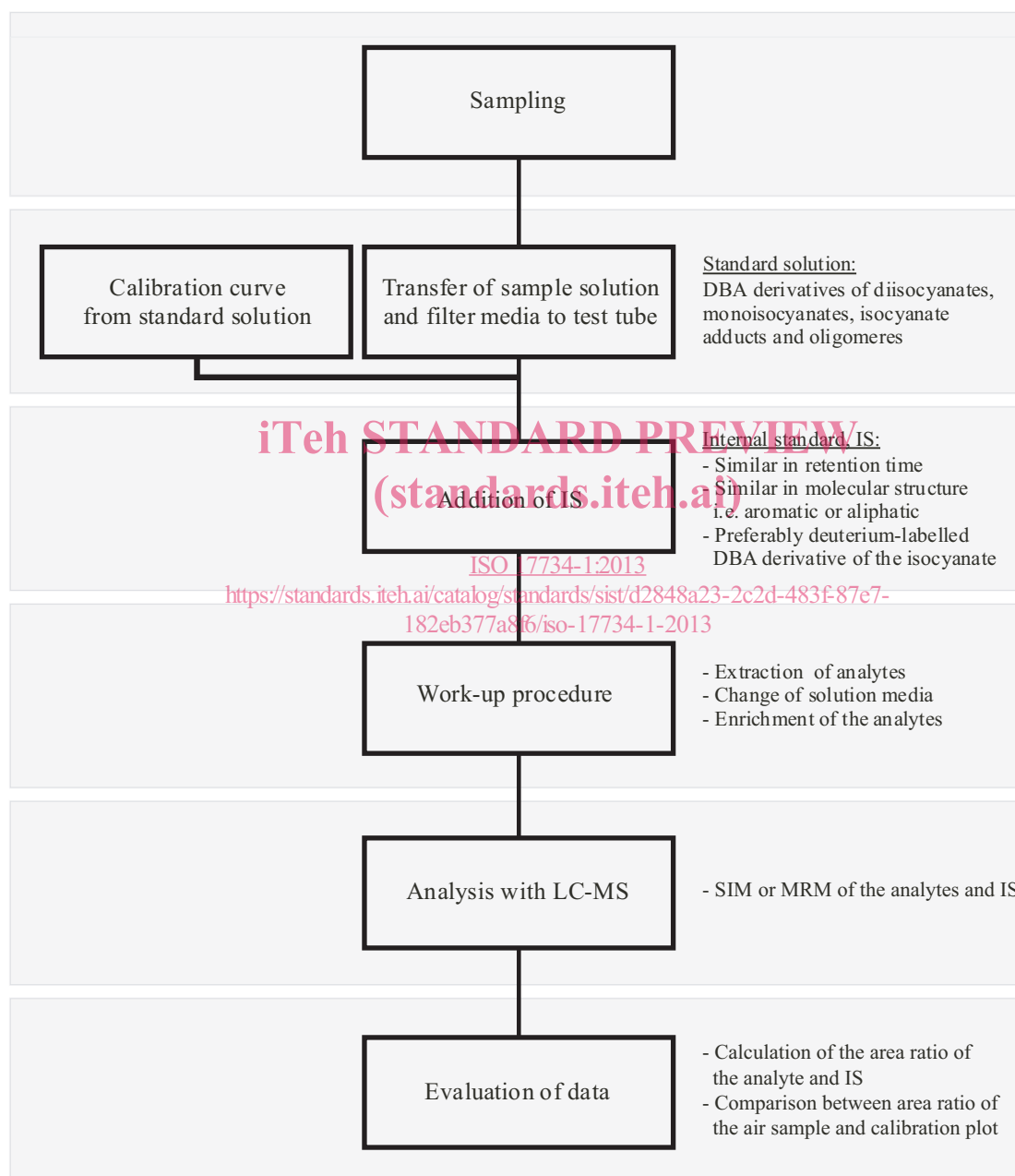


Figure 1 — Principle of the described method

4 Reagents and materials

4.1 DBA reagent.

Analytical grade di-*n*-butylamine is commercially available.

4.2 Solvents.

The reagent solvent, typically toluene, and other solvents, such as acetonitrile, isooctane, and methanol, should be of liquid chromatographic quality.

4.3 Formic acid, concentrated formic acid, analytical grade.

4.4 Acetic acid, concentrated acetic acid, analytical grade.

4.5 Sulfuric acid 5 mM, 0,27 ml of concentrated sulfuric acid (98 %) is added to 1 000 ml of water.

4.6 Reagent solution.

In a 1 l volumetric flask, dilute 1,69 ml of DBA in toluene and make up to the mark. The solution is stable and no special care during storage is necessary.

4.7 Reagent solution, for solvent-free sampler.

4.7.1 Solution 1: 0,74 mol·l⁻¹ DBA

Mix 80 ml methanol and 12,5 ml DBA in a 100 ml volumetric flask. Then while stirring, slowly add 4,16 ml of acetic acid to the flask. Finally, add methanol to the flask, and make up to the mark.

4.7.2 Solution 2: 1,5 mol·l⁻¹ DBA.

Mix 60 ml methanol and 25 ml DBA in a 100 ml volumetric flask. Then while stirring, slowly add 8,32 ml of acetic acid to the flask. Finally, add methanol to the flask, and make up to the mark.

4.8 HPLC mobile phases.

4.8.1 LC-MS.

The weak mobile phase (mobile phase A) consists of water/acetonitrile (95/5 volume fraction) and 0,05 % formic acid. The strong mobile phase (mobile phase B) consists of water/acetonitrile (5/95 volume fraction) and 0,05 % formic acid. The mobile phases are degassed prior to use.

4.8.2 LC-CLND.

The weak mobile phase (mobile phase C) consists of water/methanol (95/5 volume fraction) and 0,05 % formic acid. The strong mobile phase (mobile phase D) consists of water/methanol (5/95 volume fraction) and 0,05 % formic acid. The mobile phases are degassed prior to use.

5 Preparation of standard solutions

5.1 Reference compounds

Reference compounds are necessary for LC-MS determination of isocyanate derivatives. For the commercially available isocyanates, the DBA derivatives are easily prepared by direct derivatization with DBA. DBA derivatives for the isocyanates not commercially available can be made from the bulk

material or from the thermal decomposition of PUR or urea-based resins used at the work place. Alternatively, standard solutions can be purchased (see [Annex C](#)).

5.2 Di-*n*-butylamine (DBA) derivatives of isocyanates

5.2.1 Preparation of isocyanate-DBA derivatives of commercially available isocyanates

Many frequently used isocyanates are commercially available from companies supplying laboratory chemicals such as HDI, 2,4-TDI and 2,6-TDI, 4,4'-MDI, 4,4'-HMDI, 1,5-NDI, IPDI, Phi, MIC, EIC, PIC, and BIC. The purity of the isocyanates varies, and some contain isomers.

Calibration standards are made by spiking accurately weighed amounts or volumes (ca 0,1 mmol) of isocyanates in 100 ml of isooctane. A 1 ml volume is added to 100 ml of toluene containing 0,01 mol·l⁻¹ of DBA (ca 0,01 μmol·ml⁻¹ of the DBA derivative).

The procedure for the synthesis of derivatives is as follows:

- 1) Dilute 6 mmol of isocyanate in 2 ml of isooctane.
- 2) Dissolve 60 mmol of DBA in 20 ml of isooctane.
- 3) Add the isocyanate solution to the DBA solution dropwise under continuous stirring.
- 4) Evaporate the reaction mixture to dryness in a rotating evaporator.
- 5) Dry the residue under vacuum to remove excess DBA.

It is also possible to prepare the isocyanate-DBA derivatives by collecting the thermal degradation products of the corresponding carbamate esters in an impinger flask containing a DBA solution (as specified in [5.2.3.3](#)).

5.2.2 Preparation of ICA and MIC-DBA

When urea is thermally degraded, ICA is formed.

Heat an amount of urea (20 mg) to about 300 °C in a glass tube. Collect the degradation products in an impinger flask containing DBA in toluene (0,5 mol·l⁻¹). Wash the toluene solution containing the ICA-DBA derivatives with water, whereupon the organic phase is evaporated in a vacuum centrifuge and the residue is dissolved in methanol. Characterize the solution, as described in [5.2.4](#).

The same procedure can be applied for preparation of MIC-DBA derivatives, by collecting thermal degradation products of 1,3-dimethyl urea.

5.2.3 Preparation of deuterium-labelled isocyanate-DBA derivatives

5.2.3.1 Internal standards

For accurate LC-MS quantifications, it is important to use proper internal standards, not only to compensate for variations during the work-up procedure, but also to compensate for fluctuation in the MS instrument response. Ideally, each analyte should have its own deuterium-labelled analogue. For isocyanate-DBA determinations, it is possible to use DBA derivatives of deuterium-labelled isocyanates or d₉- and d₁₈-DBA derivatives of the isocyanates as internal standards.

The quality of the quantification is influenced by the number of deuterium substitutions in the internal standard (less deuterium in the molecule result in higher precision). Having the deuterium on the isocyanate, and not on the DBA, has advantages when performing structural identification using MS and MS/MS. It is then possible to distinguish between labelled and non-labelled fragments that originate from the isocyanate itself. Therefore, the ideal internal standards are the DBA derivatives of the deuterium-labelled isocyanates. However, they are labour intensive to prepare, and they are only available for a few isocyanates.

The deuterium-labelled d_9 - and d_{18} -DBA derivatives are easy to prepare, and any technical isocyanate or thermal degradation product can be derivatized and used as internal standard.

5.2.3.2 DBA derivatives of deuterium-labelled isocyanates

Dissolve a 10 mmol aliquot of the deuterium-labelled corresponding amine in 20 ml of toluene. Thereafter, add 150 μ l pyridine and 40 ml of 5 mol·l⁻¹ NaOH. Then add 1,5 ml of ethyl chloroformate dropwise under continuous stirring. After 10 min, the toluene phase is separated, and the solvent is evaporated.

Place the residue containing the formed amine carbamate ester (10 μ l) in a glass tube. Heat the tube to about 300 °C. By connecting the tube to an impinger flask, containing 0,5 mol·l⁻¹ DBA in toluene, the formed deuterium-labelled isocyanate is collected as a DBA derivative. Evaporate the solvent, and dissolve the residue in methanol to an appropriate concentration. Characterize the solution, as described in 5.2.4.

5.2.3.3 d_9 -DBA and d_{18} -DBA derivatives of the isocyanates

Prepare the deuterium-labelled d_9 - and d_{18} -DBA derivatives by dissolving accurately weighed amounts of the isocyanates in 10 ml of 0,1 mol·l⁻¹ d_9 -DBA or d_{18} -DBA in toluene.

Prepare the deuterium-labelled derivatives of ICA and MIC by placing 20 mg of urea for ICA and 1,3-dimethyl urea for MIC in a glass tube. Heat the tube to about 300 °C and collect the formed ICA and MIC in impinger flasks containing 0,1 mol·l⁻¹ d_9 -DBA or d_{18} -DBA in toluene. Evaporate the solutions containing the isocyanate d_9 -DBA or d_{18} -DBA derivatives to dryness, and dissolve the residues in methanol. Characterize the solution, as described in 5.2.4.

5.2.4 Characterization

The solutions are diluted in methanol to appropriate concentrations and characterized on the LC-MS and quantified on the LC-CLND. This technique is nitrogen specific and any nitrogen-containing compound can be used as external standard, e.g. caffeine. The technique is used in several applications. [13][14][15] Quantification of volatile isocyanate-DBA derivatives can also be made by using GC-TSD.

5.3 DBA derivatives of bulk isocyanates

5.3.1 Preparation

Technical isocyanates used in industry are typically available in mixtures such as oligomers in polymeric MDI, biuret-, isocyanurate-, and allophanate-adducts, and prepolymeric forms of isocyanates. These isocyanates are typically multifunctional.

If product data sheets are available and correct, standard solutions for the technical-grade isocyanates can be prepared in the same way as described in 5.2.1, by adding a known amount of bulk isocyanate to a toluene solution of DBA. If data regarding the composition and concentrations of different isocyanates are of poor quality or missing, the bulk material shall be characterized.

The procedure for the technical-grade isocyanate is as follows:

- 1) Add an aliquot of 10 mg of the isocyanate product to a 10 ml vial containing 0,5 mol·l⁻¹ DBA.
- 2) Sonicate the solution and evaporate it to dryness and then dissolve it in methanol.
- 3) Further dilute the solution with methanol to appropriate concentrations.
- 4) Characterize the solution, as described in 5.3.2.

5.3.2 Characterization

If the isocyanates that are present in the bulk are known or reference compounds are available, calibration standards can be prepared, as described in 5.2.1.

If the isocyanates that are present in the bulk are unknown, qualitative data are obtained with full-scan chromatograms for DBA-derivatized bulk material. The obtained structural data together with the LC-CLND data make it possible to calculate the concentrations of the different components in the solution. The characterized bulk sample solution is used as a calibration standard for LC-MS.

When prepolymeric forms or complex isocyanates are to be determined, it can be difficult to quantify each individual isocyanate using LC-MS. However, one or more components can be used as index compounds. The total isocyanate group (NCO) concentration of the bulk is obtained by titration with DBA (see Reference [16]) and standard solutions can be prepared (dilution). The concentration of isocyanates in the air sample is estimated by comparison of peak areas. This can be performed with the assumption that the composition of the bulk material reflects the composition of the airborne isocyanates. The obtained result gives the concentration of the total isocyanate content in the air. However, detection limits are increased by the factor of the ratio of the total isocyanate concentration and the assumed concentration of the index isocyanate. Still, in most cases, levels below 1/10 of the threshold limit value (TLV) are possible to determine.

5.4 DBA derivatives of isocyanates in thermal decomposition products of polyurethane (PUR) or urea-based resins

5.4.1 Preparation

During the thermal decomposition of PUR or urea-based resins, isocyanates that are not commercially available are formed. PUR or urea-based materials can be thermally decomposed at appropriate temperatures. The emitted degradation products are collected in impinger flasks (filters in series) containing 0,5 mol·l⁻¹ DBA. The solutions are evaporated to dryness, and the residues are dissolved in methanol.

5.4.2 Characterization

Qualitative data are obtained with LC-MS. The obtained structural data together with the LC-CLND data make it possible to calculate the concentrations of different components in the solution. The characterized and diluted sample solution is used as a calibration standard for LC-MS.

5.5 Stability

Solutions of isocyanate-DBA derivatives (MDI, 2,4- and 2,6-TDI, HDI, IPDI, PhI, BIC, PIC, EIC, MIC, and ICA) have been found stable in toluene, acetonitrile, and methanol for 6 mo when stored at 8 °C. NDI-DBA has limited stability and shall be freshly prepared and quantified before use as a calibration standard.

6 Apparatus

6.1 Sampler, can be either an impinger (6.1.3) followed by a filter (6.1.1) connected in series to the sampling pump (6.1.5) through a vapour trap (6.1.7) or a solvent-free sampler (6.1.4) connected to the sampling pump (6.1.5).

6.1.1 Filter.

Use a 13 mm glass fibre filter (binder free) with a pore size of 0,3 µm.

6.1.2 Filter holder.

Use a 13 mm polypropylene filter holder with luer-lock connections.

6.1.3 Midget impingers.

A midget impinger consists of a tapered inlet tube. Match the two parts so that the distance between the inlet and the receiver bottom is 1 mm to 2 mm. The filter holder is attached to the outlet of the impinger,

by using an impinger with a luer-lock fitting on the outlet. Alternatively, the filter holder is attached to the outlet of the impinger by flexible tubing.

6.1.4 Solvent-free sampler.

Cut three kinds of glass fibre filters from a glass fibre filter sheet (binder free) with a pore size of $0,3\ \mu\text{m}$:

- 1) rectangular filter, $2,5\ \text{cm} \times 5,7\ \text{cm}$;
- 2) rectangular filter, $1,4\ \text{cm} \times 5,7\ \text{cm}$;
- 3) round filter, 13 mm diameter.

To prepare the sampler, the different filters are mounted in the tube and the filter holder unimpregnated. The inside of the tube is lined with filter 1 ($2,5\ \text{cm} \times 5,7\ \text{cm}$), so that it covers the inner walls of the tube. Filter 2 ($1,4\ \text{cm} \times 5,7\ \text{cm}$) is folded as a "V" and also placed inside the tube to increase the collection efficiency, by increasing the number of channels in the tube.

The dry sampler is then impregnated by dropwise adding 1,5 ml 1,4 M DBA-acetic acid in methanol to the filters at the top of the tube. After impregnation, the tube is dried by blowing nitrogen through the tube for evaporation of methanol.

The round-end filters (diameter of 13 mm) are impregnated by adding $100\ \mu\text{l}$ 0,7 M DBA-acetic acid solution, and dried under nitrogen in open cassettes. When solvent evaporation is complete, the end filters are mounted on the denuder. Then the complete sampler is sealed at both ends. Impregnation and drying of the filters are performed in a container filled with nitrogen to avoid contamination.

Alternatively, the sampler can be purchased (see [Annex C](#)).

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Dimensions in millimetres

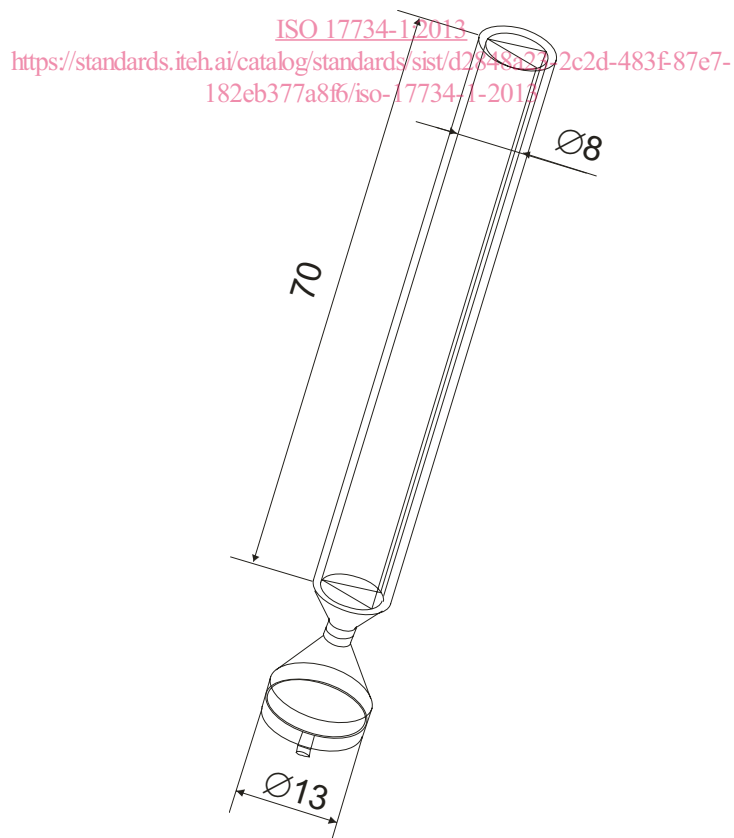


Figure 2 — Solvent-free sampler