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**ISO 17734-2**

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

## Part 2: Amines and aminoisocyanates using dibutylamine and ethyl chloroformate derivatives

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

*Partie 2: Amines et aminoisocyanates par les dérivés de la dibutylamine et du chloroformate d'éthyle*



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# Foreword

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International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 2.

The main task of technical committees is to prepare International Standards. Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

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.

ISO 17734-2 was prepared by Technical Committee ISO/TC 146, *Air Quality*, Subcommittee SC 2, *Workplace Atmospheres*.

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

In many applications, when considering isocyanates as a workplace contaminant, there is also a need to investigate the presence of aminoisocyanates and amines. During thermal decomposition of polyurethane (PUR), not only isocyanates, but also amines and aminoisocyanates, are formed [1], [2], [3], [4], [5], [6].

The determination of isocyanates in the work environment using DBA as a reagent has been demonstrated to be a robust method (ISO 17734-1). Using the DBA-method and derivatization with ethyl chloroformate in the following work-up procedure makes simultaneous determination of amines, aminoisocyanates and isocyanates possible [6], [7].

For quantification of amine and aminoisocyanate derivatives, reference compounds are necessary, but are only available for a few diamines. Aminoisocyanates can not be analysed directly because they react with themselves. In this method, a nitrogen-specific detector has been used for quantification of amine and aminoisocyanate derivatives 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 [6].

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

## Part 2:
## Amines and aminoisocyanates using dibutylamine and ethyl chloroformate derivatives



## 1 Scope

This part of ISO 17734 gives general guidance for the sampling and analysis of airborne amines and aminoisocyanates in workplace air. It is strongly recommended that the determination of amines and aminoisocyanates is made together with the determination of isocyanates in air, using DBA as a reagent (ISO 17734-1).

The method can be used for simultaneous determinations of amines, 4,4'-methylenediphenyldiamine (4,4'-MDA), 2,4- and 2,6-toluenediamine (2,4-, 2,6-TDA) and 1,6-hexamethylenediamine (1,6-HDA), and compounds containing both isocyanate and amine groups, 4,4'-methylenediphenyl aminoisocyanate (4,4'-MAI), 2,4-, 4,2- and 2,6-toluene aminoisocyanate (2,4, 4,2, 2,6-TAI), 1,6-hexamethylene aminoisocyanate (1,6-HAI). The method is suitable for collecting amines and aminoisocyanates in both the gas and particle phases. The instrumental detection limit for the amines is about 50 fmol and for the aminoisocyanate, it is about 3 fmol. For a 15-l air sample, this corresponds to 0,4 $ng \cdot m^{-3}$ for TDA and 0,03 $ng \cdot m^{-3}$ for TAI.

## 2 Normative references

The following referenced documents are indispensable for the application 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 16200-1:2001, *Workplace air quality — Sampling and analysis of volatile organic compounds by solvent desorption/gas chromatography — Part 1: Pumped sampling method*

ISO 5725-2:1994, *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* (including Technical Corrigendum 1:2002)

## 3 Principle

The method permits the simultaneous sampling and analysis of amines, aminoisocyanates and isocyanates. Only amines and aminoisocyanates are discussed in this part, because isocyanates are considered in ISO 17734-1.

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 $mol \cdot l^{-1}$ of di-*n*-butylamine (DBA) in toluene, and the filter is a glass fibre filter with no binder. After sampling, deuterium-labelled amine-ethyl chloroformate (ET) and 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 liquid chromatography (LC) and electrospray (ESP) mass spectrometric (MS) detection, monitoring positive ions. Quantification is made by monitoring selected ions.

Quantification and qualitative determinations can be performed using different LC-MS techniques. LC-CLND (chemiluminescent nitrogen detection) or for aromatic isocyanates, aminoisocyanates and amines LC-UV (ultraviolet detection) can be used for the determination of higher concentrations. Reference materials can be characterized using LC-MS/CLND. For characterization of volatile compounds, a GC-thermoionic specific detector (TSD) can also be used.

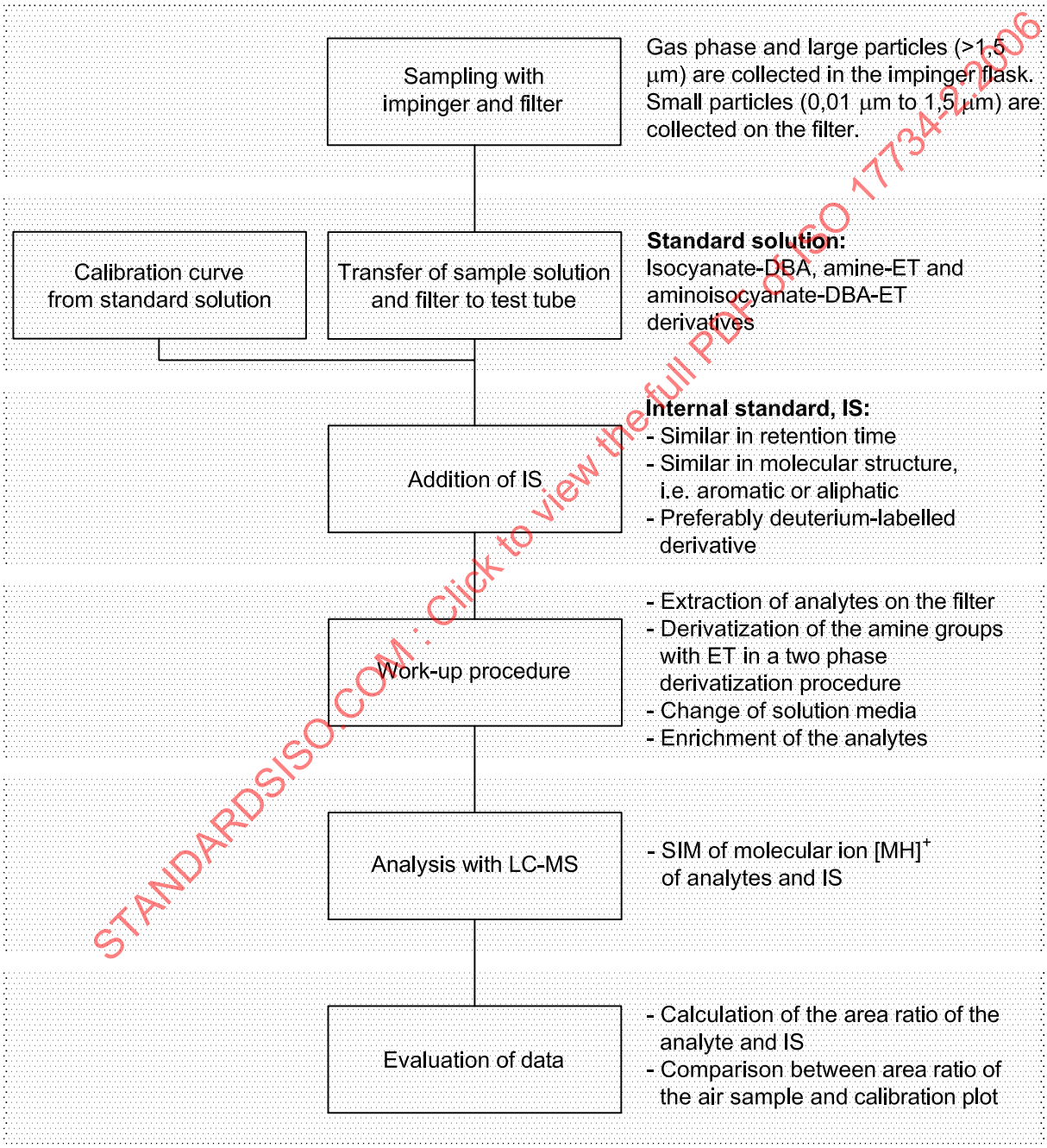





**Figure 1 — Principle of the described method**

# 4 Reagents and materials

## 4.1 DBA reagent.

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

## 4.2 Ethyl chloroformate reagent.

Analytical grade ethyl chloroformate is commercially available.

## 4.3 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.

## 4.4 Sodium hydroxide, 5 mol $l^{-1}$.

Dissolve 200 g of NaOH in water in a beaker, then transfer the solution to a 1-l volumetric flask, and make up to the mark.

## 4.5 Pyridine, analytical grade.

## 4.6 Solvents.

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

## 4.7 Formic acid, concentrated formic acid, analytical grade.

## 4.8 Ethanol, absolute, extra pure 99,5 %.

## 4.9 HPLC mobile phases.

### 4.9.1 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/methanol (5/70/25 volume fraction) and 0,05 % formic acid. The mobile phases are degassed prior to use.

### 4.9.2 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 Standard solutions

## 5.1 Reference compounds

Reference compounds are necessary for LC-MS determination. For the commercially available amines, the ET derivatives are easily prepared by direct derivatization with ethyl chloroformate (ET) for the use as calibration standards. The aminoisocyanate derivatives are prepared by reacting one of the isocyanate groups with DBA and the other group with ethanol. The mixed derivatives formed must be characterized before using as calibration standards. Isocyanate, aminoisocyanate and amine derivatives for compounds that not are commercially available can be made from the bulk material or from the thermal decomposition of PUR. Alternatively, standard solutions can be purchased.

## 5.2 Preparation of amine and deuterium-labelled amine derivatives

Calibration standards are made by spiking accurately weighed amounts (ca 0,1 mmol) of amines in 100 ml of toluene. The solution is further diluted to ca 0,01 µmol $ml^{-1}$. 5-ml toluene solutions are spiked with volumes of the amine solutions appropriate for the construction of a calibration curve. The work up procedure is then performed; this is described in 8.2.

Synthesis of derivatives:

— Dissolve a 10 mmol aliquot of the amines and the deuterium-labelled amines in 20 ml of toluene. Thereafter, add 150 µl pyridine and 40 ml of 5 $mol \cdot l^{-1}$ NaOH. Then add 1,5 ml of ethyl chloroformate dropwise under continuous stirring.

— After 10 min, separate the toluene phase.

— Evaporate the reaction mixture to dryness in a rotating evaporator, and dry the residue under vacuum.

## 5.3 Aminoisocyanate derivatives

### 5.3.1 Preparation

Dissolve 0,5 mmol of the isocyanates in 50 ml isooctane. Add 0,5 mmol of DBA dissolved in isooctane under continuous stirring to the isocyanate solutions. After 30 min, add excess ethanol to the solutions. Allow the mixtures to react for 16 h. Evaporate the solutions to dryness and dissolve in methanol.

To produce both the isomers for the 2,4-TAI, prepare another solution by first allowing the isocyanate solution to react with 0,5 mmol of ethanol during 16 h. Then add excess DBA to the solution. Evaporate the solution to dryness and dissolve in methanol. The solution is characterized as described in 5.3.2.

### 5.3.2 Characterization

Dilute the solutions in methanol to appropriate concentrations and characterize them on the LC-MS and quantify them on the LC-CLND. This technique is nitrogen specific and any nitrogen-containing compound (e.g. caffeine) can be used as external standard. The technique is used in several applications [8], [9], [10].

## 5.4 Thermal decomposition products of polyurethane (PUR)

### 5.4.1 Preparation of mixed isocyanate, amine and aminoisocyanate derivatives

During the thermal decomposition of, e.g. PUR, isocyanates, aminoisocyanates and amines are formed that are not commercially available. PUR-based material can be thermally decomposed at appropriate temperatures. Collect emitted degradation products in impinger flasks (filters in series) containing 0,5 $mol \cdot DBA \cdot l^{-1}$ and follow this by the work up procedure described in 7.2. The solution is characterized as described in 5.3.2.

### 5.4.2 Characterization

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

## 5.5 Stability of the amine and aminoisocyanate derivatives

Solutions of amine-ET and ET-DBA-aminoisocyanate derivatives (MDA, 2,4- and 2,6-TDA, HDA, MAI, 2,4-, 4,2- and 2,6-TAI and HAI) have been found stable in toluene, acetonitrile and methanol for six months.

# 6 Apparatus

## 6.1 Sampler.

Sample the air with an impinger flask followed by a filter.

### 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. A luer-lock fitting is attached to the outlet of the impinger.

### 6.1.4 Sampling pump.

Use a sampling pump with a calibrated flow rate of 1 $l \cdot min^{-1}$.

### 6.1.5 Tubing.

Use rubber tubing of suitable length and of appropriate diameter to ensure a leak-proof fit to both the pump and the sampler outlet.

### 6.1.6 Vapour trap.

Use a vapour trap with an internal diameter of 17 mm and a length of 140 mm, filled with charcoal (with a medium particle size < 3 mm), between the sampler and the sampling pump.

## 6.2 Flow meter.

Use a portable flow meter capable of measuring the appropriate flow rate with acceptable accuracy.

## 6.3 Liquid chromatographic system.

In this method, a micro-LC system is used in order to improve the sensitivity, to minimize the maintenance on the MS and to minimize the consumption of the mobile phase. The micro-LC system is described in the following paragraphs. If desired, this system can be replaced by a conventional LC-system.

### 6.3.1 Autosampler.

#### 6.3.1.1 LC-MS.

On-column focusing is performed by partially filled loops (typically 10 µl total volume) of 2 µl loop injections between 4+4 µl of 50/30/20 water/acetonitrile/methanol. Any commercially available autosampler capable of making partially filled loop injections and making sample injections of acceptable accuracy and precision can be used.

#### 6.3.1.2 LC-CLND.

On-column focusing is performed by partially filled loops (typically 10 µl total volume) of 2 µl loop injections between 4+4 µl of 50/50 methanol/water. Any commercially available autosampler capable of making partially filled loop injections and making sample injections of acceptable accuracy and precision can be used.

#### 6.3.2 Pumping system (LC-MS and LC-CLND).

An HPLC-pump capable of gradient elution with a flow rate of 100 µL $min^{-1}$ is required.

#### 6.3.3 Analytical column (LC-MS and LC-CLND).

An HPLC-column capable of separating the different analytes is required.

EXAMPLE An example of a suitable column is a PepMap® $C_{18}$ [1] (50 × 1,0 mm with 3 µm particles).

#### 6.3.4 Tubing.

Use short (< 40 cm) tubing with a small internal diameter (typically ID < 0,1 mm).

#### 6.3.5 Detectors.

##### 6.3.5.1 LC-MS.

Any modern MS equipped with a robust and stable electrospray interface will have the necessary performance. The MS detection is performed with atmospheric pressure ionization, monitoring positive ions. For quantification, selected ions are monitored. Full spectra are obtained using continuum scans (typically 50-1 500 amu) for identification of unknown analytes. If wanted, a UV-detector can be used in series, prior to the MS. The UV-detector needs to be equipped with a micro flow cell (typically 300 nl) to minimize peak band broadening.

##### 6.3.5.2 LC-CLND.

Use a detector which is specific for bound nitrogen.

## 7 Air sampling

### 7.1 Pre-sampling laboratory preparation

#### 7.1.1 Cleaning of sampling equipment

Impingers should be taken apart and soaked in alkaline cleaning solution for a minimum of 2 h. The upper part must be rinsed with an alkaline cleaning solution, pure water and finally deionized water. If the nozzle is clogged, place it in an ultrasonic bath, and then continue with the cleaning procedure. The lower part should be cleaned in a laboratory dishwasher. Both parts should be dried in an oven.

The filter cassettes and the gaskets should be immersed in ethanol in a glass beaker, sonicated for at least 15 min, rinsed with deionized water and dried in an oven.

#### 7.1.2 Preparation of reagent solution and extraction solution tubes

Prepare test tubes containing 10 ml of 0,01 mol·$l^{-1}$ DBA as the reagent solution for the impingers. If the gas phase and the particulate phase are to be collected separately, prepare test tubes containing 10 ml of 0,01 mol $l^{-1}$ DBA as extraction solution tubes for the filters.



1) PepMap® is an example of a suitable product available commercially. This information is given for the convenience of users of this part of ISO 17734 and does not constitute an endorsement by ISO of this product.

## 7.2 Pre-sampling field preparations

Assemble the sampling system with the filter cassette containing the glass fibre filter coupled to the outlet of the impinger. Transfer the reagent solution to the impinger.

Calibrate the pumps with the impinger-filter sampling system in line, using a portable flow meter. Fill the impinger with the appropriate amount of reagent solution during calibration. The sampling rate should be 1 l $min^{-1}$.

## 7.3 Collection of air samples

### 7.3.1 Sampling

In order to relate measurement results to occupational exposure limit values, take samples in the worker's breathing zone. In order to illustrate risks of being exposed, take stationary samples at every place at the worksite where isocyanates can be emitted into the air. It is also important to include operations that are not frequently performed, for example repair and maintenance. Differences in materials and batch-to-batch variations are factors that also should be taken into account when sampling. A sufficient number of samples must be collected in order to make a representative exposure assessment.

### 7.3.2 Impinger-filter sampling

Position the sampling system, either attached to the worker with the inlet in the breathing zone for personal sampling, or stationary for area sampling. Connect the pump to the sampling system, and place a charcoal vapour trap in line between the pump and the sampling system in order to protect the pump from the solvent vapour. Make sure that the equipment does not disturb the work operation, and that the impinger can be held in a vertical position during the whole sampling period.

When ready to begin sampling, switch on the pump. Record the time of sampling. At the end of the sampling period, measure the flow and turn off the pump. Transfer the impinger solution to a test tube and immerse the glass fibre filter into either the sampling solution or an extraction solution tube using tweezers. If the filter is transferred to an extraction solution, it is possible to determine the amount of isocyanates in the particulate phase that passes through the impinger (i.e. particles approx. 0,01 µm to 1,5 µm), separately from the gas phase and large particles (>1,5 µm) sampled in the impinger. For an illustration of the sampling procedure, see Figure 2. The volume drawn through the sampler is calculated from the sampling time and the average sampling flow. The total sampling time is limited (about 30 min), unless the reagent solution is refilled during sampling.

## 7.4 Blanks

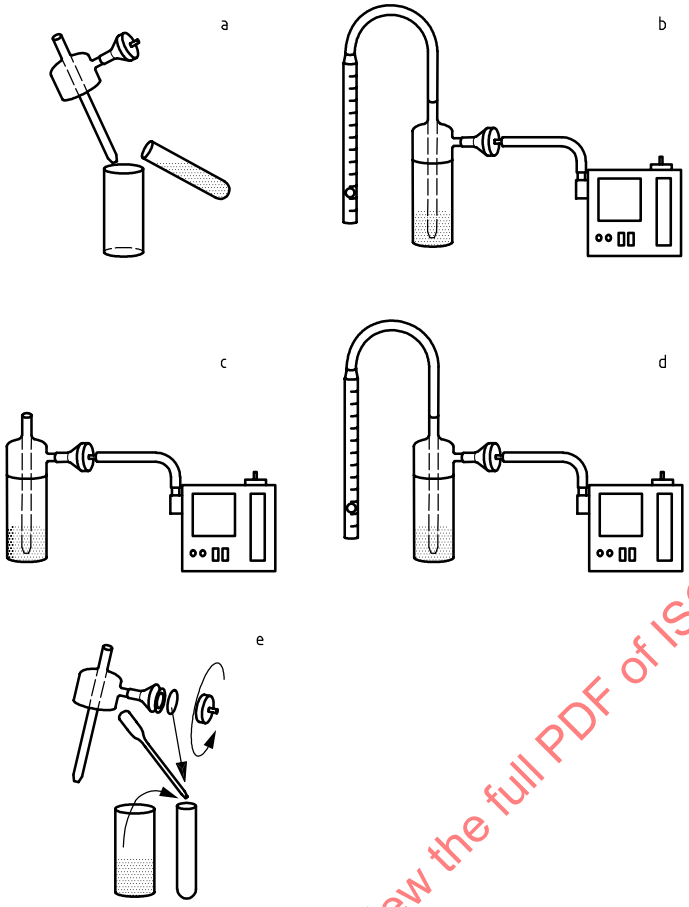
From every series of samples, there should be an appropriate number of field blanks collected. Field blanks are samples that have been handled exactly like the other samples out in the field, except that no air has been drawn through.

## 7.5 Raw material

From each work-site, it is desirable to collect samples of the raw material suspected of emitting amines, aminoisocyanates and isocyanates during the work operation. Collecting and subsequent laboratory testing of materials that are known or are suspected of emitting amines, aminoisocyanates and isocyanates is useful for assessing the exposure. The testing may consist of extraction, heating or other processing of the material, as similar to the original work operation as possible.

## 7.6 Shipment of samples

The test tubes containing the DBA-toluene samples should be shipped in individual plastic cases and preferably kept in an upright position. The sampling solution tubes should be placed well apart from any raw material collected.




a The impinger solution is transferred to the impinger flask.

b The airflow is measured and the sampling pump is calibrated to 1 l/min.

c Air sampling.

d The airflow is measured.

e The impinger solution is transferred to a test tube. The filter is either transferred to the impinger solution tube or to an extraction solution tube.

**Figure 2 — Illustration of the sampling procedure**



## 8 Laboratory sample preparation

### 8.1 Sample sequence

In each sample sequence (typically 50 samples), a number of samples consist of field blanks, two chemical blanks, two internal standard blanks and an appropriate number of calibration standards. Internal standard blanks are reagent solutions from the same batch as the reagent solution used for air sampling spiked with internal standard in the work-up procedure. Chemical blanks are pure toluene with no addition of internal standard in the work-up procedure.

### 8.2 Work-up procedure

For preparation of calibration standards, aliquots of 10 ml toluene solutions, containing 0,01 mol $l^{-1}$ DBA, are spiked with the amine derivatives and the aminoisocyanate derivatives to concentrations appropriate for the calibration curve. For simultaneous isocyanate determination, the isocyanate-DBA derivatives are also added to the standard solutions (see ISO 17734-1).

Upon receiving samples from the field, add deuterium-labelled amine derivatives (internal standard) to the air samples, to the standard solutions, to the field blanks and to the internal standard blanks. For simultaneous isocyanate determination, the deuterium-labelled isocyanate derivatives are also added to the solutions (see ISO 17734-1). Place the samples in an ultrasonic bath for 15 min. If the sample solutions contain filters, place the samples in a centrifuge for 10 min (3 000 r/min). Remove the sample solutions from the filters with a pipette into new test tubes. Carbamate esters are formed by a two-phase derivatisation procedure by the addition of 3 ml of 5 mol $l^{-1}$ NaOH, 10 µl pyridine and 50 µl ethyl chloroformate. The samples are shaken for 15 min and the organic phase is separated and evaporated to dryness. The residues are dissolved in 0,5 ml acetonitrile and placed in an ultrasonic bath for 15 min.

## 9 Instrumental settings

### 9.1 HPLC program (LC-MS)

For simultaneous determination of amine, aminoisocyanate and isocyanate derivatives, the following mobile phase composition can be used:

— Flow rate: 100 µl $min^{-1}$;

— 0 - 20 min: Linear gradient from 40 % mobile phase B to 80 % mobile phase B;

— 20 - 25 min: Re-equilibrate at 40 % mobile phase B.

If single or a few derivatives are to be determined, isocratic elution or gradient elution with appropriate mobile phase composition can be performed.

### 9.2 HPLC program (LC-chemiluminescent nitrogen detector) (LC-CLND)

— Flow rate: 100 µl $min^{-1}$;

— 0 - 20 min: Linear gradient from 40 % mobile phase D to 100 % mobile phase D;

— 20 - 25 min: Re-equilibrate at 40 % mobile phase D.

Depending on the properties of the analytes in the sample, stronger, weaker or isocratic elution can be used.

### 9.3 Mass spectrometer

Settings of the MS depend greatly on which type of instrument that is used. Optimization is normally performed by the introduction of flow at 100 µl $min^{-1}$ of mobile phase containing aromatic and aliphatic amine and aminoisocyanate derivatives. Optimal settings vary for the analytes and the ions to be monitored. Practical settings are not the optimum for all of the compounds to be studied.

For quantification, selected ions are monitored, e.g. the molecular ion $[MH]^+$, but other typical ions can be used:

For the DBA derivatives, typical formed ions are $[MH]^+$, $[(DBA)H]^+$ ($m/z$ = 130), $[(DBA)CO]^+$ ($m/z$ = 156), $[MH-129]^+$ and $[MNa]^+$.

Typical ions for the amine derivatives are $[MH]^+$, $[MNa]^+$, $[M-46]^+$ and $[M-92]^+$.

Typical ions for the aminoisocyanate derivatives are $[MH]^+$, $[MNa]^+$, $[M-46]^+$, $[M-129]^+$, $[(DBA)H]^+$ ($m/z$ = 130) and $[(DBA)CO]^+$ ($m/z$ = 156) (see Example 4 in Annex B).

For identification of unknown isocyanates, full spectra are obtained using continuous scans (typically 50-1 500 amu).

# 10 Data handling

## 10.1 Identification

For identification, the retention times of sample peaks in the selected ion chromatograms are compared to the standards and the internal standards.

## 10.2 Calibration curves

The peak areas of the amine and the aminoisocyanate derivatives and the internal standard are measured, and the ratio is calculated. The ratio versus the concentration is plotted. The coefficient of correlation should be better than 0,98.

## 10.3 Quantification

Quantification is accomplished by comparing the area ratio of the sample peak and internal standard to the calibration plot.

# 11 Determination of performance characteristics

## 11.1 Introduction

The measurement of the concentration of amines, aminoisocyanates and isocyanates in workplace air has associated with it an uncertainty that may be expressed as overall uncertainty, see EN 482 [11] or Reference [12]. Thus, an uncertainty assessment has to be performed according to one or other of these definitions of uncertainty. In both cases, this consists of the determination of uncertainty contributions evaluated by means of laboratory and simulated field tests or from existing information. The values obtained of the measurement uncertainty may then be compared with pre-set criteria, for example those in EN 482 [11], or defined in national or international legislation.

## 11.2 Relevant uncertainty contributions and criteria

| Uncertainty contribution | Quantity | Subclause | Criterion |
|---|---|---|---|
| *Sample volume* | $V_{sam}$ | 11.3.2 | |
| Sample flow – calibration | $q_{cal}$ | | Relative uncertainty < 2 % |
| Sample flow – variation | $\Delta q$ | | < 5 % |
| Sampling time | $t$ | | Relative uncertainty < 0,1 % |
| Knowledge of temperature during sampling | $T$ | | Relative uncertainty < 4 % |
| Knowledge of pressure during sampling | $p$ | | Relative uncertainty < 2 % |
| *Analyte mass* | $m_{sam}$ | 11.3.3 | |
| Analyte stability during storage | $k_{AS}$ | | No significant difference between results of analysis of samples before and after storage |
| Reaction/ Extraction efficiency | $E_{RE}$ | | > 90 % at the limit value with a relative uncertainty of < 3 % |
| Mass of isocyanate in calibration standards | $m_{CS}$ | | Relative uncertainty < 2 % |
| Calibration lack-of-fit | LOF | | Relative residuals over the calibration range < 3 %; at the limit value < 2 % |
| Response drift between calibrations | $D_R$ | | < 3 % |
| Analytical precision | $r$ | | < 1 % |
| Selectivity | $s$ | | Resolution factor > 1 |
| *Blank level* | $m_{BL}$ | 11.3.4 | < 50 ng with a relative uncertainty of < 5 % |
| *Between-laboratory variations* | bl | 11.3.5 | Relative uncertainty < 7,5 % |



## 11.3 Assessment of performance characteristics, following the detailed approach in Reference [12]

### 11.3.1 Collection efficiency – relative to particle size distribution

For a complete description of the performance requirements and tests to be performed, see Reference [12].

### 11.3.2 Air sampling

#### 11.3.2.1 Sampling volume

The sampled volume of air is calculated on the basis of measuring the sample flow rate before and after sampling as specified in ISO 16200-1:

$$V_{sam} = \frac{(q_{start} + q_{end})}{2} \cdot t \qquad (1)$$

where

$q_{start}$ is the sample flow rate at the beginning of the sampling period (usually in millilitres per minute);

$q_{end}$ is the sample flow rate at the end of the sampling period;

$t$ is the sampling time (in minutes).

The uncertainty in the volume of air sampled is built up of contributions from

a) the measurements of the flow rates before and after sampling,

b) the measurement of the sampling time, and

c) variations in the flow rate during the sampling period,

and may be expressed as

$$\frac{u^2(V_{sam})}{V_{sam}^2} = \frac{u^2(q_{start}) + u^2(q_{end})}{(q_{start} + q_{end})^2} + \frac{u_t^2}{t^2} + \frac{u_{var,q}^2}{\left[\frac{(q_{start} + q_{end})}{2}\right]^2} \quad (2)$$

where the last term represents the uncertainty contribution due to flow rate variations during sampling.

#### 11.3.2.2 Sampling time

The sampling time, $t$, can be measured to within ± 0,5 min. For a sampling time of 8 h, the relative uncertainty due to the measurement of $t$ is about 0,1 % and is negligible.

#### 11.3.2.3 Variations in flow rate during sampling

The flow rate during sampling is unknown. The uncertainty due to variations in the flow rate during sampling can be estimated by assuming a uniform distribution as

$$u_{var,q}^2 = \frac{(q_{start} - q_{end})^2}{12} \quad (3)$$

#### 11.3.2.4 Conversion of sample volume to STP

For the conversion of concentrations to STP, knowledge is required of the actual mean temperature and pressure during sampling. Uncertainties in values of $T$ and $p$ used for conversion may be obtained from:

a) actual measurements, taking into account the uncertainty in the calibration of temperature and pressure sensors used as

$$u^2 = u_{cal}^2 + \frac{s_{meas}^2}{n} \quad (4)$$

where

$u_{cal}$ is the uncertainty due to calibration of the sensor;

$s_{meas}$ is the standard deviation of the temperature/pressure measurements;

$n$ is the number of temperature/pressure measurements; and

b) knowledge of extremes of temperature and pressure during sampling, assuming these to be uniformly distributed.

For example, if the temperature extremes are known to be $T_{min}$ and $T_{max}$, the uncertainty in $T$ may be calculated from

$$u_T^2 = u_{cal}^2 + \frac{(T_{max} - T_{min})^2}{12} \quad (5)$$

Generally, the first term will be negligible compared to the second.

#### 11.3.2.5 Combined uncertainty of sample volume

The above uncertainty contributions are combined to give the uncertainty in the sample volume converted to SPT as

$$\frac{u^2(V_{sam,\,SPT})}{V_{sam,\,SPT}^2} = \frac{u^2(V_{sam})}{V_{sam}^2} + \frac{u^2(T)}{\bar{T}^2} + \frac{u^2(p)}{\bar{p}^2} \quad (6)$$

where

$\bar{T}$ is the mean temperature during sampling; and

$\bar{p}$ is the mean pressure during sampling.

### 11.3.3 Analysis

#### 11.3.3.1 Sampled mass

The mass of isocyanate in the air samples may be expressed as:

$$m_{sam} = \frac{m_{anal}}{E_{coll} \cdot \Delta S \cdot k_{AS} \cdot E_{RE}} \quad (7)$$

where

$E_{coll}$ is the collection efficiency;

$\Delta S$ is the sampler variability;

$k_{AS}$ is the analyte stability in the sample;

$E_{RE}$ is the reaction/extraction efficiency;

$m_{anal}$ is the uncorrected analytical mass of isocyanate in the analytical sample.

#### 11.3.3.2 Analyte stability

The analyte stability shall be experimentally established for storage under conditions (time, temperature, environment) typical to the individual laboratory. Tests shall be performed at an isocyanate level corresponding to a concentration equivalent to the Limit value.

At time $t = 0$ and time $t$, $n$ samples each shall be analysed under repeatability conditions ($n \geqslant 6$). For both times, the samples shall be randomly picked from a batch of representative samples in order to minimize possible systematic concentration differences. As a test of (in)stability, a $t$-test will be performed (95 % confidence, two-sided). The uncertainty of the stability determination consists of contributions from

a) desorption (random part of desorption efficiency),

b) calibration (random part of calibration),

c) analytical precision, and

d) inhomogeneity of the sample batch.

As such, the contribution of the determination of $k_{AS}$ will already be incorporated in other contributions and needs not to be taken into account.

#### 11.3.3.3 Reaction/Extraction efficiency

The reaction/extraction efficiency of isocyanate and its uncertainty are typically obtained from replicate measurements on certified reference materials (CRM) of the isocyanate or of its reaction product(s). The uncertainty due to incomplete reaction/extraction for the isocyanate level corresponding to the limit value is calculated from contributions of

a) the uncertainty in the concentration of the CRM,

b) the standard deviation of the mean recovery,

c) the bias between the mass of isocyanate in the CRM and the mean mass of isocyanate determined as:

$$\frac{u_{E_{RE}}^2}{E_{RE}^2} = \frac{u_{m_{CRM}}^2}{m_{CRM}^2} + \frac{s^2(\bar{m}_{DE})}{\bar{m}_{DE}^2} + \frac{(\bar{m}_{DE} - m_{CRM})^2}{m_{CRM}^2} \quad (8)$$

where

$m_{CRM}$ is the certified mass of isocyanate in CRM;

$u_{m_{CRM}}$ is the uncertainty in the certified mass of isocyanate in CRM;

$\bar{m}_{DE}$ is the mean mass of isocyanate determined;

$s(\bar{m}_{DE})$ is the standard deviation of the mean of the replicate measurement results.

The latter term, representing the uncertainty due to a significant bias between certified and determined mass, may be ignored if

a) the bias is statistically insignificant at the 95 % level,

b) a correction is applied for the bias.

If a CRM is not available, the material with the highest metrological quality available should be used.

#### 11.3.3.4 Uncorrected analytical mass of compound

The uncertainty in the uncorrected analytical mass of a compound is determined by

a) the uncertainty in the concentrations of the calibration standards used,

b) the lack-of-fit of the calibration function,

c) drift of detector response between calibrations,

d) the precision of the analysis,

e) the selectivity of the chromatographic system.

#### 11.3.3.5 Calibration standards

The uncertainty of the concentration of isocyanate in the calibration standards used depends on the type of calibration standard used.

For calibration standards consisting of solutions in toluene or acetonitrile, the uncertainty will be built up of contributions from

— the purity of isocyanate; this is generally known from manufacturer's specifications as a minimum purity $p$, e.g.

  — $p = 99$ %, the relative uncertainty due to impurity is given by $(100 - p)$ %; or

  — $p \geqslant 99$ %, the relative uncertainty can be estimated assuming a uniform distribution as

$$u_{\text{pur}}^2 = \frac{(100 - p)^2}{12} \tag{9}$$

— the uncertainties in the weighings of compounds and solutions, i.e. the uncertainty of the balance used.

The latter contribution is generally expressed for differential weighings as

$$u_{\text{weigh}}^2 = 2u_{\text{bal}}^2 \tag{10}$$

where $u_{\text{bal}}$ is the uncertainty of the balance used.

If this method is used for the determination of other compounds besides isocyanate, the concentration of isocyanate in the chemicals used and its uncertainty shall be established and used in the above uncertainty assessment.

#### 11.3.3.6 Lack-of-fit of calibration function

The uncertainty due to lack-of-fit of the calibration function can be calculated for the relevant concentration (corresponding to a mass of isocyanate sampled at the limit value) from residuals of a calibration function obtained by a least-squares linear regression weighted in the concentration of isocyanate in the calibration standard as

$$u_{\text{LOF}}^2 = \frac{\left(m_{\text{regr}} - m_{\text{std}}\right)^2}{m_{\text{std}}^2} = \rho^2 \tag{11}$$

where

$m_{\text{regr}}$ is the mass of isocyanate calculated from the regression equation at the level of the calibration standard corresponding closest to the mass of isocyanate representing a sample at the limit value;

$m_{\text{std}}$ is the mass of isocyanate present in the corresponding calibration standard;

$\rho$ is the relative residual for the particular concentration level.

NOTE The lack of fit of the calibration function will contribute to the uncertainty due to incomplete extraction or reaction if the latter's efficiency is significantly different from 1. In that case – irrespective of whether or not a correction for incomplete reaction/extraction is applied – the uncertainty due to lack of fit of the calibration function needs not to be taken into account in the uncertainty assessment.

#### 11.3.3.7 Drift in detector response

The uncertainty due to response drift, $D_R$, can be estimated from data on the relative differences in responses between subsequent calibrations as

$$u_{D_R}^2 = \frac{\left(r_n - r_{n\text{-}1}\right)^2}{12\left(\frac{r_n + r_{n\text{-}1}}{2}\right)^2} \tag{12}$$

where

$r_n$ is the detector response for a calibration standard corresponding closest to the mass of isocyanate representing a sample at the limit value; and

$n$ is the number of replicate analyses.

#### 11.3.3.8 Precision of the analysis

The uncertainty due to the (im)precision of the analysis is determined by analysis under repeatability conditions of calibration standards of the same composition; a minimum of 6 replicate analyses shall be performed. The uncertainty is then calculated as

$$u_r^2 = \frac{s_{\text{anal}}^2}{r^2} \tag{13}$$

where

$s_{\text{anal}}$ is the standard deviation of the replicate responses;

$r$ is the mean response.

In the uncertainty assessment, this contribution is already incorporated in contributions from the determination of desorption efficiency and needs not be taken into account.

#### 11.3.3.9 Analytical selectivity

The separation system used (liquid chromatographic column, gradient program) shall be optimized in order to minimize uncertainty due to (unnoticed) co-elution of potential interferents.

The resolution, $R$, of the liquid chromatographic system used – given by Equation (14) - shall be better than 1. In that case, the maximum uncertainty due to co-elution is 2,5 %. The typical uncertainty contribution will then be ± 0,7 %.

$$R = \frac{\Delta t_r}{0{,}85\left(w_B + w_I\right)} \tag{14}$$

where

$\Delta t_r$ is the difference in retention time of isocyanate and interferent (in seconds);

$w_B$ is the peak width at half height of the peak (in seconds), with subscript B referring to isocyanate;

$w_I$ is the peak width at half height of the peak (in seconds), with subscript I referring to interferent.

#### 11.3.3.10 Combined uncertainty in the analytical mass of isocyanate

The above contributions are combined to give the uncertainty of the analytical mass of isocyanate excluding the uncertainty due to imprecision as $m_{\text{std}}$.

$$\frac{u^2(m_{\text{anal}})}{m^2_{\text{anal}}} = \frac{u^2_{\text{std}}}{m^2_{\text{std}}} + u^2_{\text{LOF}} + u^2_{D_{\text{R}}} + u^2_{\text{sel}} \tag{15}$$

#### 11.3.3.11 Combined uncertainty in the sampled mass of isocyanate

The contributions given in 11.3.3.4 to 11.3.3.8 and 11.3.3.10 are combined to give the uncertainty of the mass isocyanate in the air sample as

$$\frac{u^2(m_{\text{sam}})}{m^2_{\text{sam}}} = \frac{u^2(m_{\text{anal}})}{m^2_{\text{anal}}} + \frac{u^2_{E_{\text{RE}}}}{E^2_{\text{RE}}} \tag{16}$$

### 11.3.4 Mass of compound in sample blank

The mass of isocyanate in a sample blank is determined by analysis under repeatability conditions of a series of sample blanks; a minimum of 6 replicate analyses shall be performed. The uncertainty is then calculated using the slope of the calibration function extrapolated to the blank response level as

$$u^2(m_{\text{BL}}) = \frac{s^2_{\text{BL}}}{b_{\text{BL}}} \tag{17}$$

where

$s_{\text{BL}}$ is the standard deviation of the replicate analytical results;

$n$ is the number of replicate analyses;

$b_{\text{BL}}$ is the slope of the calibration function at the blank response level.

If the blank response is below 3 times the noise level of the detector at the retention time of isocyanate, then the blank level and its uncertainty shall be calculated from the detector noise level using the slope of the calibration function extrapolated to zero response assuming a uniform distribution as

$$m_{\text{BL}} = \frac{3r_0}{2b_0} \tag{18}$$

$$u^2(m_{\text{BL}}) = \frac{9r_0^2}{12} \tag{19}$$

where

$r_0$ is the noise level;

$b_0$ is the slope of calibration function at zero response.

### 11.3.5 Between-laboratory uncertainty contributions

The procedures described above are not restrictive but allow for possible variations in approaches between laboratories. The resulting additional uncertainty contributions can be quantified by performing interlaboratory comparisons involving

— the complete measurement procedure inclusive of sampling, and

— the analytical part of the measurement procedure.

Interlaboratory comparisons shall be organized in accordance with ISO 5725-2 using samples of sufficient homogeneity to assure that the contribution to the between-laboratory uncertainty due to inhomogeneity is negligible. In practice, an uncertainty due to inhomogeneity of $< 2$ % will usually be sufficient.

### 11.3.6 Combined uncertainty

The combined uncertainty of the isocyanate concentration in the air sampled is obtained by combination of contributions given in Equations (6), (14), (18), (19) adding the between-laboratory uncertainty (if considered appropriate) as

$$u_{\mathrm{c}}^2\left(C_{\mathrm{m}}\right) = u^2\left(m_{\mathrm{sam}}\right) + u^2\left(m_{\mathrm{BL}}\right) + u^2\left(V_{\mathrm{sam,\,SPT}}\right) + u_{\mathrm{bl}}^2 \tag{20}$$

where $u_{\mathrm{bl}}$ is the between-laboratory uncertainty contribution.

### 11.3.7 Expanded uncertainty

The expanded uncertainty in $C$ at the 95 % confidence level is obtained by multiplying $u_{\mathrm{c}}(C_{\mathrm{m}})$ with a coverage factor of 2.

### 11.3.8 Uncertainty from performance criteria

When combining the uncertainties specified for the performance characteristics (12.2), a worst-case situation will result. The resulting combined relative uncertainty, calculated as described in 11.3.6 will be about 10 %. The expanded uncertainty will be about 20 %.

# Annex A
(informative)

# Performance characteristics

## A.1 Published uncertainty estimates

The following data on uncertainty contributions has been obtained from literature, surveys conducted in the course of the validation of the currently described method and estimations.

| Uncertainty contribution | Uncertainty (%) | Comments |
|---|---|---|
| *Sample volume* | 4 | For a 15 min air sample at a flow rate of 1 l $min^{-1}$ |
| Sample flow – calibration | 2 | Calibration instrument specification |
| Sample flow – variation | 3 | Estimation |
| Sampling time | 0,2 | |
| Temperature during sampling | 1 | Estimation |
| Pressure during sampling | 1 | Estimation |
| *Analyte mass (weighing)*<br>*Analyte mass (CLND)* | 3<br>11 | The amine content in calibration standards is determined by weighing of compounds. The aminoisocyanate content is determined by LC-CLND |
| Analyte stability during storage | negligible | References [13],[14] and [15] |
| Reaction/ Extraction efficiency | 6 | Estimation, no data is available |
| Mass of amines in calibration standards (weighing)<br>Mass of aminoisocyanates in calibration standards (CLND) | 1<br>10 | The amine content in calibration standards is determined by weighing of compounds. The aminoisocyanate content is determined by LC-CLND [16] |
| Calibration lack-of-fit | 1 | |
| Response drift between calibrations | negligible | Instrumental drift is corrected by using internal standards [6], [17] |
| Analytical precision | 2 | |
| Selectivity | negligible | LC-MS provides highly selective determinations |
| *Blank level* | negligible | |
| *Between-laboratory variations* | 10 | Estimation, no data is available |



## A.2 Combined uncertainty

The combined uncertainty for the amine concentration is estimated to 12 %. The combined uncertainty for aminoisocyanate concentration is estimated to 16 %.

## A.3 Expanded uncertainty

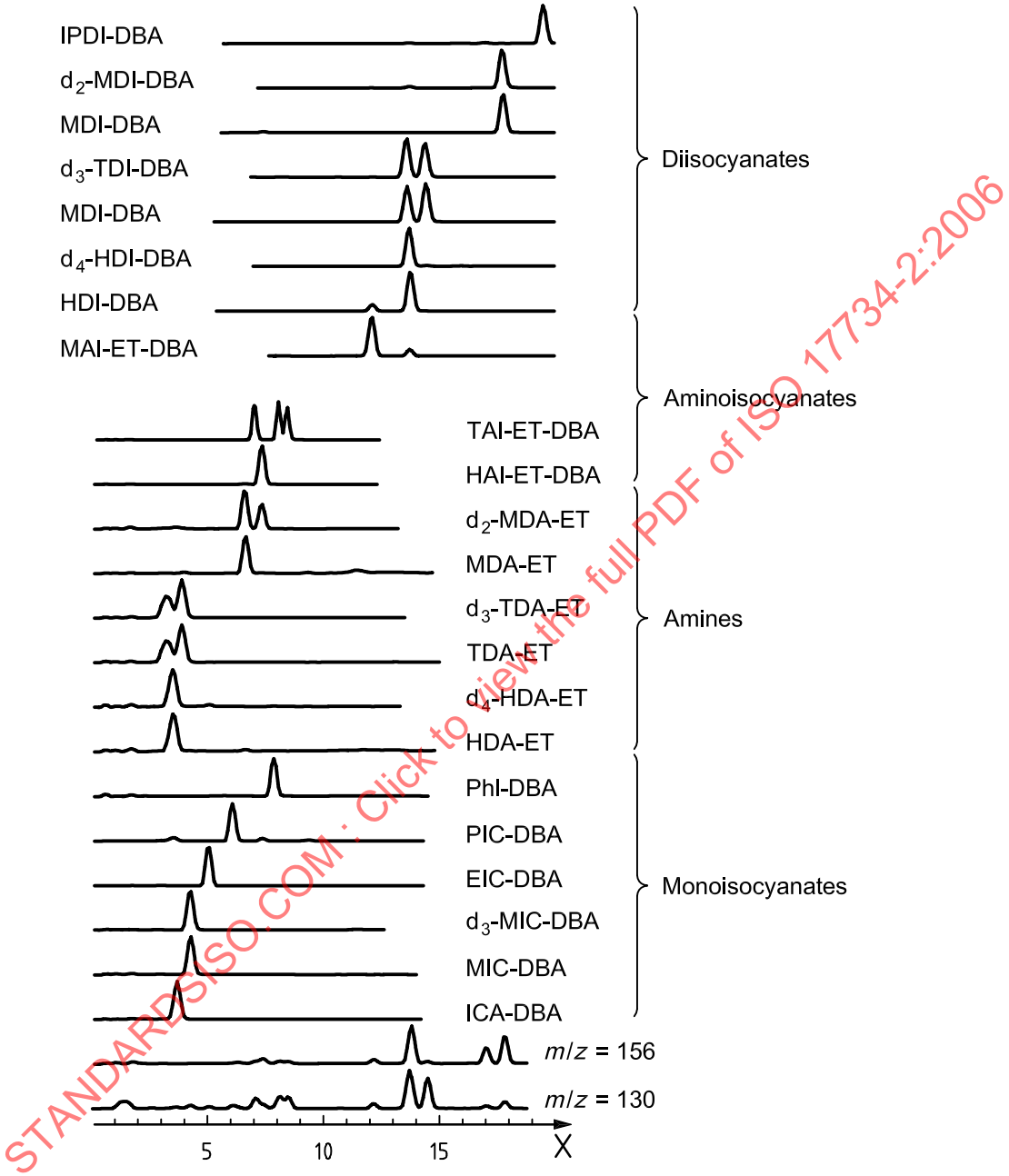
By using a coverage factor of 2, the expanded uncertainty for the amine and aminoisocyanate concentrations is 24 % and 32 %, respectively. There will be an additional uncertainty contribution, so far not accounted for, from the collection efficiency, if collection according to a sampling convention is required.

# Annex B
(informative)

# Examples

## B.1 Example 1: Standard solution

See Figure B.1.






**Key**

X $t_R$, min

SIR monitoring of 22 different molecular ions [$MH^+$] and the $m/z$ = 130 and 156 amu ions was performed. Peak heights in terms of retention time $t_R$ were adjusted to 100 % (arbitrary scale).

**Figure B.1 — LC-MS of a solution containing 0,15 µg $ml^{-1}$ of different isocyanate-DBA, aminoisocyanate-ET-DBA, amine-ET derivatives**