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Workplace atmospheres — Determination of total isocyanate groups in air using 1-(9-anthracenylmethyl)piperazine (MAP) reagent and liquid chromatography

Air des lieux de travail — Dosage des groupements isocyanates totaux dans l'air par réaction avec la 1-(9-anthracénylméthyl)pipérazine (MAP) et par chromatographie en phase liquide

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Foreword

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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 17735 was prepared by Technical Committee ISO/TC 146, *Air quality*, Subcommittee SC 2, *Workplace atmospheres*.

Introduction

This International Standard specifies the use of 1-(9-anthracenylmethyl)piperazine (MAP) to measure monomeric and oligomeric isocyanate species in workplace atmospheres. MAP was designed to improve the reliability of identification of isocyanate species in sample chromatograms and to improve the accuracy of quantification of these species relative to established reagents. The high performance liquid chromatography (HPLC) analysis uses a pH gradient to selectively accelerate the elution of MAP derivatives of oligomeric isocyanates that might be unobservable in an isocratic analysis. The performance of MAP has been compared to other reagents used for total isocyanate analysis (Reference [7]). MAP has been found to react with phenyl isocyanate (used as a model isocyanate) as fast or faster than other reagents commonly used for isocyanate analysis. The UV response of MAP derivatives is comparable to that of 9-(methylaminomethyl)anthracene (MAMA) derivatives and considerably greater than other commonly used reagents [approximately three times greater than 1-(2-methoxyphenyl)piperazine (1-2MP) derivatives of aromatic isocyanates and 14 times greater than 1-2MP derivatives of aliphatic isocyanates]. The compound-to-compound variability of UV response per isocyanate group for MAP derivatives is smaller than the variability of any other commonly used reagent/detector combination (the coefficient of variation is 3,5 % for five model isocyanates). This results in accurate quantification of detectable non-monomeric isocyante species based on a calibration curve generated from analysing standards of monomeric species. The monomeric species used for calibration is generally the one associated with the product being analysed, but others could be used due to the very small compound-to-compound response variability of the MAP derivatives. The intensity of fluorescence response of MAP derivatives is comparable to that of MAMA derivatives and considerably greater than other reagents (e.g. approximately 30 times more intense than that of tryptamine derivatives). The compound-to-compound variability in fluorescence response has been found to be smaller than that of MAMA derivatives but larger than that of tryptamine derivatives (MAMA = 59 % coefficient of variation, MAP = 33 % coefficient of variation, and tryptamine = 16 % coefficient of variation for 5 model isocyanates). The compound-to-compound fluorescence variability of MAP derivatives is considered too great for accurate quantification of non-monomeric isocyanate species based on calibration with monomer standards. However, the sensitivity of the fluorescence detection makes it especially suitable for quantification of low levels of monomer, and the selectivity is very useful to designate an unidentified HPLC peak as a MAP derivative. MAP derivatives also give a strong response by electrochemical detection. The pH gradient used in the HPLC analysis selectively accelerates the elution of amines (MAP derivatives are amines), and is very strong (it accelerates MDI more than 100-fold). Re-equilibration to initial conditions is almost immediate. Many oligomeric species can be measured in the 30 min MAP analysis that may be unobservable in a much longer isocratic analysis.

MAP has been used in several studies comparing it side-by-side with other methods. Reference [8] found MAP impingers and NIOSH 5521 impingers (comparable to MDHS 25) to give comparable results in spray painting environments. Reference [8] used MAP reagent, but the pH gradient was not employed. Reference [9] compared MAP impingers with several other impinger methods (NIOSH 5521 and NIOSH 5522) and the double filter method. The average MAP oligomer value was substantially higher than the other impinger methods and slightly higher than the double filter method. The pH gradient was used in these MAP analyses.

The MAP method is currently available as NIOSH Method 5525 (Reference [11]). The performance characteristics of the method have been evaluated in Reference [12].

Workplace atmospheres — Determination of total isocyanate groups in air using 1-(9-anthracenylmethyl)piperazine (MAP) reagent and liquid chromatography

1 Scope

This International Standard gives general guidance for the sampling and analysis of airborne organic isocyanates in workplace air.

This International Standard is appropriate for a wide range of organic compounds containing isocyanate groups, including monofunctional isocyanates (e.g. phenyl isocyanate), diisocyanate monomers (e.g. 1,6-hexamethylene diisocyanate (HDI), toluene diisocyanate (TDI), 4,4'-diphenylmethane diisocyanate (MDI), and isophorone diisocyanate (IPDI), prepolymers (e.g. the biuret and isocyanurate of HDI), as well as intermediate products formed during production or thermal breakdown of polyurethane.

In mixed systems of HDI and IPDI products, it is impossible to identify and quantify low levels of IPDI monomer using this International Standard, due to coelution of IPDI monomer with HDI-uretidinedione.

The useful range of the method, expressed in moles of isocyanate group per species per sample, is approximately 1×10^{-10} to 2×10^{-7}.

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 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*

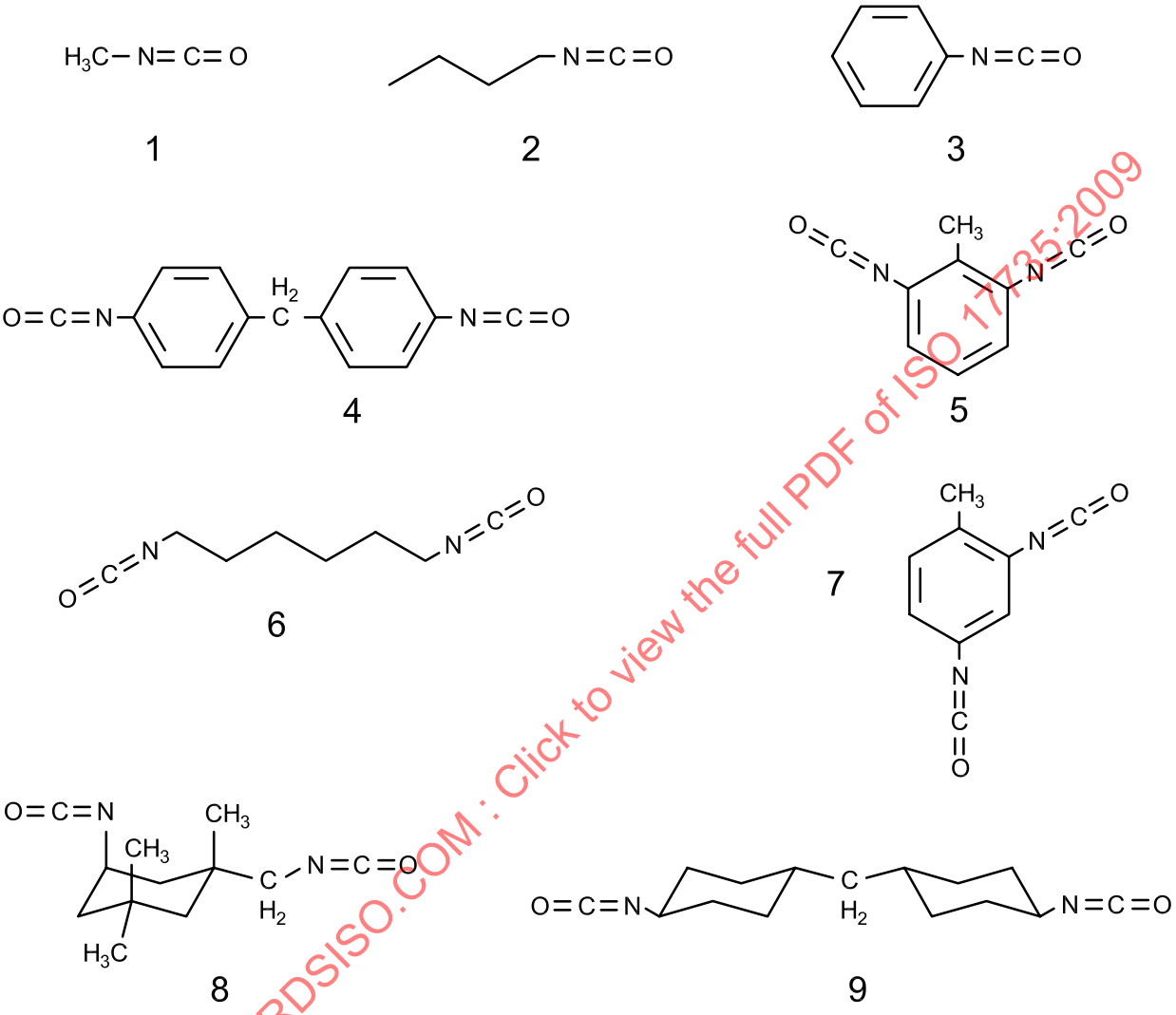
ISO 16200-1, *Workplace air quality — Sampling and analysis of volatile organic compounds by solvent desorption/gas chromatography — Part 1: Pumped sampling method*

3 Principle

A measured volume of air is drawn through either an impinger containing a solution of 1-(9-anthracenylmethyl)piperazine (MAP), a filter impregnated with MAP, or a sampling train consisting of an impinger followed by an impregnated filter. The choice of sampler depends on the chemical and physical characteristics of the airborne isocyanate (Reference [13]). If an impinger is used, the solution is subjected to solid-phase extraction (SPE) and the eluate is concentrated and analysed by reverse phase high performance liquid chromatography (HPLC) with ultraviolet (UV) absorbance and fluorescence (FL) detection in series. If an impregnated filter is used for sampling, it is extracted with solvent either in the field after completion of sampling or in the laboratory. Waiting to extract the filter until after the sample has been received by the analytical laboratory is acceptable only for analysis of isocyanates collected as vapour. This solution is filtered and analysed by HPLC/UV/FL. Isocyanate-derived peaks are identified based on their UV and FL responses and by comparison with the chromatogram of a derivatised bulk isocyanate product if available. Quantification of compounds for which analytical standards are available (generally monomers) is achieved by comparison

of the FL peak height of the sample peak with the FL peak height of standard matching solutions. Quantification of compounds for which analytical standards are not available is achieved by comparison of the UV area of the sample peak with the UV area of the appropriate monomer standard (i.e. the monomer from which the isocyanate product is derived).

Structures of some common diisocyanate monomers are shown in Figure 1.



Key

1 methyl isocyanate
2 butyl isocyanate
3 phenyl isocyanate
4 4,4'-MDI
5 2,6-TDI
6 HDI
7 2,4-TDI
8 IPDI
9 HMDI

Figure 1 — Structures of some common isocyanates

4 Reagents and materials

CAUTION — Observe appropriate safety precautions when preparing reagents. Carry out preparations under a fume hood to avoid exposure to solvents, isocyanates or other volatile reagents. Wear nitrile gloves when manipulating reagents and solvents.

During the analysis, unless otherwise stated, use only reagents of HPLC grade or better, and water of HPLC grade.

4.1 MAP reagent

MAP is prepared by the reaction of 9-(chloromethyl)anthracene with piperazine as shown in Figure 2.

The procedure using HPLC grade solvents is as follows.

Dissolve 10,8 mmol (2,47 g) of 9-(chloromethyl)anthracene (98 % mass fraction) in 25 ml methylene chloride. Place this solution in a dropping funnel.

Dissolve 54,4 mmol (4,69 g) of piperazine (99 % mass fraction) and 21,8 mmol (3,04 ml) of triethylamine (99,5 % mass fraction) in 37 ml methylene chloride. Place this solution in a 250 ml 2-necked round-bottomed flask with a magnetic stirring bar.

While stirring this solution, add the 9-(chloromethyl)anthracene solution dropwise over a 30 min period. Rinse down the dropping funnel with an additional 10 ml of methylene chloride. Allow the reaction to continue while stirring for at least 2 h.

Using a separating funnel, wash the reaction mixture three times with 130 ml water by shaking vigorously for 1 min. Discard the emulsion that forms after the first wash, which contains primarily an impurity and not MAP. Discard the aqueous washings.

Place the washed MAP solution in a weighed round-bottomed flask. Allow the methylene chloride to evaporate under a steady stream of nitrogen. Weigh the flask with the residue to obtain an approximate yield. This crude MAP can be safely stored in a freezer until further purification.

MAP is purified by column chromatography followed by sublimation. Using a glass chromatography column of internal diameter approximately 50 mm, add a slurry of silica gel in toluene until the silica gel bed is approximately 80 mm deep. Wash the sides of the column down with toluene and allow the toluene to run through the column until the toluene is even with the silica gel surface.

Dissolve the crude MAP in 80 ml of toluene. Sonicate the mixture for 5 min and filter through filter paper. Save the filtrate. Resuspend the residue in 20 ml toluene, sonicate for 5 min, and filter through filter paper. Discard the residue. Combine the filtrates and carefully load them onto the top of the silica gel bed. Pass an additional bed volume of toluene through the column. Discard the toluene eluate.

Begin to elute with ethyl acetate. Begin collecting 20 ml fractions in disposable vials with caps lined with polytetrafluoroethylene (PTFE). Monitor the fractions by spotting 1 µl of each on a thin layer chromatography (TLC) sheet (see below) and viewing the intensity of the spot under UV light after the solvent has evaporated. This procedure indicates the presence of compounds in the fraction, which may or may not be MAP. Elute with ethyl acetate until the yellow colour has been eluted, which requires about 400 ml ethyl acetate. The MAP should be completely retained on the column at this point. After elution of the yellow colour, begin eluting with methanol, which requires 1,0 l to 1,5 l methanol.

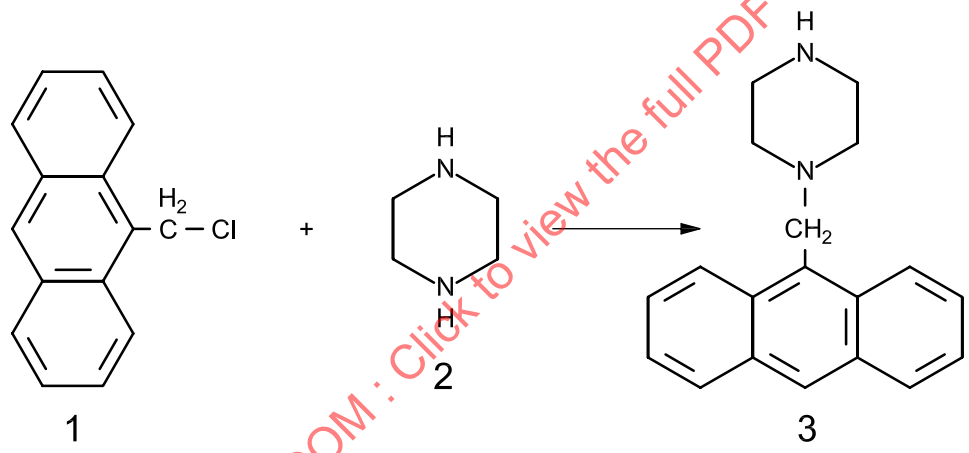
The elution of the MAP can be readily followed by TLC. A portion of the fractions that had given a significant spot on the TLC sheet are analysed by TLC to determine which fractions contain MAP.

The TLC procedure is carried out as follows. Use commercially available TLC sheets coated with silica gel and containing a fluorescent indicator. A portion of a sheet measuring 100 mm × 30 mm is adequate. Spot aliquots of volume 1 µl of several fractions adjacent to each other approximately 15 mm from the bottom of the sheet and place the sheet in a small jar containing methanol 10 mm deep. Cover the jar and allow the

methanol to climb up the sheet to 5 mm from the top. Remove the sheet and allow the methanol saturating the sheet to evaporate. MAP produces a dark spot when viewed under short wavelength UV light which glows under irradiation with long wavelength UV. Identify the MAP spot by comparing the retention factor, R_f, of the aliquot spots with the R_f of a MAP standard.

Based on TLC analyses, combine the fractions containing pure MAP. Weigh a round-bottomed flask to be used for rotary evaporation. Add the combined fractions to the flask, but do not exceed half the volume of the flask at any given time. Heat the evaporator bath to 35 °C to 40 °C and use water aspirator vacuum. After evaporation and trace solvent removal from all of the combined MAP fractions under high vacuum, weigh the flask and its contents to assess the yield.

Purify the MAP powder further by sublimation. Dissolve the MAP in a small volume of methylene chloride (< 20 ml) and transfer the solution to a sublimation apparatus. Allow the methylene chloride to evaporate under a gentle stream of nitrogen, keeping the MAP below the level of the bottom of the coldfinger. When the methylene chloride has evaporated, seal the vessel and reduce the pressure with a vacuum pump to 6,67 mPa[1)] or less. Begin a slow flow of cold water through the coldfinger and place the sublimation vessel in a wax bath maintained at 125 °C to 130 °C. Sublimation takes many hours and may need to continue overnight. Sublimation is complete when there is no further growth of MAP crystals on the coldfinger and the small amount of material remaining at the bottom of the apparatus appears constant. When complete, remove the crystals from the coldfinger with a spatula. A typical yield is 2,236 g (74 % mass fraction). The melting point of the MAP is 146 °C to 147 °C. The purity of MAP as assessed by HPLC is typically 99 % mass fraction.



Key

1 9-(chloromethyl)anthracene
2 piperazine
3 MAP

Figure 2 — Preparation of MAP

4.2 Reagent solutions

4.2.1 Impinger solution

Butyl benzoate, 99 % mass fraction, is used as the impinger solvent. The butyl benzoate is further purified by passing it through a bed of chromatography-grade silica gel. Dissolve MAP in the butyl benzoate to make a 1×10^{-4} mol/l solution (27,6 mg/l). Store the solution in a refrigerator until use.

1) 1 Pa = 7,5 torr.

4.2.2 Solution for filter impregnation

MAP is dissolved in acetonitrile to make a solution of 2 mg/ml. Store in a freezer until use.

4.2.3 Filter extraction solution

MAP is dissolved in acetonitrile to make a 1×10^{-4} mol/l solution (27,6 mg/l). Store in a freezer until use.

4.2.4 Stability of reagent solutions

It is best to make filter-spiking solution immediately before use, but this solution can be stored for up to 2 weeks in a freezer. The impinger and filter extraction solutions are stable for at least 1 month in a refrigerator.

4.3 Standard matching solutions

The UV detector response is nearly identical for all MAP-derivatised isocyanate groups. This allows the use of the MAP-derivatised monomer of the isocyanate product of interest as a standard for quantification of the other unknown oligomeric MAP-derivatised species in the chromatogram. A calibration curve, plotting UV response as a function of number or concentration of isocyanate groups, can then be used to quantify the oligomeric species for which there is no standard available. For this reason, it is conceptually simpler to use standard matching solutions quantified in terms of their concentration of isocyanate groups rather than in terms of mass concentration of isocyanate compound.

An equivalent is the amount of substance of isocyanate compound containing a mole of isocyanate group or the amount of substance of MAP-derivatised isocyanate compound containing a mole of bound MAP groups. The equivalent mass of an isocyanate compound is the relative molecular mass divided by the number of isocyanate groups per molecule, n. The equivalent mass of a MAP-derivatised isocyanate compound is the relative molecular mass divided by the number of MAP groups per molecule. The number of isocyanate groups, irrespective of their attachment, can be measured in moles per litre. Table 1 lists relative molecular masses and equivalent masses for common isocyanates and their MAP derivatives.

4.3.1 Preparation of monomer derivatives

Accurately weigh approximately 0,5 mmol (1 milliequivalent) of diisocyanate or 1 mmol (1 milliequivalent) of a monoisocyanate and record the amount of substance to four decimal places. Dissolve in 10 ml of toluene. Weigh approximately 1,2 mmol of MAP (20 % mass fraction excess) and record the amount of substance to four decimal places. Dissolve in 20 ml of toluene. While stirring the MAP solution, add the isocyanate solution dropwise over a period of 10 min to 15 min. Continue to stir for at least 1 h. Tightly cover the solution and store overnight in a freezer to maximise precipitation of product. Collect the precipitate using a Büchner funnel. Wash the precipitate several times with cold toluene to remove residual MAP, then wash it several times with cold hexane to displace the toluene. Transfer the solid derivative to a preweighed 20 ml disposable vial. Subject the vial to high vacuum until constant mass is obtained and seal with a PTFE-lined cap. Yields are typically $>$ 95 % mass fraction and purity is sufficient to use this material for standard matching solutions. Experience shows that when stored in the dark in a freezer, these derivatives are stable for several years.

4.3.2 Preparation of standard solutions of monomer derivatives for HPLC analysis

Of a MAP derivative, weigh approximately $5,0 \times 10^{-5}$ mol (monoisocyanate) or $2,5 \times 10^{-5}$ mol (diisocyanate) ($5,0 \times 10^{-5}$ equivalents) into a 10 ml one-mark volumetric flask, ISO 1042[1], class A. Dissolve in several millilitres dimethyl formamide (DMF) and fill to the mark with DMF. Methylene chloride can be used instead, if desired, for MAP derivatives that are very soluble in methylene chloride (aliphatic diisocyanates and 2,4-TDI). The stock solutions are of approximate concentration $5,0 \times 10^{-3}$ mol/l (monoisocyanate) or $2,5 \times 10^{-3}$ mol (diisocyanate). Store the stock solutions in a freezer. Working standards are made by dilution into acetonitrile, with the highest concentration standard being approximately $2,0 \times 10^{-4}$ mol/l (monoisocyanate) or $1,0 \times 10^{-4}$ mol/l (diisocyanate). Other concentrations can be made by serial dilution, typically the lowest concentration being approximately 1×10^{-7} mol/l (monoisocyanate) or $0,5 \times 10^{-7}$ mol/l (diisocyanate). These stock solutions and dilutions are stable for up to 3 months when stored in a refrigerator.

Table 1 — Relative molecular masses and equivalent masses of some common isocyanates and their MAP derivatives

Compound	Short form	Relative molecular mass	Equivalent mass m[eq]	MAP derivative relative molecular mass	MAP derivative equivalent mass
1-(9-Anthracenylmethyl)piperazine	MAP	276,38	276,38	—	—
Methyl isocyanate		57,05	57,05	333,43	333,43
Butyl isocyanate		99,13	99,13	375,51	375,51
Phenyl isocyanate		119,12	119,12	395,50	395,50
1,6-Hexamethylene diisocyanate 1,6-diisocyanatohexane	HDI	168,20	84,10	720,96	360,48
Toluene diisocyanate (both 2,4- and 2,6-diisocyanatotoluene)	TDI	174,16	87,08	726,92	363,46
Isophorone diisocyanate 1-isocyanato-3-isocyanatomethyl-3,5,5-trimethylcyclohexane	IPDI	222,29	111,14	775,05	387,52
4,4'-Diphenylmethane diisocyanate Di-(4-isocyanatophenyl)methane	4,4'-MDI	250,26	125,13	803,02	401,51
Hydrogenated MDI Methylenebis(cyclohexyl-4-isocyanate) 4,4'-Dicyclohexylmethane diisocyanate	HMDI	262,35	131,18	815,11	407,56
Isocyanate group	NCO	42	42	—	—

4.3.3 Preparation of standard solutions of monomer derivatives for solid-phase extraction (SPE)

Evaluate recovery of MAP-derivatised monomers through solid-phase extraction (SPE) cartridges periodically.

Stock solutions in DMF cannot be used to make SPE standards because even low concentrations of DMF appear to cause premature elution of MAP derivatives. Standards to be passed through an SPE cartridge should be derived from methylene chloride stock solutions. MAP-derivatives of aliphatic diisocyanates and 2,4-TDI are quite soluble in methylene chloride. MAP derivatives of 2,6-TDI and MDI are less soluble. All MAP-derivatives except the MAP derivative of MDI are sufficiently soluble to prepare 1×10^{-3} mol/l (monoisocyanate) or $0{,}5 \times 10^{-3}$ mol/l (diisocyanate) stock solutions. A stock solution of concentration 2×10^{-4} mol/l can be made for the MAP derivative of MDI. These stock solutions can be further diluted into butyl benzoate to simulate impinger solutions.

4.3.4 Preparation of derivative solutions of bulk isocyanate products

This procedure has been found to be suitable for HDI- and IPDI-based products, and may be suitable for other products as well.

Weigh approximately 0.5 g of bulk isocyanate product into a 7 ml vial. Then add 4,5 g (3,4 ml) methylene chloride to this, and mix until the solution is homogeneous. Determine the density of this stock solution, unless subsequent analyses are for qualitative purposes only. Dilute the stock solution 1 → 100 (10 µl → 1 ml) in methylene chloride. Mix until the solution is homogeneous, then immediately add 25 µl of this dilution to 975 µl of 5×10^{-4} mol/l MAP in acetonitrile. It is important to make this second dilution into the derivatising solution as quickly as possible because dilute solutions of free isocyanates are not stable. Allow this final solution to react overnight in the dark. The next day, add 5 µl of acetic anhydride and allow to react at least 2 h at room temperature or overnight in a refrigerator before analysing by HPLC.

4.4 HPLC mobile phase

The HPLC analysis utilises a pH gradient. The weak and the strong mobile phases have identical aqueous:organic compositions. They differ only by the pH of the aqueous portions. The fluorescence response of the MAP derivatives is greatly affected by the mobile phase pH. Therefore, an acidic solution is added to the mobile phase between the analytical column and the detectors to give a fluorescence response independent of the mobile phase pH.

4.4.1 Mobile phase buffer solutions

To 3 840 ml water is added 46,1 g 85 % mass fraction phosphoric acid (approximately 27 ml, 0,4 mol) and 15,1 ml 96 % mass fraction formic acid (0,4 mol). The initial pH should be approximately 1,6. Add triethylamine (99,5 % mass fraction) to this solution in 10 ml aliquots, mixing after each addition, until a total of 117 ml triethylamine has been added. The pH should be 6,0. Adjust to 6,0 with additional triethylamine if necessary. Split this solution into two 2 l portions. To one of these portions, add 33,5 ml concentrated HCl. Mix thoroughly. The final pH should be 1,6. To the second 2 l portion, add 33,5 ml water. This procedure yields buffers that contain approximate concentrations of 0,1 mol/l phosphoric acid and 0,1 mol/l formic acid, one with a pH of approximately 1,6 and the other with a pH approximately 6,0. Experience shows that the buffers are stable for 6 months when stored in a refrigerator.

4.4.2 Primary mobile phases

The weak mobile phase (mobile phase A) is produced by mixing 65 % volume fraction acetonitrile and 35 % volume fraction pH 6,0 buffer. The strong mobile phase (mobile phase B) is produced by mixing 65 % volume fraction acetonitrile and 35 % volume fraction pH 1,6 buffer. The mobile phases are filtered through 0,45 µm nylon filters. Degas the mobile phase prior to use either by vacuum degassing or by helium sparging. Experience shows that the primary mobile phase is stable for 6 months if evaporation is prevented.

4.4.3 Post-column acid mobile phase

Dilute 35 ml of 85 % mass fraction phosphoric acid to 350 ml final volume with water. Mix this dilute phosphoric acid with 650 ml acetonitrile. Filter the solution through a 0,45 µm nylon filter. Degas the mobile phase prior to use either by vacuum degassing or by helium sparging. Experience shows that the acid mobile phase is stable for 6 months if evaporation is prevented.

5 Apparatus

5.1 Sampler

The choice of sampler depends on the chemical and physical properties of the airborne isocyanate (Reference [13]). If little is known about the physical and chemical nature of the isocyanates in the atmosphere, then the sampler should consist of a midget impinger followed by a MAP impregnated filter (ISO/TR 17737[3]). If the isocyanate is present only as vapour, either a MAP-coated filter or a midget impinger may be used. If isocyanate species are present in particles < 2 µm in diameter (condensation or combustion aerosol), then filters should be used. If isocyanate is present in particles > 2 µm (e.g. spray painting), the recommended choice of impinger or filter depends on the reactivity of the aerosol. Typically aerosols containing aliphatic isocyanates react relatively slowly and can be collected using a MAP-impregnated filter as described in Reference [14]. Aerosols > 2 µm containing aromatic isocyanates, such as those generated by MDI spray operations (Reference [15]), frequently react fast and shall be collected using an impinger. If both particles < 2 µm and relatively fast-curing particles > 2 µm are present, then the recommended sampler consists of an impinger followed by a MAP-coated filter.

5.1.1 Filters

The filter material should be glass fibre (binder-free) and the filter should allow no more than 5 % mass fraction breakthrough of the aerosol being sampled. The choice of filter size and filter holder is primarily dependent on the physical state of the isocyanate. Vapour and relatively small aerosol can be collected efficiently with any of the common filter samplers (e.g. open- or closed-face 37 mm polystyrene cassettes, 13 mm polypropylene filter holders). For relatively large aerosol ($>$ 20 µm), an inhalation sampler [e.g. that developed by the Institute of Occupational Medicine (IOM), UK] is recommended. Because isocyanates are strong sensitisers, it is appropriate to measure isocyanate in particles that will be deposited anywhere in the respiratory tract, i.e. the inhalable fraction (Reference [16]).

5.1.2 Midget impingers

A midget impinger consists of a graduated receiver and a tapered inlet tube. The two parts should be matched so that the distance between the inlet and the receiver bottom is 1 mm to 2 mm. Non-spill impingers are commercially available.

5.2 Sampling pump

The sampling pump shall be able to pump up to 2 l/min and shall fulfil the requirements of EN 1232[6] or equivalent.

5.3 Tubing

Use plastic, rubber, or other suitable tubing about 900 mm long and of appropriate diameter to ensure a leak-proof fit to both the pump and the sampler outlet. Clips shall be provided to secure the sampler and the connecting tubing to the wearer. It has been observed that impinger solvents (in particular, toluene) can leach substances from the tubing that ultimately interfere in the sample analysis. It is not known if butyl benzoate leaches interfering compounds from tubing under normal sampling conditions, but it has been found that the problem with toluene is greatly reduced when using fluoroelastomer[2)] tubing. Therefore, fluoroelastomer tubing is recommended for impinger sampling. A short length of fluoroelastomer tubing inserted before the plastic, rubber or other suitable tubing[3)] is sufficient.

5.4 Flowmeter

A portable flowmeter capable of measuring the appropriate volume flow rate to within $\pm$ 5 % is used in the field. This flowmeter is calibrated against a primary standard before taking it into the field.

5.5 Filtration and solid-phase extraction equipment

HPLC solvent is filtered through a solvent-resistant vacuum filtration apparatus using 0,45 µm nylon filters prior to use. Filter samples are passed through 0,45 µm PTFE syringe filters prior to analysis. Impinger samples are subjected to solid-phase extraction (SPE) using a SPE vacuum manifold. Disposable PTFE liners are inserted into the ports of the vacuum manifold to eliminate sample contamination. SPE cartridges of capacity 6 ml containing 500 mg silica gel are inserted into the inlets of the disposable PTFE liners.

2) Fluran is an example of a suitable product available commercially. This information is given for the convenience of users of this International Standard and does not constitute an endorsement by ISO of this product.

3) Tygon R-3603 is an example of a suitable product available commercially. This information is given for the convenience of users of this International Standard and does not constitute an endorsement by ISO of this product.

5.6 Liquid chromatographic system

5.6.1 Autosampler

Any commercially available autosampler capable of making sample injections of acceptable accuracy and precision.

5.6.2 Pumping system

An HPLC capable of gradient elution is required. It is preferable that the HPLC system have highly inert fluid paths [polyetheretherketone (PEEK) or titanium] after the point of sample introduction. If the HPLC has stainless steel fluid paths after the point of sample introduction, it is desirable to replace as much of this as possible with PEEK tubing.

5.6.3 Analytical column

The analytical column is 150 mm × 4,6 mm with the stationary phase consisting of 5 µm C8 high purity silica[4]. The use of a short, replaceable guard column containing the same stationary phase in front of the analytical column is advisable.

5.6.4 Column oven

The analytical column is contained within a column oven maintained at 30 °C, or at least 5 °C above ambient temperature.

5.6.5 Post-column acid delivery pump

An HPLC pump is used that is capable of delivering a single mobile phase at 0,7 ml/min into a mixing tee immediately downstream of the analytical column. Because the back pressure is low downstream of the analytical column, a pulse dampener may be necessary between this pump and the mixing tee to get pulse-free delivery.

5.6.6 Detectors

This method uses two detectors in series for identification and quantification: a variable wavelength UV/visible absorbance detector (UV) and a fluorescence detector. It is preferable to use a fluorescence detector with a xenon source because of the wider range of excitation wavelengths available. However, a fluorescence detector with a deuterium source is acceptable.

6 Air sampling

6.1 Pre-sampling laboratory preparation

6.1.1 Cleaning of sampling equipment

Reusable sampling apparatus shall be carefully cleaned prior to use. Impingers containing residual butyl benzoate should be rinsed with acetone, allowed to dry, and, if necessary, soaked in a non-chromate/concentrated sulfuric acid-based cleaning solution. After 30 min, thoroughly rinse with water and dry in an oven. IOM stainless steel cassettes (not the entire sampler body) are immersed in methylene chloride in a small beaker, sonicated for 15 min, removed and allowed to dry. They are then rinsed with water, immersed in 6 mol/l nitric acid for 30 min (with 10 min sonication), rinsed with water, and oven dried.

4) C8 Inertsil is an example of a suitable product available commercially. This information is given for the convenience of users of this International Standard and does not constitute an endorsement by ISO of this product.

6.1.2 Preparation of MAP-coated filter samplers

With a microlitre syringe, in an area free of isocyanates, add the appropriate volume of the 2 mg/ml filter impregnation solution to a glass-fibre or quartz-fibre filter so that the reagent coverage is 1,0 µg/mm^2 (e.g. 250 µl for a 25 mm filter). After allowing solvent to evaporate, store in a freezer until ready for use. When ready to use, load the filters into the appropriate filter holder.

6.1.3 Preparation of extraction solution jars

Distribute an appropriate amount of MAP filter extraction solution (1×10^{-4} mol/l MAP in acetonitrile) into a wide-mouth jar with a PTFE-lined cap. The size of the jar and amount of solution will depend on the filter size being used and the nature of the sampler. For IOM samplers, the entire stainless steel cassette is submerged in extraction solution, requiring 10 ml solution. If only the filter is being extracted, considerably less solution is required (e.g. 5,0 ml for 37 mm filters).

6.2 Pre-sampling field preparation

6.2.1 Calibration of pump

Calibrate the pump with a representative sampling train in line, using a portable flow meter. If an impinger is used, it shall contain the appropriate solution during calibration.

6.2.2 Preparation of samplers

Prepare for sampling in an area free of isocyanates. If using impingers, add 15 ml of the 1×10^{-4} mol/l solution of MAP in butyl benzoate to each impinger. Connect the outlet of each loaded sampler to a sampling pump using appropriate tubing, ensuring that no leaks can occur. Switch on the pump, attach the calibrated flow meter to the sampler inlet, and set the appropriate flow rate. Switch off the pump and seal the sampler during transport to the sampling site.

6.3 Collection of air samples

6.3.1 Filter sampling

In an area free of isocyanates, attach the sampler to the worker close to the breathing zone. Place the sampling pump on the worker's belt or another secure location. When ready to begin sampling, switch on the pump. Record the time at the start of the sampling period. Draw a measured volume of air at a sampling rate of 1 l/min to 2 l/min (1 l/min for 37 mm filter cassettes, 2 l/min for IOM samplers). The minimum recommended volume is 1 l. The maximum volume depends on the ability of the pump to continue without significant drop in air sampling rate, but 960 l (an 8 h shift at 2 l/min) should be achievable. The filter may become clogged from heavy loading, resulting in a drop in the flow rate. If this is suspected, test the flow rate of the sampler. Terminate sampling and consider the sample to be invalid if the volume flow rate is not maintained to within ± 5 % of the nominal value throughout the sampling period. At the end of the sampling period, measure the volume flow rate, turn off the sampling pump, and record the time. If it is possible that some of the isocyanate collected was in particulate form, place the filter immediately in a jar containing filter extraction solution. If there is good reason to believe only isocyanate vapour was collected, the filter need not be extracted prior to shipment to the laboratory. Calculate the mean flow rate by averaging the flow rate measurements throughout the period and calculate the volume of air sampled by multiplying the flow rate in litres per minute by the sampling time in minutes.

6.3.2 Impinger sampling

The specifications of 6.3.1 regarding attachment of the sampling apparatus to the worker, the need to monitor flow rate, and the calculation of air volume apply to impinger sampling as well. Sample at a flow rate of 1 l/min. Maintain the impinger in an approximately vertical position during sampling. Because the impinger solvent (butyl benzoate) is non-volatile, there is no need to refill the impinger during sampling. At the completion of sampling, transfer the impinger solution to a vial or jar with a PTFE-lined cap.

6.3.3 Sampling with an impinger followed by a filter

If the environment is suspected to contain both particles < 2 µm (which are not collected efficiently by impingers) and relatively fast curing particles > 2 µm (which are believed to be derivatised inefficiently on an impregnated filter), then a sampling train consisting of an impinger followed by an impregnated filter should be used. After sampling, the filter can either be placed in the impinger solution or separately in a filter extraction jar with a PTFE lined closure.

6.4 Blanks and negative controls

Artifacts derived from the MAP reagent can be important chromatographic interferences when isocyanate levels are very low. Blanks and negative controls are used to identify these peaks as reagent artifacts. Field reagent blanks are samples that have been handled identically to other samples except that no air has been drawn through them. One field reagent blank should be provided for every 10 samples, with a minimum of three per sample set. Negative controls are samples that have been handled identically to other samples except that air has been drawn through the sampler in a location near the actual sampling but where no analyte is expected. Three of these per sample set are recommended to identify any chromatographic interferences associated with air sampling.

6.5 Bulk products

It is desirable to collect at least 3 ml of each bulk product when these are available at the worksite. Isocyanate-containing products are very useful for qualitative identification of isocyanate species in the samples. Non-isocyanate products might contain compounds that react with the derivatising reagent and give interfering peaks in the sample chromatograms. Therefore, derivatisation and analysis of these non-isocyanate bulk products could be helpful in identifying unknown peaks in the sample chromatograms that would otherwise be mistaken for isocyanates.

6.6 Shipment of samples

Place the vials containing impinger solutions, the jars containing filter extraction solutions, or unextracted filters, in a cooler. It is helpful if the cooler is fitted with a device to provide support to samples during transport, such as an expanded polystyrene tray with holes cut out to fit the sample vials or jars securely. In any event, glass containers should be kept upright during transport and prevented from coming into contact with each other. Include numerous cool packs to keep the samples cool during shipment. To eliminate any possibility of contamination, it is recommended that bulk products not be shipped in the same container as the air samples. If bulk products are shipped with the samples, they shall be packed in a manner that ensures they cannot contaminate the samples. Place the cooler in a sturdy box that meets the requirements for shipment of hazardous substances. Samples should be shipped in accordance with the IATA *Dangerous goods regulations* (Reference [17]). Accordingly, impinger samples in butyl benzoate do not need to be shipped as hazardous materials. Filter samples extracted in the field in acetonitrile do need to be shipped following the appropriate regulations for acetonitrile.

6.7 Filter test samples

Upon receiving samples from the field, add 5 µl of acetic anhydride to each sample. This procedure converts the excess MAP reagent into an acetamide derivative. This procedure is carried out because the acetylated MAP exhibits much less tailing in the subsequent HPLC analysis than free MAP and because injection of large quantities of excess reagent has been reported to be detrimental to the analytical column. Let the samples react at least 2 h at room temperature or overnight in a refrigerator before proceeding. Attach the outlet of a 0,45 µm PTFE syringe filter to a disposable PTFE liner in one of the ports of the SPE vacuum manifold. Place an empty polypropylene syringe barrel in the inlet of the syringe filter. Add the extraction solution into the syringe barrel. Force the sample through the PTFE filter using either positive or negative pressure and collect in a 20 ml vial. Record the final test sample volume as V_f, the volume used to extract the filter. Store the samples in a freezer prior to analysis. It is recommended that samples be analysed within 1 month to minimise the problem of reagent-derived chromatographic interferences that grow into the sample over time.

6.8 Impinger test samples

The total volume of the impinger sample is 15,0 ml and is designated V_t. A 5,0 ml test portion of the impinger sample is analysed and is designated V_{tp}. Attach a 6 ml SPE cartridge containing 500 mg silica gel in the inlet of the syringe filter. Condition the SPE cartridge with 2 ml butyl benzoate, bringing the liquid level down to the top of the sorbent bed. Add the 5,0 ml test portion to the SPE cartridge, adjusting the vacuum of the SPE manifold to obtain a flow of 1 ml/min to 2 ml/min and stopping when the liquid level reaches the top of the sorbent. Add 6 ml methylene chloride directly to the SPE cartridge and elute until the liquid level reaches the top of the sorbent. Discard all eluate collected up to this point.

Add 3 ml of a mixture of nine volumes of acetonitrile and one volume of methanol to the SPE cartridge. Elute at 1 ml/min to 2 ml/min until the liquid level reaches the top of the sorbent. Collect the eluate in a pre-weighed 7 ml vial. Add 3 ml of methanol to the SPE cartridge. Elute and collect the eluate in the same vial. Reduce the volume to approximately 1 ml by evaporation with a gentle stream of nitrogen. Determine sample volume using sample mass and the density of acetonitrile (0,786 g/ml). Record the final test sample volume as V_f. Store the samples in a freezer prior to analysis.

7 HPLC analysis

7.1 Instrumental settings

Set the HPLC flow rate to 1,5 ml/min. Set the post-column acid delivery pump to 0,7 ml/min. Maintain the analytical column at 30 °C, or at least 5 °C above ambient temperature.

Wavelength settings that are given are optimal for the instrument on which this method was developed. However, optimal settings on other instruments may be slightly different. Set the UV detector to 253 nm. If using a fluorescence detector with a xenon source, set the excitation wavelength to 368 nm. This wavelength provides the best selectivity and is therefore best for confirmation that a peak represents a MAP derivative. If maximum sensitivity is required, set the excitation wavelength to 250 nm. If using a fluorescence detector with a deuterium source, set the excitation wavelength to 250 nm. If the fluorescence detector allows selection of emission wavelength, set the emission wavelength to 409 nm. Set the autosampler to inject 30 µl of the sample and to include a needle wash between samples.

7.2 HPLC programme

The HPLC programme can be tailored to achieve the best combination of peak resolution and short analysis time for a particular isocyanate product. Sample injection should occur at 100 % mobile phase A, but the length of hold at 100 % A and the steepness of the initial gradient depends on the nature of the earliest eluting isocyanates. The HPLC programme given in Table 2 is very strong and intended to ensure elution of as many isocyanate species as possible. It is recommended as an initial programme to determine the latest eluters in a particular product. Generally, the 13 min hold at 100 % B can be shortened and the gradient made more shallow to increase resolution of peaks.

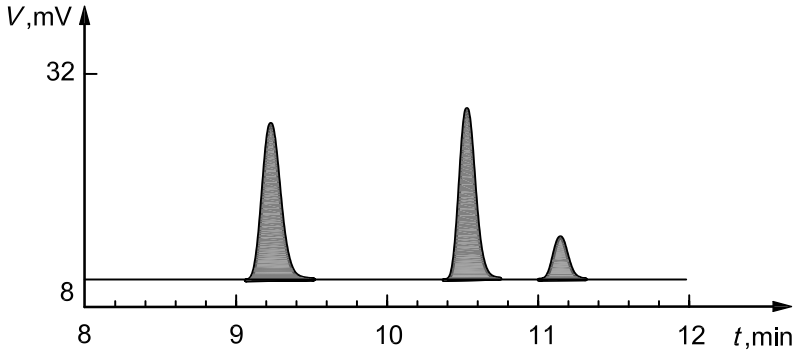
Table 2 — HPLC programme

Time min	Stage end point
0 to 4	100 % mass fraction mobile phase A[a]
4 to 17	Linear gradient from 100 % mobile phase A[a] to 100 % mobile phase B[b]
17 to 30	Hold 100 % mobile phase B[b]
30 to 36	Re-equilibrate at 100 % mobile phase A[a]

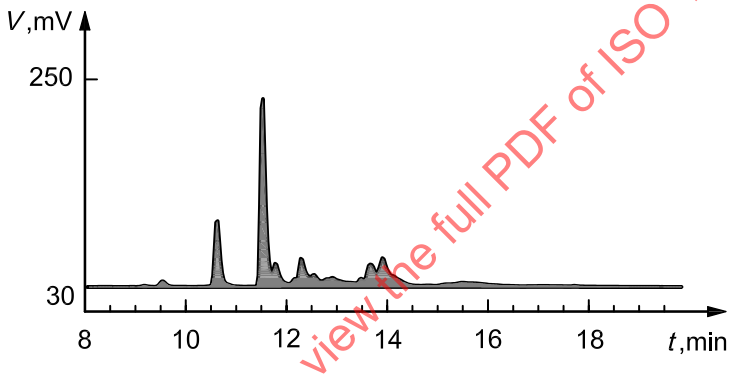
[a] 65 % Volume fraction acetonitrile + 35 % volume fraction pH 6,0 buffer (4.4.1).

[b] 65 % Volume fraction acetonitrile + 35 % volume fraction pH 1,6 buffer (4.4.1).

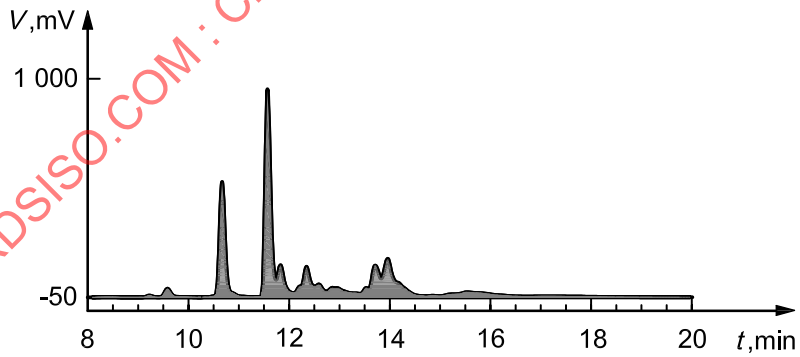
Sample chromatograms of MAP-derivatised isocyanates are given in Figure 3.



a) fluorescence chromatogram of 2,5 × 10^{-7} mol/l HDI-MAP (9,22 min) and IPDI-MAP (two isomers, 2,5 × 10^{-7} mol/l total)



b) UV chromatogram of bulk product containing HDI- and IPDI-based oligomers



c) fluorescence chromatogram of bulk product containing HDI- and IPDI-based oligomers

Key

t time

V potential difference

Figure 3 — Example chromatograms of MAP-derivatised isocyanates

8 Data handling

8.1 Monomer measurement

Isocyanate species for which pure analytical standards are available, such as diisocyanate monomers, can be measured by comparing the response of the peak at the correct retention time with the calibration curve generated by analysing standards. Although either the UV or the fluorescence detector can be used for quantification of monomers, and either peak height or peak area can be used, fluorescence peak height is recommended for quantification. The fluorescence detector is more sensitive and more selective than the UV detector. Peak height is preferable to peak area, especially in the presence of closely eluting interfering peaks. Confirmation of the identity of the monomer is achieved by comparing the FL/UV response ratio of the sample peak to that of the standard giving a similar response.

NOTE The FL/UV response ratio changes somewhat at low levels due to curvature of the fluorescence calibration curve. Usually, the sample peak gives a FL/UV response ratio within 25 % of the ratio for the standard peak of comparable size.

8.2 Oligomer measurement (total detectable isocyanate)

Isocyanate species for which pure analytical standards are not available, such as oligomeric isocyanates, are measured by using the UV area of the sample peak(s) and the slope of the calibration curve generated by analysing standards. Frequently, numerous isocyanate peaks elute as an envelope of poorly resolved peaks. In this case, rather than attempting to integrate peaks individually, the entire chromatogram is integrated over the region of interest. The fluorescence chromatogram is used qualitatively to confirm the presence of MAP groups in the eluting species. In the absence of MAP-derivatised compounds, the baseline of the fluorescence chromatogram is very flat. Therefore, the fluorescence chromatogram can be useful in defining when to begin and end integration in the UV chromatogram. The fluorescence response varies too much from compound to compound to quantify isocyanate species for which standards are unavailable. However, the excitation and emission wavelengths that have been chosen make the detection very selective for MAP derivatives. Experience has shown that MAP-derivatised isocyanates have a FL/UV ratio of approximately 0,33 to 2 times that of the MAP-derivatised diisocyanate monomer. Samples should be compared to field blanks and negative controls to distinguish isocyanate peaks from reagent artifacts and other non-isocyanate peaks.

9 Calibration and quality control

9.1 Standard matching solutions

Calibrate daily with six standard matching solutions in the range of interest. Intersperse standard matching solutions among samples. The useful range of the UV detector, expressed in moles of isocyanate group per litre, is approximately 1×10^{-7} to 2×10^{-4}. The useful range of some fluorescence detectors can be modified by changing the detector sensitivity. It is generally advisable to set the sensitivity of the fluorescence detector so that its useful range corresponds to somewhat lower concentrations than that of the UV detector. This enables quantification of very low levels of monomer. However, under these conditions, MAP derivatives at high concentrations give fluorescence signals that are off-scale. Standard matching solutions should consist of the MAP derivative(s) of the appropriate diisocyanate monomer(s).

Although not specified in the method, the use of an internal standard is likely to improve the performance of the method. The internal standard can identify and/or correct such things as variability in injection volume and retention time drift. Internal standards shall not be naturally present in the sample and shall elute in regions of the chromatogram where no analytes elute. For analysis of samples containing di- and polyisocyanates, use of MAP-derivatised monoisocyanates (such as phenyl isocyanate or butyl isocyanate) would serve as good internal standards as they elute between the reagent peak and the diisocyanates.

9.2 Calibration curves

Prepare calibration curves (response versus c_{NCO}, the concentration of NCO groups in moles per litre, of the standard matching solutions) for both monomer and oligomer quantification. Oligomer quantification shall be accomplished using a linear calibration curve based on UV areas of the monomer derivative. Monomer quantification can be accomplished with either detector and either peak height or peak area, but fluorescence peak height is recommended. A quadratic fit may be necessary for the fluorescence calibration curve because it may show curvature at the low end.

9.3 Blank tests

Analyse two solvent blank tests (acetonitrile) at the beginning of each sample set. Use the chromatogram from the second blank test to subtract electronically from sample chromatograms. Analyse one additional solvent blank within the sample set. Analyse one field blank (containing MAP reagent) for every 10 samples, with a minimum of three per sample set. Use field blank tests and negative controls to identify non-isocyanate peaks (generally reagent artifact peaks) that are likely to appear in the samples.

9.4 Bulk products

Analyse bulk products used at the worksite that are representative of the compounds being collected in the air sample. Bulk isocyanate products should be analysed whenever available. It is advisable to analyse non-isocyanate products (such as the polyol portion of a two-part spray system) to ensure that they give rise to no interfering compounds that might be mistaken for isocyanates. Bulk products shall be derivatised with MAP prior to analysis. This procedure is specified in 4.3.4. Use of the bulk isocyanate chromatograms is advisable for qualitative confirmation of peaks observed in the sample chromatograms, especially in low level samples.

9.5 Quality control spikes

For each set of samples, spike analyte into one unused sampler for every 10 samples in the set, with a minimum of one spike per sample set. The analyte level should be appropriate to the particular sample set and/or the requirements for the analyses. This may mean spiking samplers at a level equivalent to what would be collected from an atmosphere containing analyte at the exposure limit and sampling for a period representative of the sampling time of the real field samples. The spikes may be at lower levels if this is more representative of the samples.

10 Calculations

10.1 Monomer

Generate a calibration curve, plotting peak height or peak area (preferably fluorescence peak height) as a function of c_{NCO}, the concentration of NCO groups, in moles per litre, of monomer standard matching solutions. Determine c_{NCO} in the test portion from the calibration curve.

For filter samples, the isocyanate mass, m, in micrograms per test sample, is given by:

$$m = c_{NCO}\, m_{eq}\, V_f \times 1\,000$$

where

c_{NCO} is the concentration of NCO groups, in moles per litre, in the test portion as taken from the calibration curve;

m_{eq} is the mass of the monomeric isocyanate, in grams per isocyanate equivalent (see Table 1), or mass of the NCO group, in grams per isocyanate equivalent, i.e. 42;

V_f is the final volume, in millilitres, of the test sample (see 6.7).

NOTE The unit of the final value depends on which m_{eq} is used in the equation. The final answer can be micrograms of monomer or micrograms of NCO group (for oligomer the final answer can be micrograms of NCO group or micrograms of other isocyanate-containing compounds, if the equivalent masses of those compounds are known).

For impinger samples, the isocyanate mass, m, in micrograms per test sample, is given by:

$$m = c_{NCO}\, m_{eq}\, V_f\, (V_t/V_{tp}) \times 1\,000$$

where

c_{NCO} is the concentration of NCO groups, in moles per litre, in the test portion as taken from the calibration curve;

m_{eq} is the mass of the monomeric isocyanate, in grams per isocyanate equivalent (see Table 1), or mass of the NCO group, in grams per isocyanate equivalent, i.e. 42;

NOTE The unit of the final value depends on which m_{eq} is used in the equation. The final answer can be micrograms of monomer or micrograms of NCO group (for oligomer the final answer can be micrograms of NCO group or micrograms of other isocyanate-containing compounds, if the equivalent masses of those compounds are known).

V_f is the final volume, in millilitres, of the sample test solution after SPE (see 6.8);

V_t is the total volume, in millilitres, of the butyl benzoate impinger test solution (see 6.8);

V_{tp} is the volume, in millilitres, of the butyl benzoate test portion (see 6.8).

10.2 Oligomers (total detectable isocyanate)

Because all MAP-derivatised isocyanate compounds have similar UV response factors, quantification of oligomers can be accomplished using a calibration curve generated from analysing monomer standard matching solutions, preferably the monomer from which the oligomer is derived. Generate a calibration curve, plotting UV peak area as a function of concentration of NCO groups, in moles per litre, of monomer standard matching solutions. Use the slope of the calibration curve to determine the concentration of NCO groups of a single component, a group of components, or all isocyanate components, $c_{NCO,tp}$, in moles per litre, in the test portion according to:

$$c_{NCO,tp} = A_{tp}/b_{cal}$$

where

A_{tp} is the UV area of the test portion component(s);

b_{cal} is the slope, expressed in UV area units per normal concentration, of the calibration curve.

The method measures the concentration, in moles, of oligomeric isocyanate group per volume of sample. It cannot measure micrograms of isocyanate per sample unless the equivalent mass of the component is known. Generally, there is a mixture of compounds of different and unknown equivalent masses. To convert the concentration of NCO groups, in moles per litre to micrograms of isocyanate group per sample, use the formulae in 10.1 and the equivalent mass of an isocyanate group: 42 g per equivalent. An exposure standard based on micrograms of isocyanate group per volume of air is recognised in some countries, including Australia, Finland, Ireland, Sweden, and the UK.

11 Interferences

Any non-isocyanate compound that forms a derivative with MAP is a potential interference. Any compound that elutes at or after the monomer retention time and absorbs at 253 nm may potentially interfere with isocyanate quantification by UV. Interfering compounds that do not fluoresce substantially using the prescribed excitation and emission wavelengths almost certainly do not contain MAP. Unless these compounds are amines, they should be readily separable from a MAP derivative by altering the pH gradient. MAP-derived artifacts are present in the chromatogram and can interfere when isocyanate levels are low. The

impact of the artifacts is minimised by analysing the samples within 30 days and using the blank tests and negative controls to identify the artifacts.

12 Determination of performance characteristics

12.1 Introduction

The measurement of the concentration of isocyanates in workplace air has associated with it an uncertainty that may be expressed as overall uncertainty (EN 482[5]) or expanded uncertainty (ISO/IEC Guide 98-3[4]). 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[5], or defined in national or international legislation.

This section on determination of performance characteristics is taken from ISO 17734-1[2] with relatively few changes. Although the majority of factors that give rise to uncertainty in the methods described in ISO 17734-1[2] and this International Standard are the same, differences in the methods do result in some changes to the contributing factors. Of course, the estimated or calculated values to the uncertainty contributions are frequently different.

Relevant uncertainty contributions and criteria are listed in Table 3.

Table 3 — Uncertainty contributions and criteria

Uncertainty contribution	Symbol or abbreviated term	Subclause	Criterion
Test sample volume	V_{sam}	12.2.2	
Sample flow — calibration	q_{cal}		Relative uncertainty, < 2 %
Sample flow — variation	Δq		< 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}	12.2.3	
Analyte stability during storage	$w_{NCO,s}$		No significant difference between results of analysis of samples before and after storage
Reaction/extraction efficiency	$\eta_{re/e}$		> 90 % at the limit value with a relative uncertainty, < 3 %
Mass of isocyanate in standard matching solutions	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	RD		< 3 %
Analytical precision (response)	r		< 1 %
Selectivity	s		Resolution factor, > 1
Blank level	m_b	12.2.4	< 50 ng with a relative uncertainty of < 5 %
Between-laboratory variations		12.2.5	Relative uncertainty, < 7,5 %

12.2 Assessment of performance characteristics[5)]

12.2.1 Collection efficiency relative to particle size distribution

For a complete description of the performance requirements and tests to be performed, see ISO/IEC Guide 98-3[4].

12.2.2 Air sampling

12.2.2.1 Sampling volume

The sampled volume of air, V_{sam}, usually in millilitres, is calculated on the basis of measuring the sample flow rate before and after sampling as specified in ISO 16200-1:

$$V_{sam} = \frac{(q_0 + q_f)}{2} t \quad (1)$$

where

q_0 is the sample flow rate, usually in millilitres per minute, at the beginning of the sampling period;

q_f is the sample flow rate, usually in millilitres per minute, 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;

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

This can be expressed as

$$\frac{u^2(V_{sam})}{V_{sam}^2} = \frac{u^2(q_0) + u^2(q_f)}{(q_0 + q_f)^2} + \frac{u_t^2}{t^2} + \frac{u_{var,q}^2}{\left[(q_0 + q_f)/2\right]^2} \quad (2)$$

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

12.2.2.2 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 negligible.

12.2.2.3 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

$$u_{var,q}^2 = \frac{(q_0 - q_f)^2}{12} \quad (3)$$

5) This analysis follows the detailed approach of ISO/IEC Guide 98-3[4].

12.2.2.4 Conversion of sample volume to STP

For the conversion of concentrations to standard temperature and pressure (STP, Reference [18]), 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

$$u^2 = u_{\text{cal}}^2 + \frac{s_{\text{meas}}^2}{n} \tag{4}$$

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.

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

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

Generally, the first term is negligible compared to the second.

12.2.2.5 Combined uncertainty of sample volume

The uncertainty contributions of 12.2.2.4 are combined to give the uncertainty in the sample volume converted to STP as

$$\frac{u^2\left(V_{\text{sam, STP}}\right)}{V_{\text{sam, STP}}^2} = \frac{u^2\left(V_{\text{sam}}\right)}{V_{\text{sam}}^2} + \frac{u^2(T)}{\overline{T}^2} + \frac{u^2(p)}{\overline{p}^2} \tag{6}$$

12.2.3 Analysis

12.2.3.1 Sampled mass

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

$$m_{\text{sam}} = \frac{m_{\text{anal}}}{\eta_{\text{c}}\, v_{\text{s}}\, S_{\text{a}}\, \eta_{\text{re/e}}\, f_{\text{r}}} \tag{7}$$

in which

η_{c} is the collection efficiency;

$\eta_{\text{re/e}}$ is the reaction/extraction efficiency;

f_{r} is the response factor;

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

S_a is the analyte stability in the sample;

v_s is the sampler variability.

12.2.3.2 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 times $t = 0$ and $t = t$, n samples ($n \geqslant 6$) shall each be analysed under repeatability conditions. For both times, the samples shall be randomly picked from a batch of representative samples in order to minimise possible systematic concentration differences. As a test of (in)stability, perform a t-test (95 % confidence, 2 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;

d) inhomogeneity of the sample batch.

As such, the contribution of the determination of analyte stability is already incorporated in other contributions and can be neglected.

12.2.3.3 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.

The uncertainty due to incomplete reaction/extraction can be expressed as

$$\frac{u^2_{\eta re/e}}{\eta^2_{re/e}} = \frac{u^2_{CRM}}{m^2_{CRM}} + \frac{s^2(m_{det})}{m^2_{det}} + \frac{(m_{det} - m_{CRM})^2}{m^2_{CRM}} \quad (8)$$

where

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

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

m_{det} is the mean mass of isocyanate determined;

$s(m_{det})$ is the standard deviation of the mean of the replicate measurement results.

The last 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.

12.2.3.4 Response factor

This method quantifies isocyanate species for which no analytical standards are available based on the UV response factor of the MAP derivative of the most appropriate monomeric diisocyanate. In other words, the method quantifies isocyanate species as if they have the same UV response factor as the monomer. In fact, there is small compound-to-compound variability in the UV response factor. The uncertainty in the response factor can be estimated by the variability in response factors of several different isocyanate compounds.

12.2.3.5 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 standard matching solutions 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.

12.2.3.6 Standard matching solutions

The uncertainty in the concentrations of isocyanate in the standard matching solutions used depends on the type of such solutions.

For standard matching solutions in toluene or acetonitrile, the uncertainty is composed of contributions from

a) the purity of isocyanate — this is generally known from manufacturer's specifications as a minimum purity, $w_{\mathrm{NCO,p}}$, e.g. $w_{\mathrm{NCO,p}} = 99$ % mass fraction or $w_{\mathrm{NCO,p}} \geqslant 99$ % mass fraction; in the first case, the relative uncertainty due to impurity is given by $(100 - w_{\mathrm{NCO,p}})$ %; in the second case the relative uncertainty can be estimated assuming a uniform distribution as

$$u^2_{w_{\mathrm{NCO,p}}} = \frac{\left(100 - w_{\mathrm{NCO,p}}\right)^2}{12} \tag{9}$$

b) the uncertainties in the weighings of compounds and solutions, i.e. the uncertainty of the balance used, generally expressed for differential weighings as

$$u^2_{\mathrm{weigh}} = 2u^2_{\mathrm{bal}} \tag{10}$$

in which u_{bal} is the uncertainty of the balance used.

12.2.3.7 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 standard matching solution as

$$u_{\mathrm{LOF}}^2 = \frac{\left(m_{\mathrm{regr}} - m_{\mathrm{sms}}\right)^2}{m_{\mathrm{sms}}^2} = \rho_{\mathrm{r}}^2 \qquad (11)$$

where

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

m_{sms} is the mass of isocyanate present in the corresponding standard matching solution;

ρ_{r} is the relative residual for the particular concentration level.

NOTE The lack of fit of the calibration function contributes 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 can be neglected in the uncertainty assessment.

12.2.3.8 Drift in detector response

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

$$u_{\mathrm{drift}}^2 = \frac{\left(r_n - r_{n-1}\right)^2}{12\left[\left(r_n + r_{n-1}\right)/2\right]^2} \qquad (12)$$

where r_n is the detector response for a standard matching solution corresponding closest to the mass of isocyanate representing a sample at the limit value.

12.2.3.9 Precision of the analysis

The uncertainty due to the (im)precision of the analysis is determined by analysis under repeatability conditions of standard matching solutions of the same composition; perform a minimum of six replicate analyses. The uncertainty is then calculated as

$$u_r^2 = \frac{s_{\mathrm{anal}}^2}{n\bar{r}^2} \qquad (13)$$

where

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

n is the number of replicate analyses;

$\bar{r}$ is the mean response.

In the uncertainty assessment, this contribution is already incorporated in contributions from the determination of reaction/extraction efficiency and can be neglected.