Analytical Method

Determination of isocyanates in workplace air

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ANALYTICAL METHOD

MA-376

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TABLE DES MATIÈRES

APPLICABILITY	This method is for the determination of monomeric and oligomeric HDI, MDI, TDI, IPDI and HMDI in workplace air.
STANDARD(S) [1]	See Appendix A for the isocyanate monomers. Exposure to isocyanates (monomeric and oligomeric) must be reduced to a minimum,[2] even when the exposure remains within the standards presented in Appendix A.
SAMPLING SYSTEMS	**Double-filter closed cassette:** teflon filter and glass fiber filter impregnated with 9-(N-methylaminomethyl) anthracene. For field derivatization: jar containing a solution of 1-(2-methoxyphenyl)piperazine. **Glass midget impinger** containing a solution of 1-(2-methoxyphenyl)piperazine, and closed cassette with glass fiber filter impregnated with 1-(2-methoxyphenyl)piperazine (optional)
RECOMMENDED SAMPLING VOLUME AND FLOW RATE	**Double-filter closed cassette:** 15 L at 1.0 L/min (up to 480 L at 1.0L/min if no aerosol is expected, refer to section 6.1) **Glass midget impinger** : >15 L at 1.0 L/min if: • No aerosol expected • Impinger used (refer to section 6.2 for limitations)
ANALYSIS	High performance liquid chromatography and ultraviolet detector (diode array detector (DAD)) The two filters are analyzed separately.
RANGE OF APPLICATION	See Appendix A.
RELIABILITY	See Appendix A.
ANALYTICAL UNCERTAINTY (CV_A):	See Appendix A.

1. RANGE OF APPLICATION

This method is used to determine the concentration of the monomer and oligomers of isocyanate compounds in workplace air by high performance liquid chromatography (HPLC).

The linearity of the analytical method was verified for quantities varying from 0.014 to 1.5 µg of diisocyanate for vapour analysis, which corresponds to concentrations from 0.0009 to 0.1 mg/m³ in air for a recommended sampling volume of 15 litres, and from 0.025 to 1.7 µg of diisocyanate in aerosol form, which corresponds to concentrations from 0.002 to 0.113 mg/m³ in air for a recommended sampling volume of 15 litres. The coefficient of determination (r^2) obtained during the validation of the two methods was greater than 0.996 for this range of application. Higher concentrations may be reported in applying appropriate dilutions to the samples.

The document also presents the method's operating method.

2. PRINCIPLE OF THE METHOD

Double filter

According to the parameters described in the Sampling Guide[1], a defined volume of air is sampled through a cassette containing a teflon (or polytetrafluoroethylene, PTFE) filter and a glass fiber filter (GFF) impregnated to collect isocyanates.

The isocyanates present in aerosol form are collected on the teflon filter, while the isocyanates present in vapour form react with the 9-(N-methylaminomethyl) anthracene (MAMA) impregnated on the GFF to form a urea derivative that will be stable until the laboratory determination stage.

Immediately after sampling, the teflon filter is transferred to a jar containing a solution of 1-(2-methoxyphenyl)piperazine (MOPIP) in toluene to form a urea derivative that will be stable until the laboratory determination stage.

The isocyanate vapours and aerosols are analyzed separately in the laboratory using high performance liquid chromatography and an ultraviolet detector. The method makes it possible to report a result for the monomer and oligomers of the isocyanate in question. The oligomeric result is expressed as monomeric equivalents.

Impinger

According to the parameters described in the Sampling Guide[1], a defined volume of air is sampled through an impinger containing a solution of 1-(2-methoxyphenyl)piperazine (MOPIP) in toluene to collect the isocyanates in vapour and aerosol form by stationary sampling.

When particulates smaller than 2 µm are suspected, a cassette equipped with a glass fiber filter impregnated with MOPIP reagent is placed downstream from the impinger.

Immediately after sampling, the impregnated filter is transferred to the impinger, while ensuring that it is well immersed in the MOPIP reagent in order to form a urea derivative that will be stable until the laboratory determination stage. The isocyanate vapours and aerosols are analyzed together by high performance liquid chromatography and ultraviolet detector and are expressed as total monomer. The method makes it possible to report a result for the monomer and oligomers of the isocyanate in question. The oligomeric result is expressed as monomeric equivalents.

Laboratory

Double-filter

In the laboratory, the MOPIP solution into which the teflon filter from the double filter sampler was transferred is evaporated to dryness, and the residue is dissolved in a solution of acetic anhydride in acetonitrile. This solution will be used for analyzing the isocyanate aerosols.

The vapour form of the collected isocyanates is analyzed by transferring the glass fiber filter remaining in the cassette after sampling to a desorption solution consisting of acetonitrile, dimethylformamide and an aqueous solution of triethylamine.

Impinger

The MOPIP solution found in the impinger and in which the glass fiber filter was immersed is evaporated to dryness, and the residue is dissolved in a solution of acetic anhydride in acetonitrile. This solution will be used for analyzing the total isocyanates.

Samples analysis

Aliquots of the solutions originating from the samples are then analyzed by high performance liquid chromatography with an ultraviolet (UV) detector of the diode array type (DAD). The DAD is also used for identifying the isocyanates during the analysis of the aerosol form. For the analysis of the isocyanate aerosols, the detector measures the absorption at the 245 nm wavelength, except for MDI which uses the 250 nm wavelength. During the analysis of the vapour form, the wavelength used is 254 nm for all isocyanates.

The concentration of the sample is determined by comparing the signal obtained for the samples and for a range of standard solutions. The diode array detector is also used to identify isocyanates in aerosol form.

LIMITATION – Sampling on double-filter cassette is not suitable for determining isocyanates with rapid reactivity expected in the aerosol form (possible underestimation). The use of an impinger is strongly suggested if applicable. An example of the best documented process involving isocyanate aerosols with rapid reactivity is the application of MDI-based polyurethane foam for building insulation spray foam. The reader will find more information on this subject by consulting the IRSST's Guide for Safe Use of Isocyanates – An Industrial Hygiene Approach (RG-773).[3]

Methods published by the ISO and ASTM standardization organizations are based, in whole or in part, on IRSST method 376.[4,5,6,7]

3. INTERFERENCES

For a substance to cause analytical interference, it must be retained on one of the filters or be added to it during the laboratory manipulations, have the same retention time as the isocyanate under the chromatographic conditions used, and produce absorbance at the detection wavelength. Any information about known or suspected interference during sampling must be transmitted with the sample. It should be noted that the chemical reagents MAMA and MOPIP used for stabilizing the isocyanates are likely to cause interference (degradation products) in the case where the sampler use period is not respected. It should also be noted that due to contact between the two filters in the sampler, a slight amount of MAMA reagent on the impregnated filter may be transferred to the teflon filter. As a result, contamination by MAMA (a peak) during the analysis of the aerosol form will be observed. Identification of the peaks obtained by the DAD makes it possible to disregard this peak during quantitation. A control sample is used to verify the possibilities of contamination during the entire analytical process. In this way, any contamination occurring when the cassette or impinger is opened, during transport or during analysis, will be detected. An analytical blank is also used to verify the possibility of contamination during preparation of the samples.

In the case where interferences are observed, the chromatographic conditions could be modified to obtain better separation of the peaks on the chromatogram. The use of mass spectrometry detection could be another option to get rid of the interferences.

4. MATERIAL

- Single-use impermeable gloves, intended to prevent possible contamination by hands and to protect the operator from any contact with toxic and corrosive substances (nitrile gloves recommended);
- Laboratory glassware (volumetric flasks, beakers, etc.);
- Three-neck flasks;
- Separatory funnel;
- 2-section polystyrene cassettes, 37 mm;
- Midget impinger, 25 mL;
- Plastic support pad, 37 mm;
- Glass fiber filters, 37 mm;
- Teflon filter, 5 µm, 37 mm;
- Glass jar that can contain a 37-mm filter;
- Precision volumetric pipettes with disposable tips;
- Syringes or pipettes that can sample volumes of 10 µL or more;
- Precision analytical balance;
- Vortex mixer (or equivalent);
- Evaporator and test tubes of at least 10 mL appropriate for its use;
- Vacuum generator, for example the vacuum from a laboratory hood;
- Eberbach shaker (or equivalent);
- Heating plate;
- Magnetic stirrers;
- pH meter;
- Vacuum filtration system with filters, porosity 0.2 µm;
- Syringe filters with 0.2 µm porosity filters;
- Injection vials with teflon coated septum;
- High performance liquid chromatograph, with the following components:
  - Isocratic pump;
  - Sample injection system, consisting of an injection valve and a sampling loop of different volumes;
  - UV (ultraviolet) or diode array (DAD) detector for quantitation and DAD for peak identification;
  - Zorbax C18 Bonus RP separating columns 3.5 µm, and Bonus RP 1.8 µm and Zorbax Eclipse Plus C18 1.8 µm, or equivalent columns with appropriate performance;
  - Solvent reservoirs;
  - Data acquisition system.

5. REAGENTS

The solvents for liquid chromatography must be of certified HPLC quality. All other reagents must be "ACS" (American Chemical Society) certified or of best quality, unless otherwise specified.

- **Water**, HPLC quality, for all preparations and sample dilutions [CAS 7732-18-5];
- **1-(2-methoxyphenyl)piperazine** (**MOPIP**), 98% purity [CAS 35386-24-4];
- **9-(N-methylaminomethyl) anthracene** (**MAMA**), 99% purity $C_{16}H_{15}N$ [CAS 73356-19-1];
- **Toluene**, $C_6H_5CH_3$ [CAS 108-88-3];
- **Acetonitrile,** 99.9%CH_3CN [CAS 75-05-8];
- **Dimethylformamide**, $HCON(CH_3)_2$ [CAS 68-12-2];
- **1,6-hexamethylene diisocyanate**, >99% **HDI** $C_6H_{12}(NCO)_2$ [CAS 822-06-0];
- **4,4'-methylenebisphenyl diisocyanate**, 98% **MDI** $CH_2(C_6H_4NCO)_2$ [CAS 101-68-8];
- **2,4-toluene diisocyanate**, 98% **2,4 TDI** $CH_3C_6H_3(NCO)_2$ [CAS 584-84-9];
- **2,6-toluene diisocyanate**, 97% **2,6 TDI** $CH_3C_6H_3(NCO)_2$ [CAS 91-08-7];
- **Isophorone diisocyanate**, mixture of isomers **IPDI** $C_{10}H_{18}(NCO)_2$ [CAS 4098-71-9];
- **4,4'-methylenebiscyclohexyl diisocyanate**, 90% mixture of isomers **HMDI** $CH_2(C_6H_{10}NCO)_2$ [CAS 5124-30-1];
- **Distilled or deionized water**, H_2O [CAS 7732-18-5];
- **Acetic anhydride**, $(CH_3CO)_2O$ [CAS 108-24-7];
- **Sodium acetate**, CH_3CO_2Na [CAS 127-09-3];
- **Triethylamine**, 98% $(C_2H_5)_3N$ [CAS 121-44-8];
- **Glacial acetic acid**, CH_3CO_2H [CAS 64-19-7];
- **Phosphoric acid**, H_3PO_4 [CAS 7664-38-2];
- **Dichloromethane**, CH_2Cl_2 [CAS 75-09-2];
- **Pentane**, C_5H_{12} [CAS 109-66-0];
- **Ice**.

WARNING – Concentrated acetic and phosphoric acids are corrosive and cause burns. Avoid all exposure through contact with skin or eyes. Use personal protective equipment (including appropriate gloves, face shield, or safety glasses, etc.) for all work with concentrated or dilute acids

6. SAMPLING

6.1 Double-filter system

Airborne isocyanates are sampled using a 37-mm cassette containing two filters: one teflon filter with 5 µm porosity and one glass fiber filter impregnated with 9-(N-methylaminomethyl) anthracene (MAMA) on a plastic support pad and using a sampling pump whose flow rate was previously adjusted. For each series of samples, a control cassette must be provided as field blank, coming from the same lot as the samples. This field blank sample must be handled in the same way as the cassettes used for sampling for everything involving storage and transport, whether before or after sampling, except that air is not passed through the sampler. The samples must be stored in the refrigerator. The recommended sampling parameters are described in the following table:

Flow rate	1 L/min
Volume*note*	15 L

These parameters take into account the exposure standard, the analytical method's sensitivity, and the sampling system's capacity. **It is important to respect the 15-minute sampling time in order to avoid any underestimation of isocyanates in aerosol form.**

Note: In the case where only isocyanates in vapour form are expected, the sampling duration can be extended.

Immediately after sampling, remove the teflon filter by means of tweezers and place it, sample collection side down, in a jar containing 5 mL of the solution of 1-(2-methoxyphenyl)piperazine (MOPIP) in toluene. The impregnated filter is kept in the cassette until further analysis in laboratory. Make sure that the cassette and the jar are properly identified and store them in the refrigerator until analysis. The samples retain their integrity before laboratory analysis for a period of 6 weeks at 4 °C, in the dark.

> It is important to verify whether the storage or transport of the jars containing toluene is suitable in relation to the contents of the refrigerator, case or cooler used, since toluene vapours could contaminate the contents.

6.2 Impinger

Airborne isocyanates are sampled using an impinger containing a solution of 1-(2-methoxyphenyl)piperazine (MOPIP) in toluene. A glass fiber filter impregnated with MOPIP reagent can be added downstream from the impinger to collect particulates smaller than 2 µm. The sampling is stationary sampling using a sampling pump whose flow rate was previously adjusted. For each series of samples, an impinger and a control cassette from the same lot as the samples must be provided as field blank. This field blank sample must be handled in the same way as the equipment used for sampling for everything relating to storage and transport, whether before or after sampling, except that air is not passed through the sampler. The samples must be stored in the refrigerator. The recommended sampling parameters are described in the following table:

Flow rate	1 L/min
Volume*note*	15 L (minimum)

These parameters take into account the exposure standard, the analytical method's sensitivity, and the sampling system's capacity.

Note: The 15-minute sampling time can be increased up to 180 minutes **on condition that the quantity of MOPIP solution, lost by evaporation, is kept at a suitable level of at least 5 mL**. Add as needed during sampling. High ambient temperatures during sampling can notably reduce sampling time.

Immediately after sampling, remove the glass fiber filter from the cassette (if used) by means of tweezers and place it in the impinger, while ensuring that the entire filter is immersed in the 1-(2-methoxyphenyl)piperazine (MOPIP) solution. Ensure that the impingers are properly identified and store them in the refrigerator until analysis. The solution contained in the impinger could be also transferred to a labeled vial to avoid refrigerating the impinger. The samples retain their integrity before laboratory analysis for a period of 6 weeks at 4°C, in the dark.

> It is important to verify whether the storage or transport of the impingers containing toluene is suitable in relation to the contents of the refrigerator, case or cooler used, since toluene vapours could contaminate the contents.

For more details about preparation of the sampling equipment, its calibration and the strategy used, refer to the IRSST's Sampling Guide.[1]

7. ANALYTICAL PROTOCOL

7.1 Determination of the monomer and oligomers in aerosol form

7.1.1 Cleaning of glassware

Wash the laboratory glassware with detergent and rinse thoroughly with tap water and then with demineralized or distilled water. Dry the glassware completely before using it.

7.1.2 Preparation of solutions

Buffer solution

Prepare a solution of 0.001 g/mL sodium acetate in HPLC quality water. Using a pH meter, acidify the buffer to pH 6.0 with glacial acetic acid. Filter the buffer using the vacuum filtration system with a 0.2-µm porosity filter. Decant to a glass container compatible with the HPLC analytical system. This solution can be stored for approximately 2 weeks in the refrigerator. The solution must be filtered again before each use.

Solution of 1-(2-methoxyphenyl)piperazine (MOPIP) at 0.1 mg/mL

Weigh approximately 50 mg of purified 1-(2-methoxyphenyl)piperazine and transfer it to a 500-mL flask containing toluene. Fill to the mark with toluene. Store in the refrigerator for a maximum of 3 months.

Desorption solution: 0.5% acetic anhydride

Pipette 2.5 mL of acetic anhydride into a graduated 500-mL flask containing acetonitrile.
Fill to the mark with acetonitrile. Store for a maximum of 3 months at ambient temperature in an amber bottle.

7.1.3 Preparation of standard solutions for the calibration curve

Prepare a stock solution of each isocyanate in toluene according to the table below. By diluting this stock solution, prepare standard solutions for producing a calibration curve.

Table 7.1 contains suggestions for dilutions corresponding to a percentage of the TWAEV (time-weighted average exposure value) for preparation of the calibration curve. Based on the table below, pipette the

appropriate volumes of isocyanates into 100-mL graduated flasks containing toluene to prepare the stock solution and fill to the mark with toluene. The conservation time for the stock solution and the standard solutions is approximately 4 months in the refrigerator.

Table 7.1 Preparation of calibration solutions – aerosol form

Isocyanate	Stock solution µL/100 mL	Suggested working solution (µL/100 mL toluene)				
		5%	10%	25%*	50%	100%
HDI	10	25	50	125	250	500
MDI	~20 mg	20	40	100	200	400
TDI	10	20	40	100	200	400
IPDI	25		25	50	125	250
HMDI	15		50	125	250	500

*: Concentration equivalent to 20% of the TWAEV for IPDI.

7.1.4 Processing of standard solutions

- For each standard solution, place 5 mL of the MOPIP solution in the containers for the evaporator, and add 1 mL of the standard solution.
- Shake each test tube on the Vortex and place in the evaporator.
- Evaporate to dryness and then add 1 mL of desorption solution.
- Mix again on the Vortex before filtering the solution with a syringe filter with 0.2 µm porosity filter.
- Transfer the sample to a vial for injection.

7.1.5 Preparation of quality control (QC) solutions

- Prepare QC solutions at different concentrations. If possible, use an isocyanate from a different lot than the one used for standard solutions. If not available, use one from a different preparation.
- For each of the QC solutions, in a jar containing a teflon filter, place 5 mL of MOPIP solution and 1 mL of the QC solution.
- Follow the same processing procedure as for the samples.

7.1.6 Preparation of samples

- Transfer the solution contained in the jar containing the teflon filter or the impinger to a recipient for the evaporator, by rinsing three times with toluene to recover the entire sample.
- Evaporate to dryness and then add 1.0 mL of desorption solution before shaking in the Vortex mixer.
- Filter the solution with a syringe filter with 0.2 µm porosity filter, while transferring the sample to a vial for injection.
- Follow the same procedure for the preparation of blanks and controls.

7.1.7 Chromatographic conditions

These conditions are suggested for the analysis of the monomer and oligomers of the isocyanates in aerosol form. They may vary slightly, depending on the instrument and the chromatographic column used:

Instrument: Agilent 1290 UPLC
DAD wavelength: 245 nm for HDI-TDI-IPDI
250 nm for MDI-HMDI

Table 7.2 Chromatographic conditions for the analysis of the aerosol form

Isocyanate	Column	Buffer (%)	Acetonitrile (%)	Flow rate (mL/min)	Injection (µL)
HDI	Zorbax Bonus RP, 4.6X150mm, 3.5 µm	38	62	1.0	20
MDI		38	62	1.0	20
IPDI		38	62	1.0	20
TDI		42	58	1.0	20

Start the chromatographic system and allow the baseline to stabilize for approximately one hour.

Inject the different calibration solutions in increasing order of concentration in order to produce a calibration curve with at least four concentrations (the blank solution and three calibration solutions) and measure the area of the peak of the monomer for each standard solution. Quantitation is by linear regression. The coefficient of determination (r^2) must be greater than 0.990 and a minimum number of 4 standard solutions should be respected, including the calibration blank solution.

The concentration of the oligomers is calculated from the sum of the areas of all the peaks identified as oligomers by the DAD and is reported on the linear regression line established by the calibration curve for the monomer. The results reported for the oligomers are consequently expressed as monomeric equivalents. Appendix E gives examples of UV spectra for identifying isocyanate oligomers.

7.2 Determination of isocyanates in vapour form (monomer only)

7.2.1 Cleaning of glassware

Wash the laboratory glassware with detergent and rinse thoroughly with tap water and then with demineralized or distilled water. Dry the glassware completely before using it.

7.2.2 Preparation of solutions

Buffer solution

Measure 30 mL of triethylamine in a graduated cylinder and pour into a 1-litre volumetric flask containing HPLC quality water. Fill to the mark with water. Using the pH meter, acidify the buffer to pH 3.0 with concentrated phosphoric acid. Filter the buffer using the vacuum filtration system with a 0.2-µm porosity filter. Pour into a glass container compatible with the HPLC analytical system.

This solution can be stored for approximately 2 weeks in the refrigerator. The solution must be filtered again before each use.

Desorption solution

Using a graduated cylinder, prepare a solution containing 60 mL of the triethylamine buffer solution, 140 mL of acetonitrile, and 400 mL of dimethylformamide. Mix well.

Store at ambient temperature for a maximum of 3 months.

9-(N-methylaminomethyl) anthracene (MAMA) impregnation solution

Prepare a solution of approximately 55 mg of 9-(N-methylaminomethyl) anthracene (MAMA) in 500 mL of toluene. Protect from light and store in the refrigerator. See Appendix B for the preparation of glass fiber filter impregnation.

7.2.3 Preparation of standard solutions for the calibration curve

Weigh precisely approximately 12.5 mg of the urea derivative of each isocyanate in 100 mL of dimethylformamide. Store these stock solutions in amber vials in the freezer.

These stock solutions are stable for approximately 2 years, if stored in the freezer at approximately -70°C. Check the stability of the solution at the end of the first year.

> Depending on the analytical conditions, the three isocyanates HDI, MDI and IPDI can sometimes be grouped together by preparing a single standard solution. The three isocyanates can thus be analyzed simultaneously during the vapour analysis.
>
> In the same way, 2,4-TDI and 2,6-TDI can be combined in a single solution and analyzed simultaneously.

By diluting this stock solution, prepare working solutions for producing a calibration curve.

Table 7.3 contains suggestions for dilutions corresponding to a percentage of the TWAEV (time-weighted average exposure value) for preparing the standard curve. Pipette the appropriate volumes of the stock solution of each urea derivative (HDIU, MDIU, TDIU, IPDIU and HMDIU) into 25-mL graduated flasks containing the desorption solution and fill to the mark. The storage time for the working solutions is approximately 3 months in the refrigerator.

Table 7.3 Preparation of calibration solutions – vapour form

Stock solution mg/100 mL	**Suggested working solution** (µL/25 mL desorption solution)				
	5%	10%	25%	50%	100%
~ 12.5	10	20	50	100	200

An aliquot of each working solution is transferred to a vial for injection.

7.2.4 Preparation of the solutions for the quality controls

Precisely weigh approximately 12.5 mg of the urea derivative of each isocyanate (HDIU, MDIU, 2,4-TDIU, 2,6-TDIU, IPDIU and HMDIU) in 100-mL volumetric flasks containing dimethylformamide. Fill to the mark. Divide these solutions into amber vials and store in the freezer for a maximum of 2 years. This solution will be used for the preparation of the different control solutions.

> Depending on the analytical conditions, the three isocyanates HDI, MDI and IPDI can sometimes be grouped together by preparing a single control solution. The three quality controls can thus be analyzed simultaneously during the analysis of the vapours.
>
> In the same way, 2,4-TDI and 2,6-TDI can be combined in a single control solution and analyzed together.

7.2.5 Preparation of quality control samples

The quality control samples consist of glass fiber filters impregnated with MAMA spiked with one of the previously prepared urea stock solutions.

See Appendix B for the preparation of glass fiber filter impregnation.

Place a volume of the control solution prepared above corresponding to a desired concentration on a glass fiber filter impregnated with a MAMA solution. Place the filter in a jar and process as a sample.

7.2.6 Preparation of samples and quality control samples

- Using tweezers, remove the glass fiber filter from the cassette and place it in a jar.
- Add 2 mL of the desorption solution.
- Close the jars and place them on a stirrer for 30 minutes.
- Filter the solution with a filter syringe with a 0.2-µm porosity filter, while transferring the sample to a vial for injection.
- Follow the same procedure to prepare the blanks and quality controls.

7.2.7 Chromatographic conditions

The conditions suggested for analysis of the monomer in vapour form may vary slightly, depending on the conditions of the equipment and the chromatographic column used:

Instrument:	Agilent 1290 UPLC
DAD wavelength:	254 nm
Column:	Zorbax, Bonus RP 3.0 X 100 mm 1.8 µm for HDIU, MDIU, IPDIU HMDIU Zorbax, Eclipse Plus C18 2.1 X 50 mm 1.8 µm for TDIU

Table 7.4 Chromatographic conditions for the analysis of the vapor form (monomer)

Isocyanate	Buffer (%)	Acetonitrile (%)	Flow rate (mL/min)	Injection (µL)	Temperature (°C)
HDIU	25	75	1.0	8	25
MDIU	25	75	1.0	8	25
IPDIU	25	75	1.0	8	25
TDIU	32	68	1.0	8	25
HMDIU	25	75	1.0	10	25

Start the chromatographic system and allow the baseline to stabilize for approximately one hour.

Inject the different calibration solutions in increasing order of concentration in order to produce a calibration curve with at least four concentrations (the blank solution and three calibration solutions) and measure the area of the peak of the monomer for each standard solution. Quantitation is by linear regression. The coefficient of determination (r^2) must be greater than 0.990 and a minimum number of 4 standard solutions must be respected, including the calibration blank solution.

7.3 Analysis

After the calibration curve is produced, the calibration control, reagent blank, and quality control samples as well as the samples are successively analyzed. The concentration of the monomer and oligomers in the samples is then determined by comparison of the area of the peak obtained for the sample and for the range of standard solutions.

> One calibration solution is analyzed approximately every 7 samples. If the relative standard deviation between two standard solutions varies by more than 5%, the instrument must be recalibrated, and the samples that were being analyzed when the change in sensitivity occurred must be analyzed again.

The monomer concentration determined in the sample must be in the analytical method's range of application. If the concentration of the sample is greater than the highest concentration in the range of application, an appropriate dilution of the sample with matrix matching is done, and the analysis is then performed again, taking the dilution factor into account during the calculations.

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8. CALCULATIONS

The concentration of monomer or oligomers ($Conc._{iso}$) for the air sample at ambient conditions is calculated using the following equation:

$$Conc._{iso}\ (mg/m^3) = \frac{mass_{\ iso\ extracted}\ (\mu g)}{Sampling\ volume\ (L)}$$

Where:

the 'mass $_{iso\ extracted}$' corresponds to the amount in micrograms (µg) of isocyanate in the analyzed extract, provided by the linear regression of the instrument's software. MAMA urea derivate can be converted to free isocyanate using the conversion factor provided in Table C.1. No conversion factor is necessary for MOPIP derivate as they are prepared in situ.

the 'Sampling volume' corresponds to the air volume in liters (L) sampled by the cassette or the impinger.

The results of the samples are not blank corrected with the results obtained for the field blanks . The results of the field blanks are reported as total mass (µg) of isocyanate. Refer to section 7.1.7 for details on quantification of oligomers.

The results in mg/m^3 can be converted in ppm using this equation:

$$Conc._{iso}\ (ppm) = \frac{Conc_{iso}\ mg/m^3 \times 24.45\ (g/mol)}{Molecular\ weight\ monomer\ (g/mol)}$$

9. VALIDATION PARAMETERS

9.1 Detection Limit and Quantitation Limit

The Method Detection Limit (MDL) represents the lowest concentration for a compound analyzed in a real matrix which, when subjected to all the steps of a complete method, including the chemical extractions and pretreatment, produces a detectable signal with a statistically defined reliability different from the one produced by a "blank" under the same conditions. The MDL represents the concentration equivalent to 3 times the standard deviation obtained from 10 samples spiked with a specific isocyanate standard at low concentration and subjected to the entire analytical procedure.

The Method Quantification Limit (MQL) represents the concentration equivalent to 10 times the standard deviation obtained with these same samples.

Table 9.1 Lower limits for the analysis of the aerosol form

ISOCYANATE MONOMER	DETECTION LIMIT (µg per filter)	QUANTITATION LIMIT (µg per filter)
HDI	0.004	0.013
MDI	0.003	0.010
IPDI	0.018	0.059
2,4-TDI	0.003	0.009
2,6-TDI	0.004	0.012

Table 9.2 Lower limits for the analysis of the vapour form

ISOCYANATE MONOMER	DETECTION LIMIT (µg per filter)	QUANTITATION LIMIT (µg per filter)
HDI	0.005	0.016
MDI	0.001	0.004
IPDI	0.001	0.004
2,4-TDI	0.001	0.002
2,6-TDI	0.001	0.003

9.2 Reliability

Reliability, also known as precision, relates to the dispersion of the results obtained from an experimental procedure applied repeatedly to a single sample in well determined conditions. Depending on the test execution conditions, reliability is expressed in the form of replicability or repeatability for an analytical method. Reliability corresponds to the precision of the method.

Replicability was determined from the individual results obtained for 24 samples subjected to the same analytical procedure (4 concentration levels, 6 samples per concentration level) in the same laboratory and under the following conditions: same analyst, same instrument and same day.

Repeatability was determined from the individual results obtained from 24 samples subjected to the same analytical procedure (4 concentration levels, 6 samples per concentration level) in the same laboratory and where at least one of the following aspects was different: the analyst, the instrument, the day.

Table 9.3 Precision of the analysis of the aerosol form

ISOCYANATE MONOMER	REPLICABILITY (%)	REPEATABILITY (%)
HDI	3.3	4.3
MDI	3.0	5.8
IPDI	5.7	4.5
2,4-TDI	2.1	4.6
2,6-TDI	5.9	8.1

Table 9.4 Precision of the analysis of the vapour form

ISOCYANATE MONOMER	REPLICABILITY (%)	REPEATABILITY (%)
HDI	1.1	2.3
MDI	0.5	2.0
IPDI	0.6	3.0
2,4-TDI	0.7	0.9
2,6-TDI	0.5	1.0

9.3 Accuracy

The accuracy corresponds to the degree of agreement of a measured value obtained by the true method with the true or expected value in a sample. It is determined by comparing the average result obtained by analyzing a minimum number of 10 samples of certified concentration with an entire analytical procedure to the concentration certified by a recognized organization (or one related thereto). The accuracy is measured, at a given concentration level, in the method's range of application. It is expressed in relation to the relative error.

Table 9.5 Accuracy and relative error for the vapour and aerosol forms

ISOCYANATE MONOMER	VAPOUR FORM		AEROSOL FORM	
	ACCURACY (%)	RELATIVE ERROR (%)	ACCURACY (%)	RELATIVE ERROR (%)
HDI	97.6	2.4	97.8	2.2
MDI	98.7	1.3	94.6	-5.4
IPDI	99.3	0.7	94.3	5.7
2,4-TDI	95.7	4.3	94.9	-5.1
2,6-TDI	94.1	5.9	94.9	-5.1

9.4 Recovery

Recovery rate is the relative quantity of analyte recovered in a specific matrix. The recovery rate corresponds to the difference (in percentage) between the measured concentration of a spiked sample and the measured concentration of the same unspiked sample, divided by the concentration of the added substance. This ratio takes into account the chemical transformation that occurred, if applicable.

Table 9.6 Recovery for the analysis of the aerosol form

ISOCYANATE MONOMER	RECOVERY (%)	N	CV (%)
HDI	107	35	1.9
MDI	103	35	4.1
IPDI	103	35	1.4
2,4-TDI	104	35	4.4
2,6-TDI	107	35	9.9

Table 9.7 Recovery for the analysis of the vapour form

ISOCYANATE MONOMER	RECOVERY (%)	N	CV (%)
HDI	105	35	4.5
MDI	100	35	0.47
IPDI	96	35	3.8
2,4-TDI	98	35	2.2
2,6-TDI	100	35	0.22

9.5 Measurement uncertainty (the uncertainty related to the reference materials is included)

The method's analytical measurement uncertainty (CV_a) is a combined measurement uncertainty. It is equal to the square root of the total variance obtained by adding the provided variances obtained by individual measurement uncertainties. It was calculated from the individual results of the intra-laboratory quality control obtained over a period of three years for two concentration levels.

The total or combined expanded measurement uncertainty (expanded CV_T) for the entire determination and sampling was calculated by taking into account a coefficient of variation estimated at 5% for sampling, and a probability threshold of 95%.

Table 9.8 Analytical measurement uncertainty (CV_a) and the total expanded measurement uncertainty (expanded CV_T) for the analysis of the aerosol form

ISOCYANATE MONOMER	CV_a (%)	Expanded CV_T (%)
HDI	12	25
MDI	8.8	20
IPDI	13	27
2,4-TDI	5.9	15
2,6-TDI	13	27

Table 9.9 Analytical measurement uncertainty (CV_a) and the total expanded measurement uncertainty (expanded CV_T) for the analysis of the vapour form

ISOCYANATE MONOMER	CV_a (%)	Expanded CV_T (%)
HDI	5.9	15
MDI	4.8	14
IPDI	11	23
2,4-TDI	7.5	18
2,6-TDI	7.6	18

9.6 Stability of the samples

Stability tests were performed on the samples over a period from 1 to 3 months under two storage conditions, at 22°C and 4°C, in the dark. Each type of sample (aerosol fraction and vapour fraction) was studied for the HDI, IPDI, 2,4-TDI, 2,6-TDI and MDI monomers. Also, for the aerosol fraction, two commercial bases of oligomers were subjected to the stability tests: N3200 for the oligomers of HDI, and MR541 for the oligomers of MDI.

In conclusion, for all physical forms (vapour or aerosol) and all isocyanates combined, the samples retain their integrity, in the dark, for 2 weeks at 22°C or even 6 weeks at 4°C. It is recommended that the sampling material be stored in the refrigerator at all times.

Validation of HDMI was not done for the two forms, vapour and aerosol. There are therefore no validation data available for this isocyanate.

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9. National Institute for Occupational Safety and Health (NIOSH), Isocyanates, monomeric, Method 5521, Manual of Analytical Methods, Fourth Edition, 1994.

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Appendix A – Range of application, reliability and analytical uncertainty

Table A.1 Information on the isocyanates analyzed by the method

ISOCYANATES	ACRONYM	CAS	TWAEV (STEV) mg/m³	TWAEV (STEV) ppb
1,6-hexamethylene diisocyanate	HDI	822-06-0	0.034	5
4,4'-diphenylmethane diisocyanate	MDI	101-68-8	0.051	5
Isophorone diisocyanate	IPDI	4098-71-9	0.045	5
2,4-toluene diisocyanate	2,4-TDI	584-84-9	0.036 (0.14)*	5 (20)
2,6-toluene diisocyanate	2,6-TDI	91-08-7	0.036 (0.14)*	5 (20)
Methylene bis(4-cyclohexylisocyanate)	HMDI	5124-30-1	0.054	5

* Mixture of the two isomers (2,4- and 2,6-TDI)

Table A.2 Range of application of the method

	Mass µg/sample		Concentration mg/m³		Recommended sampling volume * (L)
HDI	0.014	1.1	0.0009	0.075	15
MDI	0.018	1.7	0.001	0.11	15
TDI	0.015	1.2	0.0009	0.078	15
IPDI	0.018	1.5	0.001	0.097	15

* See section 6 for more information about the sampling volume

Table A.3 Reliability of the method – aerosol form

Isocyanates	RELIABILITY (%) Replicability	Repeatability	Analytical uncertainty (CVa) (%)
HDI	3.3	4.3	12
MDI	3.0	5.8	8.8
IPDI	5.7	4.5	13
2,4-TDI	2.1	4.6	5.9
2,6-TDI	5.9	8.1	13

Table A.4 Reliability of the method – vapour form

Isocyanates	RELIABILITY (%) Replicability	Repeatability	Analytical uncertainty (CVa)(%)
HDI	1.1	2.3	5.9
MDI	0.5	2.0	4.8
IPDI	0.6	3.0	11
2,4-TDI	0.7	0.9	7.5
2,6-TDI	0.5	1.0	7.6

Appendix B – Preparation of the sampling equipment

B.1 Preparation of the sampling equipment

The glass fiber filters must be previously be calcined in an oven at 400°C for a period of 4 hours.

B.2 Impregnation of glass fiber filters

The previously calcined GFF are impregnated with a solution of 110 mg of MAMA per litre of toluene.

- Soak the filters in this solution for 30 minutes protected from the light.
- Remove the filters from the solution and allow to dry for approximately 12 hours on a plate covered with aluminum foil, protected from the light.

Impregnated filters can be stored for approximately 3 months in the refrigerator.

B.3 Cassette assembly

- Place the filter holder smooth side upwards.
- Add the calcined and impregnated glass fiber filter wavy side upwards.
- Top off with the teflon filter.
- Press the cassette and seal it with cellulose tape.
- Label the cassette to clearly identify it, with the lot number as well as the expiry date on it.
- MAMA reagent is stable for a period of 3 months at 4°C. It is recommended that an expiry date be written on the cassette.

Note: It is possible to obtain the Iso-chek® double filter sampling system distributed by SKC (Cat # 225-9022).

B.4 Preparation of jars

The jars contain a solution of 1-(2-methoxyphenyl)piperazine (MOPIP) in toluene. This solution allows, after sampling, immediate derivatization of the isocyanates in aerosol form that are collected on the teflon filter.

- Weigh 50 mg of MOPIP in 500 mL of toluene.
- Store the solution in the refrigerator (4°C)for a maximum of 3 months.
- When shipping the sampling equipment, identify the jars with the same series of numbers as the cassettes.

B.5 Preparation of impingers

The impingers contain 15 mL of a solution of 1-(2-methoxyphenyl)piperazine (MOPIP) in toluene. This solution allows immediate derivatization of the isocyanate in aerosol form.

B.6 Impregnation of glass fiber filters for impinger analysis

The previously calcined GFF are impregnated with a solution of **100 mg** MOPIP/**100 mL** toluene.

- Soak the filters in this solution for 30 minutes protected from the light.
- Remove the filters from the solution and allow to dry for approximately 12 hours on a plate covered with aluminum foil, protected from the light.
- Proceed with the cassette assembly, omitting the teflon filter. The cassette must bear the same number as the corresponding impinger.

The impingers contain 15 mL of a solution of 1-(2-methoxyphenyl)piperazine (MOPIP) in toluene. This solution allows immediate derivatization of the isocyanate in aerosol form.

If fine particulates are suspected, a cassette containing a glass fiber filter impregnated with MOPIP is installed downstream from the impinger to collect the particulates smaller than 2 µm.

Immediately after sampling, the filter impregnated with MOPIP is transferred to the impinger by completely immersing the filter in the MOPIP solution.

Appendix C – Synthesis of the isocyanate-MAMA derivative

C.1 Synthesis of the isocyanate-MAMA derivative

C.1.1 Isocyanate solution

Collect, according to Table C.1 below, 2 mmoles of the desired isocyanate (HDI, MDI, TDI, IPDI or HMDI) with a graduated microsyringe and dissolve in 25 mL of dichloromethane.

C.1.2 MAMA solution

- Weigh approximately 1.3 g of MAMA (6 mmoles) and dissolve this solute in 25 mL dichloromethane.
- Place the MAMA solution in a 3-neck flask and the isocyanate solution in a separate funnel.
- Add the isocyanate dropwise at approximately 25°C with the MAMA by stirring with a magnetic stirrer for approximately 1.5 hours.
- Cool the resulting solution in ice.
- Filter the precipitate formed and recrystallize it in dichloromethane.
- Analysis by mass spectrometry will confirm the identification and purity of the urea derivative formed.
- The urea derivatives in powder form are stored in amber vials under a hood. For each isocyanate, one vial is identified for the preparation of standards and another will be used in the preparation of quality controls. There is no expiry date.

Table C.1 Relevant information for the preparation of isocyanate-MAMA derivatives

Isocyanate	Weight (µL)	MAMA weighed (g)	Melting point (°C)	Molecular weight of the derivative (g/mole)	Conversion factor
HDI	325	1.3	200	610	0.2754
MDI	1 g	1.2	265	692	0.3611
TDI	575	1.2	270	616	0.2823
IPDI	210	0.5		664	0.3348
HMDI	270	0.5		705	0.3721

Appendix D – MOPIP purification and storage of isocyanate bases

D.1 MOPIP purification (based on NIOSH method 5521[9])

- Place approximately 25 g of MOPIP (1-(2-methoxyphenyl)piperazine) in a beaker. The MOPIP will be in a more or less solid form and often yellowish in colour.
- Add approximately 150 mL of pentane.
- Bring it slowly to a boil on a heating plate by stirring with a glass rod.
  The MOPIP will melt in the pentane by heating. A yellowy oil layer forms in the solution.
- Carefully pour the supernatant and the whitish deposit into a clean beaker. Leave the oily yellowish layer in the beaker.
- Allow to cool slightly to avoid splashes before adding pentane to the oily layer and reheating to purify the entire compound.
- Place a watch glass over the purified portion and put on ice. In cooling, the MOPIP will become crystalline, white and light.
- Dry under vacuum. A small desiccator can be used with desiccant and allow to sit for the night.
- Pour into amber vials. If possible, store under nitrogen.

D.2 Storage of isocyanate bases

The isocyanate polymer bases are received in 1 L to 4 L containers. These bases keep well at room temperature in airtight containers under nitrogen.

To prevent their polymerization, the solutions should be transferred into smaller containers. Much of it is transferred to 125 mL bottles with a nitrogen jet to exhaust the air before sealing the bottle. The bottles are filled as completely as possible.

Small format bottles that are more easily handled on a daily basis should also be prepared.

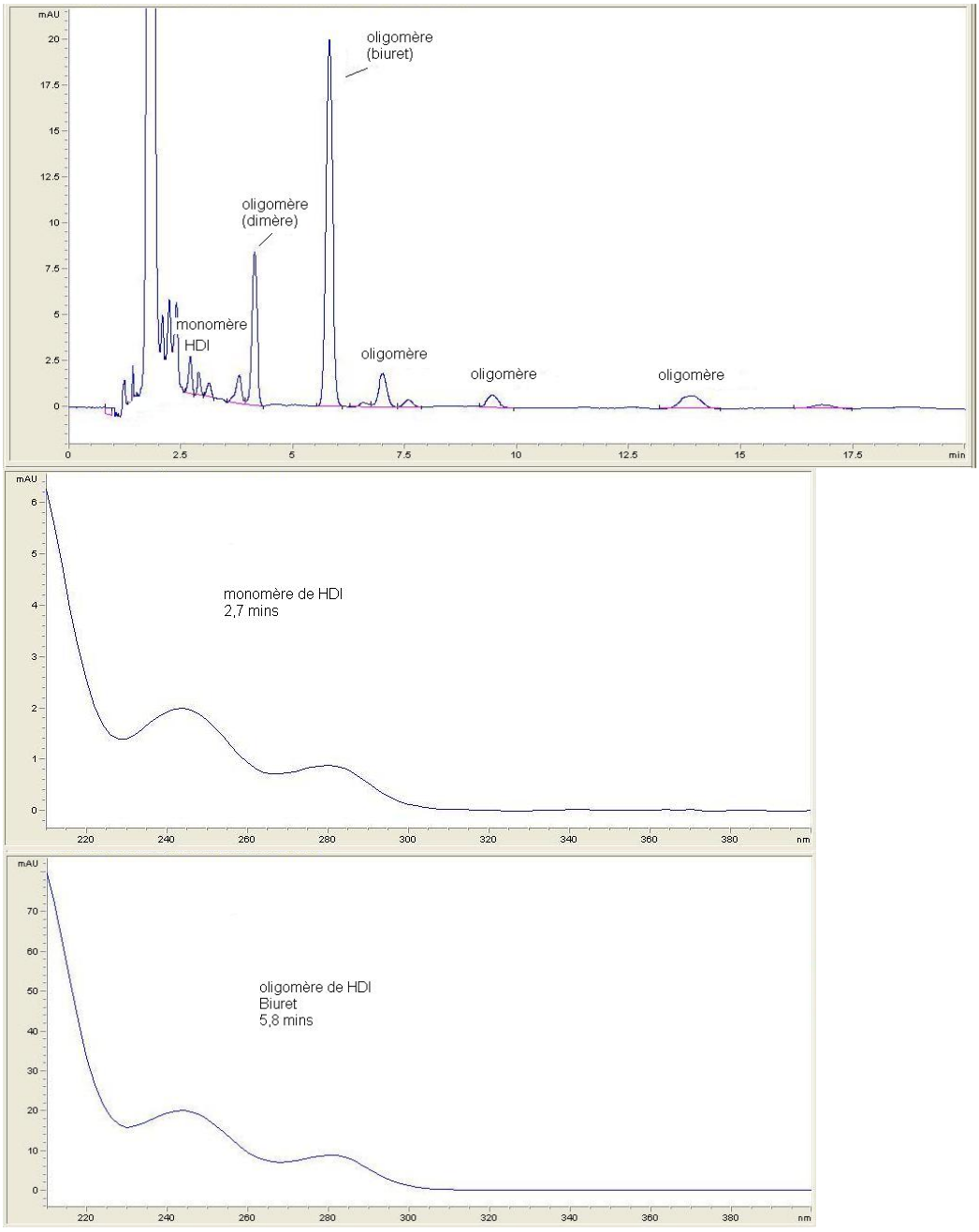
At any time, the state of polymerization of the product can be checked by inverting the bottle while observing the speed at which the bubble rises. The faster the speed, the less polymerized the product.

A layer can also form on top, showing that the base is partially polymerized.

The bases are stored at room temperature.

Appendix E – UV spectra generated by the DAD for the identification of oligomer peaks

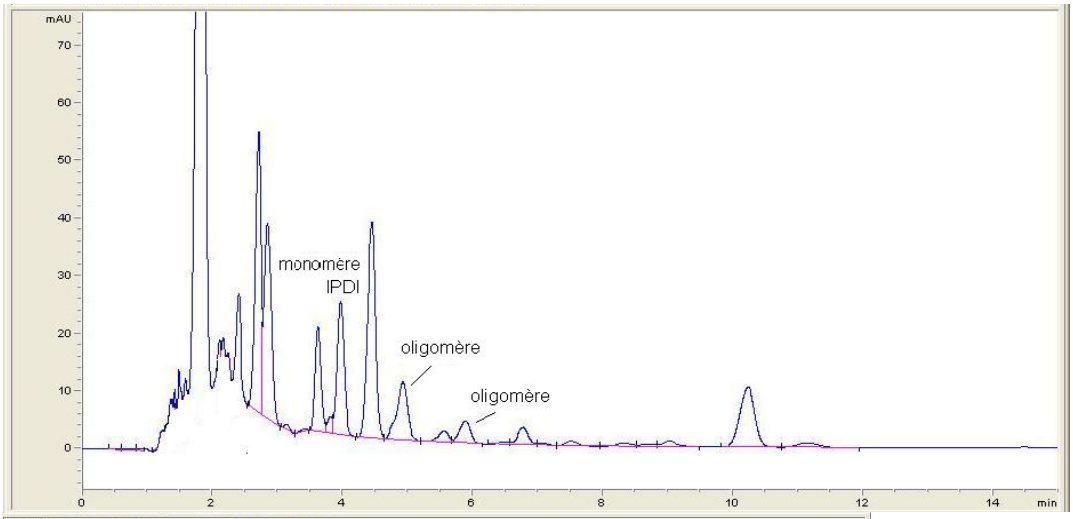
Figure E.1 Chromatogram and UV spectra – HDI aerosol form (Desmodur N3200)

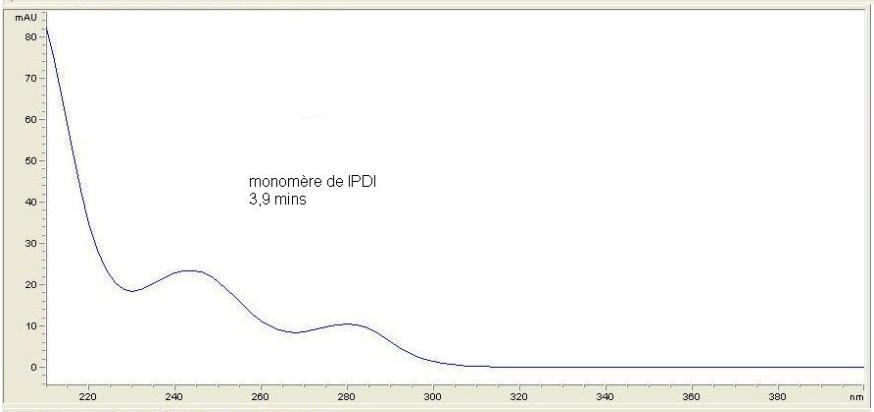


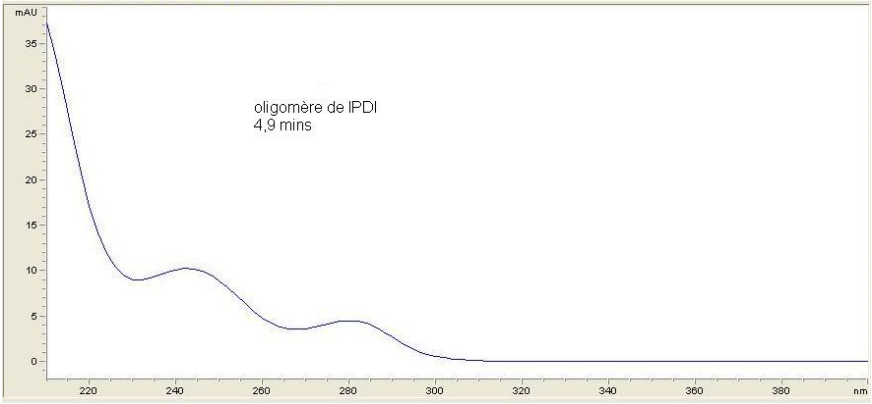
oligomer (biuret) oligomer (dimer) monomer HDI oligomer oligomer oligomer
monomer of HDI

2.7 min
oligomer of HDI
Biuret
5.8 min

Figure E.2 Chromatogram and UV spectra – IPDI aerosol form (*Powdura® Urethane Polyester* powder coating, dusts collected on filter)





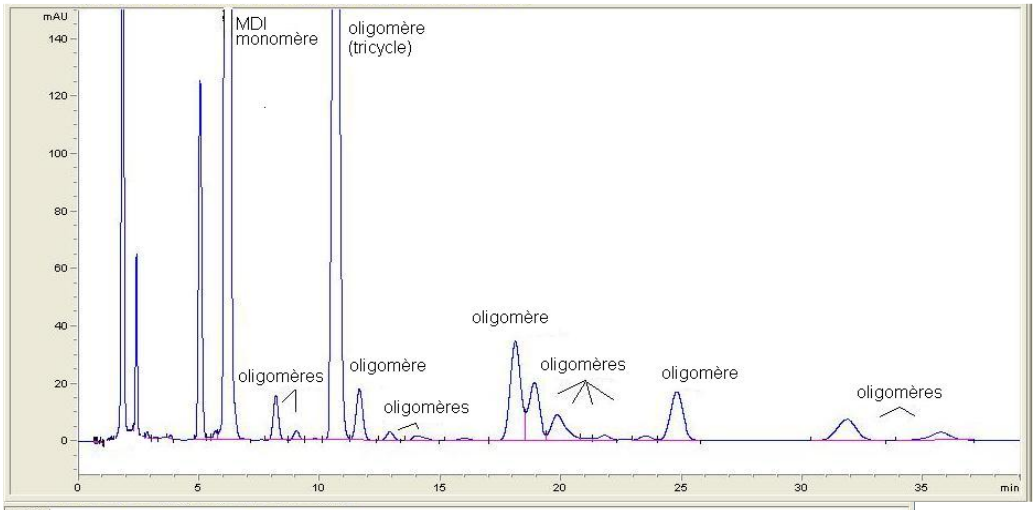


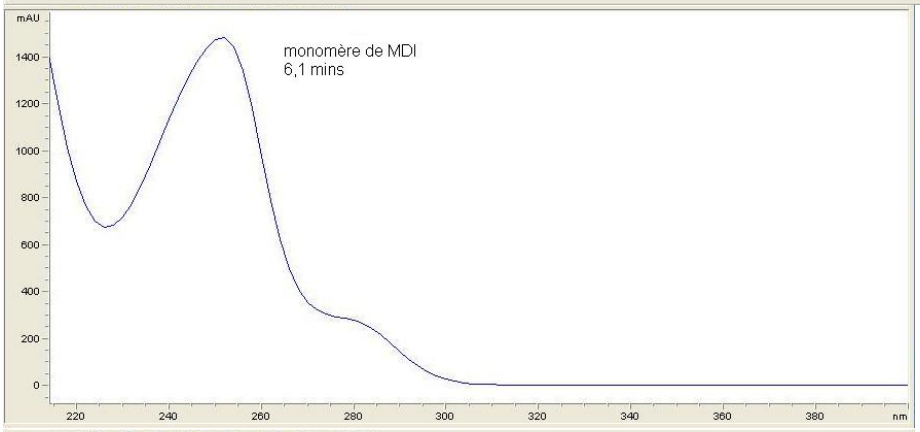
monomer IPDI oligomer oligomer

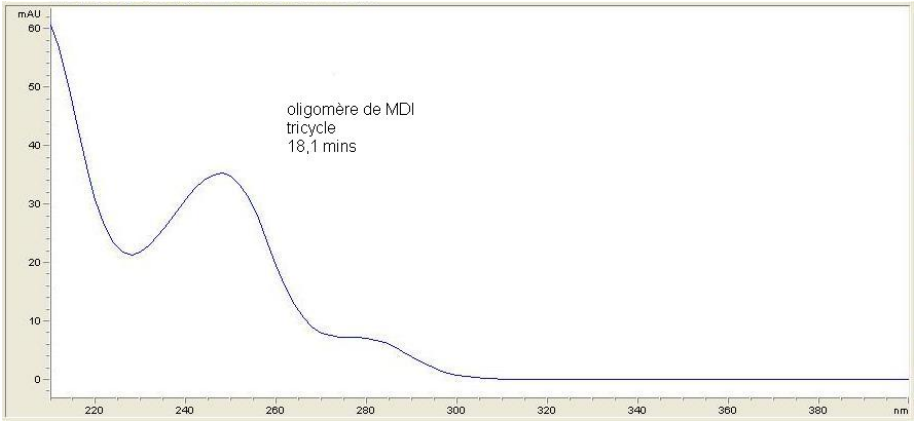
monomer of IPDI
3.9 min

oligomer of IPDI
4.9 min

Figure E.3 Chromatogram and UV spectra – MDI aerosol form (Mondur 200)



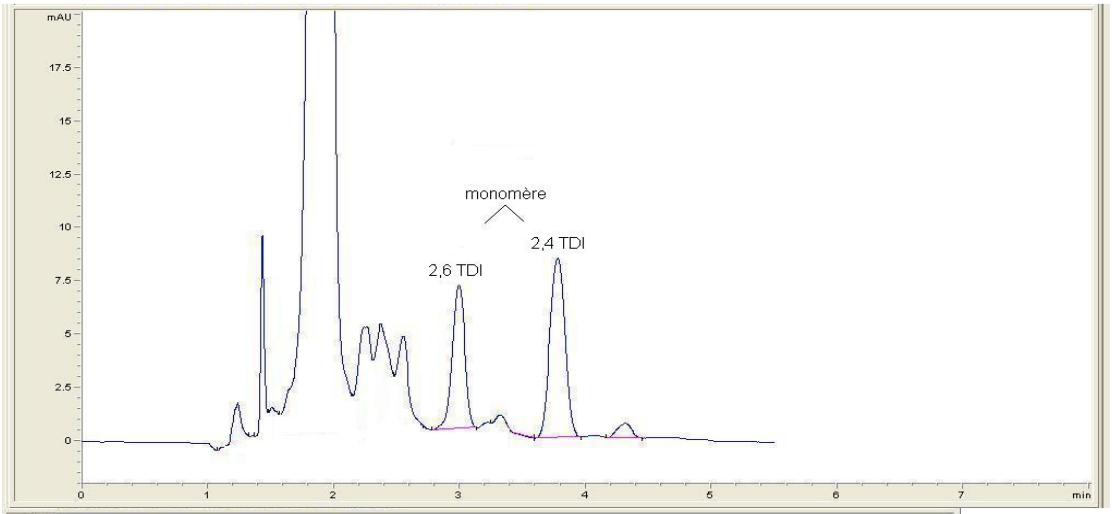


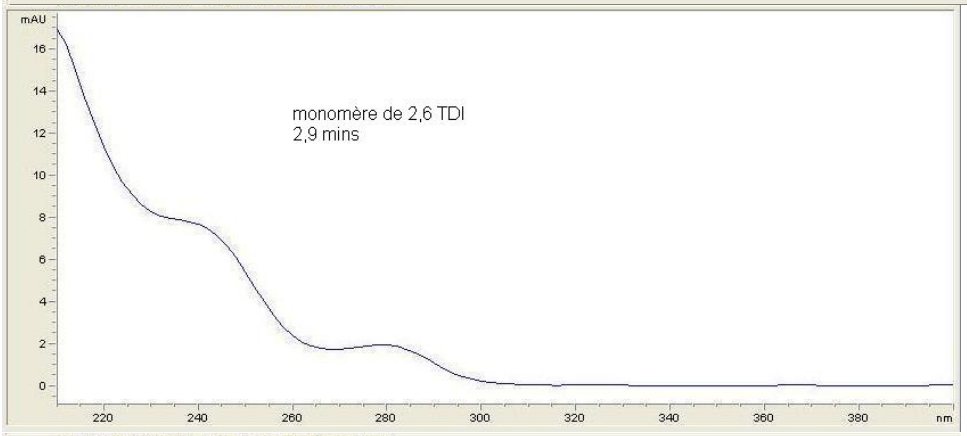


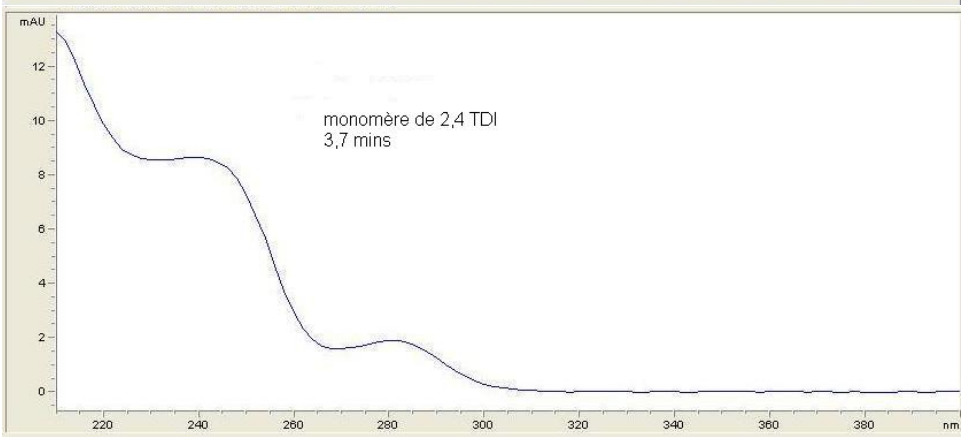
MDI monomer oligomer (tricyclic)
oligomers oligomer oligomers oligomer oligomers oligomer oligomers
monomer of MDI
6.1 min

oligomer of MDI
tricyclic
18.1 minFigure E.4 Chromatogram and UV spectra – TDI (mixture of isomers)

Figure E.4 Chromatogramme et spectres UV TDI (mélange isomères)







2,6-TDI monomer 2,4-TDI
monomer of 2,6-TDI
2.9 min

monomer of 2,4-TDI
3.7 min