DRAFT METHOD 207-2: ANALYSIS FOR ISOCYANATES BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)

Conditional Test Method (CTM) 024

1.0 Scope and Application.

1.1 Method 207-2 covers the determination of derivatized isocyanates collected by Method 207-1: Sampling Method for Isocyanates.

Compound Name	CAS No.	Limit of Quantitation[a]
2,4-Toluene Diisocyanate (TDI)	584-84-9	0.036 µg/mL
1,6-Hexamethylene Diisocyanate (HDI)	822-06-0	0.132 µg/mL
Methylene Diphenyl Diisocyanate (MDI)	101-68-8	0.036 µg/mL
Methyl Isocyanate (MI)	624-83-9	NA[b]

[a]The limit of quantitation is for the instrument conditions given in Section 11.5.1 at the 95% confidence level for seven replicate injections.
[b]The limit of quantitation for MI has not been determined.

1.2 This method is restricted to use by, or under the supervision of, analysts experienced in the use of chromatography and in the interpretation of chromatograms. Each analyst must demonstrate the ability to generate acceptable results with this method.

2.0 Summary of Method.

2.1 The impinger contents from Method 207-1 are concentrated to dryness under vacuum, brought to volume with acetonitrile (ACN) and analyzed by HPLC.

3.0 Definitions. Not Applicable.

4.0 Interferences.

4.1 Method interferences may be caused by contaminants in solvents, reagents, glassware, and other sample processing hardware. All of these materials must be routinely demonstrated to be free from interferences under conditions of the analysis by preparing and analyzing laboratory method (or reagent) blanks.

4.1.1 Glassware must be cleaned thoroughly before using. The glassware should be washed with laboratory detergent in hot water followed by rinsing with tap water and distilled water. The glassware may be cleaned by baking in a glassware oven at 400 °C for at least one hour. After the glassware has cooled, the glassware should be rinsed three times with methylene chloride and three times with acetonitrile. Volumetric glassware should not be heated to 400 °C. Rather, after washing and rinsing, volumetric glassware may be rinsed with ACN followed by methylene chloride and allowed to dry in air.

4.1.2 The use of high purity reagents and solvents helps to minimize interference problems in sample analysis.

5.0 Safety.

5.1 The toxicity of each reagent has been precisely defined. Each isocyanate can produce dangerous levels of hydrogen cyanide (HCN). The exposure to these chemicals must be reduced to the lowest possible level by whatever means available. **The laboratory is responsible for maintaining a current awareness file of Occupational Safety and Health Administration (OSHA) regulations regarding safe handling of the chemicals specified in this method**. A reference file of material safety data sheets should also be made available to all personnel involved in the chemical analysis. Additional references to laboratory safety are available.

6.0 Equipment and Supplies.

6.1 Rotary Evaporator. Buchii Model EL-130 or equivalent.
6.2 1000 ml round bottom flask for use with rotary evaporator.
6.3 Separatory Funnel. 500 mL or larger, with Teflon® Stopcock.
6.4 Glass Funnel. Short stemmed or equivalent.
6.5 Vials. 15 mL capacity with Teflon® lined caps.
6.6 Class A Volumetric Flasks. 10 mL for bringing sample to volume after concentration.
6.7 Filter Paper. Scientific Products Grade 370 Qualitative or equivalent.
6.8 Buchner Funnel. Porcelain with 100 mm ID or equivalent.
6.9 Erlenmeyer Flask. 500 mL with side arm and vacuum source.
6.10 HPLC with at least a binary pumping system capable of a programmed gradient.
6.11 Column. Alltech Altima C18, 250 mm x 4.6 mm ID, 5µm particle size (or equivalent).
6.12 Guard Column. Alltech Hypersil ODS C18, 10 mm x 4.6 mm ID, 5µm particle size (or equivalent).
6.13 UV detector at 254 nm.
6.14 Data system for measuring peak areas and retention times.

7.0 Reagents and Standards.

7.1 Reagent grade chemicals should be used in all tests. All reagents shall conform to the specifications of the Committee on Analytical Reagents of the American Chemical Society, where such specifications are available.
7.2 Toluene, $C_6H_5CH_3$. HPLC Grade or equivalent.
7.3 Acetonitrile, CH_3CN (ACN). HPLC Grade or equivalent.
7.4 Methylene Chloride, CH_2CL_2. HPLC Grade or equivalent.
7.5 Hexane, C_6H_{14}. Pesticide Grade or equivalent.
7.6 Water, H_2O. HPLC Grade or equivalent.
7.7 Ammonium Acetate, $CH_3CO_2NH_4$.
7.8 Acetic Acid (glacial), CH_3CO_2H.
7.9 1-(2-Pyridyl)piperazine, (1,2-pp). Aldrich, 99.5+% or equivalent.
7.10 Absorption Solution. Prepare a solution of 1-(2-pyridyl)piperazine in toluene at a concentration of 40 mg/300 mL. This solution is used for method blanks and method spikes.
7.11 Ammonium Acetate Buffer Solution (AAB). Prepare a solution of ammonium acetate in water at a concentration of 0.1 M by transferring 7.705 g of ammonium acetate to a 1000 mL volumetric flask and diluting to volume with HPLC Grade water. Adjust pH to 6.2 with glacial acetic acid.

8.0 Sample Collection, Preservation, Storage, and Transport.

8.1 The components from Method 207-1 must be stored at 4 °C between the time of sampling and concentration. Each sample should be extracted and concentrated within 30 days after collection and analyzed within 30 days after extraction. The extracted sample must be stored at 4 °C.

9.0 Quality Control.

9.1 The correlation coefficient for the calibration curve must be 0.995 or greater. If the correlation coefficient is less than 0.995, the HPLC system should be examined for problems, and a new calibration curve should be prepared and analyzed.
9.2 A solvent blank should be analyzed daily to verify that the system is not contaminated.
9.3 A calibration standard should be analyzed prior to any samples being analyzed, after every 10 injections and at the end of the sample set. Samples must be bracketed by calibration standards that have a response that does not vary by more than 10% of the target value. If the calibration standards are outside the limit, the samples must be reanalyzed after it is verified that the analytical system is in control.
9.4 A method blank should be prepared and analyzed for every 10 samples concentrated (Section 11.4).
9.5 A method spike should be prepared and analyzed for every 20 samples. The response for each analyte should be within 20% of the expected theoretical value of the method spike (Section 11.3).

10.0 Calibration and Standardization.

10.1 Establish the retention times for each of the isocyanates of interest using the chromatographic conditions provided in Section 11.5.1. The retention times provided in Table 11.5.1-1 are provided as a guide to relative retention times. Prepare

derivatized calibration standards (concentrations expressed in terms of the free isocyanate, Section 12.1) according to the procedure in Section 10.1.1. Calibrate the chromatographic system using the external standard technique (Section 10.1.2)

10.1.1 Preparation of calibration standards. Prepare a 100 µg/mL stock solution of the isocyanates of interest from the individual urea as prepared in Sections 11.1.1 and 11.1.2. This is accomplished by dissolving 1 mg of each urea in 10 mL of ACN. Calibration standards are prepared from this stock solution by making appropriate dilutions of aliquots of the stock into ACN. Calibrate the instrument from 1 to 20 µg/mL for HDI, TDI and MDI, and from 1 to 80 µg/mL for MI using at least six calibration points.

10.1.2 External standard calibration procedure. Analyze each derivatized calibration standard using the chromatographic conditions listed in Section 11.5.1 and tabulate peak area against concentration injected.

The working calibration curve must be verified on each working day by the measurement of one or more calibration standards. If the response for any analyte varies from the target response by more than 10%, the test must be repeated using a fresh calibration standard(s) after it is verified that the analytical system is under control. Alternatively, a new calibration curve may be prepared for that compound.

11.0 Procedures.

11.1 Preparation of isocyanate derivatives.

11.1.1 HDI, TDI, MDI.

11.1.1.1 Dissolve 500 mg of each isocyanate in individual 100 mL aliquots of $MeCl_2$, except for MDI which requires 250 mL of $MeCl_2$. Transfer a 5 mL aliquot of 1,2-pp (see Section 7.10) to each of the solutions, stir and allow to stand overnight at room temperature. Transfer 150 mL aliquots of hexane to each of the solutions to precipitate the isocyanate-urea. Using a Buchner funnel, vacuum filter the solid-urea and wash with 50 mL of hexane. Dissolve the precipitate in a minimum aliquot of $MeCl_2$. Repeat the hexane precipitation and filtration twice. After the third filtration, dry the crystals at 50 °C and transfer to bottles for storage. The crystals are stable for at least 21 months when stored at room temperature in a closed container.

Table 11.1.1-1

Molecular Weight of the Free Isocyanates and the Isocyanate-Urea

Analyte	MW (Free Isocyanate)	MW (Derivative)
1,6-HDI	168	494.44
2,4-TDI	174.16	500.56
MDI	250.25	576.65

11.1.2 MI.

11.1.2.1 To prepare a 200 µg/mL stock solution of methyl isocyanate-urea, transfer 60 mg of 1,2-pp to a 100 mL volumetric flask containing 50 mL of $MeCl_2$. Carefully transfer 20 mg of methyl isocyanate to the volumetric flask and shake for 2 minutes. Dilute the solution to volume with $MeCl_2$ and transfer to a bottle for storage. Methyl isocyanate does not produce a solid derivative and standards must be prepared from this stock solution.

Table 11.1.2.1-1

Molecular Weight of Free Methyl Isocyanate and Methyl Isocyanate-Urea

Analyte	MW (Free Isocyanate)	MW (Derivative)
MI	57.1	220.32

11.2 Concentration of Samples.

11.2.1 Transfer each sample to a 1000 mL round bottom flask. Attach the flask to a rotary evaporator and gently evaporate to dryness under vacuum in a 65 °C water bath. Rinse the round bottom flask three times each with two mL of ACN and transfer the rinse to a 10 mL volumetric flask. Dilute the sample to volume with ACN and transfer to a 15 mL vial and seal with a Teflon® lined lid. Store the vial at 4 °C until analysis.

11.3 Preparation of Method Spikes.

11.3.1 Prepare a method spike for every twenty samples. Transfer 300 mL of the absorption solution to a 1000 mL round bottom flask. Transfer 1 mL of a 100 µg/mL standard containing the isocyanate-ureas of interest. Follow the procedure outlined in Section 11.2.1 for sample concentration. This will result in a method spike with a theoretical concentration of 10 µg/mL.

11.4 Preparation of Method Blanks.

11.4.1 Prepare a method blank for every ten samples by transferring 300 mL of the absorption solution to a 1000 mL round bottom flask and concentrate as outlined in Section 11.2.1.

11.5 Chromatographic Analysis.

11.5.1 Chromatographic Conditions.

Column:	C18, 250 mm x 4.6 mm ID, 5µm particle size.
Mobile Phase:	Acetonitrile/Ammonium Acetate Buffer.
Gradient:	10:90 (v/v) ACN:AAB to 60:40 (v/v) ACN:AAB over 30 minutes.
Flow Rate:	2 mL / min.
UV Detector:	254 nm.
Injection Volume:	50 µL.

Table 11.5.1-1

Retention Times of the Four Isocyanates

Compound	Retention Time (minutes)
MI	10.0
1,6-HDI	19.9
2,4-TDI	27.1
MDI	27.3

11.5.2 Analysis.

11.5.2.1 Analyze samples by HPLC, using conditions established in Section 11.5.1.

11.5.2.2 The width of the retention time window used to make identifications should be based upon measurements of actual retention time variations of standards over the course of a day.

Three times the standard deviation of a retention time for a compound can be used to calculate a suggested window size; however, the experience of the analyst should weigh heavily in the interpretation of the chromatograms.

11.5.2.3 If the peak area exceeds the linear range of the calibration curve, the sample should be diluted with ACN and reanalyzed.

12.0 Data Analysis and Calculations.

12.1 Conversion from isocyanate to the urea. The equation for converting the amount of free isocyanate to the corresponding amount of isocyanate-urea is as follows:

$$\text{Amount of the urea} = \text{Amount of free isocyanate} * \left(\frac{\text{Molecular weight of the isocyanate-urea}}{\text{Molecular weight of the isocyanate}} \right) \quad \text{Eq. 207-2-1}$$

The equation for converting the amount of isocyanate-urea to the corresponding amount of free isocyanate is as follows:

$$\text{Amount of the isocyanate} = \text{Amount of isocyanate-urea} * \left(\frac{\text{Molecular weight of the isocyanate}}{\text{Molecular weight of the isocyanate-urea}} \right) \quad \text{Eq. 207-2-2}$$

12.2 Calculate the correlation coefficient, slope and intercept for the calibration data using the least squares method for linear regression. Concentrations are expressed as the x-variable and response is expressed as the y-variable.

12.3 Calculate the concentration of isocyanate in the sample:

$$\text{Concentration } (\mu g/mL) = \frac{(\text{Sample Response (Area)} - \text{Intercept})}{\text{Slope}} \quad \text{Eq. 207-2-3}$$

12.4 Calculate the total amount collected in the sample by multiplying the concentration (µg/mL) times the final volume of ACN (10 mL).

$$\text{Amount Isocyanate } (\mu g) = \text{Concentration } (\mu g/mL) \times \text{Final Volume } (10 \text{ mL}) \quad \text{Eq. 207-2-4}$$

12.5 Calculate the concentration of isocyanate (µg/dscm) in the stack gas.

$$\text{Concentration of isocyanate} = K \frac{\text{Total Isocyanate in Sample (ug)}}{V_{m(std)}} \quad \text{Eq. 207-2-5}$$

where:

$K = 35.31\ ft^3/m^3$ if $V_{m(std)}$ is expressed in English units.

$= 1.00\ m^3/m^3$ if $V_{m(std)}$ is expressed in metric units.

$V_{m(std)}$ = Volume of gas sample as measured by a dry gas meter; corrected to standard conditions, dscm (dscf).

13.0 Method Performance.

13.1 Method detection limit (MDL) concentrations were obtained by determining the standard deviation of the area count for seven replicate injections and then multiplying the standard deviation for seven replicate injections by the student's t-valve at the 95% confidence level (3.143).

13.2 This method has been tested for linearity over the range from 2 x MDL to 500 x MDL.

14.0 Pollution Prevention. Not Applicable.

15.0 Waste Management. Not Applicable.

16.0 References. Not Applicable.

17.0 Tables, Diagrams, Flowcharts, and Validation Data. Not Applicable.