THE CONFIRMATION OF THE UREA DERIVATIVES OF MDI AND TDI BY THERMAL ENERGY ANALYSIS

Method no.:	33
Recommended minimum sample concentration for confirmation:	1 µg/sample MDI or TDI
Procedure:	Air samples are collected and analyzed as recommended in OSHA Organic Division Method No. 18 - Diisocyanates: 2,4-TDI and MDI (Ref. 4.1.). Following routine analysis, samples which exceed the OSHA PEL are submitted in a graduated evaporative concentrator for confirmation. The sample is evaporated to dryness and rediluted with chloroform. Excess nitro reagent is removed by extraction and the sample is subjected to normal-phase HPLC separation where the diisocyanate derivative peaks are collected. The isolated components are concentrated by evaporating the HPLC mobile phase to dryness. The sample is diluted with toluene and analyzed by GC/TEA.
Minimum sample concentration required for detection:	0.13 µg/sample for TDI 0.15 µg/sample for MDI
Status of method:	A confirmatory procedure which has been developed and reviewed by the Organic Methods Evaluation Branch.

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1. General Discussion

1.1. Introduction

The OSHA air sampling procedure for toluene-2,4-diisocyanate (TDI) and methylene bisphenyl isocyanate (MDI) recommends the use of a bubbler containing 15 mL of 0.0002M *p*-nitrobenzyl-N-n-propylamine (nitro reagent) in toluene. Both MDI and TDI readily react with nitro reagent to form urea derivatives (Ref. 4.1.).

The OSHA procedure for the routine analysis of the nitro reagent derivatives of MDI and TDI (MDIU and TDIU) recommends using reversed-phase high pressure liquid chromatography (HPLC) with ultraviolet (UV) detection at 254 nm (Ref. 4.1.). This procedure is fast, precise, reliable, and convenient.

Mass spectrometric (MS) confirmation of air samples which exceed the OSHA PEL is often difficult because the MS method has a relatively high detection limit and the mass spectra are usually complicated. Because the MS procedure uses the direct insertion probe (DIP) method to introduce the sample, the analyst must isolate and concentrate the analytes by repetitive HPLC separation and peak collection. This process is time consuming and usually inconvenient.

The highly selective Thermal Energy Analyzer (TEA) has been in use to determine N-nitrosamines for several years and its operation has been described (Ref. 4.2.). Recently, a TEA retrofit modification which also permits the detection of analytes containing one or more nitro groups has been made commercially available. The main components of the retrofit package are pyrolyzers which can attain higher temperatures than those of the unmodified TEA. The principle of operation is similar for both detectors - the chromatographed analyte passes through a pyrolyzer where the appropriate moieties (-NNO or -ONO) decompose to liberate NO. The NO enters a reaction chamber where it undergoes a chemiluminescent reaction with ozone and is detected. Since MDIU and TDIU both contain nitro groups, this method which utilizes a modified TEA was developed to confirm samples which exceed the OSHA PEL.

When the analytes were subjected to separation by gas chromatography (GC) nitro reagent, MDIU and TDIU all gave a single sharp peak at the same retention time on a 3-ft glass SP-1000 GC column. It became apparent that MDIU and TDIU decomposed at normal GC temperatures. Melting point determinations, performed on solid MDIU and TDIU, gave visible evidence of decomposition. Both diisocyanate derivatives melted at about 120°C and changed to a black, tar-like material at about 200°C. A subsequent GC/MS study has shown a thermal decomposition product of TDIU to be nitro reagent and this is the likely source of the TEA response. Therefore, GC techniques cannot be used for the separation of MDIU and TDIU but GC/TEA can provide a means to detect the isolated analyte.

The effects of increasing the GC pyrolyzer temperature on detector response were studied. It was found that maximum detector response for the analytes occurred at 875°C (Table 3.4.). The response at 875°C is approximately 14 times that observed at 750°C. No TEA response was observed at 600°C.

The maximum temperature that the HPLC pyrolyzer can attain is 800°C. The intact analytes can be separated by HPLC but the detector response is poor at reduced pyrolyzer temperatures. Therefore, HPLC/TEA does not seem to be a viable technique for samples containing low levels of MDIU or TDIU.

Because, at this time, it does not seem possible to separate and then simultaneously confirm the analyte at sufficiently low levels, a compromise method is recommended. More than 99% of the excess nitro reagent in air samples can be removed by a simple acid extraction. Following nitro reagent removal, the sample is separated into its components by normal-phase HPLC. The individual analytes are collected after they pass through a UV detector. The analytes are concentrated by evaporating the mobile phase to dryness. The sample is diluted with toluene and analyzed by GC/TEA. Even though the recommended method is not direct, the need for repetitive separation and collection is eliminated because the GC/TEA method has a lower detection limit than the MS/DIP procedure.

It is unlikely that an interference will have the same retention time on both reversed and normal-phase HPLC columns and also the same GC retention time as the decomposed analyte. It is possible that a UV interference will elicit a TEA response but it is unlikely that the degree of response will be the same for both detectors.

Data have been collected on MDI and TDI samples subjected to reversed-phase HPLC/UV, normal-phase HPLC/UV and GC/TEA analysis. The data are presented in Table 3.5.

This alternative method is not intended for routine analytical use. It was developed to confirm high results obtained by the routine reversed-phase HPLC/UV method. The new method has a sufficiently low detection limit to confirm results well below the OSHA PEL when the recommended air sampling method is followed.

1.2. Detection limit of the analytical procedure (GC/TEA)

The detection limit of the GC/TEA analytical procedure is 81 pg for TDI and 92 pg for MDI per GC/TEA injection. This is the amount of analyte which will give a peak whose height is about five times the height of the baseline noise (Section 3.1.).

1.3. Minimum sample concentration required for detection

The minimum sample concentration required for detection is 0.13 µg per TDI sample and 0.15 µg per MDI sample. This is equivalent to 7 $\mu g/m^3$ for TDI and 8 $\mu g/m^3$ for MDI based on the recommended air volume of 20 L.

1.4. Advantages

1.4.1. This method has a lower detection limit than the MS/DIP procedure.

1.4.2. This procedure is less tedious than the MS/DIP method because it eliminates the need for multiple HPLC runs to isolate and concentrate the analyte.

1.4.3. The cost of the recommended instrumentation is less for this method than for the MS/DIP procedure.

1.4.4. It is possible to quantitate results obtained by use of this method.

1.5. Disadvantages

1.5.1. The analytes can not be simultaneously separated and confirmed by use of this method.

1.5.2. Unlike the MS/DIP procedure, the molecular structure of the compound in question is not obtained through use of this method.

2. Analytical Method

2.1. Apparatus

2.1.1. HPLC apparatus equipped with UV detector, sample injector and chart recorder. The UV detector used in this work was a Waters Associates Model 440 Absorbance Detector. The detector was equipped with a 50-cm length of 0.23-mm i.d. stainless steel tubing attached to the outlet of the sample cell for peak collection.

2.1.2. HPLC analytical column capable of separating MDIU and TDIU. The column used in this work was a 25-cm x 4.6-mm Dupont Zorbax CN column.

2.1.3. Electronic integrator or other suitable means to determine peak areas.

2.1.4. Graduated evaporative concentrators, 10 mL, Kontes or equivalent.

2.1.5. Temperature controlled water bath equipped with nitrogen stream evaporative needles.

2.1.6. Vortex mixer, Scientific Products Deluxe Mixer 58220 or equivalent.

2.1.7. Laboratory centrifuge, IEC HN-SII Centrifuge, or equivalent.

2.1.8. Vials, 2-mL with Teflon-lined caps.

2.1.9. Gas chromatograph.

2.1.10. Thermal Energy Analyzer equipped with an Explosives Analysis Package, Thermo Electron Corp., Waltham, Mass.

2.1.11. GC column capable of resolving the analyte decomposition product from potential interferences. The column used in this work was 3 ft x 1/4-in. o.d. (2-mm i.d.) glass, on-column injection, with 10% SP-1000 on 80/100 Supelcoport. The glass column was modified to conform to the GC-TEA interface.

2.1.12. Dewar flasks, for liquid nitrogen.

2.1.13. Pipets, disposable Pasteur type.

2.1.14. Assorted miscellaneous laboratory equipment.

2.1.15. Stopwatch.

2.2. Reagents

2.2.1. Analytical standards, see Section 3.3. of OSHA Organic Division, Method No. 18, Diisocyanates: 2,4-TDI and MDI (Ref. 4.1.).

2.2.2. Methanol, isopropanol, isooctane, toluene, acetonitrile, chloroform, and n-propanol, HPLC grade.

2.2.3. Phosphoric acid, 1% in deionized water by volume, reagent grade.

2.2.4. Liquid nitrogen.

2.2.5. Helium and nitrogen, GC grade.

2.2.6. Oxygen, medical grade.

2.3. Standard preparation

2.3.1. Prepare MDIU and TDIU standards, diluted with acetonitrile, as described in Section 3.3. of OSHA Organic Division, Method No. 18 Diisocyanates: 2,4-TDI and MDI (Ref. 4.1.).

2.3.2. Place 1.00 mL of each standard from the working range into a 10-mL concentrator tube. Evaporate the standard to dryness using a heated water bath (55°C) and a gentle nitrogen gas stream. Do not allow the standard to stand in the water bath for an extended time following solvent evaporation.

2.3.3. Allow the concentrator tube to return to room temperature and then add 1.00 mL of chloroform.

2.3.4. Add 5 mL of 1% v/v phosphoric acid to the concentrator tube and then mix the contents of the tube using a vortex mixer for 30 seconds. The phosphoric acid serves to extract nitro reagent from the organic to the aqueous phase.

2.3.5. Separate the aqueous and organic phases by centrifuging the concentrator tube.

2.3.6. Remove and discard the aqueous (upper) phase with a disposable pipet. Using a clean pipet, transfer the organic (lower) phase to a small vial and then tightly seal the vial with a Teflon-lined cap. Be careful not to transfer aqueous with the organic phase. The standard is now ready for HPLC/UV analysis and component isolation.

2.4. Sample preparation

About 0.5 mL of each sample to be confirmed should be submitted in a graduated evaporative concentrator. The sample should contain at least 1 µg of analyte. The person requesting the confirmation should provide the suspected concentration and identity of the analyte in question. The sample should be stored in a freezer until analysis.

2.4.1. Record the volume of the sample in the graduated concentrator to two decimal places. Evaporate the sample to dryness using a heated (55°C) water bath and a gentle nitrogen

gas stream. Do not allow the sample to stand in the water bath for an extended time following solvent evaporation.

2.4.2. Allow the concentrator tube to return to room temperature and then add 1.00 mL of chloroform. If the sample to be confirmed contains low levels of the analyte, 0.50 mL of chloroform may be substituted to give a more concentrated solution.

2.4.3. Add 5 mL of 1% v/v phosphoric acid to the concentrator tube and then mix the contents of the tube using a vortex mixer for 30 s. The phosphoric acid serves to extract nitro reagent from the organic to the aqueous phase.

2.4.4. Separate the aqueous and organic phases by centrifuging the concentrator tube.

2.4.5. Remove and discard the aqueous (upper) phase with a disposable pipet. Using a clean pipet, transfer the organic (lower) phase to a small vial and then tightly seal the vial with a Teflon-lined cap. Be careful not to transfer aqueous with the organic phase. The sample is now ready for HPLC/UV analysis and component isolation.

2.5. HPLC/UV analysis

2.5.1. Normal-phase HPLC conditions

column:	Dupont Zorbax CN (25 cm x 4.6 mm) or equivalent
mobile phase:	75/15/10 (v/v/v) isooctane/isopropanol/methanol
flow rate:	1 mL/min
UV detector:	254 nm (fixed wavelength)
injection volume:	25 µL
chromatogram:	Figure 3.2.

2.5.2. HPLC separation and peak collection

2.5.2.1. Determine the retention time for each analyte using standards of similar concentration as those suspected in the samples.

2.5.2.2. Isolate each analyte by collection of the HPLC column effluent at the appropriate time using a 10-mL graduated concentrator tube. The use of excessive tubing and/or valves to collect the analyte is not recommended. The 50-cm length of tubing described in Section 2.1.1. has a dead volume of 21 µL and the transfer time from the sample cell to the collection point is 1 s. Therefore, the transfer time from the sample cell to the collection point is insignificant when the recommended apparatus is used.

2.5.2.3. Evaporate the collected analyte to dryness using a heated (55°C) water bath and a gentle nitrogen gas stream. Do not allow samples to stand in the water bath for an extended period of time following solvent evaporation.

2.5.2.4. Allow the concentrator tubes to return to room temperature and then add 0.20 mL toluene to each tube. Mix the contents of each tube using a vortex mixer.

2.5.2.5. Reinject each collected standard and sample to insure that proper peak collection technique has been used. When using Zorbax CN analytical column, toluene will not present a chromatographic interference.

2.6. GC/TEA analysis

2.6.1. GC conditions

column:	3 ft x 1/4-in. o.d. (2-mm i.d.) glass, on-column injection, 10% SP-1000 on 80/100 Supelcoport.
injector temperature:	250°C
column temperature:	240°C
GC/TEA interface temperature:	250°C
helium (carrier gas) flow rate:	30 mL/min
injection volume:	5 µL

2.6.2. TEA conditions

GC pyrolyzer temp.:	875°C
oxygen flow rate:	0.5 mL/min
pressure:	0.5 mm Hg
cold trap temp.:	-130°C (n-propanol and liquid N_2)
chromatogram:	Figure 3.3.

2.7. Analysis notes

Results of this method are quantitative. Confirmation of suspected MDIU and TDIU in air samples depends on the comparison of results obtained by the reversed-phase HPLC/UV method, the normal-phase HPLC/UV method and the GC/TEA method (Section 3.5.).

2.7.1. Measure UV and TEA detector response with an electronic integrator or other suitable means.

2.7.2. Compare samples to standards of similar concentration. This is easy to do because the suspected concentration of samples is known prior to confirmation.

2.7.3. Use an external standard procedure to prepare a calibration curve using at least three standard solutions of different concentrations. Prepare the calibration curve daily. Calibrate the integrator to report results in µg/mL.

2.8. Interferences

2.8.1. Nitro reagent is an interference in the GC/TEA analysis of TDIU and MDIU. Excess nitro reagent is removed by phosphoric acid extraction prior to HPLC/UV analysis. The potential interference of nitro reagent is further reduced by HPLC separation of the analytes prior to peak collection. The analysis of blank samples will confirm the absence of nitro reagent.

2.8.2. Any compound having the same retention time as the analytes and giving a TEA response is a potential interference. Generally, HPLC or GC parameters can be changed to circumvent an interference. An interference can often manifest itself by causing a difference in expected results. If the reversed-phase HPLC/UV, the normal-phase HPLC/UV and GC/TEA results do not compare within experimental error, then a chromatographic interference is possible.

2.9. Calculations

The following section applies to both HPLC/UV and GC/TEA results.

2.9.1. Use the integrator value, in µg/mL, for reference only. More reliable results are obtained by use of a calibration curve. The detector response, for each standard, compared to its equivalent concentration in µg/mL and the best straight line through the data points is determined by linear regression.

2.9.2. Determine the concentration, in µg/mL, for a particular sample by comparing its detector response to the calibration curve.

2.9.3. HPLC/UV

Corrected µg/mL diisocyanate =

$$\mu g / mL \text{ from Section 2.9.2.} \times \frac{\text{volume from Section 2.4.2}}{\text{volume from Section 2.4.1}}$$

2.9.4. GC/TEA

Corrected µg/mL diisocyanate =

$$\mu g / mL \text{ from Section 2.9.2.} \times \frac{\text{volume from Section 2.4.2}}{\text{volume from Section 2.4.1}}$$

2.9.5. Discussion

If the reversed-phase HPLC/UV, normal-phase HPLC/UV and GC/TEA results all agree, within experimental limits, then the sample results are confirmed. If the results do not agree, within limits, then the sample results are not confirmed. If no peak, with the same retention time as the analyte, is observed upon normal-phase HPLC/UV analysis, the sample results are not confirmed. If the appropriate normal-phase HPLC/UV results are obtained but no GC/TEA peak with the proper retention time is observed, then the sample results are not confirmed.

2.10. Safety precautions

2.10.1. Sample and standard preparations should be done in a fume hood. Avoid exposure to diisocyanates.

2.10.2. Avoid skin contact with liquid nitrogen and the solvents.

2.10.3. Avoid exposure to solvent vapors.

2.10.4. Wear safety glasses in all laboratory areas.

2.10.5. Check to be sure that the TEA exhaust is connected to a fume hood.

3. Backup Data

The chromatograms in this section were generated by the analysis of MDIU and TDIU, however, all calculated results and amounts were presented as free MDI and TDI.

3.1. Detection limit of the analytical procedure (GC/TEA)

The GC/TEA chromatogram shown in Figure 3.1. represents the detection limit for TDIU and MDIU. Twenty-five microliters of an acid extracted standard containing 0.13 µg/mL TDI in chloroform was subjected to normal-phase HPLC/UV analysis and the TDIU peak was collected. The HPLC mobile phase was evaporated and the residue was diluted with 0.20 mL of toluene. The GC/TEA chromatogram was generated by the injection of 5 µL of the toluene solution.

25 µL x 0.13 µg/mL TDI = 3.25 ng TDI
3.25 ng/0.20 mL = 16.25 ng/mL TDI
5 µL x 16.25 ng/µL TDI = 81 µg TDI

Therefore, the GC/TEA detection limit for TDI is 81 µg per injection.

Because the TEA response is molar, the detection limit for MDIU may be calculated.

$$\frac{\text{638 (MW for MDIU)}}{\text{562 (MW for TDIU)}} \times 81 \text{ pg TDI} = 92 \text{ pg MDI}$$

The detection limit for MDIU is 92 pg per injection.

The detection limit is that amount of analyte which will give a peak whose height is about 5 times the height of the baseline noise.

3.2. Minimum sample concentration required for detection

The following sample concentrations will provide the necessary quantities for GC/TEA detection and the concentrations are more than adequate for HPLC/UV detection.

The minimum sample concentration required for detection is 0.13 µg/sample for TDI and 0.15 µg/sample for MDI. This is equivalent to 7 $\mu g/m^3$ for TDI and 8 $\mu g/m^3$ for MDI based on the recommended air volume.

The volumes recommended in Section 3.1. were used to determine the minimum concentration required for detection.

3.3. GC/TEA chromatogram

Twenty-five microliters of an acid extracted standard containing 2.5 µg/mL TDI in chloroform was subjected to normal-phase HPLC/UV analysis and the TDIU peak was collected. The HPLC mobile phase was evaporated and then the residue was diluted with 0.20 mL toluene. The chromatogram shown in Figure 3.3. was generated by the injection of 5 µL of the toluene solution.

3.4. The data in Table 3.4. were generated by the GC/TEA analysis of the same sample using different GC pyrolyzer temperatures. The TEA response at 750°C was assigned a value of 1.0 and the response at other temperatures was calculated relative it.

Table 3.4.
The Effects of GC Pyrolyzer
Temperature on TEA Detector Response

pyrolyzer temperature, °C	TEA response
600	0.0
700	0.23
750	1.0
800	3.1
850	9.4
875	14
900	12

3.5. The data in Table 3.5 were obtained from the reversed-phase HPLC/UV, normal-phase HPLC/UV and GC/TEA analysis of different MDI and TDI samples.

Table 3.5.
Comparison of Diisocyanate Results (µg/mL)

sample	analyte	reversed-phase	normal-phase	GC/TEA
QC	MDIU	4.0	4.4	6.9[1]
QC	MDIU	6.2	6.4	7.1[1]
QC	MDIU	8.1	8.8	10.6[1]
air	TDIU	5.2	3.3	3.8[1]
air	TDIU	3.8	2.6	2.9[1]
air	TDIU	0.77	0.4	0.6
standard	MDIU	13.5	13.3	12.6
standard	MDIU	10.8	11.4	10.9
standard	MDIU	8.1	9.4	8.0
standard	MDIU	5.4	4.9	5.0
standard	MDIU	2.7	2.5	3.2
standard	TDIU	0.26	0.28	0.49
standard	TDIU	0.79	0.95	0.67
standard	TDIU	1.3	1.1	1.1
standard	TDIU	2.5	2.5	2.6
standard	TDIU	6.6	6.6	6.6
QC	MDIU	5.8	6.7	6.8
QC	MDIU	3.8	3.5	4.5
air	MDIU	16	15	15
air	MDIU	26	27	26
air	MDIU	3.0	3.0	3.4
air	TDIU	4.0	6.8	5.6

[1] The solvent used for the final dilution was changed from chloroform to toluene because of the volatility of chloroform.

The reverse-phase result was divided by the normal-phase result, the reversed-phase HPLC/UV result was divided by the GC/TEA result, and the normal-phase HPLC/UV result was divided by the GC/TEA result. These calculations were performed for each set of data and the average for each calculation is shown below.

$$\frac{\text{average reversed-phase HPLC/UV}}{\text{normal-phase HPLC/UV}} = 1.06$$

$$\frac{\text{average reversed-phase HPLC/UV}}{\text{GC/TEA}} = 0.972$$

$$\frac{\text{average normal-phase HPLC/UV}}{\text{GC/TEA}} = 0.941$$

When the above calculations were performed on individual samples which contained more than 1 µg of analyte and were diluted with toluene, 40 of 42 individual results were within the range of 0.75 to 1.25 (±25%). These data indicate that results from reversed-phase HPLC/UV, normal-phase HPLC/UV and GC/TEA analysis of the same sample should be within ±25% of each other if the samples contain at least 1 µg of analyte and the recommended analytical procedures are followed. Therefore, samples should be reported as confirmed only when the results of the recommended analytical procedures are within ±25% of each other. The ±25% figure is presented without rigorous statistical argument.

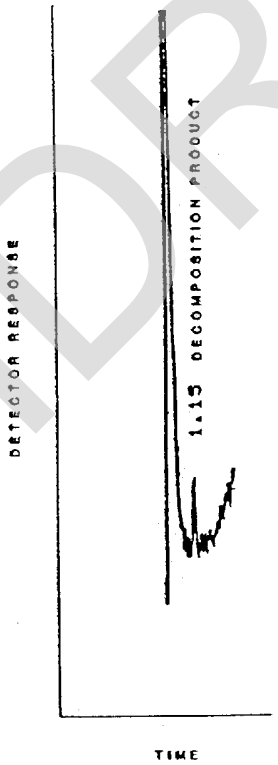

Figure 3.1. GC/TEA detection limit for the
decomposition product of the nitro reagent derivatives of MDI and TDI.

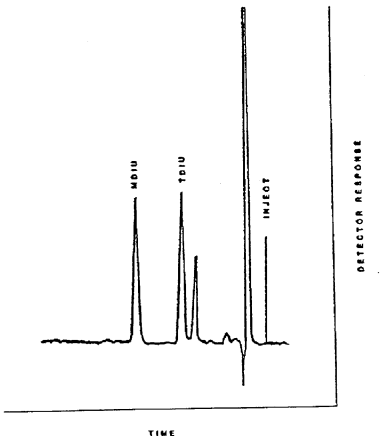

Figure 3.2. Normal-phase HPLC/UV chromatogram for MDIU and TDIU.

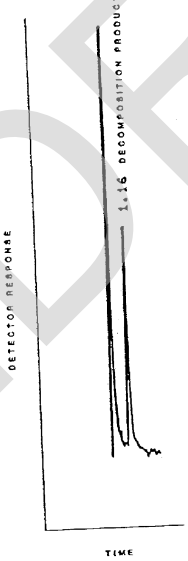

Figure 3.3. GC/TEA chromatogram for the decomposition product of the nitro reagent derivatives of MDI and TDI.

4. References

4.1. Cummins, K. Diisocyanates - 2,4-TDI and MDI (Method 18, Organic Methods Evaluation Branch, OSHA Analytical Laboratory, Salt Lake City, Utah). Unpublished (2-80).

4.2. Hendricks, W. Volatile Nitrosamine Mixture I (Method 27, Organic Methods Evaluations Branch, OSHA Analytical Laboratory, Salt Lake City, Utah). Unpublished (2-81).