Issued: 4/15/00
**Revised 02/16/04**

# Analysis of Isocyanates

# Liquid Chromatography — Diode Array/MSD

## 1.0 Scope and Application

1.1 This method is applicable for the determination of isocyanates prepared by derivatization of analytes extracted from solids, soils, filters, and foams or collected in impinger solutions. The following isocyanates, may be determined by this method: 2,4-toluene diisocyanate (CAS 584-84-9), 2,6-toluene diisocyanate (CAS 91-08-7), 1,6-hexamethylene diisocyanate (CAS 822-06-0), 1,6-hexamethylene diisocyanate biuret (CAS 4035-89-6), 1,6-hexamethylene diisocyanate trimer (CAS 28182-81-2, isophorone diisocyanate (CAS 4098-71-9), 2,4'-methylene diphenyl diisocyanate (CAS 5873-54-1) and 4,4'-methylene diphenyl diisocyanate (CAS 101-68-8).

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

1.3 The toxicity of each reagent has been precisely defined. The exposure to these chemicals must be reduced to the lowest possible level by whatever means available. The laboratory maintains a current awareness file of OSHA regulations regarding safe handling of the chemicals specified in this method. A reference file of material safety data sheets is available to all personnel involved in chemical analysis. Refer to the Chemical Hygiene Plan for additional information regarding laboratory safety.

## 2.0 Summary of Method

2.1 The extract obtained from the solid sample, i.e. soil, foam, etc., is evaporated to dryness and re-dissolved in 90:10 Acetonitrile:Dimethyl sulfoxide [TDI,MDI] or 70:30 Acetonitrile:Dimethyl sulfoxide [HDI, IPDI].

2.2 Samples obtained from impingers are evaporated to dryness and re-dissolved in the appropriate Acetonitrile:Dimethyl sulfoxide solution.

2.3 Filter samples which have been field desorbed with Acetonitrile:Dimethyl sulfoxide are analyzed directly. If other solvents are used, the extract is evaporated to dryness and re-dissolved in the appropriate Acetonitrile:Dimethyl sulfoxide solution.

## 3.0 Interferences

3.1 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 the conditions of the analysis by preparing and analyzing laboratory method (or reagent) blanks.

3.1.1 Glassware must be thoroughly cleaned prior to use.

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

3.2 Compounds existing in the sample matrix that pass through the LC column and have an absorbance at the monitored wavelength may be a chemical interference. Changing the chromatographic conditions may allow a separation of peaks suitable for determination of the isocyanates. It is especially important in these cases to confirm the positive identification of isocyanates with matrix fortified samples.

3.3 Under the chromatographic conditions, HDI monomer and 2,6-TDI co-elute; consequently, if the two compounds

are thought to be present then a mass selective detector must be employed.

## 4.0 Apparatus and Materials

4.1 High Performance Liquid Chromatograph (HPLC):

4.1.1 Hewlett Packard HP-1100 LC, equipped with a Diode Array and MS detectors.

4.1.2 Phenomenex Luna 3μ C8(2) 100Å 100 X 2.00 mm, Analytical Part No. 00D-42486-B0

4.1.3 Phenomenex Security Guard Cartridge 4 X 2.0 mm Part No. AJO-4350

4.2 Vials, 20-mL with Teflon®-lined caps.

4.3 Volumetric flask, 5-mL.

4.4 Analytical balance: Capable of weighing to the nearest 0.1 mg.

## 5.0 Reagents

5.1 Water, Fisher Scientific Optima grade, Cat. No. W7-4, or equivalent: Water must be free of interferences at the IDL for each analyte.

5.2 Acetonitrile, Fisher Scientific HPLC grade, Cat. No. A998-4, or equivalent.

5.3 Methylene chloride, Fisher Scientific Optima grade, Cat. No. D151-4, or equivalent.

5.4 Hexane, Fisher Scientific Optima grade, Cat. No. H303-1, or equivalent.

5.5 Ammonium acetate, Aldrich Cat. No. 24,019-2.

5.6 Acetic acid, glacial, Fisher Scientific Cat. No. $A38^{S}$-212.

5.7 1-(2-Pyridyl)piperazine (1,2-PP) solution, Aldrich Cat. No. 15,127-0.

5.8 1-(2-Methoxyphenyl)piperazine (1,2-MP, 1,2-MOPP) solution, Aldrich Cat. No. M2,260-1

5.9 Ammonium acetate buffer solution (AAB), 0.1 M: Prepared by weighing out 7.705 g of ammonium acetate and diluting to 1 L with HPLC grade water. Adjust the pH to 6.2 with glacial acetic acid.

5.10 Dimethyl sulfoxide, Baxter HPLC grade, Cat. No. 081-1.

## 6.0 Sample Collection, Preservation, and Handling

6.1 Samples must be stored at 4°C prior to analysis. Samples must be concentrated within 30 days of extraction and should be analyzed as soon as possible.

## 7.0 Procedure

7.1 Sample Preparation:

7.1.1 Samples that are soils, solids, or foams are extracted using the Accelerated Solvent Extractor or Soxhlet technique.

7.1.1.1 Extract 10.0 g of sample using standard Soxhlet procedures except, 40 mg of 1,2-PP must be added to the methylene chloride in the receiving flask. Derivatization of the isocyanate will occur during extraction. In the case of the ASE, the reagent, dissolved in 2 mL of solvent, is added to the receiving vessel during the

set up of the extractor.

7.1.1.2 Quantitatively transfer the extract to a 5-mL volumetric flask and gently evaporate to dryness using a stream of nitrogen.

7.1.1.3 Re-dissolve the extract in the appropriate Acetonitrile:Dimethyl sulfoxide solution and dilute to the mark.

7.1.2 Toluene impinger samples should be received with the isocyanates already derivatized and in toluene. The sample should be evaporated to dryness and redissolved in the appropriate Acetonitrile:Dimethyl sulfoxide solution. To insure that all of the toluene has been removed the following solvent exchange procedure is recommended.

7.1.2.1 Solvent Exchange
The impinger sample (in its entirety) is placed in a concentrator vessel (example: Kuderna-Danish, Rapid Vap) and concentrated to less than 2 mL (less than 1 mL is preferable for toluene) with the settings for the concentrator as follows:

TEMPERATURE SETTINGS – 70 DEGREES C
TEMPERATURE ACTUAL – 65 DEGREES C
VORTEX ACTION – 90
TIME SETTINGS – 15 min intervals (~4-5 will be followed)

Add 50 mL of a 95:5 acetonitrile: dimethyl sulfoxide mixture directly in to the vessel. Gently swirl the concentrator tube so that the 95:5 mixes in its completely with the small amount of sample left in the concentrator vessel. Place the vessel in the concentrator and resume concentrating at the same settings as before until the extract reaches <1 mL or near dryness.

Qualitatively transfer the sample into a 5 mL volumetric flask* with the appropriate Acetonitrile:Dimethyl sulfoxide solution and mixed.

Transfer 1-2 mL of the final sample into a 2-mL autosampler vial and store the vial at 4° C until analysis is performed.

* If samples are known to be very low in concentration or if a very low MDL is necessary, a smaller final volume maybe necessary.

7.1.3 Filter samples are desorbed in the field by addition of either 5 mL of the appropriate Acetonitrile:Dimethyl sulfoxide solution and are ready for analysis. If other solvents are used for field desorption, the extract is evaporated to dryness and re-dissolved in the appropriate Acetonitrile:Dimethyl sulfoxide solution. The volume used for desorption is dictated by the concentration expected.

7.2 Instrument Conditions:

7.2.1 Fill the reservoirs with the solvents listed below:

| | |
|---|---|
| Pump B | acetonitrile (section 5.2) |
| Pump C | ammonium acetate buffer (section 5.9) |

7.2.2 Install the LC column taking care not to overtighten the plastic fittings (fingertight is sufficient). A guard column, complementary to the column, should be installed prior to the analytical column.

7.2.3 Start the pumps and allow the column to equilibrate for 30 min. Make sure all fittings are leak-free.

7.2.4 Set the liquid chromatograph and detectors to the following parameters:

**Pump:**

Stop Time 20 min
Post Time 5.00 min
Flow 300 µL/min
Oven Temperature 40°C

**Injector:**

Injection Volume 5.0 µL Draw Speed 200 µL/min

| **Diode Array Detector Settings:** | | |
|---|---|---|
| | Stop Time | as Pump: 20.00 min |
| | Post Time | Off |
| | Peak Width | 0.050 min |
| | Sampling | 0.320 s |
| | Autobalance | On |
| **Signals:** | | |
| | Sample, A | 254 nm |
| | Sample, C | 240 nm |
| | Stored | Yes |
| | Band Width | 4 nm |
| | Reference C | 490 nm |
| | Band Width | 40 nm |
| **Spectrum:** | | |
| | Store | All in Peak |
| | From | 220 nm |
| | To | 500 nm |
| | Step | 2 nm |
| | Threshold | 1.0 mAU |
| | UV Lamp required | Yes |
| | VIS Lamp required | No |
| | Autobalance Prerun | No |
| | Autobalance Postrun | No |

| **MSD Scan** | |
|---|---|
| Stop Time | as Pump: 20.00 min |
| APCI | Positive |
| Mass Range | Varies with compound of interest |
| Gas Temperature | 300°C |
| Vaporizer Temp. | 350°C |
| Drying Gas | 6.0 L/min |
| Neb. Pressure | 60 psig |
| Vcap | 2500 V |
| Cornona | 4.0 µA |
| Data Storage | Condensed |
| Fragmentor | Disable Ramp |

| MSD SIM Parameters | | | |
|---|---|---|---|
| | Group Name | Sim Ions | Fragmentor |
| | TDI-PP | 501.40, 338.1 | 150 |
| | HDI-PP | 495.00 | 150 |
| | HDI Biuret-PP | 968.7, 805.5 | 150 |
| | HDI Trimer-PP | 994.5, 831.5 | 150 |
| | IPDI-PP | 549.3 | 150 |
| | MDI-PP | 577.00 | 150 |
| Gain EMV – 3.0 | SIM Resol – Low | Polarity - Positive | Actual Dwell - 229 |

**Time Table:**

| Time [min] | %B | %C | mL/min |
|---|---|---|---|
| 0 | 35 | 65 | 0.300 |
| 0.5 | 35 | 65 | 0.300 |
| 10.00 | 70 | 30 | 0.300 |
| 11.00 | 70 | 30 | 0.300 |
| 16.01 | 35 | 65 | 0.300 |

7.3 Calibration:

7.3.1 Establish the retention times for each of the isocyanates of interest using the chromatographic conditions provided above. The retention times shown in Table 1 are to be used as guidance only.

7.3.2 Make suitable dilutions of the isocyanate-1, 2-PP derivative standard solutions to calibrate the LC. Suggested concentrations are 0.5, 1, 5, 10, 20,and 50 ng/µL.

7.3.3 Analyze 5.0 µL of each calibration standard and record the area of each peak and the concentration of the compound. Figure 1 shows a chromatogram of a typical mixed isocyanate-PP standard.

7.3.4 The ChemStation will construct a calibration curve for each analyte. The calibration is acceptable if the $r^2$ value is greater than 0.99 (correlation coefficient r > 0.995).

7.3.5 The ChemStation calibration curves must be set to linear with forced origin on the MSD section in order to obtain the required LOQ.

7.4 Continuing Calibration:

7.4.1 A 10.0-ng/µL calibration check solution must be analyzed every 12 hours or after 20 samples. The response of each compound must be within ± 10% from the response predicted by the calibration curve or else that parameter is considered to be out of control.

7.4.2 If a parameter is out of control, check the HPLC system and correct any problems, prepare and analyze a fresh calibration check solution, or clean the detector.

7.4.3 If the parameter is still out of control, prepare and analyze a new five-point calibration.

7.5 HPLC Analysis:

7.5.1 Analyze the samples using the HPLC conditions listed in section 7.2.4.

7.5.2 If the response for any of the isocyanate ureas exceeds the initial calibration range of the HPLC system, the sample must be reanalyzed following dilution with the appropriate Acetonitrile:Dimethyl sulfoxide solution.

7.5.3 Samples containing <1 µg/mL of the analyte should be quantified using MSD data.

7.6 Calculations:

7.6.1 The concentration of each isocyanate urea is given directly in the sample run report printed by the ChemStation based upon the current calibration data for that analyte. The value is usually given in ng/µL.

7.6.2 Convert the result to concentration of the underivatized isocyanate using the following equation:

$$C_{Iso},\ ng/\mu L = \frac{C_{Iso\text{-}Urea} * MW_{Iso}}{MW_{Iso\text{-}Urea}}$$

Where,

$C_{Iso\text{-}Urea}$ = the concentration of the derivatized isocyanate from the sample run report,

$MW_{Iso}$ = the molecular weight of the free isocyanate (from Table 1), and

$MW_{Iso\text{-}Urea}$ = the molecular weight of the derivatized isocyanate (Table 1).

7.6.3 Multiply the value by the dilution factor, if necessary. This dilution factor must take into account changes in volume of the solution during preparation, unless included elsewhere in the calculations.

7.6.4 The concentration should be converted for solids as follows:

$$C_{Iso},\ mg/Kg = \frac{C_{Iso} * 5.0\ mL}{W_s}$$

Where,

$W_s$ = the dry weight of the sample in grams (from SP-3).

## 8.0 Quality Control

8.1 Required instrument QC is found in the following sections:

8.1.1 There must be an initial calibration of the HPLC/DAD system as specified in section 7.3.

8.12 The HPLC/DAD system must meet continuing calibration criteria each 12 hr as specified in section 7.4.

8.2 The laboratory will on an ongoing basis analyze a reagent blank, a matrix spike, and a matrix spike duplicate for each analytical batch to assess accuracy.

8.2.1 The concentration of the matrix spike is generally the level of the mid-range standard.

8.2.2 The accuracy is defined as the percent recovery of the matrix spike analyses. The percent recovery should be within the range 80 - 120%, or else a matrix interference will be suspected.

8.2.3 The relative percent difference of the duplicates should be no greater than ±25%.

## 9.0 Method Performance

9.1 The method detection limit (MDL) is defined as the minimum concentration of a substance that can be measured and reported with 99% confidence that the value is above zero. The MDL concentrations are obtained for each analyte by measuring seven replicates of a low-level solution brought through the entire analytical procedure. The MDL is three times the standard deviation of the measured value for each analyte. The MDLs are obtained on at least a yearly basis, and the most recent determinations are listed in Table 1.

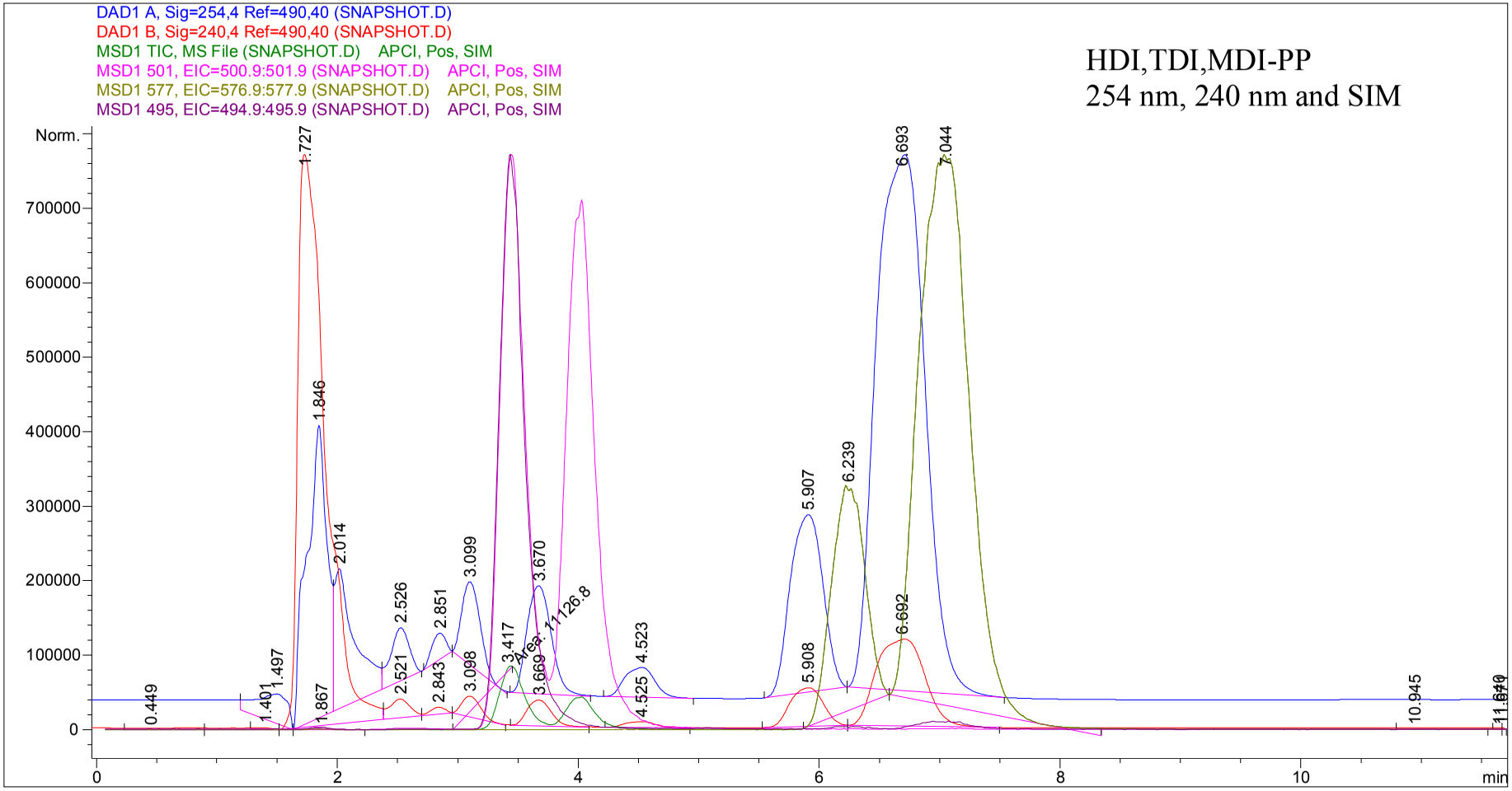
**Table 1 Typical Retention Times (DAD/MSD) and IDLs**

| Parameter | Retention Time* | IDL** | MWISO | MWISO-PP Urea | Monitoring Ion |
|---|---|---|---|---|---|
| 1,6-HDI | 3.098/3.417 | 0.0060(0.00215) | 168.0 | 494.4 | 495 |
| HDI Biuret | 8.21/89.38 | 0.049(0.024) | 478 | 967 | 968.7/805.5 |
| HDI Trimer | 9.14/9.34 | 0.037(0.015) | 504.5 | 993.5 | 994.5/831.5 |
| IPDI (2 isomers) | 5.20(7.09)/5.39(7.29) | 0.033(0.0135) | 222.3 | 548.3 | 549.3 |
| 2,4-TDI | 3.669/4.021 | 0.003(0.0010) | 174.2 | 500.6 | 501 |
| 2,6-TDI | 3.098/3.417 | 0.005(0.0017) | 174.2 | 500.6 | 501 |
| 4,4'-MDI | 6.692/7.044 | 0.073(0.0318) | 250.3 | 576.7 | 577 |
| 2,4'-MDI | 5.908/6.239 | 0.073(0.0318) | 250.3 | 576.7 | 577 |
| *typical UV/MSD retention times | | | **as PP derivative (as Isocyanate) in ng/μL | | |

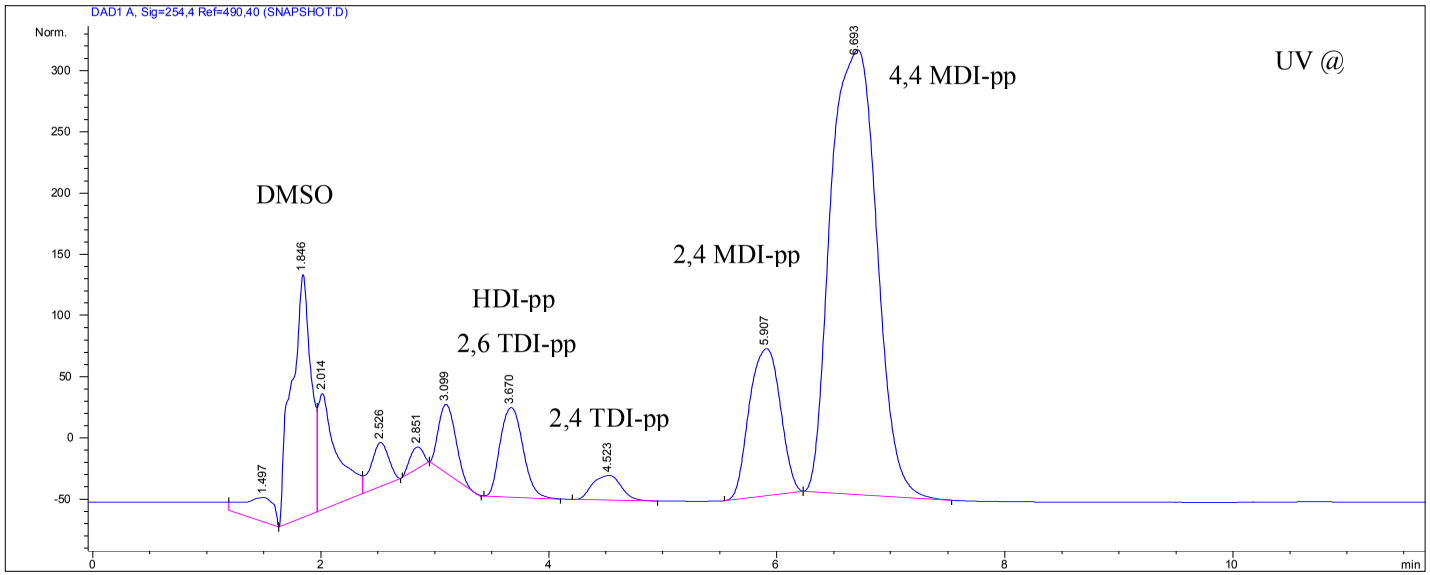
## 10.0 References

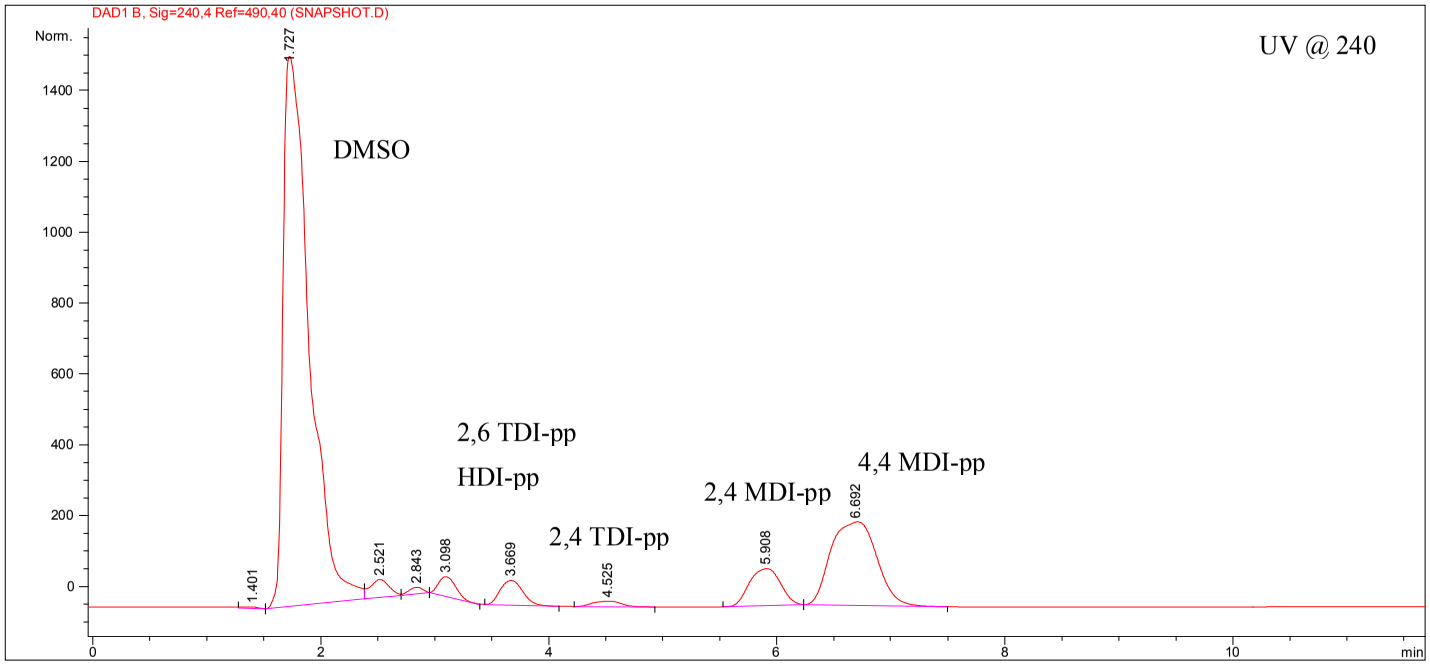
**10.1 Draft Method 207**
**10.2 Conditional Test Method 024**

**Figure 1** **Liquid chromatograms of a 10.0-ng/µL solution of Isocyanate-PP Ureas.**



Issued: 4/15/00
**Revised 02/16/04**

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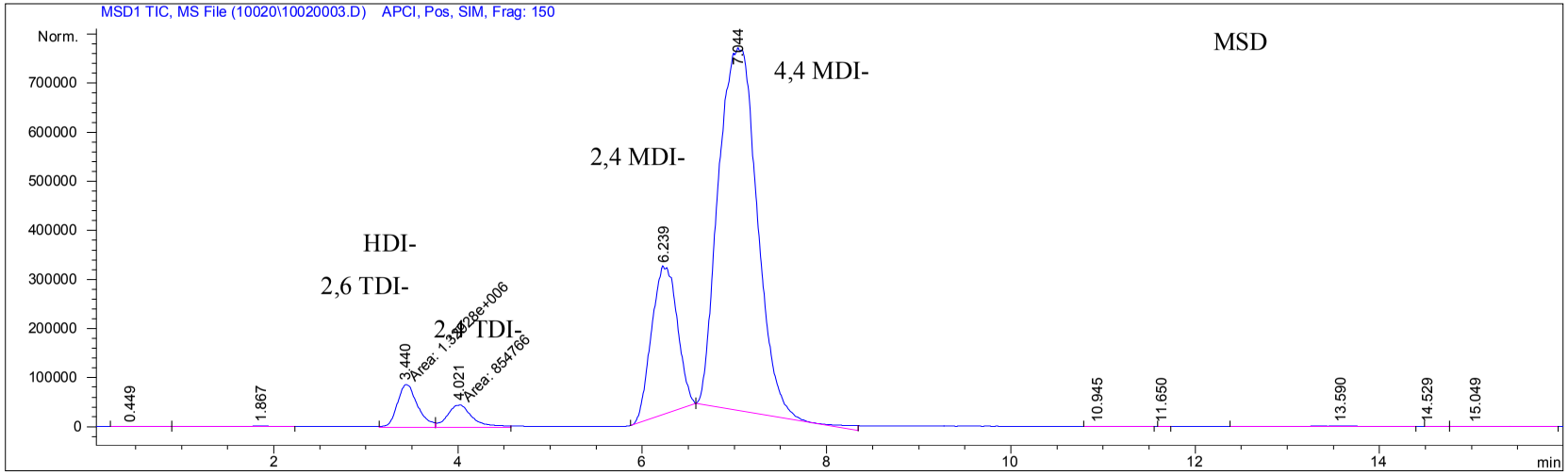
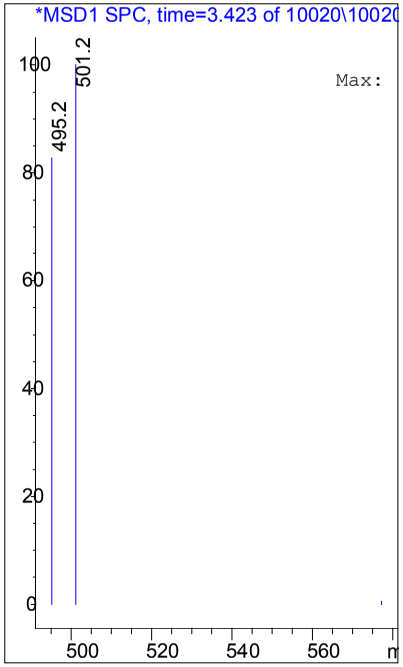
Issued: 4/15/00
**Revised 02/16/04**

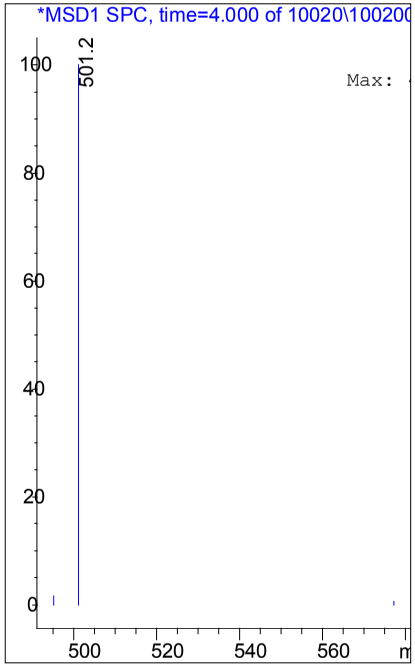



495.2 - HDI-

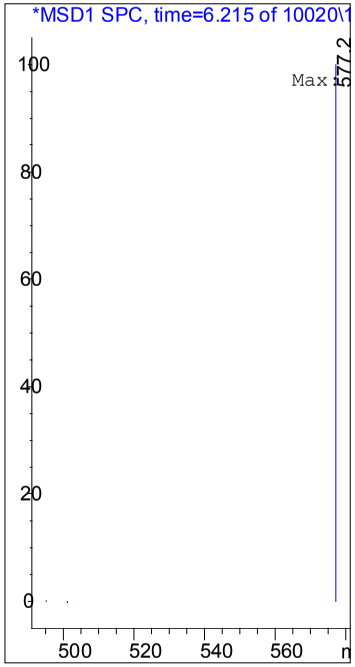
501.2 - 2,6 TDI-



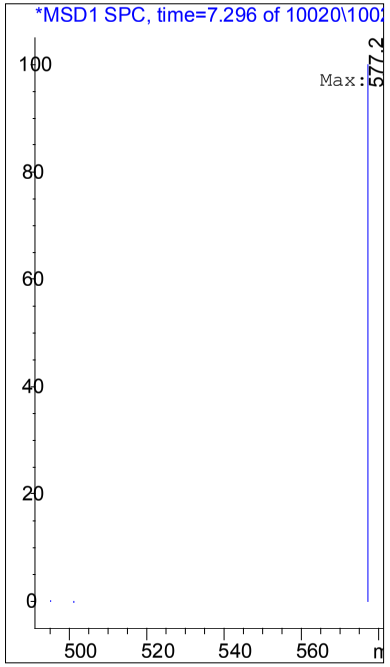

501.2 - 2,4 TDI-



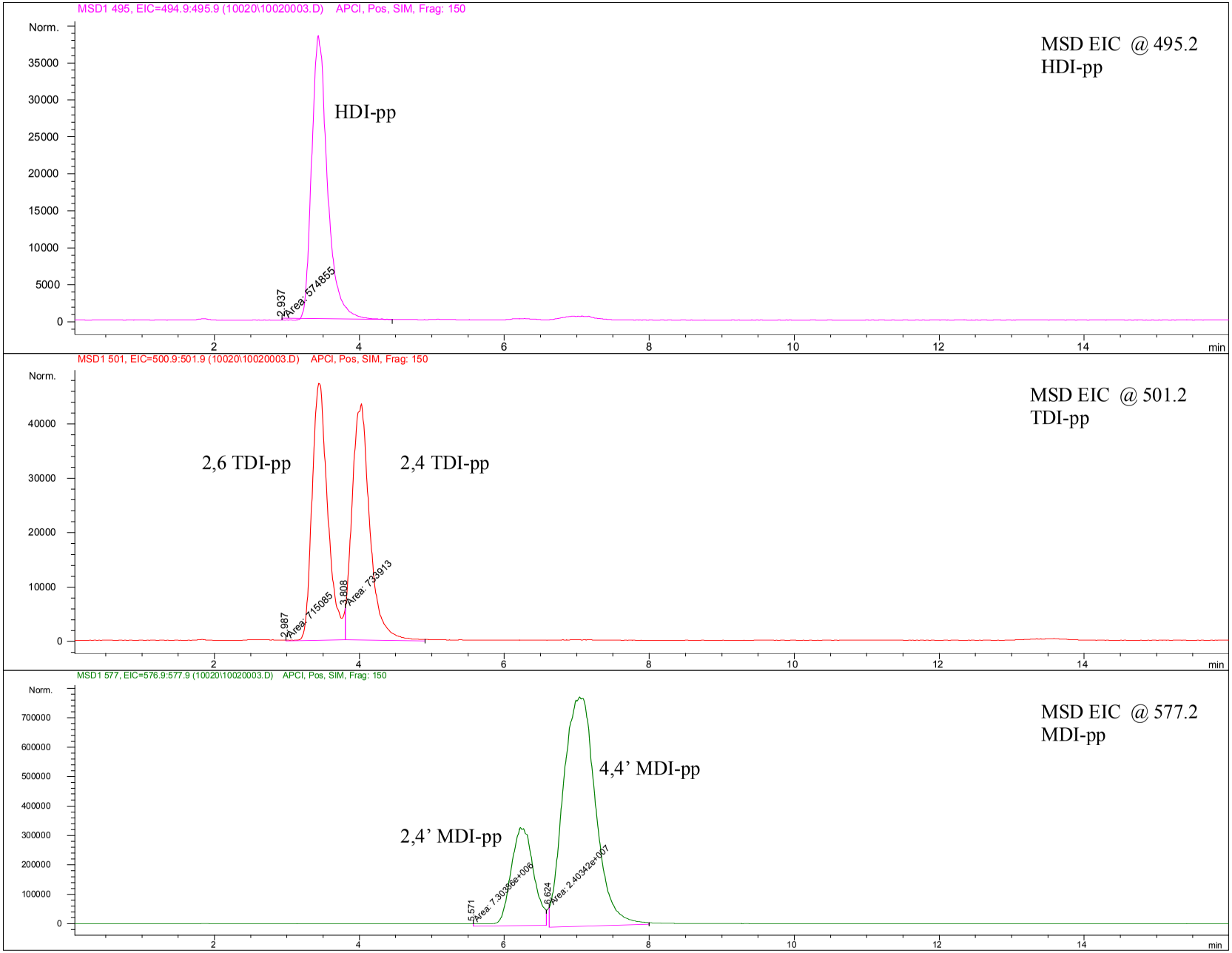

577.2 - 2,4 MDI-




577.2 - 4,4 MDI-



Issued: 4/15/00
**Revised 02/16/04**




**Isocyanates-11**