ISOCYANATES, TOTAL (MAP) 5525

Formula: R-NCO MW: Appendix C CAS: Table 1 RTECS: Table 1

METHOD: 5525, Issue 1 **EVALUATION: PARTIAL** **Issue 1: 15 March 2003**

OSHA: Table 1
NIOSH: Table 1
ACGIH: Table 1

PROPERTIES: Table 1

NAMES & SYNONYMS: Table 2

SAMPLING

SAMPLER: FILTER (glass fiber, impregnated); or IMPINGER; or IMPINGER + FILTER (see Table 3)

FLOW RATE: 1 to 2 L/min

VOL-MIN: 1 L @ 5 ppb
-MAX: 500 L

SHIPMENT: Cold; impinger solution: vial with PTFE-lined cap; filter: wide-mouth jar with PTFE-lined cap, containing 5.0 mL MAP extraction solution

SAMPLE STABILITY: Extracted filters: store cold and process as soon as possible; prepared samples: at least 3 months at -10° C (freezer) in the dark

REAGENT STABILITY: MAP on filters: 6 months at -10° C (freezer) in dark; MAP solutions: irreversible precipitation occurs within a few weeks in 2 mg/mL solutions

BLANKS: Minimum of 3 field controls, 3 field blanks per set

BULKS: Necessary for oligomer analysis

ACCURACY

RANGE STUDIED: Not studied

BIAS: Not determined

OVERALL PRECISION ($\hat{S}_{rT}$): Not determined

ACCURACY: Not determined

MEASUREMENT

TECHNIQUE: HPLC, UV/FLUORESCENCE DETECTION

ANALYTE: MAP derivatives of isocyanates

SAMPLE PREP: Impinger samples: solid-phase extraction (SPE); filter samples: acetylate with acetic anhydride

INJECTION VOLUME: 30 µL

MOBILE PHASE: 65:35 (v/v) acetonitrile:triethylammonium phosphate/formate (100 mM in both), pH gradient 6.0 to 1.6, 1.5 mL/min.

POST-COLUMN SOLUTION: 65:35 (v/v) acetonitrile- 4.4 N phosphoric acid, 0.7 mL/min.

COLUMN: Reversed phase Inertsil™ C 8, 150 x 4.6 mm, 5-µm, titanium frits in a constant temperature column oven set at 30°C.

DETECTORS: UV @ 253 nm; fluorescence (xenon lamp-ex: 368 nm, em: 409 nm; or deuterium lamp-ex: 254 nm, em: 409 nm).

CALIBRATION: MAP derivatives of monomeric isocyanates, with FL or UV for monomer; mandatory use of UV peak area for oligomer.

RANGE: 0.5 nmole NCO per species to 300 nmole total NCO per sample (e.g., 0.04 to 25 µg HDI per sample).

ESTIMATED LOD: 0.2 nmole NCO per species per sample (0.2 nmole NCO equals 0.017 µg HDI per sample).

PRECISION ($\bar{S}_r$): Filters: HDI 0.05; 2,4-TDI 0.06; MDI 0.06 Impingers: not determined.

APPLICABILITY: The useful air concentration range for this method is approx. 1.4 to 840 µg/m^3 NCO total isocyanate group for a 15-L air sample. This is equivalent to 0.4 ppb to 250 ppb for a diisocyanate. The method determines the air concentration of monomeric and oligomeric isocyanates. The applicability of the method depends on the correct choice of sampler for a given environment [1,2].

INTERFERENCES: Any non-isocyanate compound that reacts with MAP. Any compound that elutes at or after the monomer retention time and absorbs at 253 nm may potentially interfere with isocyanate quantification by UV. Interfering compounds that do not fluoresce using 368 nm excitation and 409 nm emission but give an interfering UV signal can be separated by altering the pH gradient.

OTHER METHODS: NIOSH Method 5522 [3] is for monomer and total isocyanate. NIOSH Method 5521 [4] is for monomer and aliphatic oligomeric isocyanate. A chapter in the NIOSH Manual of Analytical Methods, a related journal article, and the references therein describe other methods and method selection criteria [1,2].

REAGENTS:

For Glass-fiber Filter (GFF) Sampling and Analysis

1. 1-(9-anthracenylmethyl)piperazine (MAP) [5,6] impregnated filters.
   a. 37-mm: 1.1 mg MAP/filter.
   b. 25-mm: 0.5 mg MAP /filter.
   c. 13-mm: 0.13 mg MAP/filter.
2. Extracting solution: $1x10^{-4}$ M MAP in acetonitrile (2.76 mg MAP per 100 mL acetonitrile).
3. Acetic anhydride (HPLC grade).*
4. Acetonitrile (HPLC grade).*

For Impinger Sampling and Analysis

5. Impinger solution: $1x10^{-4}$ M MAP [5,6] in butyl benzoate (2.76 mg per 100 mL butyl benzoate).
6. Butyl benzoate: Purified by running through a silica gel column.
7. Methylene chloride (HPLC grade).*
8. Acetonitrile (HPLC grade).
9. Methanol (HPLC grade).*
10. 90:10 acetonitrile-methanol.

Chromatography

11. Phosphoric acid, 85% (99.999%).*
12. Formic acid (ACS reagent grade).*
13. Acetonitrile (HPLC grade).
14. Triethylamine (99.5%).*
15. Hydrochloric acid (concentrated).*
16. 100 mM phosphate-formate buffer, pH 6.0 and pH 1.6, and acetonitrile-buffer mobile phases (APPENDIX A).
17. Post-column phosphoric acid (4.4 N) and acetonitrile-acid post-column addition mobile phase (APPENDIX B).
18. Monomer standards in acetonitrile: MAP-derivatives, $2x10^{-8}$ N to $3x10^{-5}$ N (NCO groups per liter). Synthesis of MAP-derivatives (APPENDIX C).

- See SPECIAL PRECAUTIONS

EQUIPMENT:

1. Sampler: Glass-fiber filter, Type A/E Binder-Free, 13- or 37-mm (Gelman or equivalent), coated with appropriate amount of MAP [5,6] (see Reagents) in an opaque cassette filter holder with cellulose backup; or 25-mm glass-fiber filter coated with MAP (see Reagents) in an IOM sampler with stainless steel cassette; or impinger.
2. Personal sampling pump, capable of sampling at 1 to 2 L/min with flexible connecting tubing.
   NOTE: Avoid leaching of plasticizer into solvent during impinger sampling. For impingers, Fluran™ tubing is acceptable, PVC tubing is not.
3. Wide-mouth glass jars (20-mL) with PTFE-lined cap for extraction and transport.
4. Insulated shipping container with ice packs.
5. High performance liquid chromatograph (HPLC) with binary solvent and gradient capabilities, PEEK tubing, and post-column addition pump, interfaced with an autoinjection system, employing 150x4.6-mm Inertsil™ C8, 5-µm column with titanium frits, in a column oven set to 30 °C (or a minimum of 5 degrees above ambient). Adaption of method to a narrower bore column is acceptable.
6. UV detector set to 253 nm.
7. Fluorescence (FL) detector with either (a) Xenon source: excitation 368 nm, emission 409 nm; or (b) Deuterium source: excitation 254 nm, emission 409 nm.
8. Volumetric flasks, glass, various sizes.
9. Visiprep™ or equivalent solid phase extraction (SPE) manifold with drying attachment and disposable valve liners.
10. Supelclean™ LC-Si or equivalent SPE tubes (0.5g) with 6-mL reservoir.
11. Disposable 20-mL glass vials with PTFE-lined caps.
12. Microsyringes.
13. HPLC vials.
14. Pipets, disposable glass.
15. PTFE syringe filters (0.45-µm pore size).
16. Forceps for handling filters.
17. Sealing bands.
18. Top-loading balance , 2 to 3 decimal place.
19. Analytical balance, 5 place.

SPECIAL PRECAUTIONS: Diisocyanates are highly toxic and known respiratory irritants. Follow manufacturer's guidelines when handling pure products. Follow hazardous material shipping requirements when transporting. Preparation of urea derivatives, samples, and standards should be done in a hood to avoid exposure to isocyanate and solvent vapors. Toxicity of MAP is unknown. Acetic anhydride is a severe irritant; acetonitrile is flammable and an irritant. Methylene chloride is an irritant and suspected carcinogen. Methanol is toxic and flammable. Strong acids are toxic and corrosive. Triethylamine is toxic and corrosive.

SAMPLING:

NOTE 1: Use sampling conditions listed below for the sampler selected: glass fiber filter (1); impinger (2); impinger followed by glass fiber filter (3). See Table 3 for examples of exposure scenarios and which sampler to choose. Refer to the chapter on isocyanates [1] for more detailed information on how to choose the appropriate sampler.

NOTE 2: Field controls are made by sampling in an area near the analyte sampling location but where one would not expect to find analyte. These samplers are then treated exactly like the samples from this point on.

NOTE 3: Field blanks are samplers handled exactly the same as samples except that no air is drawn through them.

1. Filter sampling
   a. Calibrate each sampling pump with a representative sampler in line.
      NOTE: When sampling aerosol with aerodynamic diameter >20 µm, an IOM sampler with stainless steel cassette or other suitable inhalable sampler should be used [1,2].
   b. Attach filter cassette to sampling pump with flexible tubing.
   c. Sample at a rate of 1 to 2 L/min (2 L/min for the IOM) for a total sample of at least 1 L.
   d. Immediately after sampling, place filter in a wide-mouth jar with a PTFE-lined screw cap containing 5.0 mL of 1 x 10^{-4} M MAP in acetonitrile. Secure screw cap with a sealing band.
      NOTE 1: If an IOM sampler is used, place entire stainless steel cassette and filter in the jar with 10.0 mL of extracting solution instead of 5.0 mL, recap and swirl to wet all surfaces of the IOM sampler.
      NOTE 2: If collecting vapor-only samples, since in-field extraction is not necessary, ship the unextracted samplers with ice packs to the lab for analysis.
   e. Store samples in a refrigerator and ship the samples with ice packs.
   f. Obtain a bulk sample (at least 3 mL) and a Material Safety Data Sheet (MSDS) for products used in the process giving rise to exposure (e.g., for a two-part polyurethane coating system, a sample of both the isocyanate product and the polyol product). These bulk samples are necessary for oligomer analysis. Ship bulks in a separate container from the air samples.
   g. Obtain a minimum of three field controls and three field blanks per sample set or at least one field control and one field blank for every ten samples. These controls and blanks are necessary for distinguishing between artifacts and isocyanates.

2. Impinger sampling
   a. Calibrate each sampling pump with a representative sampler in line.
   b. Transfer 15.0 mL of 1 x 10^{-4} M MAP in purified butyl benzoate to each impinger and connect impinger to sampling pump with flexible tubing that is free of phthalate plasticizer (Fluran™ tubing is acceptable, PVC tubing is not.)
   c. Sample at 1 L/min for a total sample of at least 1 L.
   d. Using a disposable pipet, transfer the entire contents of the impinger to a 20-mL vial with a PTFE-lined screw cap. Secure screw cap with sealing band.
   e. Store samples in a refrigerator and ship the samples with ice packs.
   f. Obtain bulk samples and MSDSs as described in step 1.f.
   g. Obtain a minimum of three field controls and three field blanks per sample set or at least one field control and one field blank for every ten samples. These controls and blanks are necessary for distinguishing between artifacts and isocyanates.

3. Impinger + filter sampling
   a. Sample with an impinger followed by a MAP-coated filter when impinger sampling is desired (e.g., a highly reactive aerosol) but particles < 2 µm are suspected to be present (e.g., condensation or combustion aerosol). Particles < 2 µm are not collected efficiently by impingers [1,2].
   b. Attach the inlet of the filter cassette to the outlet of the impinger and the outlet of the filter cassette to the pump with flexible tubing. The portion of the tubing connecting the impinger to the filter should be free of phthalate plasticizer (Fluran™ tubing is acceptable, PVC tubing is not.)
   c. Sample at a rate of 1 L/min for a total sample size of at least 1 L.
   d. After sampling, treat the impinger solution as described in step 1.d. If a combined measure of the total isocyanate from the two stages is desired, place the filter in the impinger sample vial. If separate measurements of the two stages are desired, place the filter in a separate jar containing MAP-acetonitrile extraction solution as in step 1.d.
   e. Store samples in a refrigerator and ship the samples with ice packs.
   f. Obtain bulk samples and MSDSs as in step 1.f.
   g. Obtain a minimum of three field controls and three field blanks as described in steps 1.g. and 2.g.

SAMPLE PREPARATION:

NOTE 1: For correct sample preparation, follow directions below for type of sampler used: glass fiber filter (4); impinger (5); impinger followed by glass fiber filter (6).
NOTE 2: Refrigerate all samples immediately upon receipt.

4. Filter and filter extract
   a. For vapor-only samples received without in-field extraction, follow the procedure in step 1.d. of filter sampling and allow the samples to extract overnight in a refrigerator before proceeding.
   b. For samples that are extracted in-field, acetylate with 5 µL of acetic anhydride upon receipt of samples. For samples that are extracted in the lab, acetylate with 5 µL of acetic anhydride after overnight extraction. Let these react at least two hours at room temperature or overnight in the refrigerator before proceeding.
   c. Attach the outlet of a 0.45-µm PTFE Luer-lock syringe filter to the SPE vacuum manifold with disposable liner. Place an empty polypropylene syringe barrel in the inlet of the syringe filter. Add the entire extraction solution into the syringe barrel.
   d. Force the sample through the PTFE filter using either positive or negative pressure and collect in a glass vial with a PTFE-lined cap.

5. Impinger
   a. Attach a 6-mL SPE cartridge containing 500 mg silica gel to a solid-phase extraction (SPE) vacuum manifold with disposable liners.
   b. Condition the SPE cartridge with 2 mL butyl benzoate, bringing the liquid level down to the top of the sorbent bed.
   c. Add a 5.0-mL aliquot of sample solution to the SPE cartridge, adjusting the vacuum of the SPE manifold to obtain a flow of approx. 1 to 2 mL/min and stopping when the liquid level reaches the top of the sorbent.
   d. Add 6 mL methylene chloride to the SPE cartridge. Elute the cartridge at 1 to 2 mL/min until the liquid level reaches the top of the sorbent. Discard all eluate collected up to this point.
   e. Add 3 mL of 9:1 acetonitrile-methanol to the SPE cartridge. Elute at approx.1-2 mL/min until the liquid level reaches the top of the sorbent. Collect the eluate in a pre-weighed glass vial.
   f. Add 3 mL of methanol to the SPE cartridge. Elute and collect the eluate in the same vial as in step 5.g.
   g. Use a stream of nitrogen to reduce the volume to exactly 1.0 mL. Reconstitute to volume if necessary by addition of acetonitrile. Determine exact volume using the sample weight and the density of acetonitrile (0.786 g/mL).

6. Impinger + Filter
   a. Follow steps under Sample Preparation step 5 for the impinger part of the sample.
   b. Follow steps under Sample Preparation step 4 for the filter part of the sample.

CALIBRATION AND QUALITY CONTROL:

7. Calibrate with six working standards in the range of interest. Prepare liquid standards with concentrations in terms of moles isocyanate group per liter (normality) in the range 3×10^{-5} N to 2×10^{-8} N. Intersperse standards among samples. For filter samples and impingers, liquid standards should consist of the MAP derivative(s) of the appropriate diisocyanate monomer(s) in acetonitrile. A concentrated stock solution of the MAP derivatives of isocyanate monomers can be made in methylene chloride. (Solubility: 1×10^{-3}N to 5×10^{-3}N). See APPENDIX C for synthesis of MAP derivatives to be used as standards. For filter samples only, to mimic samples in artifact content, a MAP impregnated filter similar to the filter used for the sample can be placed in 5.0 mL of each level of standard, and then treated the same as the samples starting at step 4.
8. Prepare calibration graphs (response vs. normality of standard solution) for both the UV and fluorescence detectors.
   a. The calibration curve prepared from either the UV or the FL detector may be used to quantify the monomer. A quadratic fit may be used for the fluorescence calibration curve if curvature is seen at the low end.
   b. A linear calibration curve using UV peak area **must be** used to quantify the oligomer because the UV response for monomer and oligomer is the same while the FL response is not.
9. Analyze two solvent blanks at the beginning of each sample set. Using the second blank chromatogram, subtract this chromatogram electronically from sample chromatograms. This improves the UV baseline of sample chromatograms and facilitates accurate integration of peaks in low level samples. Analyze one additional solvent blank within the sample set.
10. Analyze a minimum of three field controls and three field blanks or at least one field control and one field blank for every ten samples. Use the field controls and field blanks to identify non-isocyanate peaks (generally reagent artifact peaks) that are likely to appear in the samples. If the blanks are consistent and the sample set is analyzed soon after sampling, subtraction of a field blank instead of the solvent blank from the sample data may make interpretation of the data simpler.
11. Analyze bulk isocyanate products whenever available, but if oligomer is to be quantified, analysis of the bulk products used at the worksite **must** be done. Bulk isocyanate chromatograms are useful for qualitative confirmation of peaks observed in the sample chromatograms. It is also advisable to analyze bulk non-isocyanate products (such as the polyol portion of a two-part spray system) in the same manner to ensure that they give rise to no interfering compounds that may be mistaken for isocyanates. Bulk products must be derivatized with MAP prior to analysis. An appropriate dilution of the bulk in methylene chloride based on the manufacturer's NCO content, followed by immediate derivatization of an aliquot of this diluted bulk in 5×10^{-4} M MAP in acetonitrile must be done. After reacting overnight, acetylate this bulk-MAP reaction mixture with acetic anhydride as in step 4.b. APPENDIX D is a protocol for a bulk dilution/derivatization with MAP that works well for most representative bulks. This bulk-MAP sample can then be analyzed on the HPLC.
12. Analyze three quality control blind spikes per sample set.

MEASUREMENT:

13. Set HPLC and detectors to conditions and settings given on pages 5525-1 and 5525-2. Note that in addition to a mobile phase flow of 1.5 mL/min, there is also a post column addition of 0.7 mL/min of 65:35 (v/v) acetonitrile-4.4 N phosphoric acid. Keep the column at constant temperature in an oven at 30°C. Equilibrate the entire system for at least forty minutes before the start of the daily analysis, using mobile phase B (65:35 (v/v) acetonitrile-pH 1.6 buffer), running post-column acid mobile phase also.
14. The HPLC gradient program can be customized to optimize the separation of the particular isocyanate species. An example of a gradient program to start with is:

    0 - 4 min: 100% mobile phase A (65:35 (v/v) acetonitrile-pH 6 buffer)
    4 - 17 min: Linear gradient from 100% mobile phase A to 100% mobile phase B (65:35 (v/v) acetonitrile-pH 1.6 buffer)
    17 - 30 min: Hold 100% mobile phase B
    30 - 36 min: Re-equilibrate at 100% mobile phase A

    NOTE 1: A gradient that provides better resolution for aliphatic isocyanate products is given in Bello et al. [7].

NOTE 2: For isocyanate products that essentially contain only monomer, the gradient can be truncated after the monomer elutes. After truncation, a two minute hold using pH 1.6 mobile phase before returning to initial conditions will ensure baseline stability for the next sample run.

NOTE 3: If there is an interfering peak in the area of interest that is not influenced by pH, changing the length of hold at the beginning of the run should move the interference peak away from the area of interest.

NOTE 4: For multi-component monomers such as TDI and HMDI and for multi-component isocyanate products that contain monomer and oligomer, changing the hold at the beginning, changing the gradient ramp, placing a hold in the middle of the ramp, or a combination of all of the above may help in separation of the individual components for a specific product.

15. Inject a 30-µL sample aliquot.
16. Monomer measurement:
    Isocyanate species for which pure analytical standards are available, such as diisocyanate monomers, can be measured by comparing the response of the peak at the correct retention time with the calibration curve generated by analyzing standards. Either UV peak height or area or fluorescence peak height or area can be used for quantification of monomer. The fluorescence detector is more sensitive, so it is usually better for measuring monomers at low levels. Peak height is usually preferable to peak area, especially in the presence of closely-eluting interfering peaks. Confirmation of the identity of the monomer is achieved by comparing the FL/UV response ratio of the sample peak to that of the standard giving similar response.
    NOTE: The FL/UV ratio may change somewhat at low levels. The sample peak should give a FL/UV response ratio within 15% of the ratio for the standard peak of comparable size.
17. Oligomer measurement (total isocyanate):
    Isocyanate species for which pure analytical standards are not available, such as oligomeric isocyanates, must be measured by using the UV area of the sample peak(s) and the slope of the linear portion of the calibration curve generated by analyzing monomer standards. Frequently, numerous isocyanate species elute as an envelope of poorly resolved peaks. In this case, rather than attempting to integrate peaks individually, the entire chromatogram is integrated over the area of interest. Because the fluorescence baseline is not disturbed by the gradient and the fluorescence detector is selective for MAP-derivatized compounds, the sample fluorescence chromatogram can be very useful for determining when to begin and end integration of peaks in the UV chromatogram. The fluorescence chromatogram is used qualitatively to confirm the presence of MAP groups in the eluting species. The fluorescence response varies too much from compound to compound to quantify isocyanate species for which standards are unavailable. However, the excitation and emission wavelengths that have been chosen make the detection very selective for MAP derivatives. Experience has shown that MAP-derivatized isocyanates will have a FL/UV ratio of approximately 0.33 to 2 times that of the MAP-derivatized monomer.

CALCULATIONS:

18. Monomers:
    Generate a calibration graph usually using fluorescence, plotting peak area or peak height as a function of concentration (normality, isocyanate milliequivalents per milliliter) of monomer standards. Determine the normality of the analyzed sample aliquot from the calibration graph.

a. For impinger samples use the following formula:

$$M = N \cdot Wt_{eq} \cdot V_f \cdot \left(V_t / V_{al}\right) \cdot 1000, \mu g / sample$$

where:

M = mass of isocyanate species (µg/sample),

N = the normality (isocyanate milliequivalents per milliliter) of the analyzed sample solution as taken from the calibration curve,

Wt_{eq} = the equivalent weight of the monomeric isocyanate in milligrams per isocyanate milliequivalent,

V_f = the final volume of SPE solution in milliliters,

V_t = the 15-mL volume of the total sample, and

V_{al} = the volume of the aliquot analyzed.

b. For filter samples use the following formula:

$$M = N \cdot Wt_{eq} \cdot V_f \cdot 1000, \mu g / sample$$

where:

M = mass of isocyanate species (µg/sample)

N = the normality (isocyanate milliequivalents per milliliter) of the analyzed sample solution as taken from the calibration curve,

Wt_{eq} = the equivalent weight of the monomeric isocyanate in milligrams per isocyanate milliequivalent, and

V_f = the final volume of the sample solution in milliliters.

19. Oligomers (total isocyanate):
Identification of oligomers is best done by following the flow chart presented in Appendix E. The bulk chromatogram is used as the comparison standard when identifying which peaks to include in the quantification. Quantification of oligomers must be accomplished using a UV area calibration curve generated from analyzing monomer standards, preferably using the monomer on which the oligomer is based. Generate a calibration curve, plotting UV peak area as a function of concentration (normality, isocyanate milliequivalents per milliliter) of monomer standards. Use the slope of the linear portion of the calibration curve to determine the normality of a single component, a group of components, or all isocyanate components in the analyzed sample aliquot.

$$N = \frac{UV}{slope_{cal}}$$

where:

N = the normality of sample component(s),

UV = the UV area of sample component(s), and

$slope_{cal}$ = the slope of the calibration curve in units of UV area per normality.

The method measures normality of oligomeric isocyanate group in the analyzed sample. It cannot measure µg per sample unless the molecular weight of the component is known. Generally, there is a mixture of compounds of different and unknown molecular weights. To deal in micrograms per sample, use the formulae given above for monomer quantification and the equivalent weight of the particular monomer of interest. This value is the monomer mass equivalent - the mass of the monomer that would contain the same number of isocyanate groups as was found in the sample. Alternatively, the micrograms of isocyanate group per sample can be calculated using the formulae and entering 42 for the equivalent weight.

EVALUATION OF METHOD:

Shelf Life of MAP, MAP Solutions and MAP-Coated Filters:

Pure, solid MAP is stable for years when stored in a freezer. Concentrated solutions of MAP (concentrated stocks or filter-spiking solutions) are not very stable and should be kept in the freezer for a maximum of one week [7,8]. MAP-coated glass-fiber and quartz-fiber filters are stable for 1 week at room temperature and 6 months in the freezer [7,8]. Artifacts derived from MAP are present in the chromatogram of MAP-coated filter samples. Although the levels are low, these impurities dictate the method LOD when they coelute with an analyte. The level of artifacts observed is not correlated well with storage time. Room temperature storage results in greater artifact levels than storage in the freezer. MAP is known to be light sensitive. It is not clear what role exposure to light plays in the formation of the chromatographic interferences. It is recommended that exposure to light be minimized when handling MAP solutions and MAP-coated filters. No formal study has been conducted regarding the shelf life of impinger solutions of MAP in butyl benzoate. However, no problems have been observed when the impinger solutions are used within one week of being made. Impinger samples are noted to have fewer artifacts than filter samples. Although this may be due in part to greater stability of the dilute MAP solutions versus MAP on filters, it may also be attributable to the generally lower amount of reagent used in impinger samples as well as the solid-phase extraction procedure to which impinger samples are subjected.

Stability of MAP-Isocyanate Derivatives:

Pure, solid MAP-derivatized isocyanate monomers are stable for years when stored in the freezer. MAP-derivatized monomers and oligomers of aliphatic isocyanates are very stable in acetonitrile (the sample solvent) for at least one year [7]. Samples containing TDI-MAP gave results 16-24% lower than the original values when reanalyzed after storage for nine months in the freezer [8].

Filter Materials:

Comparison of quartz-fiber and glass-fiber filters showed no significant differences in recovery of MAP-derivatized monomers from the filters. Comparison of stainless steel and cellulose backup pads showed no significant reagent loss with either. Neither backup material contributed to the artifact production in the filter sample [8].

Filter Versus Impinger Sampling:

A side-by-side comparison of MAP-coated IOM filters and MAP impingers, conducted in autobody shops using aliphatic isocyanate products [9], showed no significant difference in the performance of these two samplers. It can be concluded from this study that losses of relatively slow-curing aliphatic isocyanates collected on filters due to curing reactions are minimal when filters are extracted in the field immediately after sampling. It also appears from this study that the IOM's better aspiration efficiency for relatively large aerosol did not result in increased collection efficiency compared to the midget impinger. This suggests that an inhalable sampler is not necessary in this type of environment. In general, the appropriate choice of sampler - filter, impinger, or impinger and filter in series - is dictated by the particular exposure scenario [1,2].

PEEK Versus Stainless Steel HPLC Columns:

PEEK and stainless steel HPLC columns were compared with respect to efficiency, tailing, and carryover. No significant differences in performance were observed [8]. Therefore, stainless steel columns are considered acceptable. However, because MAP derivatives are known to adsorb onto stainless steel surfaces, it is recommended that the frits of the column be constructed of more inert material, i.e., PEEK or titanium.

pH Gradient:

Method 5525 uses pH-gradient HPLC to enable measurement of relatively weakly retained monofunctional isocyanates and diisocyanate monomers as well as relatively strongly retained oligomeric isocyanates in the same analysis. Several characteristics of the pH gradient have been evaluated [10]. The pH gradient is selective, only accelerating strongly basic compounds (all compounds containing a MAP group are strongly basic). The strength of the pH gradient was evaluated by comparing the degree to which retention is

shortened in the strong mobile phase relative to the weak mobile phase. A model monofunctional isocyanate (octyl isocyanate) eluted 14 times faster and a model diisocyanate (MDI) eluted several hundred times faster using the strong mobile phase. Re-equilibration time is only a few minutes when switching from the strong mobile phase back to the weak mobile phase. Detector baselines are very stable during the gradient. The fluorescence baseline is unchanged and the UV_{253} baseline is disturbed less than 1 mV. The UV response of MAP derivatives has been found to be independent of the pH gradient. However, the fluorescence response is highly dependent on the pH of the mobile phase. This is corrected by post-column addition of acid to the mobile phase prior to detection.

Compound-to-Compound UV Response Variability:

Evaluation of UV response for several MAP-derivatized isocyanates, including monofunctional isocyanates and diisocyanates [5,7] showed a compound-to-compound RSD of 3.0-3.5% at the maximum 254-256 nm and an RSD of 1.0-2.2% at the less intense but more selective wavelengths of 352 nm, 368 nm, and 388 nm. In other studies, the UV response of MDI triisocyanate oligomer varied from 6% higher than the mean of several monomers [7] to about 10% lower than the MDI monomer [8]. The overwhelming majority of the data suggests very little variability in UV response among MAP-derivatized isocyanate compounds, supporting the validity of quantifying non-monomeric isocyanate species by using the UV area of the monomer standard.

Recovery of MAP-Derivatized Monomers:

Quartz-fiber and glass fiber filters were spiked with HDI-MAP, 2,4-TDI-MAP, and MDI-MAP at 630 ng NCO group per component per filter along with 500 µg MAP. Recovery ranged from 97-98% for the quartz-fiber filters and 97-99% for the glass-fiber filters [8]. In another study, MAP-coated quartz-fiber filters were spiked with a mixture of HDI, 2,4-TDI, and MDI at nominal levels of 21, 63, 210, 630, and 2100 ng NCO group per component. Recoveries averaged 93% for HDI, 91% for 2,4-TDI, and 92% for MDI with no correlation between recovery and spiking level over the range studied. As part of an International Organization for Standardization (ISO) intercomparison study, two commercial products containing only monomeric isocyanates were studied [8]. Levels spiked on filters were 2100, 1050, 525, and 105 ng NCO group per filter, corresponding to 2X, 1X, 0.5X, and 0.1X the U.K STEL level. For Desmodur H (HDI monomer), the mean recoveries were 104% at 2X, 103% at 1X, 102% at 0.5X, and 99% at 0.1X. For Desmodur T-80 (a mixture of 2,6-TDI and 2,4-TDI), the mean recoveries were 101% at 2X, 104% at 1X, 103% at 0.5X, and 108% at 0.1X. Bello et al. found HDI monomer recovery averaging 108% in the range of 0.45 to 42 ng NCO per filter and, in a separate study, a mean recovery of 100% in the range of 8 to 250 ng NCO per filter [7].

Recovery of Total Isocyanate Group:

Losses of isocyanates can occur at several stages in the sampling and analytical process [1,2]. Bello et al. evaluated total reactive isocyanate group (TRIG) recoveries from spiked filters and impingers as well as recoveries from HPLC analysis [7]. Desmodur N3300 (HDI isocyanurate) was derivatized with MAP and spiked onto quartz-fiber filters. Extraction and analysis found recovery ranging from 91-93% compared to directly analyzed product for spiking levels ranging from 90 ng to 2.34 µg NCO per filter. Similarly, a bulk product containing HDI isocyanurate and IPDI oligomers was derivatized with MAP, spiked into MAP-containing impingers, processed by solid-phase extraction, and analyzed by HPLC. The recovery of the TRIG varied from 92-106% for the HDI isocyanurate in the range 18-2500 ng NCO/mL and 76-89% for the IPDI oligomer in the range of 16-1900 ng NCO/mL. Recoveries from HPLC analysis were determined by comparison with isocyanate content obtained by bulk product titration. The products investigated and their respective recoveries were Bayer N100 (HDI biuret) 75%, Bayer N3300 (HDI isocyanurate) 94%, Bayer N3400 (HDI isocyanurate + uretidinedione) 95%, Bayer Z4470 (IPDI oligomers) 100%, and B26-2 (HDI isocyanurate + IPDI oligomers) 96%. These HPLC recoveries were conducted at high analyte levels. It is recognized that quantitative recoveries of complex products are not possible at low levels because many components fall below the limit of detection [7]. As part of an International Organization for Standardization (ISO) intercomparison study, two commercial products containing oligomeric isocyanates were studied [8]. Levels spiked on filters were 2100, 1050, 525, and 105 ng NCO group per filter, corresponding to 2X, 1X, 0.5X, and 0.1X the U.K. STEL level. For Desmodur N3300 (HDI isocyanurate), the mean recoveries were 52% at 2X, 43% at 1X, 35% at 0.5X, and 28% at 0.1X. These results are very different than those obtained by Bello et al. [7] and require re-examination. For Suprasec 5030 (polymeric MDI), the mean recoveries were 65% at 2X, 62% at 1X, 59% at 0.5X, and 54% at 0.1X. Studies are underway to account for missing isocyanate in oligomeric products.

Limit of Detection/Limit of Quantification:

The limit of detection (LOD) and limit of quantification (LOQ) were determined by spiking several MAP-derivatized monomers onto MAP filters in the range of 0.01 to 3.0 nanoequivalents, extracting the filters with acetonitrile, acetylating and analyzing by HPLC. The method LOD is estimated to be about 0.2 nanoequivalents NCO per sample and the LOQ 0.5 nanoequivalents NCO per sample for both fluorescence height and UV area [8]. As an example, these are equivalent to an LOD of 17 ng per sample and an LOQ of 42 ng per sample for HDI monomer. Bello et al. estimated the method detection limit to be 8 ng per sample for HDI monomer [9]. The instrumental LOD is substantially lower than the method detection limit, but is not achievable because of low levels of reagent-derived artifacts that are present in the chromatogram. Occasionally an analyte peak will coelute with a relatively large artifact peak, raising the LOD and LOQ substantially. Our experience is that IPDI monomer isomers coelute with some HDI oligomers when both are present in a sample. This makes the IPDI monomers, which are typically present at very low levels, unmeasurable.

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METHOD WRITTEN BY:

Robert P. Streicher, Ph. D., M. Kathleen Ernst and George Y. Williamson, NIOSH/DART

TABLE 1. EXPOSURE LIMITS, PROPERTIES, CAS AND RTECS NUMBERS

Isocyanates	Exposure Limits µg/m^3*			Properties [11]	CAS #	RTECS #
	OSHA (PEL)	NIOSH (REL)	ACGIH (TLV)			
Toluene 2,6-diisocyanate (2,6-TDI)	no PEL	lowest feasible (Ca)	no TLV	liquid; d 1.225 g/mL @ 25°C; MP 19.5-21.5°C; VP 3.33 Pa (0.025 mm Hg) @ 25°C [12]	91-08-7	CZ6310000
Toluene 2,4-diisocyanate (2,4-TDI)	140 (ceiling)	lowest feasible (Ca)	36 140 STEL	liquid; d 1.214 g/mL @ 25°C; MP 20-22°C, VP 3.33 Pa (0.025 mm Hg) @ 25°C [12]	584-84-9	CZ6300000
4,4'-Methylene-bis(phenyl isocyanate) (MDI)	200 (ceiling)	50; 200 (10 min ceiling)	51	solid (fused); d 1.180g/mL @ 25°C; MP 42-44°C; VP 6x10^{-4} Pa (4.5x10^{-6} mm Hg) @ 25°C [12]	101-68-8	NQ9350000
1,6-Hexamethylene diisocyanate (HDI)	no PEL	35; 140 (10 min ceiling)	34	liquid; d 1.04 g/mL @ 25°C; BP 255°C; 7 Pa (0.05 mm Hg) @ 25°C [13]	822-06-0	MO1740000
Isophorone diisocyanate (IPDI)	no PEL	45; 180 (10 min ceiling)	45	liquid; mixture of isomers, d 1.049 @ 25°C; MP -60°C [14]; VP 0.04 Pa @ 20°C (0.0003 mm Hg) [12]	4098-71-9	NQ9370000
4,4'-Methylene-bis(cyclohexyl isocyanate) (HMDI)	no PEL	No REL; 110 (10 min ceiling)	54	liquid; mixture of isomers, d 1.066 g/mL @ 25°C; VP 0.13 Pa @ 20°C (0.001 mm Hg) [15]	5124-30-1	NQ9250000
1,5-Naphthalene diisocyanate (NDI)	no PEL	40;70 (10 min ceiling)	no TLV	solid flakes; MP 129-131°C [12]; VP 0.4 Pa @ 25°C (0.003 mm Hg) [12]	3173-72-6	NQ9600000

* 1 ppb = 7.12 µg/m^3 TDI, 10.24 µg/m^3 MDI, 6.88 µg/m^3 HDI, 9.09 µg/m^3 IPDI, 10.73 µg/m^3 HMDI, 8.60 µg/m^3 NDI

TABLE 2. SYNONYMS OF COMMON ISOCYANATES

Isocyanate Acronym	Common Chemical Name	Synonyms
2,4-TDI	Toluene 2,4-diisocyanate	2,4-Diisocyanatotoluene; 2,4-diisocyanato-1-methylbenzene; isocyanic acid, 4-methyl-m-phenylene ester; methyl-m-phenylene isocyanate; 4-methyl-1,3-phenylene diisocyanate; tolylene 2,4-diisocyanate
2,6-TDI	Toluene 2,6-diisocyanate	2,6-Diisocyanatotoluene; 2,6-diisocyanato-1-methylbenzene; 2-methyl-1,3-phenylene diisocyanate; tolylene 2,6-diisocyanate
MDI	Methylenebis-(phenyl isocyanate)	4,4'-Diisocyanatodiphenylmethane; 4,4'-diphenylmethane diisocyanate; isocyanic acid, methylene di-p-phenylene ester; 1,1'-methylenebis(4-isocyanatobenzene); 4,4'-methylenebis(phenyl isocyanate); methylenebis(4-phenyl isocyanate); methylenediphenyl diisocyanate; methylenedi-p-phenylene diisocyanate
HDI	Hexamethylene diisocyanate	1,6-Diisocyanatohexane; 1,6-Hexanediol diisocyanate; HMDI; hexane-1,6-diisocyanate ; 1,6-hexylene diisocyanate; isocyanic acid, hexamethylene ester
IPDI	Isophorone diisocyanate	5-Isocyanato-1-(isocyanatomethyl)-1,3,3-trimethylcyclohexane; 3-isocyanatomethyl-3,5,5-trimethylcyclohexylisocyanate; isocyanic acid, methylene(3,5,5-trimethyl-3,1-cyclohexylene) ester; isophorone diamine diisocyanate
HMDI	4,4'-Methylenebis-(cyclohexyl isocyanate)	Hydrogenated MDI

TABLE 3. EXAMPLES OF EXPOSURE SCENERIOS AND WHICH SAMPLER TO CHOOSE

Sampler	Sampling Section in Method	Exposure Scenario
GFF: 0.1 mg MAP/cm^2	1	Vapor only (e.g., flexible foam manufacture using TDI monomer) Aerosols of aliphatic isocyanates (e.g., autobody refinishing using HDI- and IPDI-based polyisocyanates) Aerosols of aromatic isocyanates <2 µm (e.g., condensation aerosol from heating MDI)
Impinger: $1x10^{-4}$ M MAP in butyl benzoate (2.76 mg MAP per 100 mL)	2	Aerosols of aromatic isocyanates >2 µm (e.g., spraying of MDI polyisocyanate or cutting partially-cured MDI-based product)
Impinger + GFF: MAP impinger followed by MAP on GFF	3	Aerosols of aromatic isocyanates both <2 µm and >2 µm (mixture of mechanically generated aerosol and condensation aerosol, e.g., heating and cutting partially-cured MDI-based product)

APPENDIX A: PREPARATION OF PHOSPHATE-FORMATE BUFFER AND MOBILE PHASE

100 mM PHOSPHATE - 100 mM FORMATE BUFFER

Add 46.12 g 85% Phosphoric Acid (~27 mL) and 15.08 mL Formic Acid to 3700 mL deionized hi-pure water. The initial pH should be ~1.56. Titrate with Triethylamine (TEA) to a pH of 6.0 using a standardized pH meter. This will take between 115 and 120 mL of TEA. After the pH adjustment to pH 6.0, add additional deionized hi-pure water to bring the final volume to 4.0 L.

Split into two 2L-portions. The first portion is adjusted to pH 1.6 with concentrated HCl, monitoring the pH by pH meter. This should take between 30 and 35 mL of concentrated HCl.

The second portion remains at pH 6.0, but must have a volume of hi-pure water added that is equal to the volume of HCl added to the first portion. This will ensure that the concentration of salts in the two different pH buffers are the same resulting in less baseline change during the gradient run.

Store all buffers in the refrigerator until mixed with acetonitrile.

MOBILE PHASE: 65:35 ACETONITRILE-PHOSPHATE/FORMATE BUFFER (For both pH 6.0 and pH 1.6)

Mix 650 mL acetonitrile and 350 mL buffer. Total volume will be less than 1000 mL. The mobile phase made from pH 6.0 buffer is designated mobile phase A. The mobile phase made from pH 1.6 buffer is designated mobile phase B.

APPENDIX B: PREPARATION OF PHOSPHORIC ACID AND MOBILE PHASE FOR POST-COLUMN ADDITION

4.4 N PHOSPHORIC ACID
Add 50 mL phosphoric acid (85%) to 400 mL deionized hi-pure water and adjust to 500 mL.

65:35 ACETONITRILE-(4.4 N PHOSPHORIC ACID) MOBILE PHASE
Mix 650 mL acetonitrile with 350 mL of 4.4 N phosphoric acid.

APPENDIX C: SYNTHESIS OF MAP DERIVATIVES

Chemical	MW	EQ. WT.	MAP Derivative MW	MAP Derivative EQ. WT.
MAP: 1-(9-Anthracenylmethyl)piperazine	276.4	276.4		
HDI: Hexamethylene diisocyanate	168.2	84.1	721.0	360.5
TDI: Tolylene diisocyanate (toluene diisocyanate)	174.2	87.1	727.0	363.5
MDI: 4,4'-Methylenebis(phenyl isocyanate)	250.3	125.2	803.1	401.6
HMDI: 4,4'-Methylene-bis(cyclohexyl isocyanate)	262.4	131.2	815.2	407.6
MDI Trimer (MDI Triiso)	381.4	127.1	1210.6	403.5
IPDI: Isophorone diisocyanate	222.3	111.2	775.1	387.5
PhICN: Phenyl isocyanate	119.1	119.1	395.5	395.5
BuICN: Butyl isocyanate	99.1	99.1	375.5	375.5
AcMAP: Acetylated MAP			318.4	318.4

2 MAP + 1 diisocyanate yields 1 MAP-diisocyanate derivative

1. Theoretical yield 1 meq: range from 360-408 mg.
2. Weigh 1 meq of diisocyanate to four decimal places. Dissolve in 10 mL of toluene.
3. Weigh 1.2 meq of MAP (20% excess) to four decimal places. Dissolve in 20 mL of toluene.
4. While stirring the MAP solution, add the diisocyanate solution dropwise over a 10 to 15 minute period. Continue to stir for at least one hour.
5. Tightly cover the solution and store overnight in the freezer to promote maximum precipitation.
6. Using a Buechner funnel with filter paper, filter the solution to collect the precipitate.
7. Wash several times with small amounts of cold toluene to remove any remaining MAP.
8. Wash several times with small amounts of cold hexane to displace the toluene. Continue to pull air through the solid for several minutes to dry.
9. Transfer the solid derivative into a preweighed vial with Teflon cap.
10. Using the vacuum pump, apply vacuum until a stable weight is obtained.

APPENDIX D: PREPARATION OF BULK DERIVATIVES

1. Dilute bulk 1/10 in methylene chloride by wt/wt. Quickly proceed to step 2.
   a. Weigh disposable 7 mL vial empty.
   b. Add about 0.5 g bulk, weigh vial again.
   c. Add 4.5 g (3.4 mL) methylene chloride by volume addition, weigh vial again. Use this total weight of 1:10 dilution in calculating final concentration of bulk solution. Density of methylene chloride is 1.325 g/mL.

2. Weigh 1.0 mL of the 1:10 dilution of bulk to determine density. Use this density in calculating the final concentration of bulk solution.

3. Dilute the solution resulting from Step 1 1:100 (10 µL into 990 µL) in methylene chloride by vol/vol. Immediately proceed to step 4.

4. Add 25 µL of the solution resulting from Step 3 to 975 µL acetonitrile containing $5X10^{-4}$ N MAP (1:40 dilution).

5. Let react overnight in dark.

6. Add 5 µL acetic anhydride. Allow to react at least two hours at room temperature or overnight in the refrigerator before proceeding.

7. Analyze by HPLC, injecting 30 µL.

8. Total dilution is about 1:40,000.

9. Final bulk solution concentration (C_B, g/mL):

$$C_B = \frac{Wt_{bulk}}{Wt_{tot}} \cdot Dens_{1:10} \cdot DF, g/mL$$

where:

Wt_{bulk} = the weight of sample (Step 1)
Wt_{tot} = the combined weight of sample and methylene chloride (Step 1)
$Dens_{1:10}$ = the density of the initial sample preparation (Steps 1 and 2)
DF = the dilution factor (Steps 3 and 4) (as presented above, DF = 0.00025)

APPENDIX E: OLIGOMER ID FLOWCHART