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ANALYTICAL PROBLEMS ENCOUNTERED WITH NIOSH METHOD 5521 FOR TOTAL ISOCYANATES

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A recent analysis for total isocyanates in air using National Institute for Occupational Safety and Health Method 5521 presented difficulties in the identification of an oligomeric isocyanate species. Two problems were encountered during the analysis. A false negative response in the high performance liquid chromatography chromatogram was encountered in a majority of the field samples. An anomalous peak served to give a false positive in some of the field blanks and in some of the field samples. Through supplementing the ratio criterion of Method 5521 using the complete UV absorption spectrum from a photodiode array (PDA) UV detector, the two peaks were successfully identified. However, this need for additional data to identify an oligomeric isocyanate species raises the question of whether the ratio criterion of Method 5521 allows the qualitative identification of isocyanate oligomers.

It has long been established that monomeric isocyanates pose health hazards in the workplace.[1] With the advent of polymer-based products, there is less exposure to the volatile monomeric isocyanates. However, exposure to polymer-based products that contain prepolymers, or oligomers, of the monomeric isocyanates is suspected of causing the same health effects as exposure to the parent monomeric isocyanates.[2–4] Recent studies of airborne isocyanates have focused on evaluating exposure to oligomeric species.[5,6] Exposure to the total isocyanate group, which includes the free monomeric isocyanate groups and any free isocyanate group attached to the oligomers, has become an area of interest in respiratory occupational health.

Most of the analytical methods developed for the determination of isocyanates in air have been evaluated for monomeric isocyanates only.[7] The existing methods for isocyanate monomers cannot be applied to quantitation of oligomers, because standards are not available. Even standards prepared from the bulk formulation may not yield accurate data for calculating airborne isocyanates. Following the setting of a total isocyanate standard in the United Kingdom,[8] work was done by Bagon et al.[9] at the Health and Safety Executive of the United Kingdom to develop a method to evaluate total isocyanates in air. This subsequently was issued as Method 25 for the Determination of Hazardous Substances (MDHS 25).[10] National Institute for Occupational Safety and Health (NIOSH) Method 5521 for determination of isocyanates in air was adapted from MDHS 25 and is recommended for the identification and quantitation of monomers and qualitative identification of oligomers of 1,6-hexamethylene diisocyanate (HDI), 4,4′-diphenylmethane diisocyanate (MDI), and 2,4- and 2,6-toluene diisocyanate (TDI).[11] NIOSH Method 5521 is based on the principle of forming an isocyanate derivative with the oligomers, which can then be quantified by reference to the parent monomer. The ratio of response from two analytical detectors is used as the criterion for identifying oligomeric derivatives.

In Method 5521 a sample of air is drawn through an impinger containing a toluene solution of the amine reagent 1-(2-methoxyphenyl)piperazine (MOPP). Any isocyanate that is collected is derivatized to the corresponding urea in the reagent solution during sampling. The sample is analyzed by reversed-phase high-performance liquid chromatography (HPLC). Two detectors are set up in series to measure the analyte: a UV detector (242 nm) and an electrochemical (EC) detector (+0.8 V vs. Ag/AgCl). The ratio of the EC detector response to the UV detector response is calculated using the areas of the chromatographic peaks. Calibration standards of the MOPP urea derivative of the particular monomeric isocyanate being measured are analyzed to determine the characteristic ratio for the sample set. In the analysis of samples, chromatographic peaks having a ratio that falls within a window of 75–150% of the average of the ratios for the derivatized monomer standards are identified as isocyanate derivatives. The more sensitive EC detector is used for quantification.

A problem with the ratio criterion in a routine analysis of air samples from an HDI-based product was suspected after inconsistencies were observed in identifying HPLC chromatographic peaks as isocyanate-derived using the ratio criterion. The elution

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of two peaks in the HPLC chromatogram after the monomer standard indicated the possibility that the peaks could be due to oligomeric species of that monomer. However, peaks of the same retention time in several different field samples gave varying response ratios, sometimes within the ratio window and sometimes outside it. Although additional data were used in this particular analysis to supplement the ratio criterion of Method 5521, the analytical problems encountered indicate a problem with the ability of the ratio criterion to identify oligomeric species qualitatively.

The ratio responses of the isocyanate-derived peaks in the field samples and in the monomer standards were studied statistically to investigate further the ratio criterion. In addition, a statistical study of the ratio response of monomer standards was conducted over a wide range of concentrations to establish whether the ratio was independent of concentration in the linear range of the detectors.

EXPERIMENTAL

Reagents

Acetonitrile, methanol, dimethyl sulfoxide, and toluene were HPLC grade solvents from Burdick and Jackson Laboratories. 1-(2methoxyphenyl)piperazine and 1,6-hexamethylene diisocyanate were obtained from Aldrich Chemical Company. Glacial acetic acid, sodium acetate trihydrate, and acetic anhydride, all reagent grade, were from Fisher Scientific. The deionized water was prepared by passing laboratory filtered deionized water through a Peck Water Systems mixed bed ion-exchange column, carbon scavenger, and 0.2-micron polishing filter. The 0.1N sodium acetate buffer for the HPLC mobile phase was prepared by dissolving 25 g of sodium acetate trihydrate in 2 L of 1:1 methanol:double-deionized water. The pH of the solution was adjusted to 6.0 with glacial acetic acid.

Urea Derivative

The 1-(2-methoxyphenyl)piperazine derivative of 1,6-hexamethylene diisocyanate, N,N′-bis[4-(2-methoxyphenyl)-piperazine-1-carbonyl] 1,6-hexamethylenediamine (HDIU) was synthesized by adding a solution of the diisocyanate (0.002 mole) in dimethyl sulfoxide (25 mL) to a stirred solution of 1-(2-methoxyphenyl)piperazine (0.005 mole, 1 g) in dimethyl sulfoxide (25 mL). After 30 min, the stirred solution was diluted with water (300 mL), and the resulting white precipitate was filtered by vacuum filtration. The urea was dried in a vacuum oven at 75°C to remove the residual water and recrystallized by suspending the urea in 150 mL toluene at 60°C, adding just enough methanol to dissolve the urea completely and allowing the solution to stand at room temperature until crystallization was complete. The crystals were collected by vacuum filtration and dried in a vacuum oven at 35°C. The melting point was 198–200°C for HDIU.

Sampling

The field samples were collected in 25 mL midget impingers, equipped with Fluran phthalate-free tubing (from VWR Scientific), containing 15 mL of sampling medium (MOPP dissolved in toluene, 43 mg/L), at a nominal flow rate of 1.0 L/min. On completion of sampling (sampling times ranging from 1 to 7 hours), the impinger solutions were transferred to 20 mL glass vials and stored under refrigeration until analysis. For analysis each sample was acetylated with 25 μL of acetic anhydride and then evaporated under nitrogen to dryness. The residue was redissolved in 5 mL of methanol while agitating the sample in an ultrasonic water bath for 15 min.

High-Performance Liquid Chromatography

The liquid chromatograph for the HDI analysis consisted of two Waters Model 6000A reciprocating pumps, a Waters Model 600-MS system controller, a Waters Model 710B autosampler, an Applied Biosystems UV detector set at a wavelength of 242 nm, and a Bioanalytical Systems LC-4A amperometric EC detector equipped with an LC-17 oxidative flowcell (+0.8 V vs. Ag/AgCl). The column was a Supelcosil LC-8-DB column operated at a pressure of 2100 psi. The flow rate was 1.0 mL/min, the injection volume was 25 μL, and the run time was 30 min. The mobile phase was 40:60 acetonitrile:buffer (0.1M sodium acetate in 1:1 methanol:water, pH adjusted to 6.0 with glacial acetic acid), isocratic. The calibration standards were solutions of the MOPP urea derivative of the HDI monomer in methanol. Six levels were run in triplicate to obtain the EC/UV ratio for the monomer standards. The average EC/UV ratio was 5.11 with an RSD of 6.8%, yielding a EC/UV ratio range (75–150%) of 3.83–7.66 for the window for positive identification of HDI oligomeric species in this analysis. The limit of detection was 0.4 μg/sample HDI and the limit of quantitation was 1.2 μg/sample HDI.

The PDA UV detector was a Waters Model 990+ UV/VIS spectrophotometer interfaced to a NEC Power Mate 2 data system. The range scanned for this analysis was 220 to 300 nm with a resolution of 1.4 nm. The spectrum analysis mode allows viewing of a full spectrum plot of a chromatographic peak.

Quality control spikes and analyst spikes were run as part of a routine QC protocol. Analyst spikes yielded recoveries of 101.4%, 83.8%, and 94.2%. Blind spikes yielded recoveries of 98.3%, 96.6%, and 105.1%.

Bulk samples of the isocyanate-containing catalyst were derivatized with the MOPP reagent. The HPLC chromatograms were run and potential analyte peaks were identified for comparison with peaks found in the chromatograms of the field samples.

RESULTS AND DISCUSSION

The HDI samples were collected during a survey at a facility involved in spray painting operations[12] that entailed the application of two coats of paint. The base coat was a white epoxy-based primer and the top coat consisted of two components, pigment and catalyst, which were mixed in the spray gun during the

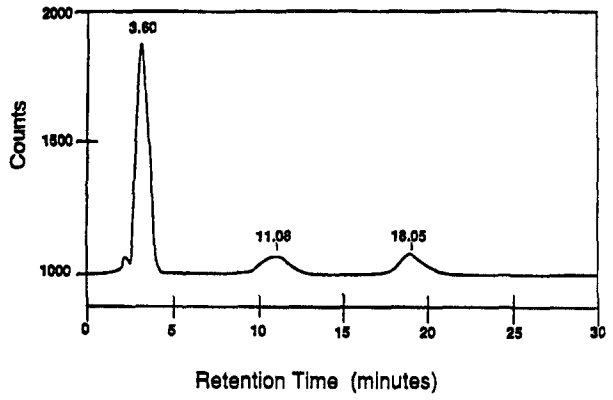

FIGURE 1. HPLC chromatogram of a typical field sample using an electrochemical detector

painting operation. The pigment component contained no HDI. According to the material safety data sheet the catalyst component contained 75% by weight oligomeric HDI (the biuret) with a maximum of 1.2% monomeric HDI.[13]

In a routine industrial hygiene survey, seven air samples were collected in areas where engineering controls had been installed and in areas that were not controlled and subsequently analyzed for HDI according to Method 5521. It was observed that the HPLC chromatograms of some of the field blanks contained a peak that eluted after the monomer standards. Quantitation of this peak yielded oligomeric isocyanate levels that ranged as high as the field samples. The site visit was repeated 4 months later, and 22 additional samples were collected. On initial analysis of the second set of samples, the HPLC chromatograms of some of the field blanks again contained a peak that yielded oligomeric isocyanate levels in the range of the field samples. This peak was seen at an EC retention time of approximately 18 min (see Figure 1). The peak was not seen in the sample of the bulk catalyst, but did appear in two of three field blanks and in a majority of the field samples.

Several possible sources of interferences were investigated to determine why the field blanks appeared to be contaminated with isocyanate analyte. Contamination of the disposable pipets used to transfer the reagent medium was ruled out by preparing reagent medium and transferring it with additional disposable pipets and also glass pipets. These reagent medium samples were then prepared for HPLC analysis according to the protocol of Method 5521. The HPLC chromatograms showed no peaks other than the reagent peaks. Contamination of the reagent medium itself was ruled out by preparing additional reagent blanks for HPLC analysis. However, the original laboratory solution was no longer available for HPLC analysis. The HPLC chromatograms of the medium and the solvents (toluene and methanol) showed no interference peaks.

Contamination at the field site was considered possible but unlikely, since the field blanks were prepared in a remote area of the facility. Contamination of the samples during preparation was ruled out by preparation of several spikes of known quantities of HDI, evaporation to dryness under nitrogen, and reconstitution in methanol. The spikes yielded >95% recovery, and the blanks analyzed with these spikes showed no interference peaks in the HPLC chromatograms. Thus, the possibility of cross contamination during the evaporation stage of sample preparation was eliminated.

The HPLC UV and EC chromatograms of the field samples each contained a peak at an EC retention time of approximately 11 min (see Figure 1). The HPLC chromatogram of the HDI-containing bulk catalyst showed a peak at an EC retention time of 11 min (see Figure 2). Two other smaller peaks were observed in the chromatogram for the bulk sample. Using the EC/UV detector response ratio window determined by the monomer standards, the 11-min chromatographic peak in the bulk sample was identified as an isocyanate derivative. This peak was not seen in any of the field blanks.

During the process of qualitatively identifying the two HPLC peaks using the ratio criterion, it was observed that neither the 11-min peak nor the 18-min peak identified in the field sample chromatograms fell consistently within the ratio window; on several occasions, the observed EC/UV ratio was substantially outside the window.

In an effort to clarify the inconsistencies observed, a Waters 990+ PDA UV detector was used to scan the HPLC chromatographic peaks of the bulk, field, and blank samples. A reference UV spectrum was established using the MOPP urea derivative of monomeric HDI in the wavelength range 220–300 nm (see Figure 3). Two maxima were observed, one at 242 nm (Method 5521 calls for measuring the UV signal at 242 nm) and a second smaller maximum at 280 nm, with a constant relative peak height. The PDA UV spectrum of each chromatographic peak in the bulk, field, and blank samples was then compared to the reference spectrum. Recently, Monteith et al.[14] used a similar protocol for analysis of HDI oligomers in spray painting operations. This group used a PDA UV detector to supplement the analytical results from Occupational Safety and Health Administration Method 47 for MDI, which uses 1-(2 pyridyl)piperazine

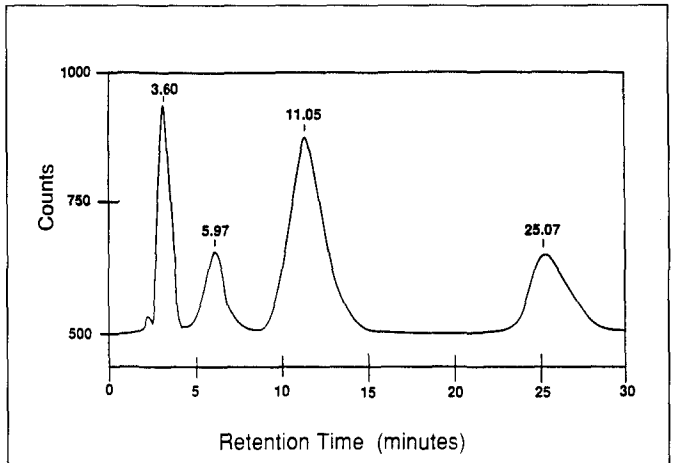

FIGURE 2. HPLC chromatogram of the HDI-containing catalyst bulk sample using an electrochemical detector

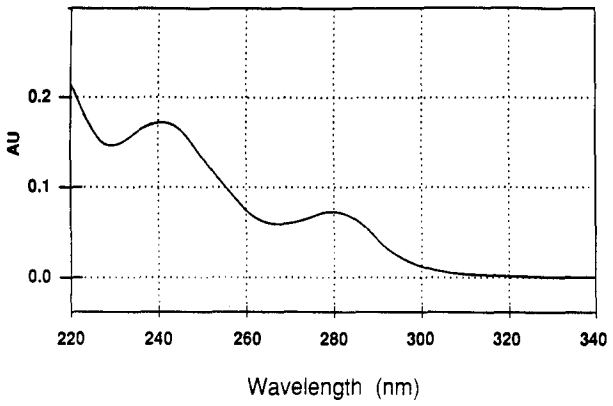

FIGURE 3. Spectrum of MOPP urea derivative of monomeric 1,6-hexamethylene diisocyanate obtained with a photodiode array detector

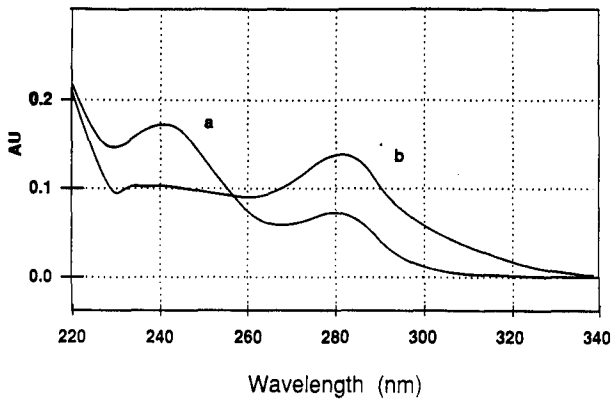

FIGURE 4. a: Spectrum of the 11-min retention time peak obtained with a photodiode array detector. b: Spectrum of the 18-min retention time peak obtained with a photodiode array detector.

as the derivatizing agent. The PDA spectra of the field samples were compared to a reference spectrum of the HDI derivative. Following this protocol, this group identified an HDI oligomer in air filter samples from HDI spray-painting operations.

In every sample containing the 18-minute peak, the UV spectrum obtained from the PDA showed only one maximum at 280 nm (see Figure 4b). It was decided to discount this peak as isocyanate-derived for two reasons: (1) the 242 nm maximum, characteristic of an isocyanate-derived species, was absent, and (2) this 18-min peak was not present in the bulk catalyst. Thus, the 18-min peak was ignored for the purposes of quantitation. This negated the peak causing the high field blanks.

The other peak tentatively identified by the ratio method to be isocyanate-derived appeared at an EC retention time of 11 min. This peak consistently exhibited the MOPP-derivatized isocyanate pattern in the UV spectrum (see Figure 4a). Possible explanations for the fact that the UV spectra confirm an oligomeric isocyanate while the ratio was somewhat out of range for the majority of the field samples (and substantially out of range for several) could be the lower sensitivity of the UV detector or the presence of a coeluting interference.

Closer examination of the results by investigating the EC/UV response ratio of the 11-min peak with decreasing analyte concentrations revealed a statistically significant trend toward higher ratios (see Figure 5). In fact, all the samples below the limit of quantitation (LOQ) yielded response ratios too high for qualitative identification as isocyanate species. The two samples with the lowest analyte concentration (near the LOD) exhibited ratios substantially outside the acceptable ratio range. The oligomer UV response at 242 nm for these two samples was only slightly above the noise level of the detector, and integration of such a signal is not very precise.

An important observation from the response ratio data for the isocyanate-derived HPLC peak was that even above the LOQ, the majority of the samples exhibited ratios outside the window (see Figure 5). For the field samples the overall average ratio for all ratios above the LOQ was 15.19, compared with 5.11 for the average ratio of the standards used for establishing the ratio range. In addition, the variability seen in the ratios of the field samples above the LOQ was significantly higher (standard deviation of 13.53) than what could be explained by the variability in the standards (standard deviation of 0.68) used to determine the ratio range. A trend of increasing variability with decreasing analyte concentration also was observed in the ratio response of the field samples. Although the isocyanate-derived

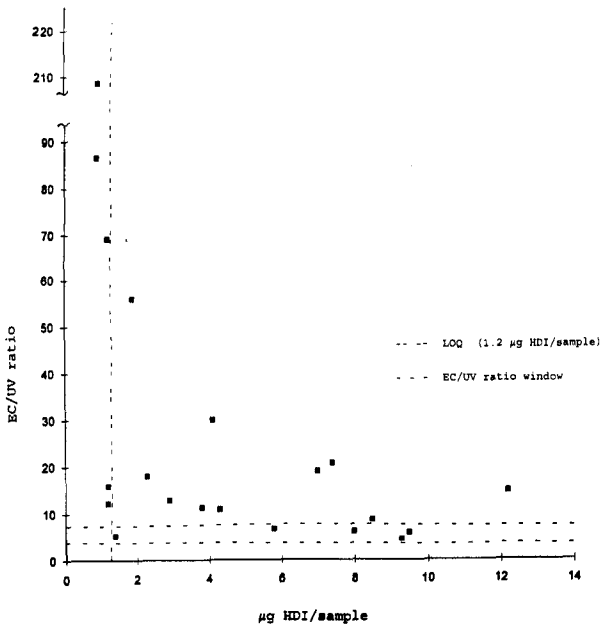

FIGURE 5. Graph of EC/UV detector response versus analyte concentration for the isocyanate-derived peak (11-min retention time) in the field samples

HPLC peak can be quantitated easily using the EC data, the variability of the ratio response limits the ability to identify the peak by its EC/UV ratio.

To investigate further the variability of the ratio response, the ratio response data for monomer standards was studied statistically. Six levels were prepared over a wide range to establish whether the ratio was independent of concentration in the linear range of the EC and UV detectors. For each standard concentration five to seven runs were performed. The average of the ratio response and the standard deviation was calculated for each level (see Table I). There was a statistically significant trend toward higher average ratios and larger standard deviations with decreasing analyte concentration. However, with the exception of the lowest concentration level, the ratio range for qualitative identification (75% to 150% of the average ratio) was wider than the standard deviation for the monomer standards.

In an effort to elucidate if one or both detectors played a major role in contributing to the overall ratio variability, the averages and standard deviations of the peak areas for each of the six standard levels were calculated for each detector. Although it was observed that at the lower concentrations both the EC and the UV detector responses exhibited high relative standard deviation, it also was observed that the EC detector response exhibited high relative standard deviation over the entire concentration range studied.

TABLE I. Ratio Response of Monomer Standards

Concentration μg HDI/Sample	*Average Ratio*	*Standard Deviation*
58.6	5.12	0.58
29.3	5.20	0.67
14.6	6.11	0.76
7.3	6.49	1.09
3.6	7.47	1.89
1.8	7.62	2.01

CONCLUSIONS

By supplementing the data obtained by the ratio criterion of Method 5521 with data from the PDA UV detector, the successful identification of an HDI oligomer was obtained. The HDI oligomer concentrations were consistent with the areas sampled in the plant: where engineering controls had been installed, the expected low levels were supported by the analytical results. Similarly, uncontrolled areas were found to have substantial levels of oligomer concentration.[12]

The use of the ratio criterion of Method 5521 was insufficient for identification of an HDI oligomer in a laboratory analysis of field samples. Supplementing the ratio criterion with data from a PDA UV detector enabled quantification of the analyte. Analyses of HDI-based products that contain oligomers can be successfully achieved using a PDA UV detector for confirmation. Method 5521, with the modification of using PDA UV data for confirmation of oligomeric species, is recommended as an analytical method for total isocyanates for HDI-based products.

The use of the full UV spectrum to identify an isocyanate is not predicted to work as well for aromatic isocyanates, such as TDI and MDI, since the aromatic rings in these compounds are sources of UV activity in addition to that due to the derivatized isocyanate groups. Thus, the protocol of adding a PDA UV detector for confirmation of isocyanate oligomer is not recommended for routine use in the analysis of aromatic isocyanate products. Method 5521 is not recommended as an analytical method for total isocyanates for TDI- and MDI-based products.

This article has presented a documented case of the failure of the ratio criterion in the analysis of total isocyanates for an HDI-based product. The question thus arises whether there are intrinsic problems with the ratio method itself as originally promulgated in MDHS 25. Additional research has been conducted to investigate this question. Urethane-bound isocyanate species derived from TDI were prepared and the ability of MDHS 25 to correctly identify and accurately quantify these species was examined. The results of this research are presented elsewhere in this issue.[15]

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