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Note

Improved high-performance liquid chromatographic method for artifact-free measurements of aldehydes in the presence of ozone using 2,4-dinitrophenylhydrazine

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A wide variety of aldehydes is present in the troposphere both as primary pollutants from combustion sources and as secondary pollutants from the photochemical oxidation of atmospheric hydrocarbons. Exposure to formaldehyde and many other of these carbonyl compounds can cause irritation to skin, eyes and upper respiratory membranes[1]. Furthermore, photolysis of formaldehyde leads to the formation of carbon monoxide and ozone[2] while the higher aldehydes can produce peroxyacyl nitrates which are suspected mutagens[3].

Numerous studies have been published which use 2,4-dinitrophenylhydrazine (2,4-DNPH) as the derivatizing reagent for the collection of carbonyl compounds in impingers and cartridges with subsequent analysis by high-performance liquid chromatography (HPLC)[4–11]. We have used the impinger technique for a number of years to detect and quantify aldehydes produced during the photooxidation of hydrocarbons in conventional smog chamber experiments. For cases in which the formation of aldehydes is monitored as function of the extent of hydrocarbon oxidation, several unknown peaks have appeared in the chromatograms and have been shown to increase in area with increasing extent of reaction. These observations for experiments using a number of different hydrocarbons have indicated that the increase in these peaks follows the formation of ozone. This result has led us to the conclusion that the unknown peaks are the result of decomposition products from the reaction of ozone with 2,4-DNPH[12]. This conclusion is consistent with a recent report by Arnts and Tejada[13,14] describing formaldehyde interferences by ozone when sampled using a cartridge technique. Under chromatographic conditions commonly reported for the analysis of the aldehydes by HPLC, two of the peaks from the decomposition products can readily co-elute with the formaldehyde hydrazone. This interference leads to a positive formaldehyde artifact when sampling in the presence of ozone. In this paper we describe an improved HPLC method for the analysis of carbonyl compounds collected using 2,4-DNHP impingers that provides excellent separation to yield artifact-free measurements.

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EXPERIMENTAL

Instrumentation

The equipment used consisted of an LDC/Milton Roy CM4000 single-pump liquid chromatograph capable of forming a low-pressure ternary gradient and an LDC/Milton Roy SM4000 variable-wavelength UV–visible detector set to 360 nm. Data were collected using a PE/Nelson PC integrator (version 5.0) operating from an IBM PC/XT compatible computer. A column heater was employed and operated at 40°C. The sample injection loop volume was 10 μl.

Column

Although several manufacturers' C_{18} columns have been tried over the years, for this application we have had the best separation and reproducibility with a single Dupont Zorbax™ ODS column (25 cm × 4.6 mm, 5 μm particle size, part No. 880952-702).

Chemicals

Methanol (HPLC grade) and acetonitrile (HPLC grade) were obtained from Burdick & Jackson and used as received. Water was doubly deionized and then distilled using a Corning Mega-Pure continuous-flow still. The 2,4-DNPH was obtained from Aldrich. Using a purification technique similar to previously published methods[5,10], 5 g of 2,4-DNPH were twice recrystallized from 200 ml of hot acidic (2 *M* HCl) absolute ethanol prior to use in either the impinger solution or for making of standards. All standards were synthesized by reacting an excess (1–2 g) of the selected carbonyl compound (highest available purity) with 200 ml of a hot acidic (2 *M* HCl) absolute ethanol or acetonitrile containing 2 g of the purified 2,4-DNPH. The precipitate was recrystallized a second time, filtered, washed with cold ethanolic 1 *M* HCl and dried in a vacuum desiccator. All standards and the 2,4-DNPH reagent were sealed in amber bottles with Teflon™-lined caps and stored in a desiccator prior to use.

Sample derivatization

Sample collection and derivatization occur in one step in the impinger. The impinger solutions were made up as previously described[5] except that cyclopentanone and/or cyclohexanone hydrazones were added as internal standards in the range 10–20 nmol/ml. Briefly, 100 ml acetonitrile and 50 mg of purified 2,4-DNPH were added to a 250-ml volumetric flask and stirred until completely dissolved. To this solution 0.05 ml concentrated H_2SO_4 and the desired level of internal standard (diluted from previously made concentrate) were aded and then filled to volume with acetonitrile. A 4-ml volume of the reagent was pipetted into the impinger which was then immersed in an ice bath. Air samples were pulled through the solution at 0.5 l/min using a Metal Bellows pump and calibrated Sierra flow controller. Following sampling, the solution was collected and analyzed directly by HPLC. For compounds with a single carbonyl group, chromatographic analysis could be performed immediately after sample collection. However, for the analysis of bi-functional compounds such as glyoxal or methyl glyoxal, the sample required heating at 70°C for 30 min to drive the reaction to completion. Alternatively, allowing the samples to stand at room temperature for 24 h provides sufficient time for complete reaction prior to analysis.

Elution gradient

A 26-min ternary gradient mobile phase (see Fig. 1) at a constant flow-rate of 1 ml/min was used as follows: (1) solvent A, water, started at 40%, was decreased linearly to 25% at 10 min, further decreased linearly to 15% at 20 min and then held constant to 26 min; (2) solvent B, acetonitrile, started at 20%, was decreased linearly to 5% at 10 min and then held constant to 26 min; (3) solvent C, methanol, was started at 40%, increased linearly to 70% at 10 min, further increasing linearly to 80% at 20 min and then held constant to 26 min. Post-gradient equilibrium time was 10 min.

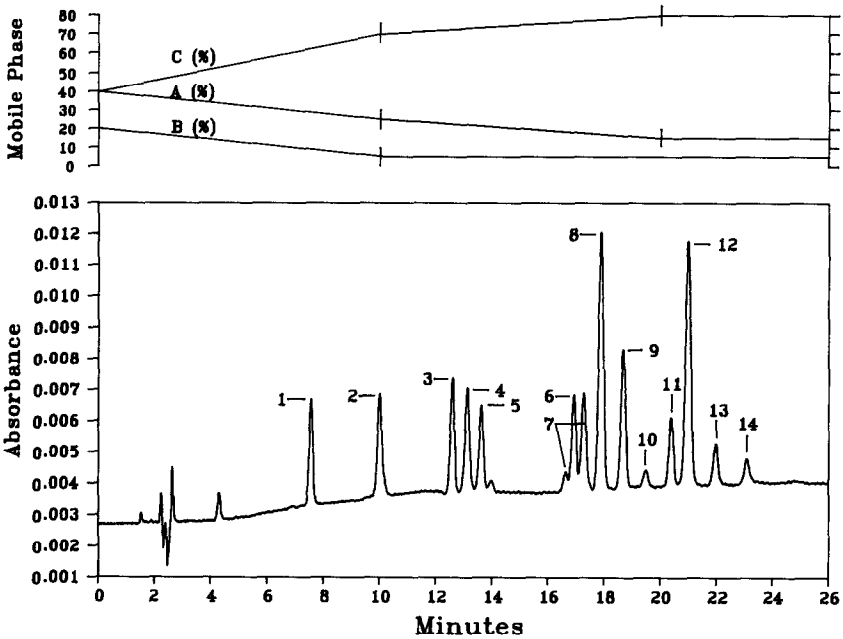

Fig. 1. (Top) Ternary gradient mobile phase: A = water; B = acetonitrile; C = methanol. (Bottom) Standard chromatogram Peaks: 1 = formaldehyde; 2 = acetaldehyde; 3 = acrolein; 4 = acetone; 5 = propionaldehyde; 6 = butyraldehyde; 7 = *anti*- and *syn*-MEK; 8 = cyclopentanone (internal standard); 9 = benzaldehyde; 10 = glyoxal; 11 = valeraldehyde; 12 = cyclohexanone (internal standard); 13 = *o*-tolualdehyde; 14 = methyl glyoxal.

RESULTS AND DISCUSSION

Initial attempts to improve the chromatography resulted from a desire to separate several of the C_3 and C_4 compounds formed during the course of the photooxidation experiments discussed earlier. These included, for example, methyl ethyl ketone (MEK) and butyraldehyde which were poorly separated under our original conditions. During the initial phase of development it was found that the peak associated with the formaldehyde hydrazone actually consisted of multiple peaks. When these interference peaks were originally observed, it was thought they might be photolysis products formed during the course of the hydrocarbon/NO_x photooxidation

since they demonstrated a time profile consistent with the formation of secondary products. Work done to identify these compounds as substituted hydroxycarbonyl derivatives proved unsuccessful. Ultimately it was established that the peaks resulted from the reaction of ozone with 2,4-DNPH.

As with many of the published methods for this HPLC analysis, we had previously used a binary mobile phase of acetonitrile and water with a gradient elution. To expand the early portion of the chromatogram, higher starting percentages of water were tried and proved successful at separating the unknown peaks. However, this modification caused the co-elution of the important C_3 compounds acetone and acrolein. It also degraded the already poor separation of the major MEK isomer peak with butyraldehyde. Efforts were then made to investigate a methanol–water gradient program. This approach was also successful at separating the interference peaks from formaldehyde (with a different elution order) but resulted in the co-elution of the C_3 peaks of acetone and propionaldehyde as well as the co-elution of the minor MEK isomer and butyraldehyde. A comparison of these results indicated that a ternary gradient using these three solvents could be developed to provide the necessary separation.

The ternary gradient separation as described above is the one presently used for most of the analyses in this laboratory. Shown in Fig. 1 is a chromatogram of a 10-μl injection of twelve-component mixture plus two internal standards. It can be seen that there is good separation of the C_3 compounds and that butyraldehyde elutes between the *syn-* and *anti*-isomers of MEK. The standard solution concentrations normally ranged from 0.5 to 50 nmol/ml, although linear response has been established up to at least 200 nmol/ml. Multipoint calibrations over two orders of magnitude in concentration yield linear plots with an r^2 value of 0.98 or better for each of the components in the twelve-component mixture. Reagent blank contributions to the analytes range from below detectable levels to the equivalent of a few ppb (v/v)[a]. Detection limits (with the HPLC signal-to-noise ratio > 3:1 and blank subtraction) are approximately 1 ppb (v/v) per component under our normal sampling conditions of a 10-l air volume into 4 ml of reagent and 10-μl injection. For air samples having lower concentrations, this detection limit can be improved considerably by both increasing the sampled air volume and concentrating the analyzing solution. Retention times are very reproducible with variations usually less than 2%. There is a slight baseline drift (approximately 0.0015 absorbance units) over the 26-min chromatogram associated with the methanol concentration but this does not contribute any additional uncertainties in the quantitative analysis. Also, it was initially feared that there might be some problems with trace acetaldehyde contamination of the methanol but this has not been a problem with the current supplier of methanol.

Fig. 2 shows a portion of two chromatograms of samples with the same concentration of formaldehyde (estimated at 140 ppb, v/v) but with different levels of ozone added. These were prepared using a dynamic dilution apparatus[13,14] where the ozone level in A was 514 ppb (v/v), while in B it was 16 ppb (v/v). It can been seen that there are several additional peaks in the high-concentration ozone sample that are not present or are greatly reduced in the low-concentration ozone sample. The quantitative data for formaldehyde using peak heights and areas were as follows: samples A,

[a] Throughout this article, the American billion (10^9) is meant.

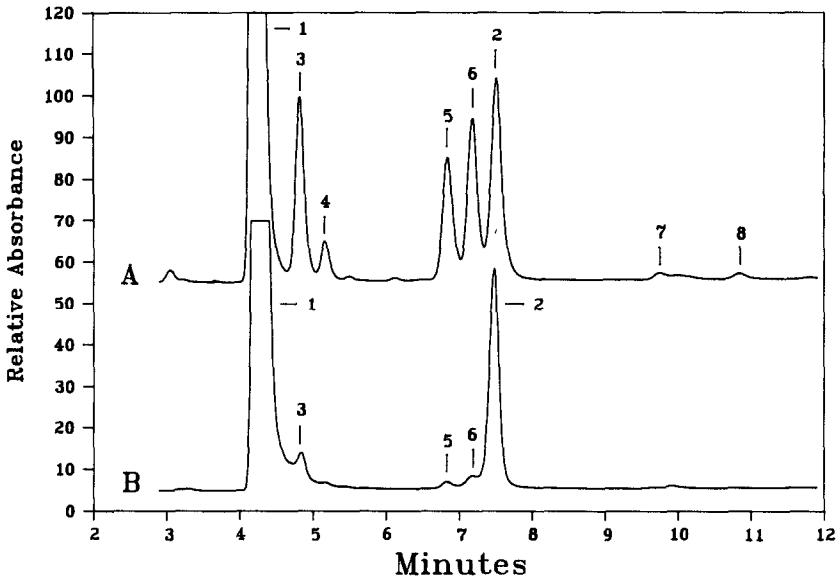

Fig. 2. Comparison of chromatograms with a constant level of formaldehyde. (A) High-level ozone (514 ppb, v/v); (B) low-level ozone (16 ppb, v/v). Peaks: 1 = 2,4-DNPH reagent; 2 = formaldehyde; 3–8 = ozone/2,4-DNPH reaction artifacts.

123 and 120 ppb (v/v); sample B, 122 and 110 ppb (v/v), respectively. These measurements indicate that the chromatographic separation of the analyte and the artifact peaks was sufficient to produce no discernable quantitative interference using peak heights or peak areas. This observation is consistent for other samples which have been tested in this manner. This data also provided evidence that there were no undetected peaks present as a function of ozone concentration that were co-eluting with formaldehyde. This has been independently verified in a series of experiments where different levels of ozone in air were bubbled into the 2,4-DNPH impinger solution as well as through solutions of the pure hydrazones and analyzing the resultant mixtures. The significant artifact peaks which developed were only in the ozone/2,4-DNPH solutions appearing directly as a function of ozone concentration and were the same as those in sample A and indicated in Fig. 2. It should be noted here that there was also some measurable degradation of the pure hydrazones but at a much lower rate than the 2,4-DNPH. In a bubbling impinger containing an excess of 2,4-DNPH, the more reactive reagent is selectively degraded over the hydrazone analytes, thus avoiding interference as a function of analyte degradation. This is consistent with the data presented in Fig. 2 from the dynamic dilution system which showed no loss of the formaldehyde when collected by impinger and with the previously reported study by Arnts and Tejada[13,14]. The high-concentration ozone sample represents a level higher than is usually seen in urban sampling and shows that this separation should be satisfactory over a broad range of ambient ozone concentrations. Other measurements from smog chamber photooxidation experiments demonstrate sufficient separation of these degradation products at ozone concentrations up

to 1 ppm (v/v). Finally, in circumstances such as direct auto exhaust sampling where the analyses of C_6 and higher aldehydes are desirable, the later portion of the gradient can be expanded over a longer period and/or run to a higher final percentage of methanol for more extensive separation of the late-eluting components.

CONCLUSIONS

The HPLC method described has been shown to provide good separation of formaldehyde from degradation products of ozone reactions with 2,4-DNPH while also providing good separation of other carbonyl compounds frequently of interest in urban sampling schemes.

DISCLAIMER

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