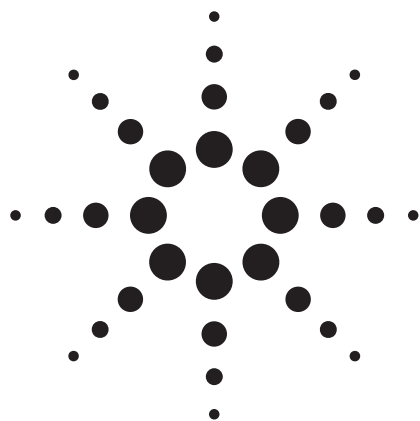


Rapid Separation and Identification of Carbonyl Compounds by HPLC



Application

Environmental

Authors

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Abstract

A fast HPLC method for the analysis of DNPH derivatized carbonyls found in air samples is demonstrated. A 1.8- μm StableBond C18 separated a standard of the major 13 DNPH derivatized carbonyls. An air sample was analyzed in under 10 minutes and found to contain several carbonyls.

Introduction

Carbonyl compounds are important constituents in urban and global atmospheres. In urban atmospheres, these compounds frequently initiate photo-

chemical smog and sustain reactions leading to ozone formation. Outdoor sources include motor vehicle exhaust and other forms of incomplete hydrocarbon combustion. Indoor sources, such as aldehydes found in insulation, tobacco smoke, furniture, and particle board, also contribute to the overall environmental concerns. Measurement of low-level atmospheric carbonyl compounds requires techniques that are sensitive and free of interferences. Unfortunately, many known carbonyl compounds in air samples (for example, formaldehyde and acetone) have no chromophores and are not detected by the UV detector. Thus, for low-molecular-weight aldehydes and ketones in air and water, HPLC methods have been developed for the in-situ derivatization of carbonyls using 2,4-dinitrophenyl-hydrazine (DNPH) (Figure 1).

Official Methods for Carbonyl Compounds

There are several official regulatory agency methods for DNPH-derivatized carbonyls based on the sample matrix (see Table 1).

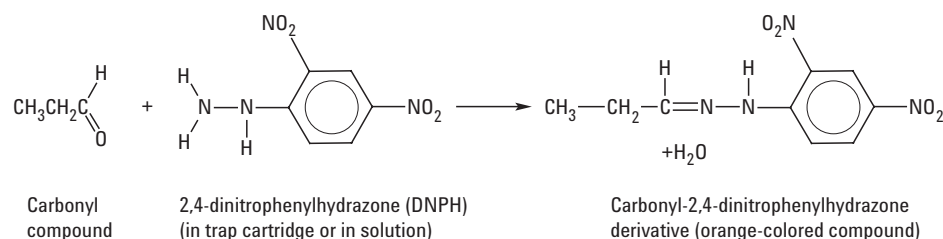


Figure 1. Chemical reaction for derivatizing of carbonyls from air or water samples.



Table 1. Methods for Analysis of DNPH-Derivatized Carbonyls

Method Number	Matrix
EPA TO-11	Ambient air
EPA 8315A	Liquid, solid, and gas samples
ASTM D5197	Ambient air
NIOSH 2016 and 2532	Ambient indoor air
EPA 554	Drinking water

EPA = U.S. Environmental Protection Agency
 ASTM = American Society for Testing Materials
 NIOSH = National Institute for Occupational Safety and Health

These methods are all variations of the general approach discussed in the experimental section. In some cases, for toxic air samples, an ozone scrubber must be installed prior to the DNPH cartridge since ozone interferes with the carbonyl-DNPH reaction.

Experimental

The carbonyl-containing air sample is passed through a silica gel cartridge that has been coated with DNPH or impinged into an acidified DNPH solution. For water samples, the reaction occurs directly by the addition of DNPH to the water, buffered at pH 3. Carbonyl compounds are rapidly

derivatized to hydrazones under these conditions. These colored hydrazones are then eluted from the trapping cartridge using acetonitrile; or for water samples, extracted from solution with methylene chloride. These extracts are then concentrated, reconstituted in a suitable solvent, and injected into a reversed-phase column for separation and quantitation.

Once the derivatized hydrazones are collected and extracted (or eluted), reversed-phase HPLC is the preferred method to analyze these compounds [1-5]. Since the hydrophobicities of the hydrazones vary widely, gradient conditions are required to elute them in a reasonable time.

Results and Discussion

Figure 2 depicts the separation of the major DNPH-derivatized carbonyl standards that are on the list of important environmental toxins. This is a commercially available standard solution (3- $\mu\text{g}/\text{mL}$) purchased from Sigma (St. Louis, MO). The Stable-Bond C18 1.8 μm 4.6 mm \times 150 mm column (pn 829975-902) showed excellent resolution,

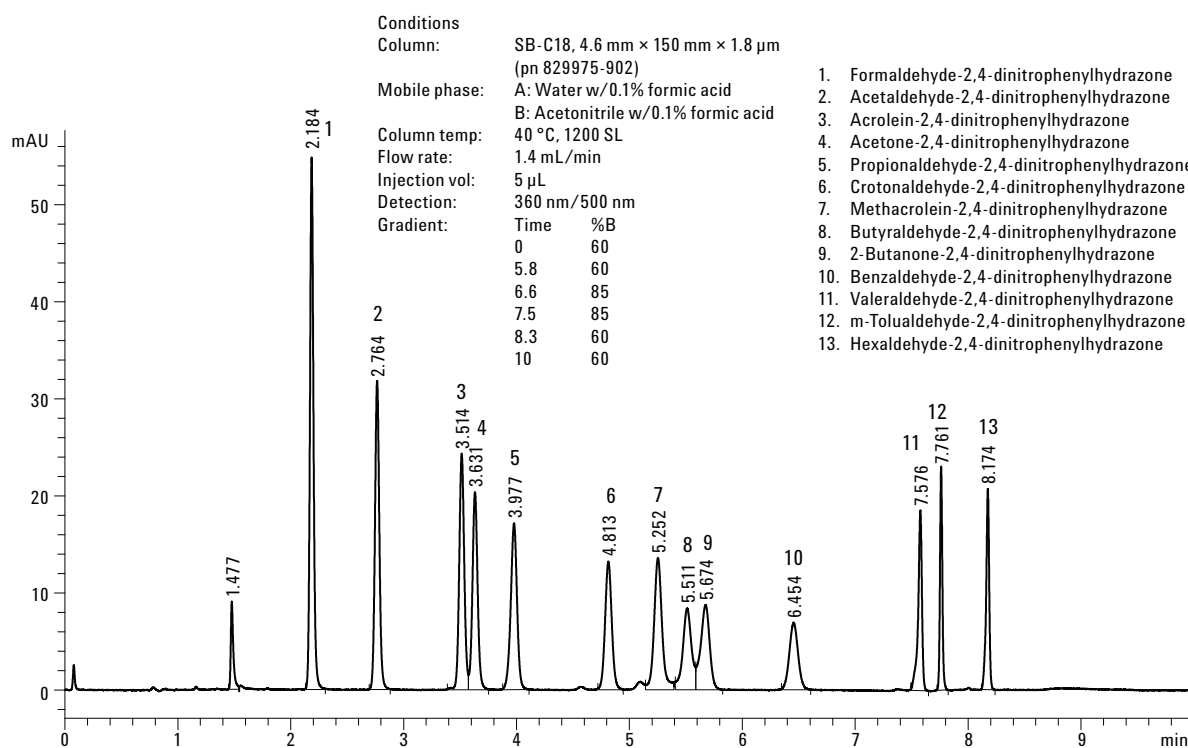


Figure 2. Commercial DNPH standard and analysis conditions.

including partial separation of peaks 8 and 9 of the standard. The separation of 2-butanone-2,4-DNPH and butyraldehyde-2,4-DNPH is noteworthy since this pair is unresolved on a number of reversed-phase columns. The high surface coverage of the C18 phase is partially responsible for the good selectivity. The initial portion of the chromatogram is isocratic, allowing easy transfer of the method to any instrument as well as potential scaling to smaller diameter columns used with mass spectroscopic detection. Higher flow-rate analyses are easily accomplished using the Agilent 1200 System; however, the maximum pressure measured throughout these gradients was approximately 370 bar.

The 60% acetonitrile initial mobile phase composition eluted these nonpolar compounds in a reasonable time. A higher aqueous mobile phase composition results in a loss of resolution of peaks 3 and 4 (acrolein-DNPH and benzaldehyde-DNPH). A higher organic composition compromises the resolution of other peaks, most readily seen in the 7-8-9 group (crotonaldehyde-DNPH, formaldehyde-DNPH, and hexaldehyde-DNPH). A wavelength of 360 nm was used. This wavelength avoids detecting extraneous peaks that usually have stronger absorbance at lower UV wavelengths. Finally, formic acid was added at 0.1 % concentration to both the water and acetonitrile. This will improve the ionization of the analytes when using MS detection, add greater stability to the column, and prevent secondary interactions, while not complicating the mobile phase preparation.

Analysis of Carbonyl Compounds in Ambient Air Samples

Outdoor air was collected on DNPH-coated traps and run through the extraction procedure. This extract was kindly provided by Vermont's Department of Environmental Conservation's (DEC) Environmental Laboratory, Waterbury, VT, USA. The extracts were directly injected into the Agilent StableBond SB-C18 column. A blank extract was also run. The blank was prepared using a collection cartridge with a DNPH loading; however, this cartridge was not carried through the entire sampling process.

Figure 3 shows the HPLC results of an actual air-sample collected near Burlington, Vermont. In this sample, small amounts of formaldehyde, acetaldehyde, and acetone were found. Some unknown peaks were observed that were also present in the blank. One compound eluted about 5.5 minutes and was attributed to unreacted DNPH; the others were unidentified.

Conclusions

Using DNPH collection and HPLC analysis, small amounts of carbonyl-containing compounds in air can be separated and measured in a short time. The Agilent StableBond SB-C18 column gave excellent selectivity and provided a fast separation of the EPA list of carbonyl compounds in about 8 minutes.

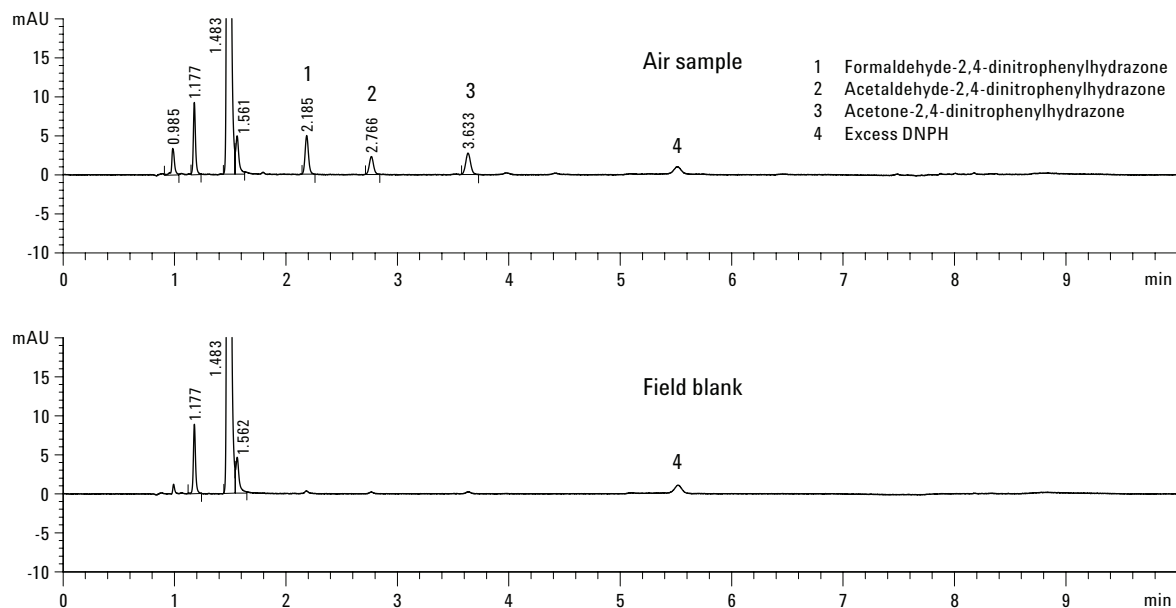


Figure 3. Burlington site top (sample) bottom (field blank) SB-C18 4.6 mm × 150 mm × 1.8 μm.

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Printed in the USA
April 30, 2008
5989-7483EN

