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Quantitative Analysis of Carbonyl-DNPH Derivatives by UHPLC/UV

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Key Words

- Accela
- Hypersil GOLD
- Carbonyls
- Environmental Analysis
- UHPLC

Goal

Develop a fast, accurate and robust method for the separation and quantitation of parts per billion (ppb) concentrations of low molecular weight carbonyls using UHPLC/UV.

Introduction

Carbonyl compounds from motor vehicle and industrial emissions are precursors to ground-level ozone, a major component of smog and strongly associated with respiratory and pulmonary problems. Moreover, several volatile aldehydes and ketones have direct adverse effects on human health and are defined as hazardous air pollutants under the Clean Air Act Amendments of 1990.[1,2] Formaldehyde, the most abundant airborne carbonyl, is classified as a probable human carcinogen by the United States Environmental Protection Agency (EPA) and designated carcinogenic to humans by the International Agency for Cancer Research (IARC).[3,4] Acetaldehyde is another abundant carbonyl pollutant that is categorized as a suspected or known carcinogen by regulatory agencies.[4,5] In accordance with the Clean Air Act, the EPA enforces ambient monitoring of three carbonyl pollutants – formaldehyde, acetaldehyde and acetone – and recommends surveillance of several others in areas with persistently high ozone levels.[6] The California Air Resources Board (CARB) requires monitoring of formaldehyde, acetaldehyde, acrolein and methyl ethyl ketone in major urban areas.[7]

Carbonyls are also sources of pollution in indoor living and working environments. Formaldehyde, a ubiquitous indoor pollutant, is released from multiple diverse sources including plywood, particle board, furniture, paper products, resins, glues, tobacco smoke, fuel-burning appliances, textiles and cosmetics. Indeed, in a recent study of over two hundred homes of non-smoking families in different U.S. cities, the median concentrations of nine carbonyls were found to be significantly higher indoors than outdoors; for formaldehyde, the median indoor and outdoor concentrations were 20.1 µg/m^3 (16 ppb) and 6.42 µg/m^3 (5 ppb), respectively.[8] Airborne formaldehyde levels of 0.1 ppm can cause irritation of the upper respiratory tract in sensitive individuals.[9] The U.S. Occupational Safety and Health Agency (OSHA) has set the legal permissible exposure limit for formaldehyde in the workplace at 0.75 ppm measured as an 8-hour time-weighted average, and established a 15-minute short-term exposure limit at 2 ppm.[9] There are currently no federal government regulations or guidelines for formaldehyde levels in residential settings, but the

California Office of Environmental Health Hazard Assessment (OEHHA) has established acute (1 hour) exposure levels of 55 µg/m^3 (44 ppb) and set both the eight-hour and chronic reference exposure levels at 9 µg/m^3 (7 ppb).[10] Carbonyls are also encountered in food and drinking water.[11] Alcohol represents a major source of acetaldehyde exposure and has been associated with increased cancer risk in individuals with aldehyde dehydrogenase deficiency.[12] Determination of exposure pathways, health outcomes and effective pollution control strategies requires sensitive and accurate methods for trace-level analysis of carbonyl compounds in a range of matrices.

Highly volatile and reactive, low molecular weight carbonyls are typically converted to stable derivatives prior to analysis. The most commonly used derivatizing agent is 2,4-dinitrophenylhydrazine, which reacts readily with carbonyls in acidic conditions to form 2,4-dinitrophenylhydrazones (DNPH) derivatives. While GC-based methods have been developed to detect these compounds, HPLC coupled with UV detection is the most widely recognized technique for the analysis of carbonyl-DNPH derivatives. EPA methods for the determination of carbonyls in ambient air (EPA TO-11), ambient indoor air (EPA 8315A Procedure 2), drinking water (EPA 554) and aqueous, soil, waste and stack samples (EPA 8315A Procedure 1) utilize DNPH derivatization and HPLC/UV analysis.[13-15] Likewise, CARB Method 1004 specifies HPLC analysis of carbonyl-DNPH derivatives with UV detection for the monitoring of aldehydes and ketones in automotive engine exhaust.[16] However, long run times, poor resolution and low separation efficiencies can limit the utility of conventional HPLC in this application. Ultra high performance liquid chromatography (UHPLC) enables faster separations and higher resolution through the use of sub-2 µm diameter particles.

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The Thermo Scientific Accela UHPLC system offers the flexibility of performing both HPLC and UHPLC separations on a single platform. The Accela™ 1250 Pump delivers precise flows and accurate gradients at an expansive range of flow rates (up to 2 mL/min) and pressures (up to 1250 bar), and accelerates method development and maximizes method flexibility through quaternary gradient capabilities. The Accela UHPLC system together with Thermo Scientific 1.9 µm Hypersil GOLD columns enables fast chromatographic separations with high efficiency and resolution. In this application note, we demonstrate fast, accurate and robust separation, detection and quantitation of ppb levels of carbonyl-DNPH derivatives using the Accela UHPLC system and high performance columns.

Materials and Methods

Sample Preparation

DNPH-derivatized carbonyl standards (100 µg/mL) were purchased from AccuStandard® (New Haven, CT, USA). Stock solutions were prepared by diluting five-fold with 60:40 acetonitrile:water (v/v). Calibration solutions, with concentrations of 98-50000 ng/mL, were prepared by serial dilution of the stock solutions in 60:40 (v/v) acetonitrile:water.

LC/UV Analysis

Instrumentation

LC separations were performed on an Accela 1250 UHPLC system with an Accela autosampler (Thermo Fisher Scientific, San Jose, CA, USA). UV absorbance was monitored at 360 nm using an 80 Hz Accela PDA detector (Thermo Fisher Scientific, San Jose, CA, USA).

LC Parameters

Separation of Carbonyls Listed in EPA Method 8315A Procedure 1

Column:	Thermo Scientific Hypersil GOLD column (2.1 × 100 mm, 1.9 µm particle size)			
Mobile Phase:	A: Water B: Acetonitrile			
Column Temperature:	40 °C			
Sample Injection Volume:	2 µL			
Gradient:	Time (min)	A %	B %	µL/min
	0.00	68.0	32.0	800
	7.00	25.0	75.0	800
	7.10	20.0	80.0	800
	9.00	18.0	82.0	800
	9.1	68.0	32.0	800

Separation of Carbonyls Listed in EPA Method 8315A Procedure 2

(a) Column:	HALO Phenyl-Hexyl (2.1 × 100 mm, 2.7 µm particle size)					
Mobile Phase:	A: Water B: Acetonitrile C: 50:50 THF:water D: Methanol					
Column Temperature:	40 °C					
Sample Injection Volume:	2 µL					
Gradient:	Time (min)	A%	B%	C%	D%	µL/min
	0.00	51.0	38.0	8.0	3.0	450
	2.00	51.0	38.0	8.0	3.0	450
	2.10	51.0	38.0	8.0	3.0	400
	4.00	43.0	40.0	14.0	3.0	400
	4.10	43.0	40.0	14.0	3.0	450
	5.00	38.0	40.0	16.0	6.0	450
	5.10	38.0	40.0	16.0	6.0	380
	7.50	30.0	40.0	16.0	14.0	380
	7.60	30.0	40.0	16.0	14.0	450
	9.00	23.0	45.0	16.0	16.0	450
	10.00	51.0	38.0	8.0	3.0	450
(b) Column:	Waters ACQUITY UPLC® BEH Phenyl column (2.1 × 100 mm, 1.7 µm particle size), Waters ACQUITY UPLC BEH C18 column (2.1 × 100 mm, 1.7 µm particle size), and Thermo Scientific Hypersil GOLD column (2.1 × 100 mm, 1.9 µm particle size)					
Mobile Phase:	A: Water B: Acetonitrile C: 50:50 THF:water D: Methanol					
Column Temperature:	40 °C					
Sample Injection Volume:	2 µL					
Gradient:	Time (min)	A%	B%	C%	D%	µL/min
	0.00	57.0	32.0	8.0	3.0	700
	2.00	57.0	32.0	8.0	3.0	700
	2.10	57.0	32.0	8.0	3.0	600
	3.00	55.0	32.0	10.0	3.0	600
	6.00	43.0	38.0	16.0	3.0	500
	8.00	40.0	38.0	16.0	16.0	500
	10.00	30.0	29.0	16.0	3.0	550

Quantitative Analysis

Column:	Thermo Scientific Hypersil GOLD (2.1 × 100 mm, 1.9 µm particle size)					
Mobile Phase:	A: Water B: Acetonitrile C: 50:50 THF:water D: Methanol					
Column Temperature:	40 °C					
Sample Injection Volume:	2 µL					
Gradient:	Time (min)	A%	B%	C%	D%	µL/min
	0.00	67.0	22.0	8.0	3.0	620
	5.00	61.0	28.0	8.0	3.0	620
	9.00	37.0	31.0	14.0	18.0	620
	13.00	35.0	31.0	14.0	20.0	620
	13.10	67.0	22.0	8.0	3.0	620

Results and Discussion

Separation of Carbonyl-DNPH Standards

The most well-established approach for the analysis of carbonyls in environmental samples relies on derivatization with 2,4 dinitrophenylhydrazine followed by separation and detection of the carbonyl-DNPH derivatives using HPLC and UV absorption. HPLC methods using conventional C18 columns packed with 3 and 5 µm particles typically require long analysis times of up to an hour and have limited resolving power. The use of sub-2 µm particle columns facilitates rapid analysis of challenging samples by improving chromatographic resolution, speed and sensitivity. Using the Accela 1250 UHPLC system, a single Hypersil GOLD™ column (1.9 µm, 2.1 × 100 mm) and a simple acetonitrile/water gradient, a mixture of the DNPH standards of 12 carbonyls targeted by EPA Method 8315A Procedure 1 was successfully separated and detected under 8 minutes (Figure 1). All the DNPH derivatives were baseline resolved and eluted in order of increasing hydrophobicity: formaldehyde, acetaldehyde, propanal, crotonaldehyde, butanal, cyclohexanone, pentanal, hexanal, heptanal, octanal, nonanal and decanal. This analysis was performed using a flow rate of 800 µL/minute, which generated back pressures up to over 1000 bar. The Accela 1250 pump is the only commercially available LC platform that is capable of handling such high operational pressures due to its very low internal back pressures.

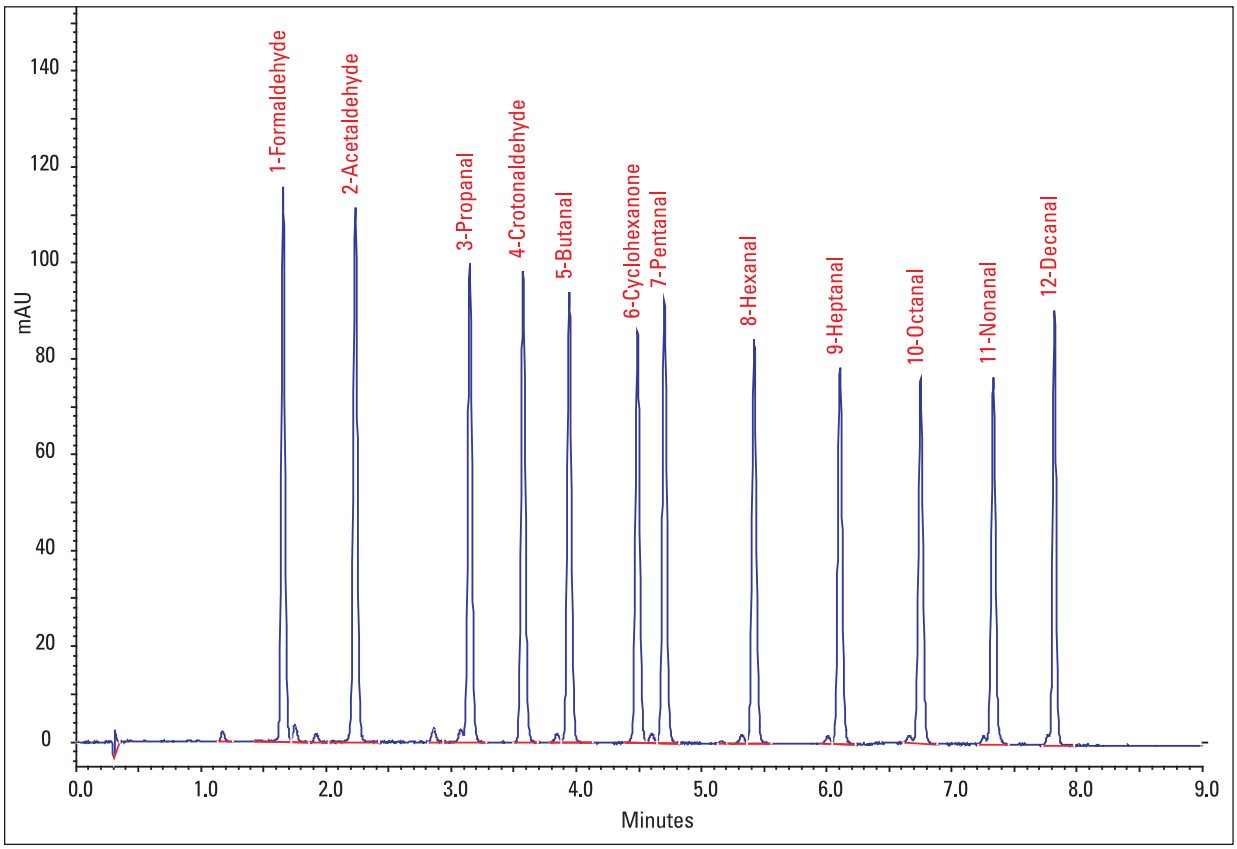

Figure 1: UHPLC separation of 12 carbonyl-DNPH derivatives at 20 µg/mL concentrations

The group of 15 carbonyls targeted by EPA Method 8315A Procedure 2 is difficult to separate and resolve by LC using conventional C18 columns and simple binary gradient systems. Higher resolution may be achieved with more complex gradients, and columns with phenyl functionalities may also help to enhance retention and improve resolution when separating difficult or complex mixtures of aromatic compounds. Both conventional and sub-2 µm columns were evaluated for the separation of the carbonyl derivatives. Figure 2a shows the gradient separation of a standard mixture of 15 carbonyl-DNPH derivatives using a HALO Phenyl-Hexyl column (2.7 µm, 2.1 × 100 mm) at 40 °C. Two organic mobile phases and THF were used, and flow rates were in the range of 380-450 µL/min. The carbonyl derivatives separated in about 8 minutes, with an elution order of formaldehyde, acetaldehyde, acetone, acrolein, propanal, crotonaldehyde, butanal, benzaldehyde, isovaleraldehyde, pentanal, o-tolualdehyde, p-tolualdehyde, m-tolualdehyde, hexanal, and 2,5-dimethylbenzaldehyde. Benzaldehyde and isovaleraldehyde were not baseline separated and the tolualdehyde isomers were not well resolved with this column. Figure 2b shows the separation of these compounds using a Waters ACQUITY BEH Phenyl column (1.7 µm, 2.1 × 100 mm) and a gradient with flow rates adjusted for UHPLC (Figure 2b). Comparable separation power was observed with the HALO Phenyl-Hexyl column and the Waters BEH Phenyl column.

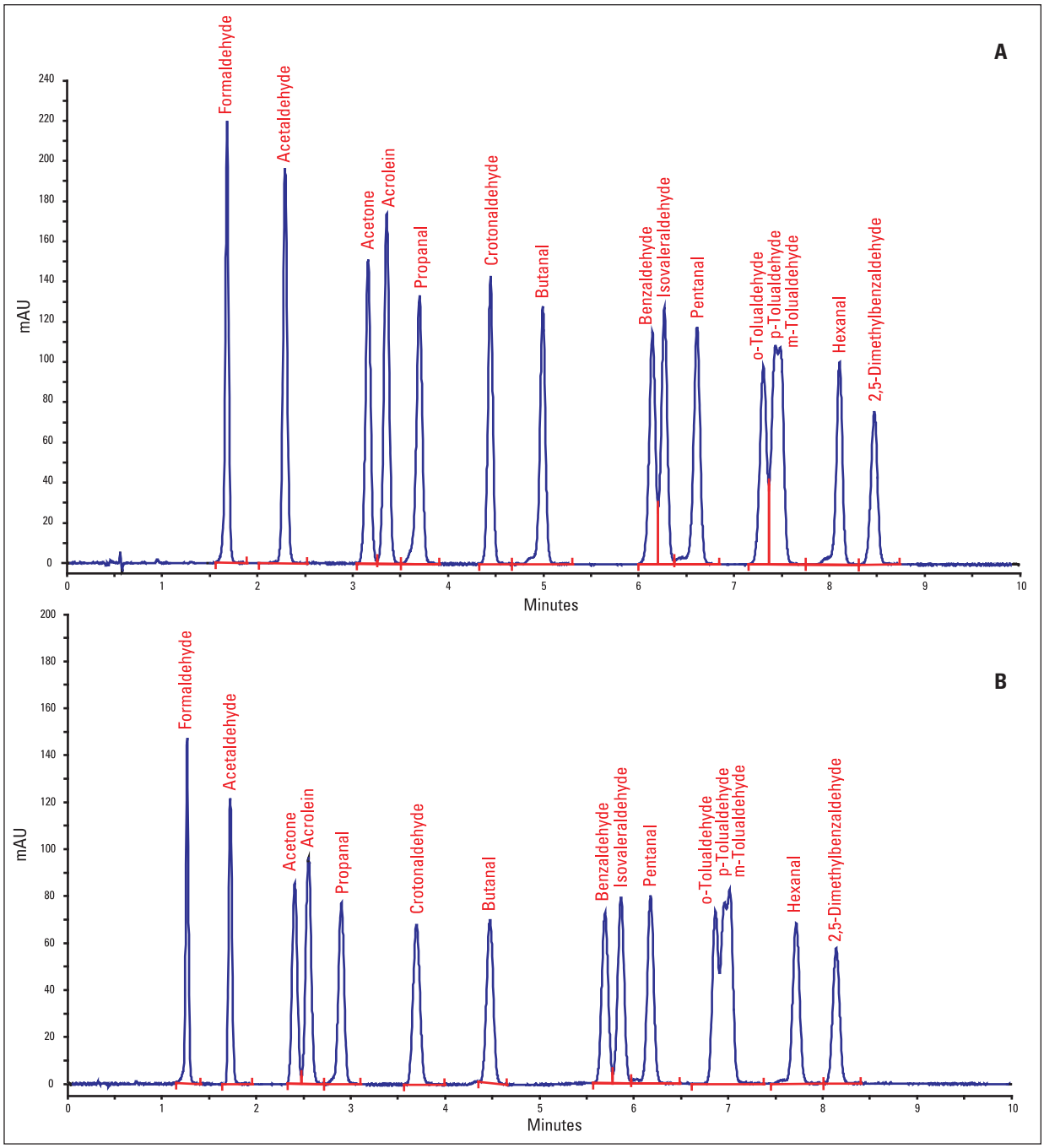

Figure 2: Separation of 15 carbonyl-DNPH derivatives at 20 µg/mL concentrations using a (A) Phenyl-Hexyl column and a (B) BEH Phenyl column

The Hypersil GOLD (1.9 µm, 2.1 × 100 mm) and Waters BEH C18 (1.7 µm, 2.1 × 100 mm) columns were also evaluated. The Hypersil GOLD column exhibited resolving power and efficiencies that were comparable to the phenyl-functionalized columns (Figure 3a), while the Waters BEH C18 column (1.7 µm, 2.1 × 100 mm) was less efficient in separating the more hydrophobic analytes (Figure 3b). The Hypersil GOLD column was selected for quantitative carbonyl analysis.

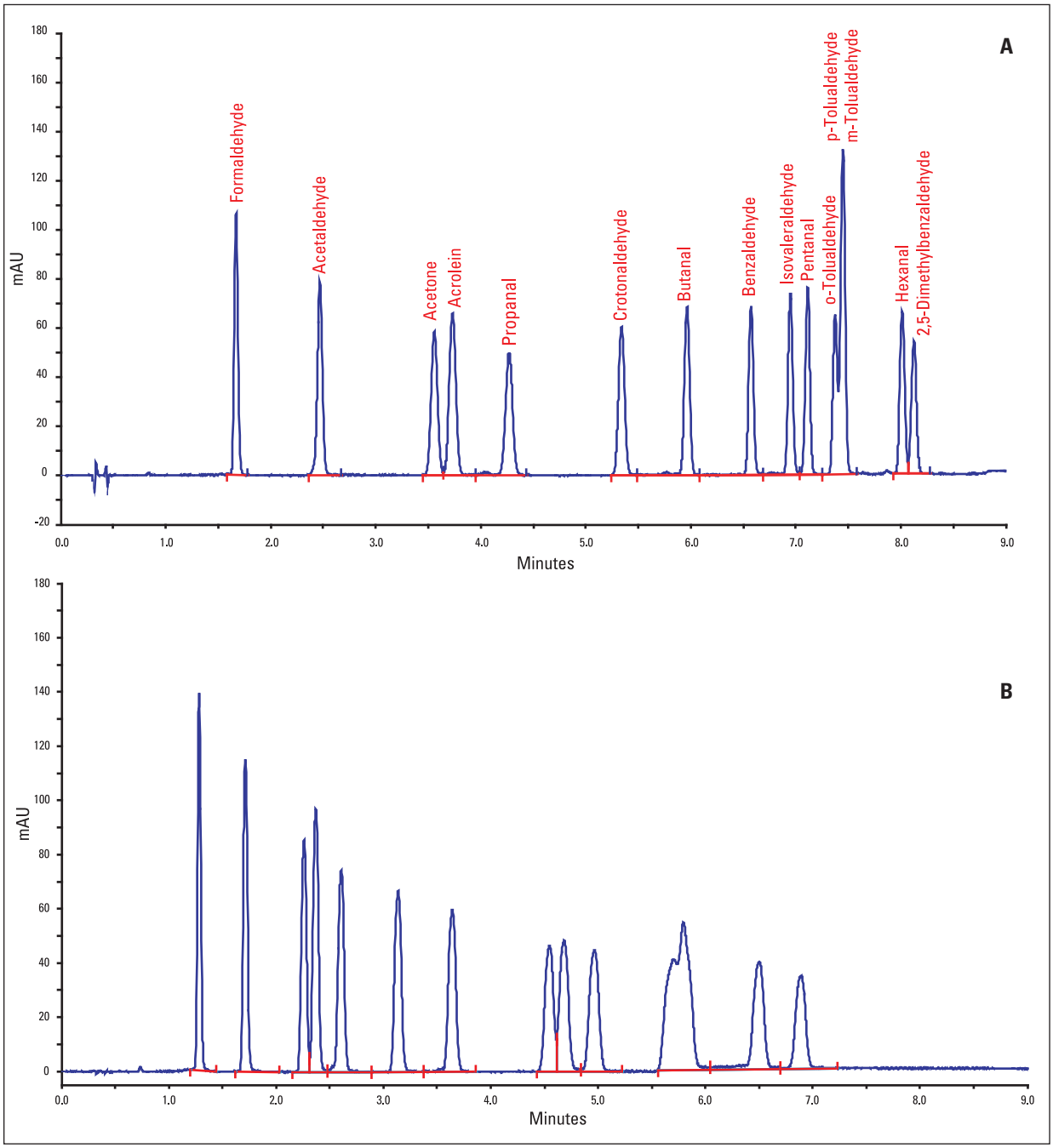

Figure 3: Separation of 15 carbonyl-DNPH derivatives at 20 µg/mL concentrations using a (A) Hypersil GOLD column and a (B) Waters ACQUITY BEH C18 column

Quantitative Analysis

Linearity and Sensitivity

Figure 4 demonstrates UHPLC separation of the 15 carbonyl-DNPH derivatives using the Hypersil GOLD column and a 13-minute gradient. All compounds but the tolualdehyde isomers were baseline resolved. While m-tolualdehyde and p-tolualdehyde co-elute, partial resolution of the o-tolualdehyde peak was achieved under these chromatographic conditions. The 13-minute gradient enabled better separation of the acetone and acrolein peaks as well as the hexanal and 2,5-dimethylbenzaldehyde peaks compared to the 8-minute gradient. This method was used for quantitative analysis of the carbonyl-DNPH standards.

The m- and p-tolualdehyde-DNPH derivatives were quantified together since they co-elute. Excellent linearity in detector response was observed over the range of 98-50000 ng/mL (ppb) (196-100000 ng/mL (ppb) for m- and p-tolualdehyde combined), with correlation coefficients greater than 0.999 for all analytes. Representative calibration curves are shown in Figure 5.

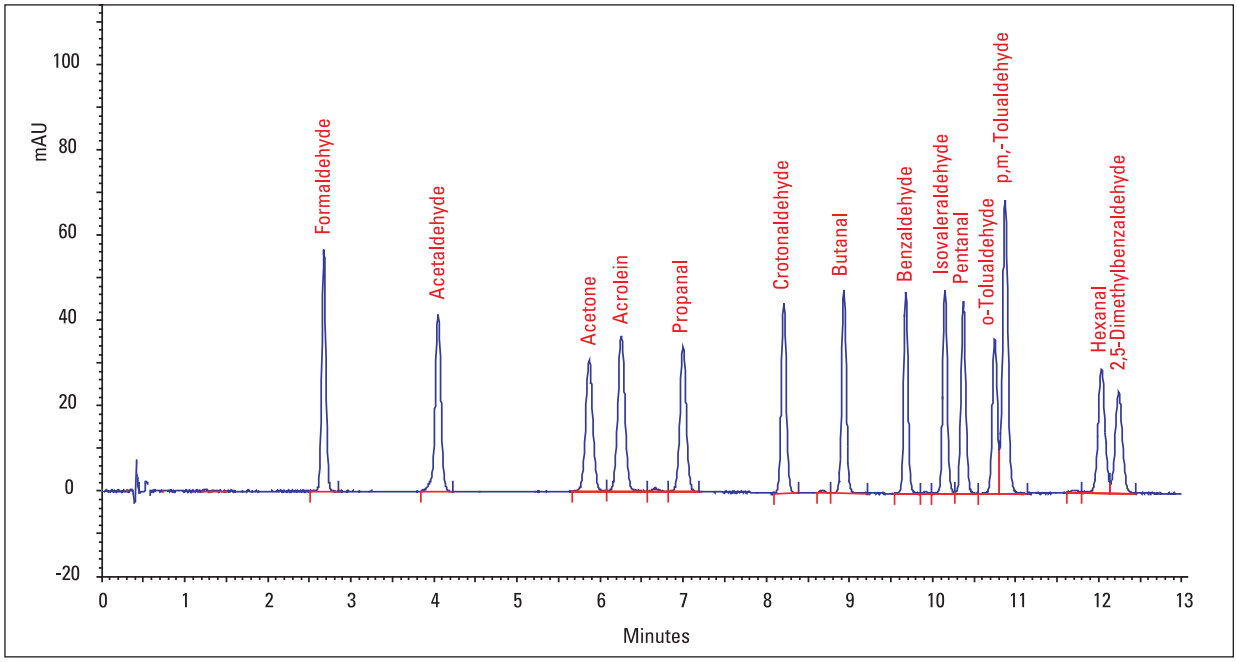

Figure 4: UHPLC separation of 15 carbonyl-DNPH derivatives at 20 µg/mL concentrations using a Hypersil GOLD column and 13-minute gradient

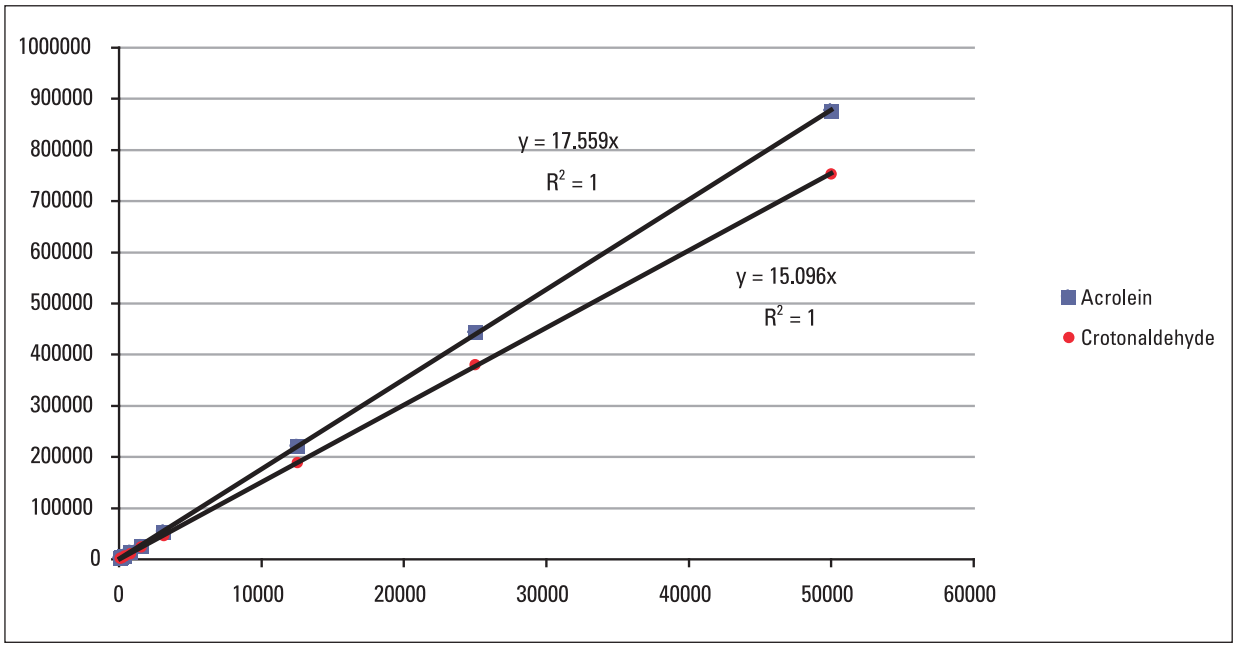

Figure 5: Representative calibration curves of acrolein-DNPH and crotonaldehyde-DNPH standards

Limits of detection (LODs) and limits of quantitation (LODs), defined as S/N ratio of 3 and 10, respectively, are shown in Table 1. LODs ranged from 33.9 to 104.5 ng/mL (ppb), and LOQs ranged from 181.2 to 396.8 ng/mL (ppb). These LODs would be sufficient for the detection of carbonyls in complex real-world samples since they are typically enriched 200-fold prior to analysis.

Reproducibility and Accuracy

Reproducibility was investigated by analyzing five replicate injections of each analyte. With four channel mixings of the solvents at various viscosities, retention time RSDs ranged from 0.52-2.22% while peak area RSDs ranged from 0.46-4.91% (Table 1), indicating excellent method reproducibility, particularly of the LC pump.

Quantitative accuracy for all carbonyl-DNPH derivatives were evaluated at two levels of concentrations, 400 ppb and 2000 ppb, using external calibration method. The accuracy of two representative analytes, benzaldehyde-DNPH and o-tolualdehyde-DNPH, were given in Table 2. The values of 96.3% and 103.6% at 400 ppb, respectively, and 99.8% and 99.9% at 2000 ppb, respectively were achieved with the UHPLC method.

Compound	LOD	LOQ	RT %RSD	Area %RSD	Linear Dynamic Range ng/mL
Formaldehyde	34.9	181.2	0.59	0.60	98-50000
Acetaldehyde	56.8	236.4	0.99	0.50	98-50000
Acetone	101.1	319.4	0.77	0.76	98-50000
Acrolein	82.1	266.7	1.53	0.64	98-50000
Propanal	85.2	281.2	2.22	0.59	98-50000
Crotonaldehyde	53.3	218.5	1.37	0.46	98-50000
Butanal	45.5	200.1	0.71	1.24	98-50000
Benzaldehyde	66.3	219.9	0.54	4.91	98-50000
Isovaleraldehyde	44.1	196.4	0.52	2.87	98-50000
Pentanal	33.9	198.2	0.60	2.65	98-50000
o-Tolualdehyde	104.5	321.1	0.75	1.99	98-50000
p,m,-Tolualdehyde	54.6	271.6	0.77	1.33	196-100000
Hexanal	62.9	318.9	0.84	0.66	98-50000
2,5-Dimethylbenzaldehyde	84.7	396.8	1.26	1.29	98-50000

Table 1: Quantitation data for 15 carbonyl-DNPH standards

Compound	400 ppb	% Accuracy	20000 ppb	% Accuracy
Benzaldehyde	385.3	96.3	1996	99.8
o-Tolualdehyde	414.4	103.6	1997	99.9

Table 2: Accuracy data for two carbonyl-DNPH standards

Conclusion

- The Accela 1250 UHPLC system offers the flexibility of performing both HPLC and UHPLC separations. With its very low internal back pressure, this system is capable of operational pressures up to 1250 bar, which is significantly higher compared to other commercial UHPLC systems.
- The Accela 1250 UHPLC system coupled with sub-2 µm Hypersil GOLD columns enabled highly efficient and reproducible separations of carbonyl-DNPH derivatives.
- Fast, accurate and robust quantitative analysis of low molecular weight carbonyls at ppb levels was achieved using Accela 1250 UHPLC followed by UV detection.
- UHPLC significantly improves resolution and speed of analysis and provides a powerful alternative to the HPLC-based procedures currently recommended by regulatory agencies for environmental monitoring of carbonyls.

References

1. http://www.epa.gov/ttn/atw/orig189.html
2. http://www.epa.gov/airtoxics/hlthef/hapindex.html
3. http://www.epa.gov/ttnatw01/hlthef/formalde.html#ref1
4. http://monographs.iarc.fr/ENG/Classification/index.php
5. http://www.epa.gov/ttnatw01/hlthef/acetalde.html
6. Technical Assistance Document for Sampling and Analysis of Ozone Precursors, EPA/600-R-98/161,1998.
7. http://www.arb.ca.gov/aaqm/toxics.htm
8. W. Liu et al. Estimating contributions of indoor and outdoor sources to indoor carbonyl concentrations in three urban areas. Atmos Environ. 2006; 40:2202-2214.
9. Formaldehyde OSHA Fact Sheet, 2002.
10. http://www.oehha.ca.gov/air/allrels.html
11. Formaldehyde. In Air Quality Guidelines, 2nd Edition, WHO Regional Office for Europe, 2001.
12. WHO Expert Committee on Problems related to Alcohol consumption, WHO Technical Report Series 944, World Health Organization, 2007.
13. Determination of Formaldehyde in Ambient Air Using Adsorbent Cartridge Followed by High Performance Liquid Chromatography (HPLC), EPA Method TO-11A, EPA/625/R-96/010b, 1999.
14. Determination of Carbonyl Compounds by High Performance Liquid Chromatography, EPA Method 8315A, 1996.
15. Determination of Carbonyl Compounds in Drinking Water by Dinitrophenylhydrazine Derivatization and High-Performance Liquid Chromatography (HPLC), EPA Method 554, 1992.
16. Determination of Aldehyde and Ketone Compounds in Automotive Source Samples by High Performance Liquid Chromatography (Method No. 1004). In California Non-Methane Organic Gas Test Procedures, California Environmental Protection Agency Air Resources Board, 2002.

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