Quantitative Analysis of Carbonyl-DNPH Derivatives by UHPLC/UV

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Abstract

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. 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 2,4-dinitrophenylhydrazones (DNPH)derivatives prior to analysis. EPA methods for the determination of carbonyls in drinking water (EPA 554),waste and stack samples (EPA 8315[a] procedure 1) and ambient indoor air (EPA 8315A Procedure 2), utilize DNPH derivatization and HPLC/UV analysis. 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.

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. 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.

Experimental

Materials and Methods

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

Separation of carbonyls listed in EPA Method 8315A Procedure 1

Column: Hypersil GOLD™ C18 column (2.1 x 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
9.10	68.0	32.0	800

Separation of carbonyls listed in EPA Method 8315A Procedure 2

Column: Hypersil GOLD C18 (2.1 x 100 mm, 1.9 µm particle size)
BEH C18 (2.1 x 100 mm, 1.7 µm particle size)
Mobile Phase: A: Water
B: Acetonitrile
C: 50:50 THF:water
D: Methanol

Gradient: D%	Time (min) µL/min	A%	B%	C%
	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: Hypersil GOLD C18 (2.1 × 100 mm, 1.9 µm particle size)
Mobile Phase: A: Water
B: Acetonitrile
C: 50:50 THF:water
D: Methanol

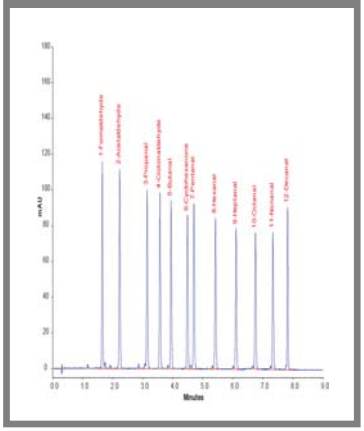
Gradient: D%	Time (min) µL/min	A%	B%	C%
	0.00	67.0	22.0	8.0
3.0	620			
	5.00	61.0	28.0	8.0
3.0	620			
	9.00	37.0	31.0	14.0
18.0	620			
	13.00	35.0	31.0	14.0
20.0	620			
	13.10	67.0	22.0	8.0
3.0	620			

Results and Discussion

Separation of Carbonyl-DNPH Standards

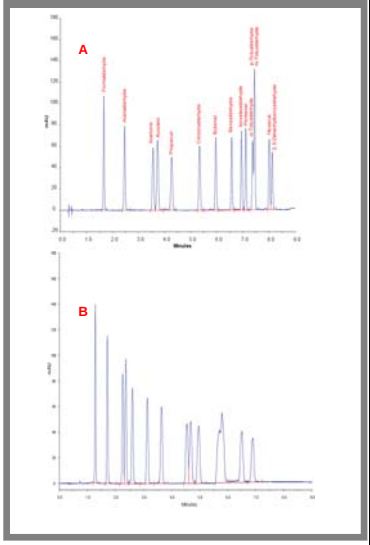
The most well-established approach for the analysis of carbonyls in environmental samples relies on derivatization with 2,4 dinitrophenylhydrazine followed by separation and detection of the carbonyl-DNPH derivatives using HPLC and UV absorption. HPLC methods using conventional C18 columns packed with 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 C18 column (1.9 µm, 2.1 × 100mm) 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. This analysis was performed using a flow rate of 800 µL/minute, which generated back pressures up to over 1000 bar.

FIGURE 1. UHPLC separation of 12 carbonyl-DNPH derivatives at 20 µg/mL concentration



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. Hypersil GOLD C18 and BEH C18 sub-2 µm columns were evaluated for the separation of the carbonyl derivatives. Figure 2 shows the gradient separation of a standard mixture of 15 carbonyl-DNPH derivatives. The carbonyl derivatives separated in about 8 minutes. The Hypersil GOLD C18 column exhibited resolving power and efficiencies that were comparable to the BEH C18 column (Figure 2b). The Thermo Hypersil GOLD C18 column was selected for quantitative carbonyl analysis

FIGURE 2. Separation of 15 carbonyl-DNPH derivatives at 20 µg/mK concentration using a (A) Hypersil GOLD C18 column and a (B) BEH C18 column



Quantitative Analysis

Linearity and Sensitivity

Figure 3 demonstrates UHPLC separation of the 15 carbonyl- DNPH derivatives using the Hypersil GOLD C18 column and a 13-minute gradient. All compounds but the tolualdehyde isomers were baseline resolved. While mtolualdehyde 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 4. 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.

FIGURE 3. UHPLC Separation of carbonyle-DNPH derivatives with 13-minute gradient for quantitation

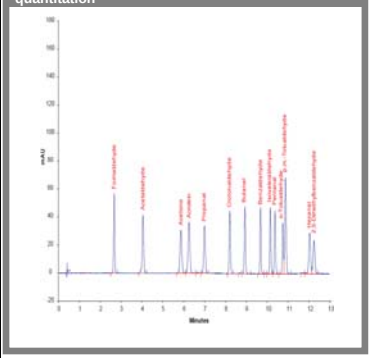
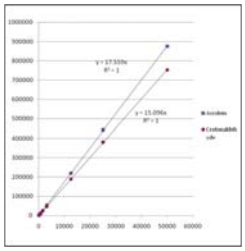


Figure 4. Representative calibration curves of acrolein-DNPH and crotonaldehyde-DNPH standards



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.

Table 1. Quantitation data for 15 carbonyl-DNPH standards

Compound	LOD	LOQ	RT % RSD	Area %RSD	Linear Dynamic Range ng/mL
Formaldehyde	34.9	181.2	0.59	0.6	98-50000
Acetaldehyde	56.8	236.4	0.99	0.5	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	45.4	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 2. Accuracy data for two carbonyl-DNPH standards

Compund	400 ppb	% Accuracy	2000ppb	% Accuracy
Benzaldehyde	385.3	96.3	1995.5	99.8
o-Tolualdehyde	414.4	103.6	1997.0	99.9

Conclusions

The Accela 1250 UHPLC system 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-2µm 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.

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