

# Method Development for Analysis of Carbonyl-DNPH Derivatives by Ultra High Performance Liquid Chromatography (UHPLC)

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Key Words: Environmental analysis, Ultra High Performance Liquid Chromatography, UHPLC, sub-2  $\mu\text{m}$  particles

## Introduction

Volatile organic carbonyl compounds from motor vehicle and industrial emissions are precursors to ground-level ozone, a major component of smog and strongly associated with pulmonary problems. Carbonyls are also sources of pollution in indoor environments (1).

An effective pollution control strategy requires sensitive and accurate methods for trace-level analysis of carbonyl compounds. While GC-based methods have been developed to detect these compounds, HPLC-UV is the most widely recognized technique for the analysis of carbonyl-2,4-dinitrophenylhydrazine (DNPH) derivatives (2, 3). However, long run times and poor resolution can limit conventional HPLC in this application.

This work presents an Ultra High Performance Liquid Chromatography (UHPLC) method that enables faster separations and higher resolution through the use of sub-2  $\mu\text{m}$  diameter particles.

## Results and Discussion

Calibration solutions of DNPH-derivatized carbonyl standards, with concentrations between 98 and 50000 ng/mL, were prepared by serial dilution in 60:40 (v/v) acetonitrile:water.

LC separations were done on a LC system that performs both HPLC and UHPLC separations. This LC system is composed of a quaternary pump that can withstand operational pressures up to 1250 bar, and an autosampler. UV absorbance was monitored at 360 nm using an 80 Hz PDA detector.

Both conventional (3-5  $\mu\text{m}$ ) and sub-2  $\mu\text{m}$  columns were evaluated for the separation of the 15 carbonyls targeted by EPA Method 8315A (3). Columns with phenyl functionalities were also evaluated. A C18 column (1.9  $\mu\text{m}$ , 2.1 x100 mm) exhibited resolving power and efficiencies comparable to the phenyl-functionalized columns and was selected for quantitative carbonyl analysis.

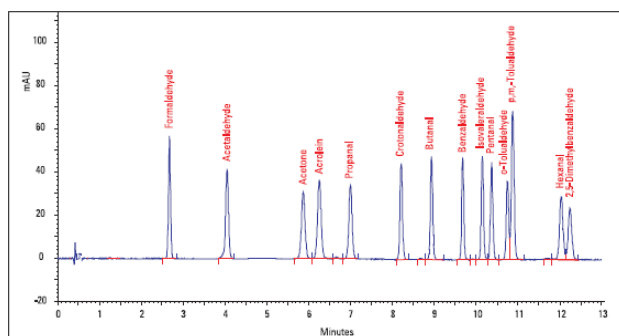
A 13 min gradient of 4 solvents (water, acetonitrile, 50:50 THF:water and methanol) was adopted. The column temperature was 40 °C and the sample injection volume was 2  $\mu\text{L}$ . All compounds but the tolaldehyde isomers were baseline resolved (Figure 1). The m- and p-tolualdehyde-DNPH derivatives were quantified together since they co-elute while

partial resolution of the o-tolualdehyde peak was achieved.

The linearity over the range of 98-50000 ng/mL presented correlation coefficients > 0.999 for all analytes. LODs ranged from 33.9 to 104.5 ng/mL and LOQs from 181.2 to 396.8 ng/mL.

Reproducibility was investigated by analyzing five replicate injections of each analyte. The retention time RSDs ranged from 0.52-2.22% while peak area RSDs ranged from 0.46-4.91%.

Quantitative accuracy for all carbonyl-DNPH derivatives were evaluated at two levels of concentrations, 400 ng/mL and 2000 ng/mL, using external calibration method. The accuracy of two representative analytes, benzaldehyde-DNPH and o-tolualdehyde-DNPH, were 96.3% and 103.6% at 400 ng/mL, respectively, and 99.8% and 99.9% at 2000 ng/mL.



**Figure 1.** UHPLC separation of 15 carbonyl-DNPH derivatives at 20  $\mu\text{g/mL}$  concentrations using a C18 column and 13-minute gradient.

## Conclusions

Fast, accurate and robust quantitative analysis of low molecular weight carbonyls at ng/mL levels was achieved.

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.

<sup>1</sup> Liu, W. et al. *Atmos Environ* **2006**, 40, 2202.

<sup>2</sup> Determination of Formaldehyde in Ambient Air Using Adsorbent Cartridge Followed by HPLC, EPA Method TO-11A, EPA/625/R-96/010b, 1999.

<sup>3</sup> Determination of Carbonyl Compounds by HPLC, EPA Method 8315A, 1996.