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## Automated Online Desorption and Analysis of DNPH Derivatives of Airborne Aldehydes and Ketones

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### ABSTRACT

The analysis of airborne aldehydes and ketones first involves collection of the analytes by passing air through a cartridge containing 2,4-dinitrophenylhydrazine (DNPH). As the air passes through the cartridge, the analytes react with the DNPH to form hydrazones which are immobilized on the cartridge. The cartridges are then eluted with solvent and the DNPH derivatives determined using HPLC with UV detection.

The GERSTEL MultiPurpose Sampler (MPS) can be configured with a special tray to hold DNPH cartridges, enabling efficient automation of the entire process of desorbing the analytes and injecting the eluate into the LC-UV system. Automating the desorption of these cartridges can significantly improve both accuracy and reproducibility. In addition, the risk of operator error is reduced. The MAESTRO software PrepAhead function enables desorption of a cartridge during the chromatographic separation of the previously injected sample. This means that the next sample is always prepared and ready to be injected as soon as the HPLC system has completed the previous analysis, ensuring maximum sample throughput.

In this report, the automation of the online desorption and analysis of DNPH derivatives by the robotic autosampler is discussed. Examination and calibration for a variety of aldehyde- and ketone-DNPH derivatives is described. Finally, DNPH cartridges used to collect samples from representative matrices are desorbed and analyzed online and the resulting data provided.

## INTRODUCTION

Aldehydes and ketones are widely used in chemical industry. Formaldehyde, for example, is used for the production of particleboard, glued wood and synthetic resins. Acetaldehyde, as well as many other aldehydes and ketones, are used as organic solvents or as intermediates for the production of a variety of products across a wide range of industries including building materials used indoors.

It has long been acknowledged that the presence of volatile aldehydes and ketones in indoor air can pose a health problem and a variety of standardized methods for the determination of formaldehyde and other carbonyl compounds in air are available. Among these are ASTM Test Method D 5197, US EPA Method To 11a, and US-EPA method IP-6A, which are all based on derivatization with 2,4-dinitrophenylhydrazine, yielding the corresponding 2,4-dinitrophenylhydrazone, followed by solvent elution and HPLC-UV determination.

The collection of air samples can be easily achieved by connecting a Supelco LpDNPH S10 cartridge to the inlet of the hand-held GERSTEL GSS sampler. By doing so, air can be sampled at a user defined flow rate for a user defined time and the carbonyl compounds collected and immobilized as DNPH derivatives on the cartridge. Automating both the desorption of the formed DNPH derivatives directly from these cartridges and their subsequent determination by HPLC-UV has been shown to enable reliable high throughput analysis of environmental air samples<sup>1</sup>.

## EXPERIMENTAL

**Materials.** All calibration stock solutions for the compounds listed in Table 1 were purchased from Cerilliant. All other reagents and solvents used were reagent grade.

The plywood used to construct a test chamber was Southern Pine Oriented Strand Board (7/16 inch thickness) purchased from a local hardware and lumber store. The interior air of a 2015 model mid-sized car was also examined.

The cartridges used for air sampling with the GERSTEL GSS sampler were 3 mL, 350 mg, LpDNPH S10 (Supelco p/n: 21026-U). Prior to their desorption, transport adapters were connected to the top of the cartridges to provide the required seal for proper delivery of the solvent through the cartridge (GERSTEL, p/n: 015575-103-00). In addition, 13 mm, 0.2  $\mu$ m, nylon syringe filters (Sigma-Aldrich p/n: Z254492-1PAK) were affixed to the bottom of the cartridge to ensure that the resulting eluent was free from any particles before being introduced into the HPLC system.

**Instrumentation.** Automated desorption was performed using a dual-head GERSTEL MultiPurpose Sampler (MPS) configured with an A300 Automated DNPH unit from Anatune as shown in Figure 1. All analyses were performed using an Agilent 1290 binary pump and thermostated column compartment, an Agilent 1260 VWD, and a Sigma-Aldrich Ascentis Express C18 column (4.6 x 50 mm, 2.7  $\mu$ m). An Agilent 6460 Triple Quadrupole Mass Spectrometer with Jet stream electrospray source was also configured in order to provide further identification of the DNPH-derivatives being monitored. Sample injections were performed using the GERSTEL MPS autosampler configured with an Active Washstation and a 6 port (0.25 mm) Cheminert C2V injection valve outfitted with a 10  $\mu$ L stainless steel sample loop.



**Figure 1.** Dual-head MultiPurpose Sampler (MPS) configured with the A300 Automated DNPH unit.

*Air Sampling.* A single DNPH cartridge was attached to the GERSTEL GSS hand-held sampler and the interior air of a constructed plywood box (2 ft. x 2 ft. x 2 ft., 7/16 inch OSB) was sampled for 1 hour at 100 mL/min. This sampling was repeated using three separate DNPH cartridges.

Immediately after sampling, all DNPH cartridges were capped with their respective end caps and stored in individual zip-sealed bags in a refrigerator at 4°C until they were desorbed and analyzed. Similar air sampling processes were performed for an extended time period (4 hours) in the plywood chamber as well as during the sampling of the interior air of a car (3 hours).

Figure 2 shows the GSS hand-held sampler with a DNPH cartridge inside the plywood test chamber.



**Figure 2.** GERSTEL GSS hand-held sampler with a DNPH cartridge inside the plywood test chamber.

*Liquid Desorption.* Following sampling, the transport adapters and syringe filters were affixed to the cartridges and the cartridges were then placed into the A300 Automated DNPH unit. Automated desorption was set up and controlled using the MAESTRO software and included the following:

1. Add 5.6 mL of 100 % acetonitrile using a 2.5 mL SPE syringe (in order to collect 5 mL of eluate).
2. Add 5 mL of air to cartridge for positive displacement.
3. Transfer 500 µL of the filtered eluate into a clean, empty, capped 2 mL autosampler vial with septum.
4. Inject 10 µL of the filtered eluate into the HPLC injection valve.

*Automated DNPH HPLC Method Parameters*

Mobile Phase:	A - Water	
	B - Acetonitrile	
Gradient:	Initial	45 % B
	15 min	60 % B
	15.1 min	45 % B
Pressure:	600 bar	
Flowrate:	1.2 mL/min	
UV Wavelength:	365 nm	
Run time:	17.5 min	
Injection volume:	10 µL (loop over-fill technique)	
Column Temp.:	30°C	

*MS2 SIM Mass Spectrometer Parameters (not required; only used for identification assistance):*

Electrospray positive mode + Agilent Jet Stream	
Gas Temperature:	350°C
Gas Flow (N <sub>2</sub> ):	5 L/min
Nebulizer pressure:	35 psi
Sheath Gas Temp:	250°C
Sheath Gas Flow:	11 L/min
Capillary voltage:	4000 V
Nozzle voltage:	500 V

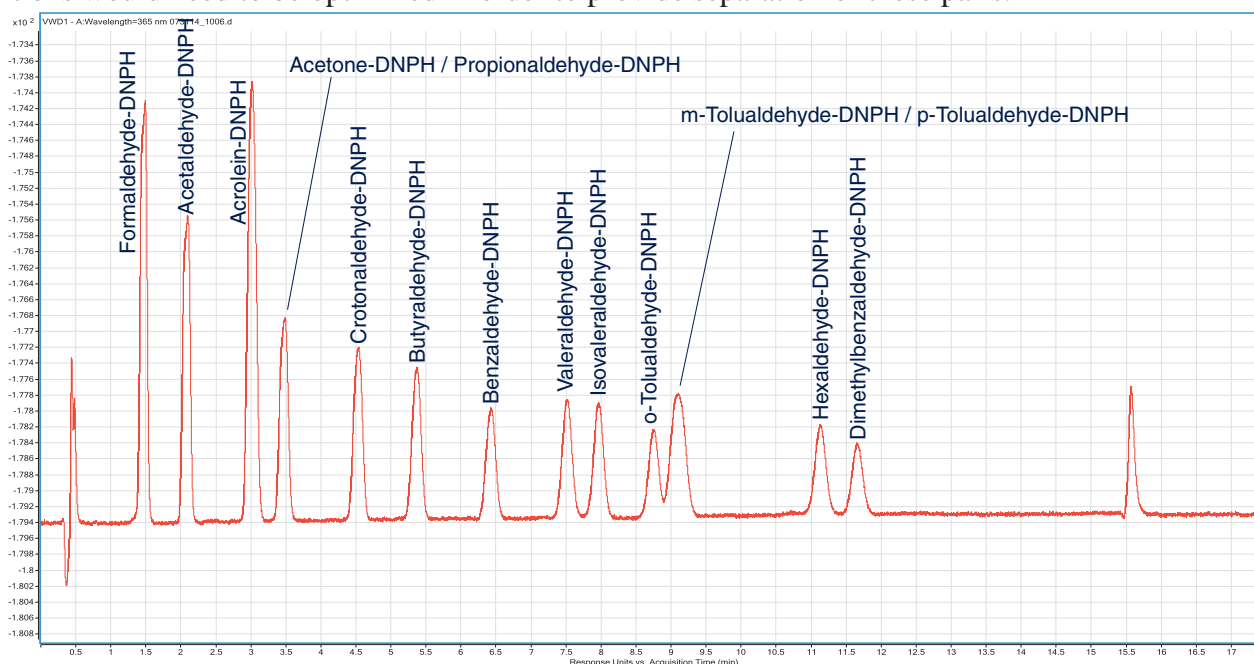
## RESULTS AND DISCUSSION

Table 1 lists the aldehyde and ketone DNPH-derivatives calibration standards with their corresponding SIM m/z's monitored for additional identification and the concentration of each calibration standard analyzed. Detailed mass spectrometric acquisition parameters are available upon request, however, the typical analysis for DNPH derivatives only requires the use of HPLC-UV. Therefore, the results from analysis of the air samples are based on the peak area responses obtained from the UV chromatograms for the samples.

**Table 1.** Calibration standards for DNPH-derivatives analyzed.

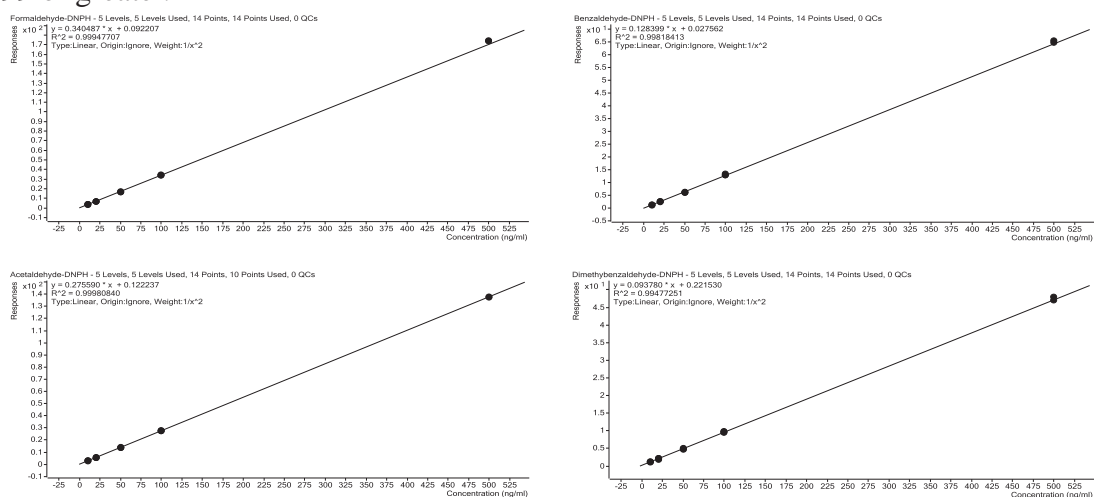
Analyte	MS2 SIM [m/z]	Concentration				
		C1 [µg/mL]	C2 [µg/mL]	C3 [µg/mL]	C4 [µg/mL]	C5 [µg/mL]
Acetaldehyde-DNPH	223	0.010	0.020	0.050	0.100	0.500
Acetone-DNPH	237	0.010	0.020	0.050	0.100	0.500
Acrolein-DNPH	235	0.010	0.020	0.050	0.100	0.500
Benzaldehyde-DNPH	285	0.010	0.020	0.050	0.100	0.500
n-Butyraldehyde-DNPH	251	0.010	0.020	0.050	0.100	0.500
Crotonaldehyde-DNPH	249	0.010	0.020	0.050	0.100	0.500
2,5-Dimethylbenzaldehyde-DNPH	313	0.010	0.020	0.050	0.100	0.500
Formaldehyde-DNPH	209	0.010	0.020	0.050	0.100	0.500
Hexaldehyde-DNPH	279	0.010	0.020	0.050	0.100	0.500
Isovaleraldehyde-DNPH	265	0.010	0.020	0.050	0.100	0.500
Propionaldehyde-DNPH	237	0.010	0.020	0.050	0.100	0.500
m-Tolualdehyde-DNPH	299	0.010	0.020	0.050	0.100	0.500
o-Tolualdehyde-DNPH	299	0.010	0.020	0.050	0.100	0.500
p-Tolualdehyde-DNPH	299	0.010	0.020	0.050	0.100	0.500
Valeraldehyde-DNPH	265	0.010	0.020	0.050	0.100	0.500

Figure 3 shows a representative chromatogram from the HPLC-UV analysis of a high calibration standard with the corresponding DNPH-derivatives labeled. As shown, the acetone- and propionaldehyde-DNPH derivatives were shown to co-elute given the chromatographic conditions used. Co-elution was also observed for the m- and p-tolualdehyde-DNPH derivatives. For methods in which these analytes are important, chromatographic conditions would need to be optimized in order to provide separation of these pairs.



**Figure 3.** Representative LC-UV chromatogram of DNPH-derivative calibration standard.

Representative calibration curves for formaldehyde-DNPH, benzaldehyde-DNPH, acetaldehyde-DNPH, and 2,5-dimethylbenzaldehyde-DNPH are shown in Figure 4. Regression analysis for all analytes resulted in R<sup>2</sup> values of 0.99 or greater.



**Figure 4.** Representative Calibration Curves from DNPH-derivative calibration standards.

Table 2 shows the results obtained following the automated desorption and HPLC analysis of the 3 hour car sample, the 1 hour plywood box samples and the 4 hour plywood box sample. Formaldehyde was shown to be the most prevalent analyte observed throughout all test samples. For the 3 hour car sample, a concentration of 125 ng/L of air (m/v) was found. Conversion to (v/v) for this value, results in a concentration of 195 ppbV, assuming 298oC and 1 atm. Three replicate 1-hour samples were taken sequentially from a single box. The first sample was taken after the box had equilibrated for 3 days, while the second and third samples were collected immediately after the preceding sample without allowing time for equilibration. Following the collection of the last 1-hour replicate, an additional sample was collected for a period of 4 hours. Comparison of the qualitative plywood box results (1 hour vs. 4 hour) shows that the amount of analyte collected on the DNPH cartridges over a given period of time gradually decreases throughout this time-course sampling examination. This seems reasonable given that the air within the plywood box chamber was continually being replaced with uncontaminated laboratory air during the series of sampling experiments performed.

**Table 2.** Results from Automated Desorption and HPLC analysis of Air samples collected using LpDNPH cartridges and GSS hand-held sampler.

Analyte	MW of Carbonyl	MW of Carbonyl Adduct	Ret. Time	Found in 3 hr Car Sampling Sample?	Calc. Conc. of Carbonyl Found	Found in 1 hr Box Sampling Sample?	Calc. Conc. of Carbonyl Found			Found in 4 hr Box Sampling Sample?	Calc. Conc. of Carbonyl Found
	[m/z]	[m/z]					[min]	[ng/L air]	1 hr Rep 1		
Acetaldehyde-DNPH	44.05	224.17	2.00	Yes	5.33	Yes	21.2	12.3	12.6	Yes	8.64
Acetone-DNPH	58.08	238.20	3.50	Yes	1.58	Yes	2.52	1.70	1.57	Yes	1.46
Acrolein-DNPH	56.06	236.18	3.00	Yes	4.27	Yes	40.3	27.8	38.9	Yes	16.2
Benzaldehyde-DNPH	106.12	286.24	6.50	Yes	2.48	Yes	9.17	5.82	5.68	Yes	2.86
Butyraldehyde-DNPH	72.11	252.23	5.30	Yes	0.826	Yes	3.41	2.05	2.03	Yes	1.49
Crotonaldehyde-DNPH	70.09	250.21	4.50	No	-	No	-	-	-	Yes	0.584
Dimethylbenzaldehyde-DNPH	134.18	314.30	11.9	No	-	No	-	-	-	No	-
Formaldehyde-DNPH	30.03	210.15	1.50	Yes	17.9	Yes	14.7	17.1	15.3	Yes	4.85
Hexaldehyde-DNPH	100.16	280.28	11.3	Yes	1.12	Yes	12.0	7.65	8.17	Yes	5.11
Isovaleraldehyde-DNPH	86.13	266.25	8.00	No	-	Yes	7.06	4.30	4.45	Yes	2.65
Propionaldehyde-DNPH	58.08	238.20	3.50	Yes	1.84	Yes	2.94	2.19	2.20	Yes	1.37
Tolualdehyde-m-DNPH	120.15	300.27	9.30	No	-	No	-	-	-	No	-
Tolualdehyde-o-DNPH	120.15	300.27	8.90	No	-	No	-	-	-	No	-
Tolualdehyde-p-DNPH	120.15	300.27	9.30	No	-	No	-	-	-	No	-
Valeraldehyde-DNPH	86.13	266.25	7.50	No	-	No	-	-	-	No	-

Precision data on DNPH tube collection of aldehydes with manual desorption and analysis is available in the literature [1]. Future studies are planned to collect precision data for the automated desorption of DNPH tubes spiked with known aldehyde standards.

Table 3 shows the reproducibility of the results from the sequential sampling from the plywood box. The first data column (n=3) includes the first sample drawn after long equilibration, whereas the second data column (n=2) shows the results from only the last two replicates collected sequentially since these were not allowed to equilibrate overnight between runs to match the conditions for the first sampling.

**Table 3.** Precision data from 1 hour air sampling of plywood box.

DNPH Derivative	Resulting %CV	
	n=3	n=2
Formaldehyde-DNPH	7.99	7.85
Acetaldehyde-DNPH	32.8	1.71
Acetone-DNPH	26.8	5.84
Acrolein-DNPH	19.3	23.7
Benzaldehyde-DNPH	28.7	1.60
Butyraldehyde-DNPH	31.7	0.630
Crotonaldehyde-DNPH	-	-
Dimethylbenzaldehyde-DNPH	-	-
Hexaldehyde-DNPH	25.4	4.68
Isovaleraldehyde-DNPH	29.5	2.39
Propionaldehyde-DNPH	17.6	0.045
Acetone-DNPH	-	-
Acrolein-DNPH	-	-
Acetaldehyde-DNPH	-	-
Formaldehyde-DNPH	-	-
Average %CV	24.4	5.38

## CONCLUSIONS

As a result of this study, we were able to show:

- Fully automated desorption from DNPH cartridges and HPLC determination of derivatized carbonyl compounds was successfully performed using the dual head GERSTEL MultiPurpose Sampler (MPS DH) combined with an HPLC-UV system.
- The air samples were reliably and reproducibly collected using a hand-held air sampler
- Previously published studies were expanded to include additional aldehyde and ketone DNPH-derivatives from plywood and car interior air samples.
- Linear calibration curves resulting in R2 values of 0.99 or greater were obtained based on injection of the calibration standards.

## REFERENCES

- [1] “Analysis of Formaldehyde and Acetaldehyde using automated DNPH derivatization”, Anatune Chromatography Technical Note No. AS109.





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