GERSTEL Application Note No. **195**, *2017*

Automated Online Desorption and Analysis of DNPH Derivatives of Formaldehyde, Acetaldehyde, and Related Carbonyl Compounds using a New Robotic Autosampler

Fredrick D. Foster, Jacqueline Whitecavage, Kurt Thaxton, John R. Stuff

GERSTEL, Inc., 701 Digital Drive, Suite J, Linthicum, MD, 21090, USA

Keywords

Sample Preparation, Lab Automation, Air Sampling, Environmental Analysis, Material Emissions, Aldehydes, Ketones

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 can be determined using HPLC with UV detection.

The new GERSTEL MPS robotic^{PRO} sampler with a dedicated tray to hold DNPH cartridges enables the entire process of eluting the analytes and injecting the eluate into the LC-UV system to be easily controlled. Automating the elution of these cartridges can result in significant improvement in accuracy and reproducibility as well as reduced potential for operator error. An integrated Balance Option allows weight data to be automatically collected following cartridge elution to further improve the accuracy of the reported results. The intuitive software includes tools that allow the elution of a cartridge to take place during the chromatographic separation of a previously injected sample to ensure maximum sample throughput.

In this report, the complete automation of the online elution, introduction of the eluate to the analysis system, and determination of the DNPH derivatives of the airborne aldehydes and ketones is presented. Performance evaluation and calibration for a variety of aldehyde- and ketone-DNPH derivatives is described. Finally, analytes from air sampled onto DNPH cartridges from representative matrices are eluted and determined online and the resulting precision data presented.

Introduction

Aldehydes and ketones are important compounds in the chemical industry. Formaldehyde is used for the production of glued wood and synthetic resins. Acetaldehyde, as well as many aldehydes and ketones, are used as organic solvents or as intermediates during the production of a variety of products in many industries.

A variety of standardized methods (such as ASTM test method D 5197, EPA method IP-6A, or ISO 16000-03) exist that require air sampling for indoor or outdoor air for the presence of formaldehyde and other carbonyl compounds. The majority of these methods use the derivatization with 2,4-dinitrophenylhydrazine yielding the corresponding 2, 4-dinitrophenyldhyrazone as shown in figure 1.

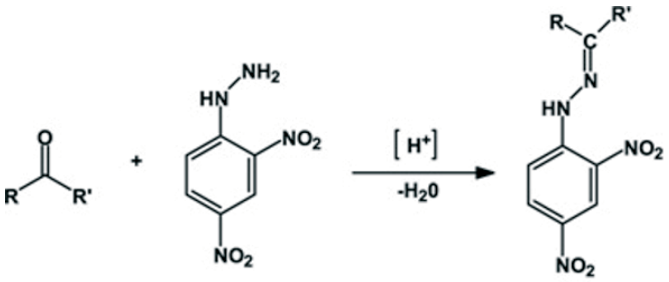

Figure 1.Derivatization using 2,4-dinitrophenylhydrazine.

The collection of air samples can be easily achieved by connecting a Waters Sep-Pak DNPH-Silica Short Plus or Supelco LpDNPH S10L cartridge to the inlet of a hand-held air sampling pump. By doing so, air can be sampled at a user defined flow rate for a user-defined time resulting in DNPH derivatives of analytes being immobilized on the cartridge. Automating the desorption/elution of DNPH derivatives collected on these cartridges and the subsequent analysis by LC-UV or LC-MS can then be used to provide high throughput analysis of environmental air samples.

Experimental

Materials. The DNPH derivative compounds listed in table 1 were purchased from Cerilliant. All other reagents and solvents used were reagent grade.

Calibration standards and QC samples were prepared by making the appropriate dilution of the high standard using acetonitrile. Calibration levels including 500, 100, 50.0, 20.0, 10.0, 5.00, 2.00, 1.00, 0.500, 0.200, and 0.100 ng/mL of each DNPH derivative were evaluated along with QC sample levels including 75.0, 15.0, and 7.50 ng/mL of each DNPH derivative.

The DNPH cartridges eluted using the GERSTEL DNPH Option were Waters Sep-Pak DNPH-Silica Plus Short cartridges, 350 mg, (Waters p/n: WAT #097500). 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) and 13 mm, 0.2 µm, nylon syringe filters (Sigma-Aldrich p/n: Z254492-1PAK) were affixed to the bottom to ensure the removal of particles from the eluent before it is introduced into the HPLC system.

Instrumentation. Automated elution was performed using an MPS robotic^{PRO} sampler with GERSTEL DNPH Option as shown in figure 2. 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 µm). An Agilent 6470 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 introduction to the LC system was performed by the GERSTEL MPS robotic^{PRO} autosampler configured with a Modular Fast Wash Station, using a 6 port (0.25 mm) Cheminert C2V injection valve outfitted with a 10 µL stainless steel sample loop.

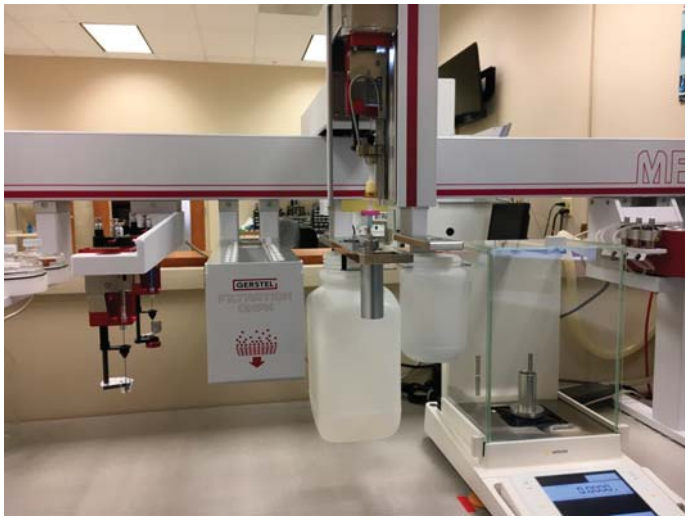

Figure 2. GERSTEL MPS robotic^{PRO} sampler with GERSTEL DNPH Option.

Liquid Desorption. Transport adapters were affixed to the top of the DNPH cartridges and syringe filters fitted with syringe needles were affixed to the bottom of the cartridges to allow the filtered eluate with the DNPH derivatives to be transferred directly into sealed vials. The DNPH cartridge assemblies were then placed into the GERSTEL DNPH Option tray. The automated elution process was controlled using the MAESTRO software and included the following steps:

1. Weigh the empty eluate collection vial.
2. Add 5 mL of 100% acetonitrile into the cartridge using a 2.5 mL SPE syringe.
3. Add 1 mL of air to cartridge for positive displacement of the remaining eluent.
4. Weigh the collection vial in order to determine the exact weight of eluent collected.
5. Combine 500 µL of the eluent with 500 µL of LCMS grade water and mix.
6. Inject 10 µL of the mixture into the HPLC injection valve.

Analysis conditions LC.

Pump:	gradient (600 bar), flowrate = 1.2 mL/min	
Mobile Phase:	A - 100 % water	
	B – 100 % acetonitrile	
Gradient:	Initial	45 % B
	15 min	60 % B
	15.1 min	45 % B
	17.5 min	45 % B
Run time:	20 minutes	
Injection volume:	10 µL (loop over-fill technique)	
Column temperature:	30°C	
UV wavelength:	365 nm	

MS2 SIM mass spectrometer parameters (not required; only used for identification assistance):

Analysis conditions MS.

Operation:	electrospray negative mode
Gas temperature:	350°C
Gas flow (N2):	5 L/min
Nebulizer pressure:	35 psi
Sheath gas heater:	250°C
Sheath gas flow (N2):	11 L/min
Capillary voltage:	- 4000 V
delta EMV:	+ 500 V

Results and Discussion

Table 1 lists the aldehyde and ketone DNPH-derivatives being analyzed with their corresponding m/z's monitored in SIM mode. Detailed mass spectrometric acquisition parameters are available upon request; however, the typical analysis for DNPH derivatives only requires the use of LC-UV.

Table 1. LC-MS method parameters for the DNPH-derivative analysis.

Compound	Mass Ion [m/z]	Dwell [ms]	Fragm. Voltage [V]	Cell Acc [V]	Polarity
m-Tolualdehyde-DNPH	299	100	50	7	Negative
Benzaldehyde-DNPH	285	100	50	7	Negative
Hexaldehyde-DNPH	279	100	50	7	Negative
Valeraldehyde-DNPH	265	100	50	7	Negative
n-Butyraldehyde-DNPH	251	100	50	7	Negative
2-Butanone(MEK)-DNPH	251	100	50	7	Negative
Methacrolein-DNPH	249	100	50	7	Negative
Crotonaldehyde-DNPH	249	100	50	7	Negative
Propionaldehyde-DNPH	237	100	50	7	Negative
Acetone-DNPH	237	100	50	7	Negative
Acrolein-DNPH	235	100	50	7	Negative
Acetaldehyde-DNPH	223	100	50	7	Negative
Formaldehyde-DNPH	209	100	50	7	Negative

Figure 3 shows a representative mass chromatogram from the analysis of a high calibration standard with the corresponding DNPH-derivatives labeled. As shown, the acetone- and acrolein-DNPH derivatives were shown to co-elute given the current chromatographic conditions. One benefit of using LC-MS to analyze DNPH derivatives is the ability to separate co-eluting peaks by their corresponding m/z ratios. Comparing the LC-UV chromatographic results shown in figure 4 to those of the mass chromatograms of figure 5, results from the analysis of acetone- and acrolein-DNPH can only be reported separately when using LC-MS. Similar separation of co-eluting peaks using LC-MS were also observed for the methacrolein and 2-butanone (MEK)-DNPH derivatives.

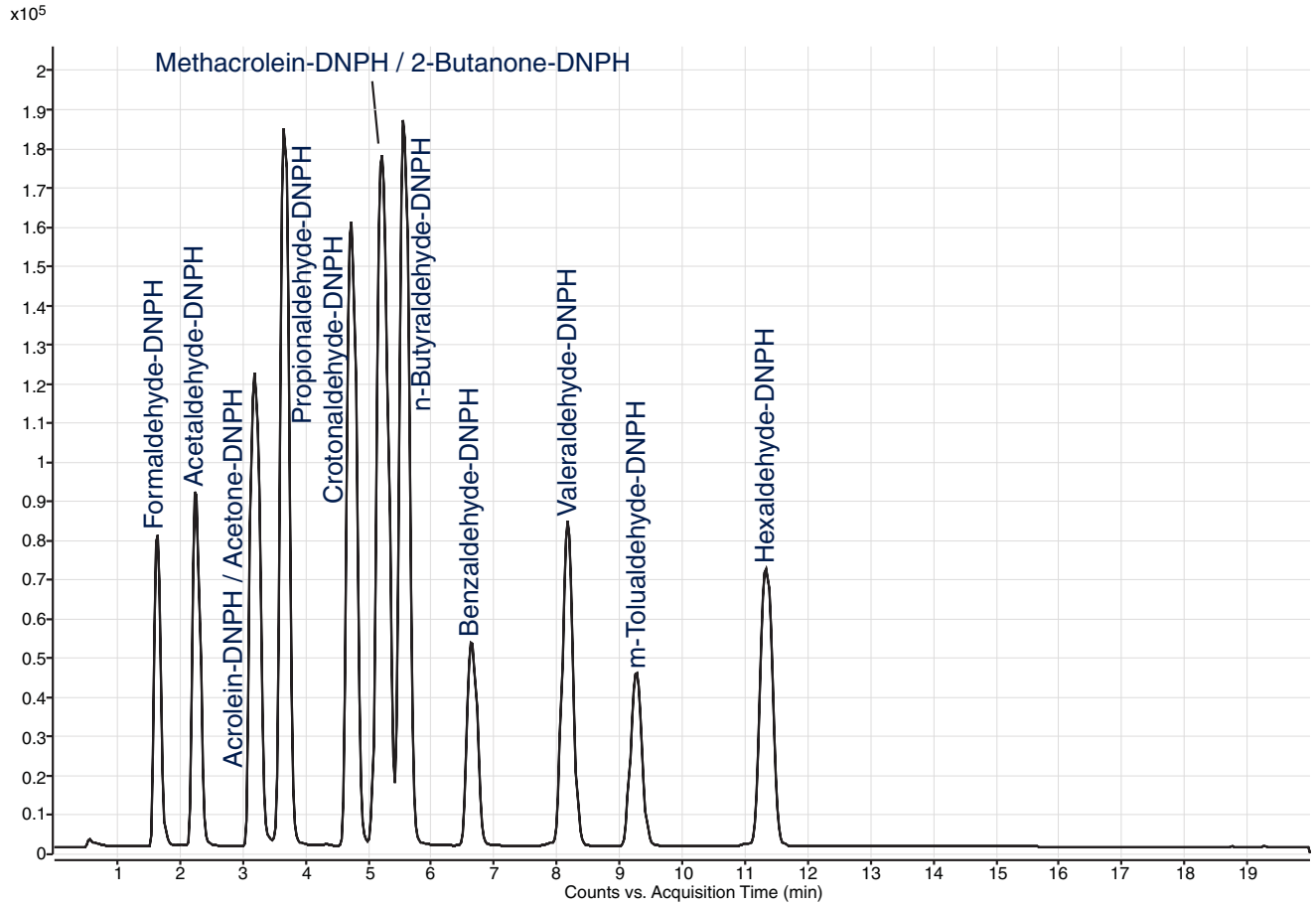

Figure 3. Representative mass chromatogram of DNPH-derivative calibration standard.

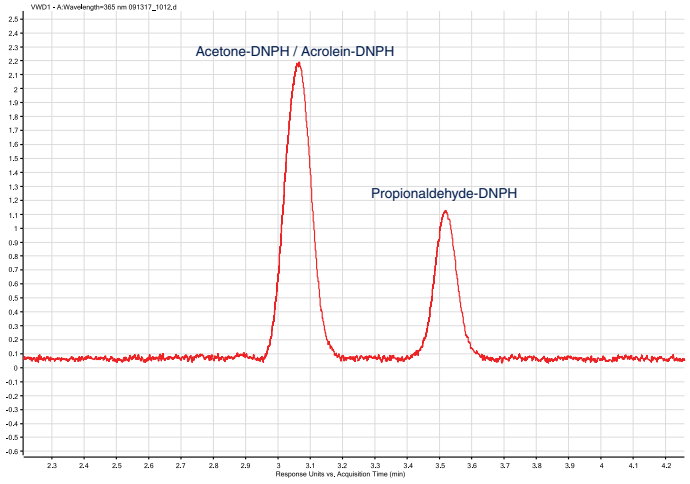

Figure 4. Representative LC-UV chromatogram of acetone-DNPH and acrolein-DNPH.

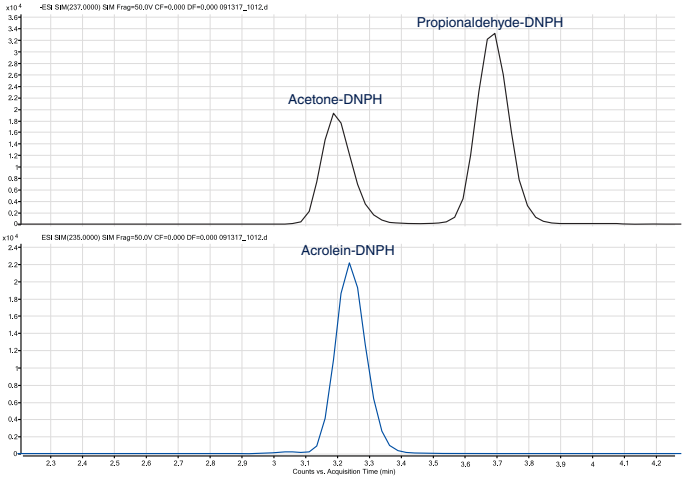

Figure 5. LC-MS overlay mass chromatograms of acetone-DNPH and acrolein-DNPH.

Representative calibration curves from the LC-UV analyses of formaldehyde-DNPH and acetaldehyde-DNPH are shown in figure 6. Regression analysis for all analytes resulted in R^2 values of 0.99 or greater. As can be seen, results for QC standards were also in excellent agreement. It is important to note that the linear range of LC-UV analysis was from 5.00 ng/mL to 500 ng/mL for the DNPH derivatives. For LC-MS analyses, the linear range was from 0.100 ng/mL to 100 ng/mL. The lower concentration of the high standard during LC-MS analyses was due to detector saturation. Depending on the goals of the DNPH analysis, the user should be aware of the linear range and limits of quantitation of the two detectors in order to choose the most appropriate detection source to fit his or her individual needs.

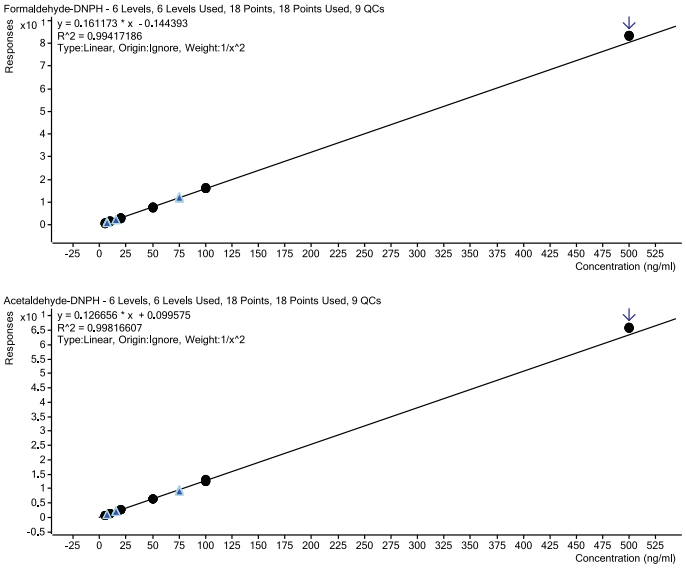

Figure 6. Representative LC-UV calibration curves for formaldehyde-DNPH (top chromatogram) and acetaldehyde-DNPH (bottom chromatogram) based on DNPH-derivative calibration standards. As can be seen, results for QC standards are in excellent agreement.

The low MS standard (0.100 ng/mL) was clearly visible above the baseline for all analytes and the overall method detection limit (MDL) for formaldehyde was calculated to be below 0.5 ng/L (0.5 µg/m3), using an elution volume of 5 mL and 1 Liter of air sampled. In normal practice, however, the overall sensitivity of the method (and the subsequent MDL's) for formaldehyde are reported to be negatively affected due to issues with sampling and the relatively high formaldehyde background observed to be present in DNPH cartridges. Consequently, when using MS detection, the resulting MDL's for formaldehyde are not significantly better than those achieved with UV detection. For other carbonyl derivatives, however, MS detection generally provides improvements in both sensitivity and MDLs.

In other aspects, such as selectivity and accuracy, the determination of DNPH derivatives benefits significantly from using MS as a detector, while using a robot for elution and injection generally benefits accuracy, productivity and laboratory throughput.

During this study, a total of 48 DNPH cartridges were eluted and weight data captured for each replicate. The graph in figure 7 shows the resulting calculated volume of acetonitrile collected from each of the 48 cartridges eluted. Precision data (%CV) for all 48 replicates averaged 1.89 %. These reproducible results would be expected when using a robotic sampler. The mean of the calculated volume of eluent collected was found to be 4.14 mL. The discrepancy from the total volume of acetonitrile applied (5 mL) is most likely due to hold up volume within the cartridge itself or within the syringe filter. Whatever the case, knowing the exact volume of acetonitrile collected allows the user to report the final concentration of aldehyde collected on the DNPH cartridges with higher accuracy.

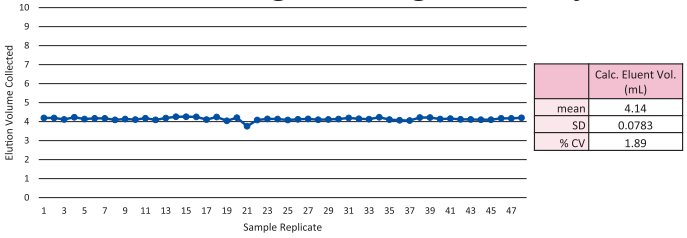

Figure 7. Precision data from the automated elution of 48 DNPH cartridges. Volumes were calculated based on the recorded eluent weight.

Conclusions

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

- Automated elution and subsequent LC-UV and LC-MS analyses of DNPH cartridges was successfully performed using the dual head GERSTEL MPS robotic^{PRO} sampler.
- Linear calibration curves resulting in R^2 values 0.99 or greater were achieved based on injection of the calibration standards.
- In some cases, MS detection enabled a deconvolution of overlapping analyte peaks that would not have been possible with UV detection.
- Precision data for the automated elution of 48 separate DNPH cartridges was found to be 1.89 %CV.
- Elution volumes were accurately calculated after determining the eluent weight, improving overall method accuracy.

GERSTEL GmbH & Co. KG
Eberhard-Gerstel-Platz 1
45473 Mülheim an der Ruhr
Germany
+49 (0) 208 - 7 65 03-0
+49 (0) 208 - 7 65 03 33
gerstel@gerstel.com
www.gerstel.com

GERSTEL Worldwide

GERSTEL, Inc.
701 Digital Drive, Suite J
Linthicum, MD 21090
USA
+1 (410) 247 5885
+1 (410) 247 5887
sales@gerstelus.com
www.gerstelus.com

GERSTEL AG
Wassergrabe 27
CH-6210 Sursee
Switzerland
+41 (41) 9 21 97 23
+41 (41) 9 21 97 25
swiss@ch.gerstel.com
www.gerstel.ch

GERSTEL K.K.
1-3-1 Nakane, Meguro-ku
Tokyo 152-0031
SMBC Toritsudai Ekimae Bldg 4F
Japan
+81 3 5731 5321
+81 3 5731 5322
info@gerstel.co.jp
www.gerstel.co.jp

GERSTEL LLP
10 Science Park Road
#02-18 The Alpha
Singapore 117684
+65 6779 0933
+65 6779 0938
SEA@gerstel.com
www.gerstel.com

GERSTEL (Shanghai) Co. Ltd
Room 206, 2F, Bldg.56
No.1000, Jinhai Road,
Pudong District
Shanghai 201206
+86 21 50 93 30 57
china@gerstel.com
www.gerstel.cn

GERSTEL Brasil
Av. Pascoal da Rocha Falcão, 367
04785-000 São Paulo - SP Brasil
+55 (11)5665-8931
+55 (11)5666-9084
gerstel-brasil@gerstel.com
www.gerstel.com.br

Awarded for the active pursuit of environmental sustainability