#AP20

EPROGEN INC

MICRA *NPS*® LC Analytical Column
Fast Separation of 2,4-DNPH on C-30

MICRA *NPS*® is a breakthrough in fast HPLC. *NPS* is ultra-pure, highly uniform non porous silica spheres which provide the LC chromatographer greatly improved mass transfer and lower detection limits. Coupled with enhanced stability and dramatically reduced solvent usage, *NPS* is the ideal analytical column to meet the ever increasing demands placed on today's analytical labs - higher productivity at a lower cost.

Data courtesy of Lamotte, S.; Potter, W.; Englehardt, H. of Universitat des Saarlandes, Saarbrucken, and Karst, U. of Westfalische Wilhelms-Univerisitat, Munster, Germany.

The use of 2,4-DNPH (dinitrophenylhydrazine) as a derivitizing agent is a well documented method for the determination of aldehydes and ketones in gas and liquid samples. The corresponding hydrazones that are formed are readily separated by reversed phase (RP) HPLC using UV detection. Due to the importance of these chemicals in industrial applications along with their production as byproducts in combustion processes, good analytical techniques are important in the detection and monitoring of these compounds.

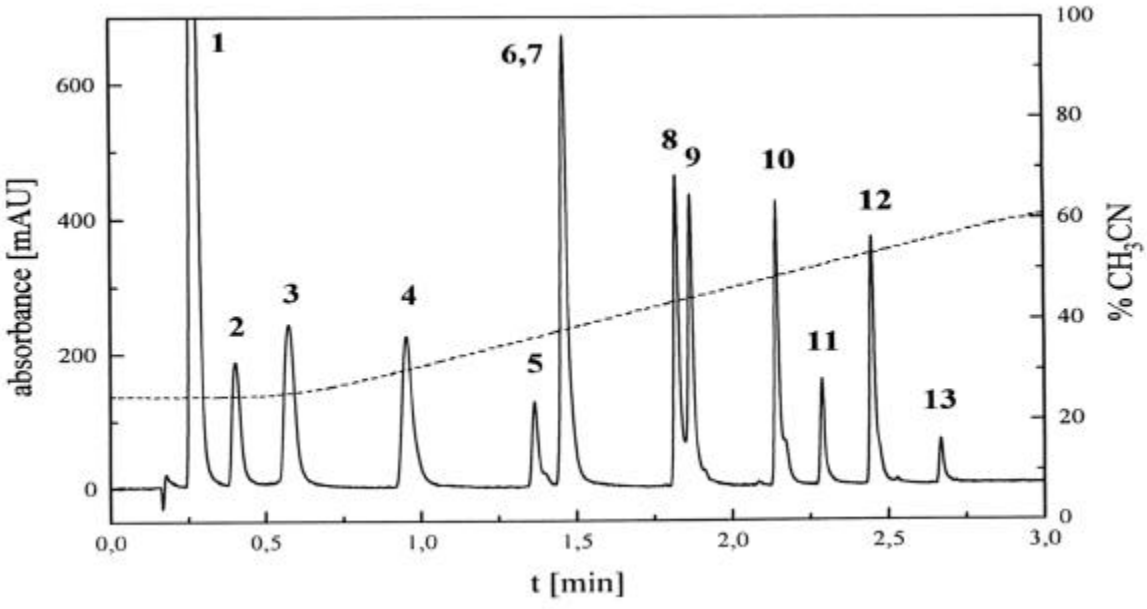
This note describes the fast and efficient HPLC method to measure aldehydes and ketones in air samples based on the use of MICRA 1.5µ *NPS*® (non-porous silica) supports. Here we compare the separation of 2,4-DNPH derivatives using analytical columns of both an *NPS* ODS-I and an extended chain *NPS* TAS support.

NPS TAS columns used in analyzing automobile exhaust show good selectivity for aliphatic and olefinic aldehydes and ketones of the same carbon chain length. In this study, selectivity was optimized by varying the temperature. This demonstration of speed for the derivatizing reaction of formaldehyde and DNPH also exemplifies the usefulness in on-line process monitoring.

Compounds for Figure 1:

1. DNPH
2. DNPA
3. Formaldehyde
4. Acetaldehyde
5. Acetone
6. Acrolein
7. Propanal
8. Crotonaldehyde
9. Butanal
10. Pentanal
11. Benzaldehyde
12. Hexanal
13. p-Tolualdehyde

Figure 1. Separation of 2,4-DNPH using MICRA 1.5µ *NPS* TAS



Analytical Conditions

Column	MICRA *NPS* TAS, 1.5µ, 4.6 x 53 mm
Detection	UV, λ = 360 nm
Mobile Phase	H_20/ACN linear gradient: 0.0 min = 25% B, 2.4 min = 62% B
Flow Rate	1.25 mL/min
Injection Volume	1.5µL
Temperature	28º C

Think small

Think fast

Think *NPS*®

High Resolution from On-line Monitoring of Gas Samples

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On-line Monitoring of Derivatization of Formaldehyde with DNPH

The use of the MICRA *NPS* TAS phase column in the separation of the hydrazones is an excellent tool for on-line monitoring of fast reactions in solution. Figures 3 & 4 below illustrate the real time accuracy in on-line measurement delivered by the speed of the *NPS* column. With chromatographic events being tracked at 16 second intervals, it is clear that the injection frequency is limited by the injection itself, and not by the instrument conditions when using the *NPS* column.

Figure 2. Formation of Formaldehyde 2,4-DNPH

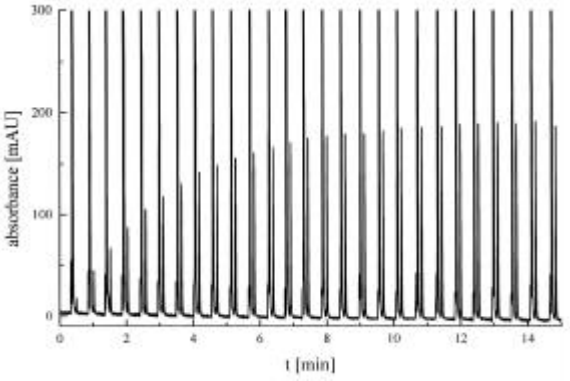
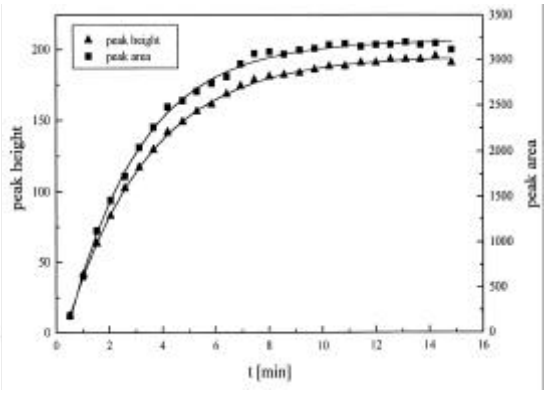


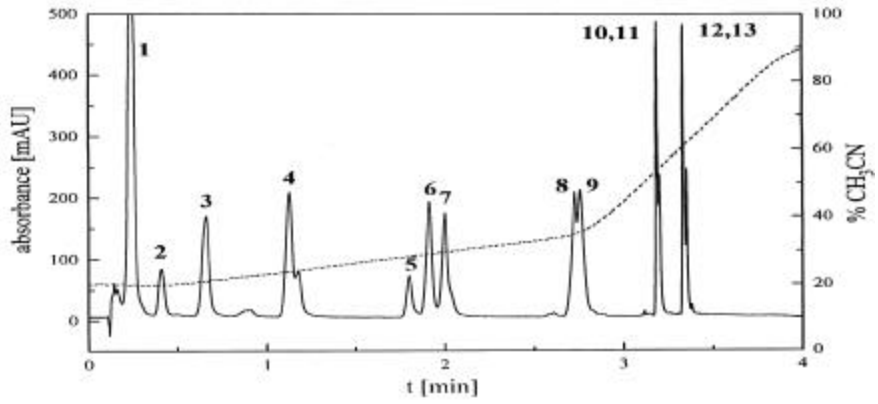
Figure 3. Peak Height & Peak Area



Figures 3 & 4 were monitored by multiple sample injection of concentrations: $5x10^{-4}$M DNPH, $5x10^{-5}$M Formaldehyde, 0.05M H_2SO_4 in Acetonitrile/H_20 (30/70)

In comparing the MICRA *NPS* ODS-I to the *NPS* TAS column, both columns reduced the average analysis time by 70% to under 4 minutes without loss in resolution. In this study, the *NPS* TAS phase was demonstrated to perform more effectively with the hydrazones of the long-chain aliphatic aldehydes.

Figure 4. Separation of 2,4-DNPH using MICRA *NPS* ODS-I



The DNPH method is becoming a recognized procedure by several standardization organizations, including the current discussion of the European Union. The *NPS* TAS column is an effective tool in this analysis for environmental and industrial testing.