

Application Note

▶ Rapid determination of DNPH derivatized carbonyl compounds

Category	Environmental analysis
Matrix	-
Method	UHPLC
Keywords	DNPH, aldehydes, carbonyls, air pollutants, ambient air
Analytes	DNPH derivatives of formaldehyde, acetaldehyde, acetone, methacrolein, propionaldehyde, benzaldehyde
ID	VEV3, 05/10 updated 09/11



Summary

This application note describes a very fast method for identifying six DNPH derivatized carbonyls in a mixture using the KNAUER PLATINblue UHPLC system. The stationary phase BlueOrchid C18 A was used to separate the carbonyl mixture. Short columns with small particles are the most suitable way for avoiding long equilibration and analysis times. Reduction of analysis time from about 20 to less than 3 minutes compared to a conventional HPLC method is achieved by using the BlueOrchid C18 A phase with a 1.8 μm particle size. A binary high pressure gradient configuration was used at a flow rate of 0.8 ml/min in combination with a 2 mm column ID and UV detection.

Introduction

Carbonyl compounds, in particular aldehydes, are reactive volatile substances. They are of concern to the public, because they are emitted as air pollutants by a large range of industrial processes and other combustion sources. In addition, they can also be found indoors when emitted from sources such as insulation, furniture or tobacco smoke.^{1,2}

Because of their adverse health effects, monitoring of these substances is important. Formaldehyde and acetaldehyde, for instance, are known for their irritating effects on animals and humans whereby formaldehyde is also carcinogenic. In particular formaldehyde emitting from wood products such as wooden toys or furniture should be observed critically. In the last few years, monitoring of indoor air pollution has gained more and more importance because individuals spend more time in indoor environments such as schools or office buildings today.³

In this work, six low molecular weight carbonyls with chemical structures shown in figure 1 are rapidly separated by UHPLC. Optimizing the speed and resolution of routine analyses by applying a UHPLC method can not only save time but also dramatically decrease eluent costs, particularly important for analyses using acetonitrile.

Low molecular weight aldehydes are highly volatile and polar substances which results in a hindered analysis by RP-UHPLC methods. Additionally, many known carbonyl compounds have no chromophores and are for this reason not detected by the UV detector. But due to their functional carbonyl group, low molecular weight aldehydes and ketones can be derivatized with several derivatization agents. The most widely used derivatization agent is 2,4 dinitrophenylhydrazine (DNPH). In acidic media, DNPH reacts with the carbonyl group to form stable hydrazones (DNPH-carbonyls) with a lower vapor pressure. The DNPH derivatization proceeds immediately and quantitatively and is therefore a feasible sample preparation method for analysis with RP-UHPLC methods. The derivatives can be separated by UHPLC on a C18 column and detected easily by UV.² The derivatization reaction leads to an orange-colored mixture of DNPH-carbonyls as shown in figure 2.

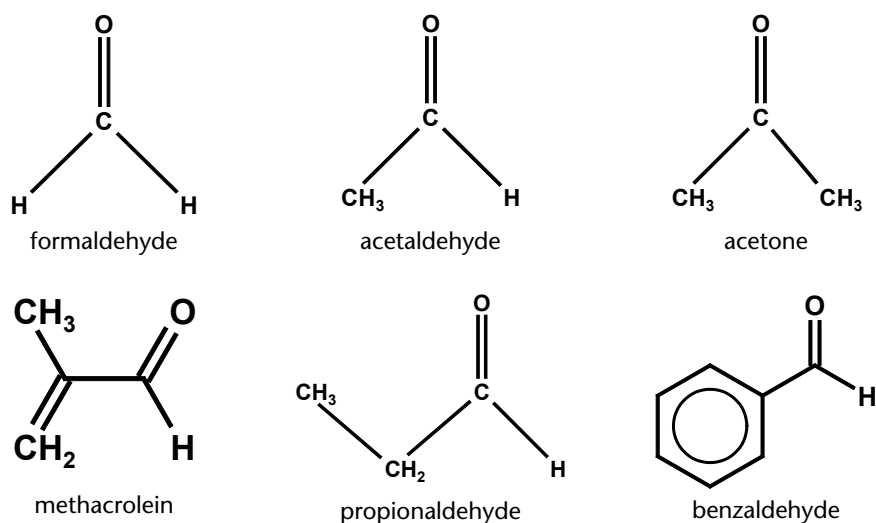


Fig. 1
Structures of the analyzed carbonyls

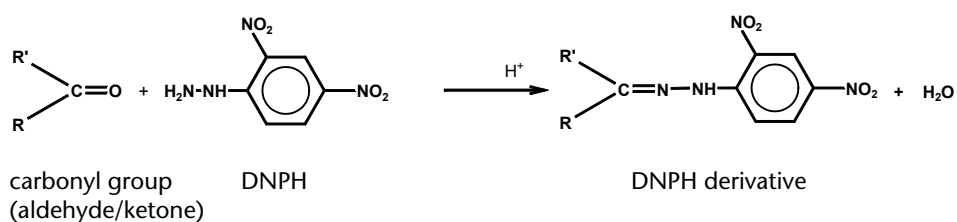


Fig. 2
DNPH derivatization reaction

Experimental Preparation of standard solution

The six carbonyls were received in a mixture of 40 ng/μl for each substance as DNPH-derivatives. A standard solution was prepared by diluting the mixture with water/acetonitrile 40:60 v/v to reach a standard concentration of 2 ng/μl for every carbonyl.

For the calibration, standard solutions are additionally prepared with concentrations of 10 and 40 ng/μl by diluting the original standard with water/acetonitrile 40:60 v/v.

Method parameters

Column	BlueOrchid C18 A 1.8 μm, 100 x 2 mm		
Eluent A	Water		
Eluent B	Acetonitrile		
Gradient	Time [min]	% A	% B
	0.0	60	40
	2.0	45	55
	4.0	0	100
	4.5	0	100
Flow rate	0.8 ml/min		
Injection volume	2 μl standard		
Column temperature	40 °C		
Detection	UV at 370 nm (50 mm cell, 50 Hz, 0.1 s)		
Analysis time	3 min		
Run time	4.5 min		

Results

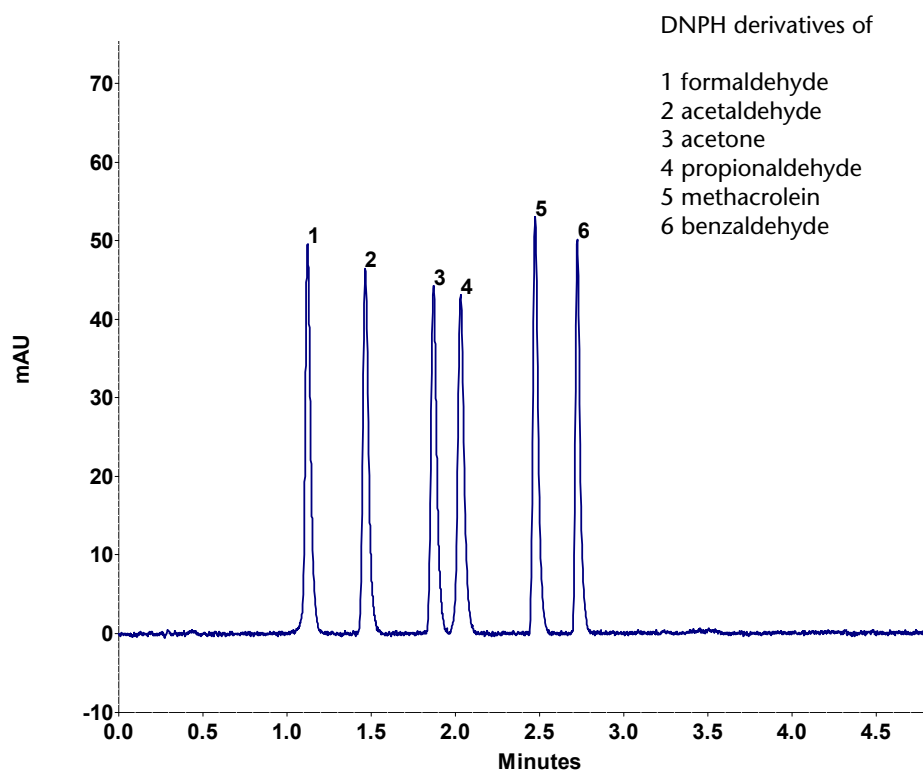


Fig. 3
Standard DNP carbonyl mixture of 4 ng

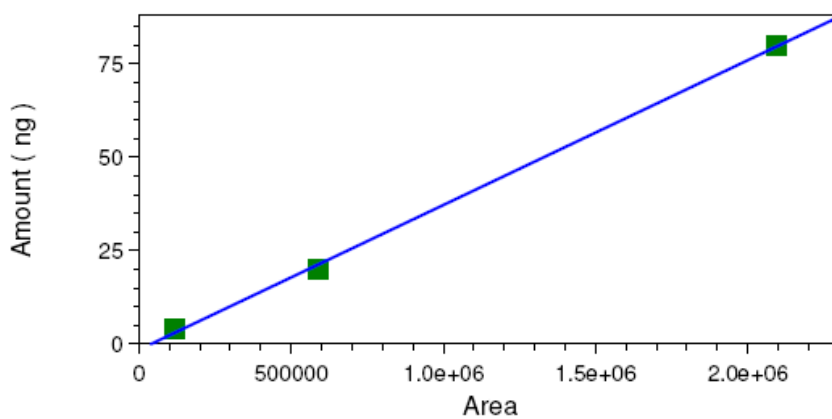


Fig. 4
Calibration curve for formaldehyde

A chromatogram of the 0.02 µg DNP carbonyl standard is shown in figure 3. All six carbonyls are baseline separated with resolution values in the range of 2.5 for the critical pair acetone and acrolein up to 7.3. The analysis time could be reduced more than six times in comparison to the method using a conventional HPLC system and larger particle sizes in the applied column.

The limits of detection (LOD) lie in the range of 0.1 ng for all six carbonyls. Calibration is realized for all analyzed compounds and the linearity (r^2) lies in the range of 0.999131 – 0.999916. As an example, the calibration curve for formaldehyde is shown in figure 4.

Method performance

Limit of detection	0.1 ng (S/N = 3)
Linearity (r^2)	0.999131 – 0.999916
Linearity range	0.1 – 80 ng

Conclusion

This application note describes a very fast method for the determination of six carbonyls. After DNPH derivatization, a separation in less than 3 minutes was possible by employing a PLATINblue UHPLC system, a BlueOrchid C18 A stationary phase and an acetonitrile gradient. The 2 mm inner diameter of the chosen column results in a comparable small amount of required eluent. One could also use a shorter column if the separation of acetone and acrolein is not of interest, because all of the other substances are separated with high resolution values. Shortening the column would lead to a shorter analysis time and lower consumption of mobile phase. By using UHPLC, long equilibration and analysis times can be avoided and a UV detection of DNPH derivatized carbonyl concentrations in the range of 4 ng can be realized by using a 50 mm flow cell.

Before analyzing carbonyls from room air, for instance, it is highly recommended to run a blank of the UHPLC system including all used chemicals and solvents and especially the derivatization agent. Otherwise the ubiquity of formaldehyde and acetone in air will result in misleadingly high quantities being detected in the analyzed samples.

References

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Physical properties of the recommended column

BlueOrchid C18 A is a polar endcapped phase for alternative C18 selectivity. For the analysis of very polar compounds, it might be necessary to use 100% aqueous eluent. BlueOrchid C18 A was especially developed for such hydrophobic and polar interactions. Due to the narrow particle size distribution, the column back pressure of all BlueOrchid columns is lower than other high speed column materials on the market.



Stationary phase	BlueOrchid 1.8 C18 A
USP code	L1
Particle size	1.8 μm
Form	spherical
pH range	2 – 9
% C	12
Endcapping	yes (polar)
Dimensions	100 x 2 mm
Order number	10BI184BOE
Stationary phase	BlueOrchid 1.8 C18 A

Recommended instrumentation



This application used the PLATINblue binary high pressure gradient UHPLC system equipped with degasser, autosampler, column thermostat, and PDA detector. PLATINblue UHPLC can also run HPLC applications. Other system configurations are also available. Please contact KNAUER to configure a system that's perfect for your needs.

Description	Order No.
PLATINblue UHPLC system	A69420
PLATINblue Pump P-1	
PLATINblue Pump P-1 with degasser	
PLATINblue Autosampler AS-1	
PLATINblue Column Thermostat T-1 Basic	
PLATINblue Detector PDA-1	
PDA-1 flow cell (10 mm, 2 µl)	
PLATINblue modular eluent tray	
PLATINblue CG data system	
PLATINblue CG PDA license	
PLATINblue stainless steel capillary kit	
+ PDA-1 flow cell (50 mm)	A64151

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