

Systematic HPLC method development and robustness evaluation of 13 carbonyl DNPH derivatives using DryLab®

Lilit Avagyan, Kate Monks; applications@knauer.net
KNAUER Wissenschaftliche Geräte GmbH, Hegauer Weg 38, 14163 Berlin; www.knauer.net



SUMMARY

In the monitoring of industrial air, the determination of carbonyl (aldehyde and ketone) emissions is crucial to prevent respiratory, pulmonological, autoimmune diseases, and cancer. According to the analytical method described in the DIN ISO 16000-3 [1], the carbonyls must be converted to their corresponding hydrazones with 2,4-dinitrophenylhydrazin (DNPH) in order to be detected via UV detector and analyzed by reversed phase HPLC. Here, the DryLab® software was used for method optimization to separate of 13 carbonyl derivatives in a standard mixture with the AZURA® HPLC system and the DNPH-column.

INTRODUCTION

The main objective of method optimization in HPLC is to define the appropriate conditions for robust, precise, and reproducible analysis. In order to save resources, a computer assisted method development can be a valuable tool. For the characterization of carbonyl content in air samples, commonly a standard mixture of 13 aldehyde and ketone DNPH derivatives is used. For precise analysis a good separation of all 13 components has to be achieved. Here, the

chromatography modelling software DryLab® with 3D Cube option was used for the optimization of the analysis of the carbonyl standard mixture. The investigation of the combined influence of gradient time, temperature and ternary eluent composition on critical resolution enabled the development of robust method conditions. Furthermore, the robustness space was investigated *in silico* and verified experimentally with a high degree of agreement.

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RESULTS

The separation of 13 carbonyls was analyzed according to the method described in DIN ISO 16000-3 [1]. The obtained chromatogram from this experiment resulted 11 peaks (Fig. 1). The peaks representing acetone-DNPH, Acroleine-DNPH, 2-Butanone-DNPH, Methacroleine-DNPH, and butyraldehyde-DNPH were not separated. In order to optimize method parameters in silico, DryLab requires measurements under 12 conditions (Fig. 2). The measurements were conducted as described below. The obtained chromatograms were fed into the DryLab software resulting in the Method Operation Design Region (MODR). The red regions in the cube represent the optimal

chromatographic conditions (Fig. 3). The selection of the best parameters from the predicted data pull are based on high resolution values. The optimal separation method was established with the solvent composition water and acetonitrile, with a column temperature at 22 °C and a gradient time of 14 min. As the results show (Fig. 4) the baseline separation of acetone-DNPH and acroleine-DNPH was reached with the resolution value of 2.69 (see suppl. results Tab. A1). The lowest resolutions were obtained between peak pairs 2-Butanone-DNPH, Methacroleine-DNPH (1.27) and Methacroleine-DNPH, n-Butylaldehyde (1.29).

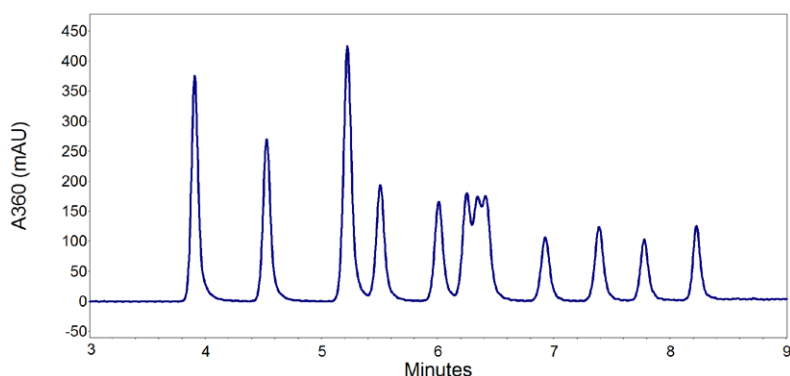


Fig. 1 Chromatogram of 13 carbonyls, measured according to ISO DIN 16000-3 method with the DNPH column with the DNPH column

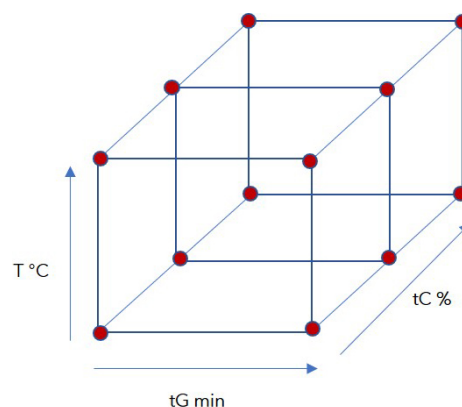


Fig. 2 DryLab 3D Cube with 12 red pointed measurement conditions

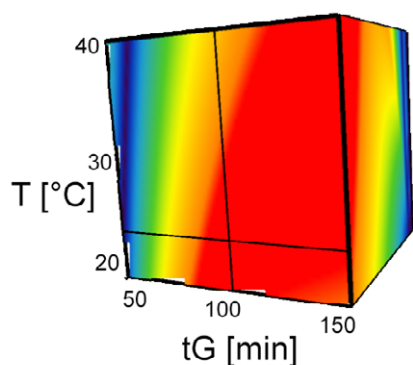


Fig. 3 MODR Method Operation Design Region

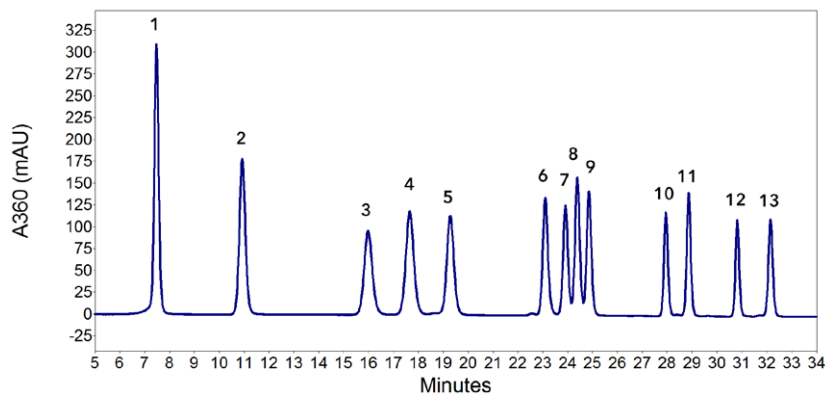


Fig. 4 Chromatogram of 13 carbonyls, measured according to DryLab® predicted method with the DNPH column

MATERIALS AND METHODS

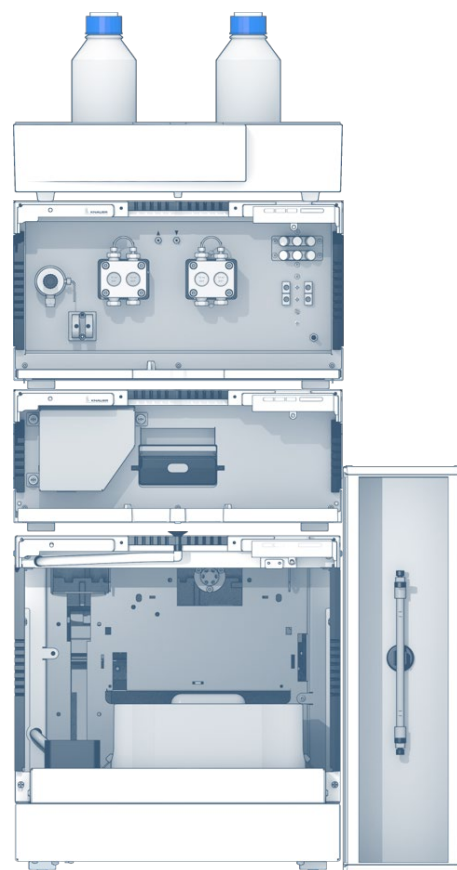
The HPLC system includes the pump AZURA® P 6.1L HPG, detector AZURA® DAD 6.1L, autosampler AZURA® AS 6.1L, column thermostat AZURA® CT 2.1. The method separation, described in DIN ISO 16000-3 [1] and following method optimization was performed on DNPH-column (150 x 3 mm). The standard with 13 aldehyde and ketone derivatives, dissolved in acetonitrile was obtained from SigmaAldrich and was diluted to a concentration of 1 µg/mL in acetonitrile. For method optimization, the DryLab® (Version 4) modeling software (Molnár-Institute, Berlin) was used. The optimal separation conditions were predicted based on 12 chromatograms. The measurements were performed by three different mobile phase compositions (100% MeOH, 50:50 MeOH:Acetonitrile, 100% Acetonitrile). Each composition was used for measurements at two different temperatures and gradient times, namely 20 and 40 °C, and 30 and 90 min respectively. The analysis of chromatograms was performed by the using of OpenLab chromatographic software. For the method optimization the column parameters, initial gradient conditions and dwell volume of the system were programmed in the DryLab® software. The chromatographic data files were converted in to AIA (*.CDF) format and loaded in the DryLab® for the calculation.

REFERENCES

[1] DIN ISO 16000-3; Indoor air - Part 3: Determination of formaldehyde and other carbonyl compounds in indoor air and test chamber air - Active sampling method (ISO 16000-3:2011)

CONCLUSION

The DryLab® software is an important part in the HPLC method optimization. Our results show, that it makes possible to define optimal separation conditions without performing of numerous unnecessary measurements. This software helps to save the time, to reduce the consumption of materials and perform ecological 'green' HPLC.



ADDITIONAL RESULTS

Tab. A1 Content of the standard solution under optimized chromatographic conditions (Fig 4)

#	Component name	Retention time	Resolution	RSD %	#	Component name	Retention time	Resolution	RSD %
1	Formaldehyde-DNPH	7.47	-	0.63	8	Methacroleine-DNPH	24.38	1.27	0.15
2	Acetaldehyde-DNPH	10.92	8.78	0.65	9	n-Bytaldehyde-DNPH	24.84	1.29	0.19
3	Acetone-DNPH	15.96	9.33	0.79	10	Benzaldehyde-DNPH	27.94	9.30	0.12
4	Acroleine-DNPH	17.63	2.69	0.47	11	Valeraldehyde-DNPH	28.85	3.00	0.38
5	Propionaldehyde-DNPH	19.26	2.81	0.53	12	m-Tolualdehyde-DNPH	30.81	6.66	0.10
6	Crotonaldehyde-DNPH	23.08	8.17	0.17	13	m-Tolualdehyde-DNPH	32.14	4.35	0.18
7	2-Butanone-DNPH	23.09	2.08	0.14					

ADDITIONAL MATERIALS AND METHODS

Tab. A2 Method parameters

Eluent A	H ₂ O _{dd}		
Eluent B	Acetonitrile		
Gradient	Time [min]	% A	% B
	0	60	40
	16	60	40
	30	40	60
	40	40	60
	41	60	40
	45	60	40
Flow rate	1 mL/min	System pressure	-
Column temperature	22 °C	Run time	45 min
Injection volume	10 µL	Injection mode	-
Detection wavelength	360 nm	Data rate	20 Hz
		Time constant	0.05 s

Tab. A3 System configuration

Instrument	Description	Article No.
Pump	AZURA P 6.1L	APH35GA
Autosampler	AZURA AS 6.1L	AAA10AA
Detector	AZURA DAD 6.1L	ADC11
Flow cell	High Sensitivity LightGuide	AMD59XA
Thermostat	AZURA CT 2.1	A05852
Column	DNPH-Column, II 100-3	15CE490E2G
Software	OpenLAB CDS EZChrom Edition	A2619-1