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Application News

Simultaneous Analysis of DNPH-Derivatized Aldehydes Using Integrated HPLC and C30 Column

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User Benefits

- DNPH-derivatized aldehydes can be analyzed using i-Series LC-2070C integrated HPLC.
- The six compounds designated under the Offensive Odor Control Law and the 15 compounds designated by EPA can be simultaneously analyzed.
- Multiple isomers of DNPH-derivatized aldehydes can be separated using Shim-pack™ SR-C30 column.

■ Introduction

Aldehydes are the source of concern regarding environmental pollution and negative effects on human health, and analytical methods for aldehydes have been established. Analysis for formaldehyde and acetaldehyde, which are designated as harmful air pollutants, were presented in Application News No. 01-00996.

This study presents the simultaneous determination of 16 carbonyl compounds, including six substances regulated under the Offensive Odor Control Law in Japan and 15 compounds listed by the U.S. Environmental Protection Agency (EPA), using a 2,4-dinitrophenylhydrazine (DNPH) derivatization coupled with high-performance liquid chromatography (HPLC). Analyses were performed using an i-Series LC-2070C integrated HPLC system, designed for robust routine operation. Chromatographic separation was achieved with a Shim-pack SR-C30 column featuring a triacontyl (C30) stationary phase. The C30 stationary phase provides enhanced shape selectivity and superior structural recognition, making it particularly suitable for the separation of highly lipophilic compounds as well as the effective resolution of structural isomers.

■ Simultaneous analysis of the six compounds designated under the Offensive Odor Control Law

The Offensive Odor Control Law[1)] designates 22 offensive odor substances and requires local governments to establish regulatory standards on respective boundary lines within the range of odor intensity from 2.5 to 3.5 on the six-level odor intensity classification. Here, analysis of the six aldehydes (Table 1) designated as offensive odor substances are presented.

Table 1 Six aldehydes designated under the Offensive Odor Control Law

• Acetaldehyde	• Propionaldehyde
• *n*-Butyraldehyde	• Isobutyraldehyde
• *n*-Valeraldehyde	• Isovaleraldehyde

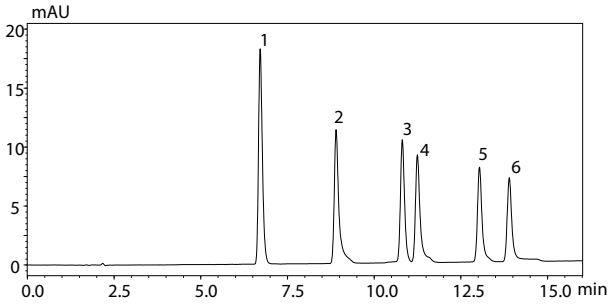
Confirming separation

A mixed standard solution of six DNPH-derivatized aldehydes (Fujifilm Wako Pure Chemical Corporation, P/N: 019-27811) was diluted with acetonitrile to prepare a mixed standard solution containing 1 mg/L of respective aldehydes. This solution was subjected to HPLC analysis under the conditions shown in Table 2. Fig. 1 shows the obtained chromatogram in which all the compounds were sufficiently separated, and the resolution between isobutyraldehyde and *n*-butyraldehyde was more than 2.0.

Table 2 Analytical conditions

System	:	i-Series LC-2070C				
Column	:	Shim-pack SR-C30 (150 mm × 4.6 mm I.D., 3 µm)*1				
Mobile phase	:	A) Water B) Tetrahydrofuran C) Acetonitrile D) Methanol				
Time program	:	Time [min]	A [%]	B [%]	C [%]	D [%]
		0.00	45.0	5.0	35.0	15.0
		16.00	23.4	2.6	51.8	22.2
		16.01	9.0	1.0	63.0	27.0
		17.00	9.0	1.0	63.0	27.0
		17.01	45.0	5.0	35.0	15.0
		24.00	45.0	5.0	35.0	15.0
Flow rate	:	1.0 mL/min				
Mixer	:	40 µL				
Column temp.	:	30 °C				
Injection volume	:	5 µL				
Vial	:	Shim-vial S, Amber glass*2				
Detection (UV)	:	360 nm				

*1 P/N：227-31218-02 *2 P/N：227-34500-53



1. DNPH-Acetaldehyde
2. DNPH-Propionaldehyde
3. DNPH-Isobutyraldehyde
4. DNPH-*n*-Butyraldehyde
5. DNPH-Isovaleraldehyde
6. DNPH-*n*-Valeraldehyde

Fig. 1 Chromatogram of mixed standard solution of six compounds (1 mg/L)

Calculation of aldehyde concentration corresponding to odor intensity

The atmospheric aldehyde concentration [ppm], which serves as the regulatory standard under the Offensive Odor Control Law, was converted into the concentration [mg/L] determined by HPLC. Table 4 shows the respective HPLC-based aldehyde concentrations $(As-At)/v$ [mg/L] converted from the concentrations C of the tested compounds in the air corresponding to the odor intensities of 2.5 and 3.5 using equation (1) and valued listed in Table 3.

$$C = \frac{2.24 \times (A_s - A_t) \times E}{MvV \times \frac{273}{273 + t} \times \frac{P}{101.3}} \quad \cdots (1)$$

Table 3 Values for conversion of concentration

C :	Atmospheric concentration of analyte[ppm]	Calculate using specified values.
As:	Amount of the analyte injected into HPLC [µg]	
At:	Operational blank for aldehyde [µg]	Calculate using 0 µg
E :	Volume of analyte [mL]	Calculate using 5 mL
M :	Molecular weight of analyte	
v :	Injection volume into HPLC [mL]	Calculate using 20×10^{-3} mL
V :	Amount of aspirated gas measured by gas meter [L]	Calculate using 30 L
t :	Temperature measured by gas meter [°C]	Calculate using 25 °C
P :	Atmospheric pressure at sample collection [kPa]	Calculate using 101.3 kPa

Table 4 Respective concentrations corresponding to odor intensity 2.5 and 3.5

	Odor intensity 2.5		Odor intensity 3.5	
	Atmospheric conc. [ppm]	Converted HPLC conc. [mg/L]	Atmospheric conc. [ppm]	Converted HPLC conc. [mg/L]
Acetaldehyde	0.05	0.54	0.5	5.4
Propionaldehyde	0.05	0.71	0.5	7.1
Isobutyraldehyde	0.02	0.35	0.2	3.5
n-Butyraldehyde	0.009	0.16	0.08	1.4
Isovaleraldehyde	0.003	0.063	0.01	0.21
n-Valeraldehyde	0.009	0.19	0.05	1.1

Calibration curve and repeatability

Above mentioned mixed standard solution of the six DNPH-derivatized aldehydes was diluted with acetonitrile to prepare mixed solutions containing 0.05, 0.1, 0.25, 0.5, 0.75, 1, 2.5, 5, 7.5, and 10 mg/L of respective aldehydes, which were then subjected to HPLC analyses. Table 5 shows the coefficients of determination of respective calibration curves and concentration ranges specified referring to the concentrations shown in Table 4. Satisfactory coefficients of determination of 0.9999 or more were obtained for all the compounds.

Furthermore, repeatabilities were verified by six consecutive analyses of standard solutions at lowest concentrations of respective calibration levels. The relative standard deviations (%RSD) of peak areas are shown in Table 6. Good repeatabilities were obtained for all the compounds.

Table 5 Calibration range and coefficient of determination for each compound

	Range of concentration (mg/L)	Coefficient of determination
Acetaldehyde	0.5 - 10	0.99999
Propionaldehyde	0.5 - 10	0.99999
Isobutyraldehyde	0.1 - 5	0.99998
n-Butyraldehyde	0.1 - 5	0.99998
Isovaleraldehyde	0.05 - 2.5	0.99991
n-Valeraldehyde	0.1 - 5	0.99998

Table 6 Peak area repeatability for each compound (n=6)

	Concentration [mg/L]	%RSD of peak area
Acetaldehyde	0.5	0.16
Propionaldehyde	0.5	0.23
Isobutyraldehyde	0.1	1.24
n-Butyraldehyde	0.1	1.41
Isovaleraldehyde	0.05	2.27
n-Valeraldehyde	0.1	1.16

■ Simultaneous analysis of 16 compounds including the 15 EPA-designated compounds

EPA 8315A[2] specifies 15 aldehydes (Table 7) in indoor air as the target analytes. Here, simultaneous analysis of these 15 compounds and additional isobutyraldehyde, totally 16 compounds are presented.

It is generally considered difficult to accomplish complete separation of these 16 compounds, as well as the six compounds designated under the Offensive Odor Control Law using ordinary C18 column since these two sets of aldehydes contain multiple isomers. Therefore, Shim-pack SR-C30 was compared with ordinary C18 through the analysis of these 16 compounds to confirm the differences in their separation selectivity.

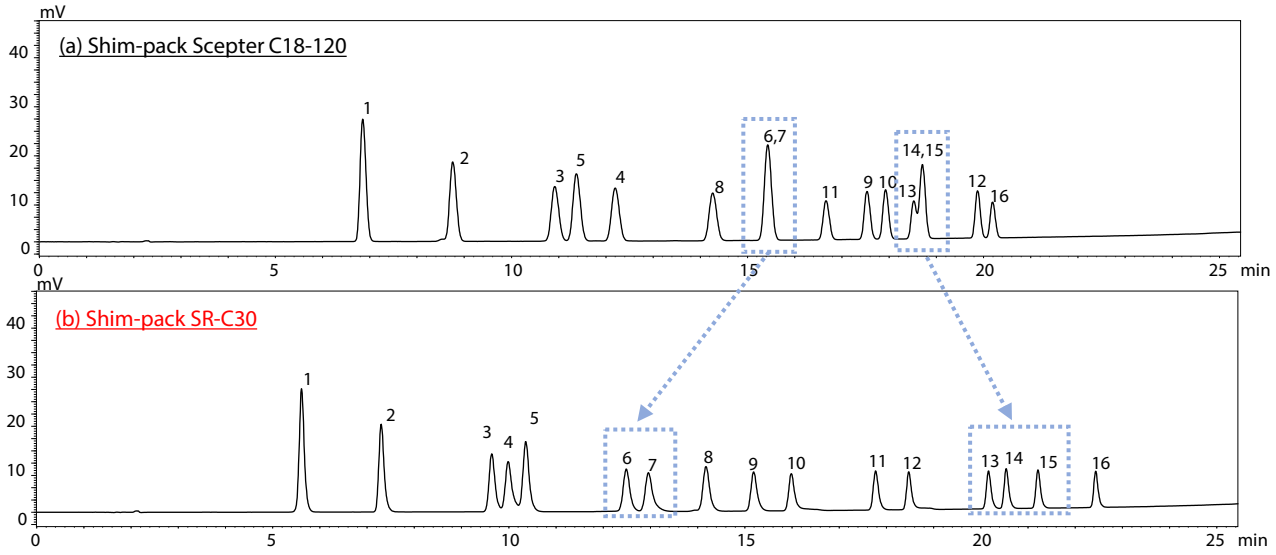
Table 7 15 aldehydes designated by EPA

• Formaldehyde	• Acetaldehyde
• Propionaldehyde	• Acrolein
• Acetone	• *n*-Butyraldehyde
• Crotonaldehyde	• *n*-Valeraldehyde
• Isovaleraldehyde	• Benzaldehyde
• *n*-Hexanal	• *o*-Tolualdehyde
• *m*-Tolualdehyde	• *p*-Tolualdehyde
• 2,5-Dimethylbenzaldehyde	

Confirming separation

A mixed standard solution of 16 DNPH-derivatized aldehydes (Fujifilm Wako Pure Chemical Corporation, P/N: 018-18231) was diluted with acetonitrile to prepare a mixed standard solution containing respective aldehydes at 1 mg/L. This solution was subjected to HPLC analysis under the conditions shown in Table 8. Two columns of Shim-pack SR-C30 and Shim-pack Scepter™ C18-120 were used for the comparison.

The resulting chromatograms are shown in Fig. 2. C30 column provided good separation of all the compounds (blue box in the figure), whereas C18 column showed incomplete separation of isomers of DNPH-derivatized butyraldehyde and tolualdehyde.



1. DNPH-formaldehyde
2. DNPH-acetaldehyde
3. DNPH-acetone
4. DNPH-propionaldehyde
5. DNPH-acrolein
6. DNPH-isobutyraldehyde
7. DNPH-*n*-butyraldehyde
8. DNPH-crotonaldehyde
9. DNPH-isovaleraldehyde
10. DNPH-*n*-valeraldehyde
11. DNPH-benzaldehyde
12. DNPH-*n*-hexanal
13. DNPH-*o*-tolualdehyde
14. DNPH-*m*-tolualdehyde
15. DNPH-*p*-tolualdehyde
16. DNPH-2,5-dimethylbenzaldehyde

Fig. 2 Comparative chromatograms of 16 mixed standard solution (1 mg/L), (a) Shim-pack Scepter C18-120, (b) Shim-pack SR-C30

Table 8 Analytical conditions

System	:	i-Series LC-2070C				
Column	:	Shim-pack SR-C30 (150 mm ×4.6 mm I.D., 3 µm)				
		Shim-pack Scepter C18-120				
		(150 mm ×4.6 mm I.D., 3 µm) *1				
Mobile phase	:	A) Water				
		B) Tetrahydrofuran				
		C) Acetonitrile				
		D) Methanol				
Time Program	:	Time [min]	A [%]	B [%]	C [%]	D [%]
		0.00	50.4	9.6	32.0	8.0
		11.50	37.8	7.2	44.0	11.0
		23.00	4.2	0.8	76.0	19.0
		24.00	4.2	0.8	76.0	19.0
		24.01	50.4	9.6	32.0	8.0
		32.00	50.4	9.6	32.0	8.0
Flow rate	:	1.0 mL/min				
Mixer	:	40 µL				
Column temp.	:	30 ℃				
Injection volume	:	5 µL				
Vial	:	Shim-vial S, Amber glass				
Detection (UV)	:	360 nm				

*1 P/N：227-31016-05

Calibration curve and repeatability

Above mentioned mixed standard solution of the 16 DNPH-derivatized aldehydes was diluted with acetonitrile to prepare solutions of respective aldehydes at concentrations of 0.05, 0.1, 0.25, 0.5, 0.75, and 1 mg/L, which were then subjected to HPLC analyses.

Table 9 shows the coefficients of determination of the respective calibration curves in the range of 0.05–1 mg/L and the %RSD of peak areas from six consecutive analyses at 0.05 mg/L. All the compounds showed good linearity of coefficients of determination 0.999 or more and good repeatability of %RSD of peak areas approximately 4% at most. Furthermore, estimated limit of quantification for each compound was approximately 0.02 mg/L, which was calculated as S/N=10 equivalent concentration using each S/N value at 0.05 mg/L.

Table 9 Coefficient of determination of calibration curve and peak area repeatability

	Coefficient of determination (0.05–1 mg/L)	%RSD of peak area (0.05 mg/L)
Formaldehyde	0.99994	0.96
Acetaldehyde	0.99997	1.60
Acetone	0.99997	1.26
Propionaldehyde	0.99989	3.06
Acrolein	0.99995	1.57
Isobutyraldehyde	0.99995	1.46
n-butyraldehyde	0.99997	4.05
Crotonaldehyde	0.99995	2.03
Isovaleraldehyde	0.99984	4.09
n-Valeraldehyde	0.99986	3.37
Benzaldehyde	0.99997	3.04
n-Hexanal	0.99985	1.68
o-Tolualdehyde	0.99997	2.47
m-Tolualdehyde	0.99995	1.87
p-Tolualdehyde	0.99993	2.02
2,5-Dimethylbenzaldehyde	0.99994	3.20

■ Conclusion

Simultaneous determination of aldehydes regulated under the Offensive Odor Control Law and by the U.S. Environmental Protection Agency (EPA) was conducted using an i-Series LC-2070C integrated HPLC system equipped with a Shim-pack SR-C30 analytical column. Excellent resolution of aldehyde isomers was achieved with the C30 column, demonstrating its high structural recognition selectivity.

A quaternary gradient system comprising water, tetrahydrofuran, acetonitrile, and methanol was employed for mobile phase delivery, enabling straightforward analysis without the need for manual mobile phase preparation. In addition, satisfactory linearity and reproducibility were confirmed for each target compound.

For the simultaneous analysis of 16 aldehydes, chromatographic performance obtained with C30 and conventional C18 columns was compared. The results demonstrated that the C30 column provided complete separation of isomeric aldehydes that could not be resolved using the C18 column. Differences in separation selectivity were further evidenced by changes in the elution order of specific aldehydes between the two stationary phases. These findings indicate that the Shim-pack SR-C30 column has strong potential to expand chromatographic applicability to aldehydes and other compounds that are difficult to separate using conventional C18 columns.

<References>

1) Ministry of the Environment: Measurement Method of Specified Offensive Odor Substances, Attached 4
https://www.env.go.jp/hourei/10/000022.html (Described in only Japanese, April 1, 2026)

2) U.S. Environmental Protection Agency METHOD 8315A
https://www.epa.gov/sites/default/files/2015-07/documents/epa-8315a.pdf (April 1, 2026)

<Related Applications>

1. 01-00996-en Analysis of Formaldehyde and Acetaldehyde in Ambient Air Using Integrated HPLC System
Application News No. 01-00996

2. L533 Simultaneous Analysis of DNPH-Derivatized Aldehydes Using Prominence-i Plus and Shim-pack Scepter PFPP-120 (Described in only Japanese)
Application News No. L533

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