Application News

No. L533

High Performance Liquid Chromatography

Simultaneous Analysis of DNPH-Derivatized Aldehydes Using Prominence™-i Plus and Shim-pack Scepter™ PFPP-120

On September 21, 2018, the method for measurement of Specified Offensive Odor Substances in Article 5 of the Ordinance of Japan's Offensive Odor Control Law was revised, and was promulgated and enforced as Ministry of the Environment Notification No. 78. This revision newly added high performance liquid chromatography (HPLC method) for concentration measurement at the border of the site and flow rate measurement at the gas emission point of six aldehyde substances (Acetaldehyde, Propionaldehyde, n-Butyraldehyde, iso-Butyraldehyde, n-Valeraldehyde, and iso-Valeraldehyde). Here, we introduce an example of analysis of these six aldehydes using the Prominence-i Plus integrated high performance liquid chromatograph and the Shim-pack Scepter PFPP-120 column, which contains the pentafluorophenylpropyl (PFPP) group.

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Simultaneous Analysis of 6 DNPH-Derivatized Aldehyde Substances

Fig. 1 shows the chromatogram obtained by simultaneous analysis of 6 DNPH-derivatized aldehydes. Table 1 shows the analysis conditions. Separation of the 6 substances was satisfactory, as the degrees of separation of the structural isomers n-butyraldehyde and iso-butyraldehyde (3 and 4 in Fig. 1) and iso-valeraldehyde and n-valeraldehyde (5 and 6 in Fig. 1) were 1.88 and 1.40, respectively.

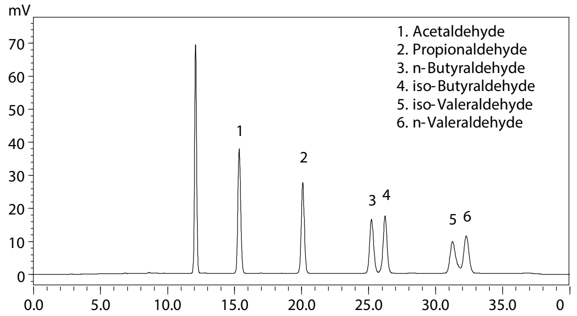

Fig. 1 Chromatogram of Mixed Standard Solution (Concentration of Each Aldehyde: 1.0 μg/mL)

Table 1 Analysis Conditions

Column	: Shim-pack Scepter PFPP-120 (150 mmL. x 4.6 mmI.D., 3 μm)
Mobile phase	: A: Water B: Methanol/Acetonitrile = 8/2 (v/v)
Time program	: B.CONC. 20 % (0 min) → 55 % (5 min) → 60 % (25 min) → 60 % (25-35 min) → 20 % (35-40 min)
Flow rate	: 1.0 mL/min
Injection volume	: 20 μL
Column temp.	: 35 °C
Detection	: UV 360 nm (D_2 Lamp)

Linearity and Repeatability

The concentration *C* of the analyte components in the atmosphere, which corresponds to the odor intensity of the respective 6 aldehyde substances designated in the regulatory standard, was converted to the concentration $\frac{As-At}{v}$ [μg/mL] obtained by the HPLC method by using Eq. 1 (Table 2 and 3). Figs. 2 to 7 show the calibration curves of the mixed standard solutions of the 6 aldehyde substances in the case of odor intensities of 2.5 and 3.5. Satisfactory linearity was obtained for all components, as the contribution ratio (R^2) was 0.9999 or higher. For iso-valeraldehyde, which has the smallest regulatory standard, the lower limit of quantitation was 0.0052 μg/mL and the lower limit of detection was 0.0017 μg/mL. The relative error (%) (n = 1) of the calibration curves at the lower limit concentration and the relative standard deviation (%RSD) (n = 6) of the peak area value, which is an index of repeatability at that concentration, were also obtained, as shown in Table 4. Relative error was 5% or less and %RSD was 1% or less for all components, confirming the accuracy of this method.

It may be noted that all of the standard solutions were diluted with acetonitrile.

$$C = \frac{22.4\,(As\text{-}At) \times E}{MvV \times \frac{273}{273+t} \times \frac{P}{101.3}} \qquad (1)$$ [1]

C	: Concentration of analyte component in atmosphere [ppm]	Calculated at respective prescribed values
As	: Volume of analyte injected in HPLC [μg]	
At	: Value of reference blank of aldehyde species [μg]	Calculated at 0 μg
E	: Amount of test solution [mL]	Calculated at 5 mL
M	: Molecular weight of analyte component	
v	: Volume injected in HPLC [mL]	Calculated at 20×10^{-3} mL
V	: Volume of suction gas measured by gas meter [L]	Calculated at 30 L
t	: Temperature in gas meter [°C]	Calculated at 25 °C
P	: Atmospheric pressure at time of sample capture [kPa]	Calculated at 101.3 kPa

Table 2 Concentrations Corresponding to Odor Intensity of 2.5

Specified offensive odor substance	Concentration in atmosphere C [ppm]	Concentration by HPLC method $\frac{As-At}{v}$ [μg/mL]
Acetaldehyde	0.05	0.54
Propionaldehyde	0.05	0.71
n-Butyraldehyde	0.009	0.16
iso-Butyraldehyde	0.02	0.35
iso-Valeraldehyde	0.003	0.063
n-Valeraldehyde	0.009	0.19

Table 3 Concentrations Corresponding to Odor Intensity of 3.5

Specified offensive odor substance	Concentration in atmosphere C [ppm]	Concentration by HPLC method $\frac{As-At}{v}$ [μg/mL]
Acetaldehyde	0.5	5.4
Propionaldehyde	0.5	7.1
n-Butyraldehyde	0.08	1.4
iso-Butyraldehyde	0.2	3.5
iso-Valeraldehyde	0.01	0.21
n-Valeraldehyde	0.05	1.1

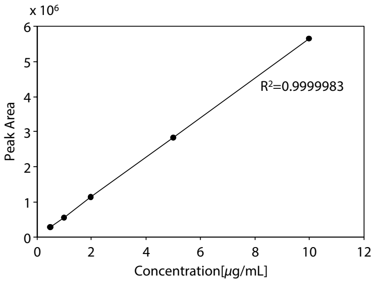

Fig. 2 Calibration Curve of Acetaldehyde (0.5, 1, 2, 5, and 10 μg/mL)

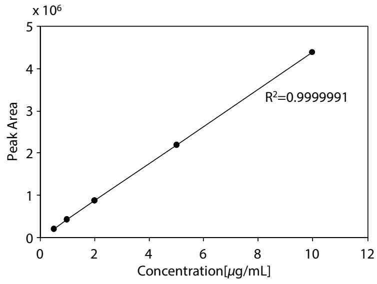

Fig. 3 Calibration Curve of Propionaldehyde (0.5, 1, 2, 5, and 10 μg/mL)

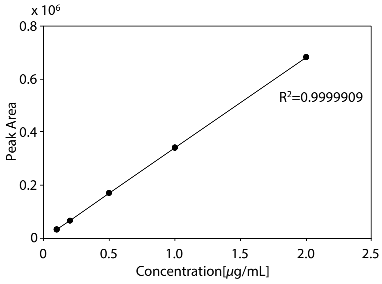

Fig. 4 Calibration Curve of n-Butyraldehyde (0.1, 0.2, 0.5, 1 and 2 μg/mL)

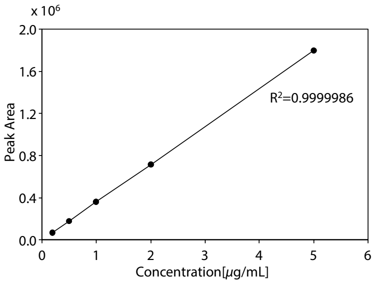

Fig. 5 Calibration Curve of iso-Butyraldehyde (0.2, 0.5, 1, 2, and 5 μg/mL)

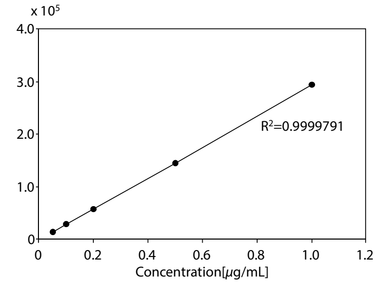

Fig. 6 Calibration Curve of iso-Valeraldehyde (0.05, 0.1, 0.2, 0.5, and 1 μg/mL)

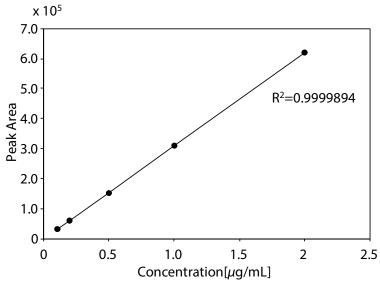

Fig. 7 Calibration Curve of n-Valeraldehyde (0.1, 0.2, 0.5, 1, and 2 μg/mL)

Table 4 Relative Error (n = 1) and %RSD of Peak Area Value (n = 6) at Each Concentration

Specified offensive odor substance	Lower limit concentration of calibration curve [μg/mL]	Relative error [%]	%RSD of peak area value [%]
Acetaldehyde	0.5	-1.3	0.089
Propionaldehyde	0.5	-1.0	0.096
n-Butyraldehyde	0.1	1.8	0.19
iso-Butyraldehyde	0.2	-0.60	0.22
iso-Valeraldehyde	0.05	1.7	0.64
n-Valeraldehyde	0.1	3.0	0.18

Simultaneous Analysis with Prominence High-Pressure Gradient System

Fig. 8 shows the chromatogram obtained by simultaneous analysis of the 6 DNPH-derivatized aldehydes using the high-pressure gradient system of the Prominence modular-type high performance liquid chromatograph. Table 5 shows the analysis conditions. The degrees of separation of n-butyraldehyde and iso-butyraldehyde (3 and 4 in Fig. 8) and iso-valeraldehyde and n-valeraldehyde (5 and 6 in Fig. 8) were 2.00 and 1.54, respectively, showing satisfactory separation of the 6 aldehydes. This results were similar to those obtained with the Prominence-i Plus (low-pressure gradient system).

Table 5 Analysis Conditions

Column : Shim-pack Scepter PFPP-120
(150 mmL. x 4.6 mmI.D., 3 μm)
Mobile phase : A: Water
B: Methanol/Acetonitrile = 8/2 (v/v)
Time program : B.CONC. 20% (0 min) → 55% (5 min) → 60%
(25 min) → 60% (25-35 min) → 20% (35-40 min)
Flow rate : 1.0 mL/min
Injection volume : 20 μL
Column temp. : 35 °C
Detection : UV 360 nm (D_2 Lamp)
Mixer volume : 0.5 mL

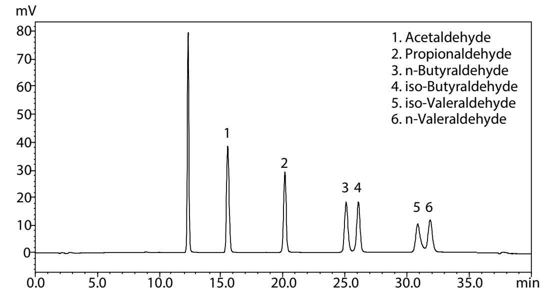

Fig. 8 Chromatogram of Mixed Standard Solution Obtained with Prominence High-Pressure Gradient System (Concentration of Each Aldehyde: 1.0 μg/mL)

Conclusion

This experiment demonstrated that separation and quantitation of a mixed standard solution of 6 DNPH-derivatized aldehydes is possible by using the Prominence-i Plus integrated high performance liquid chromatograph and Shim-pack Scepter PFPP-120 column. Satisfactory separation of the two pairs of structural isomers, n-butyraldehyde and iso-butyraldehyde and n-valeraldehyde and iso-valeraldehyde, was possible by using the Shim-pack Scepter PFPP-120, which contains the PFPP group. Similar results were also obtained with a high-pressure gradient system using a Prominence modular-type high performance liquid chromatograph, demonstrating that this system can also support the prescribed test method for the 6 aldehyde substances.

<Reference>
(1) Notification of partial revision of the measurement method for Specified Offensive Odor Substances (Sept. 2018, Ministry of the Environment Notification No. 78)

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