SHIMADZU
Excellence in Science

Application News

No.L476

High Performance Liquid Chromatography

Rapid Analysis of 2,4-DNPH-Derivatized Aldehydes and Ketones Using the Prominence-i with a Shim-pack XR-ODS Column

Application News No. L268 presented a rapid analysis of 2,4-DNPH (2,4-dinitrophenylhydrazine)-derivatized aldehydes and ketones using the LC-2010 integrated high-performance liquid chromatograph with a Shim-pack FC-ODS column. As the goal of this study was to obtain a shorter the analysis time than that using the previous method, we investigated the run conditions for 2,4-DNPH-derivatized aldehydes and ketones using the new Prominence-i integrated high-performance liquid chromatograph with a 2.2 µm particle size Shim-pack XR-ODS column. Here we present the results of simultaneous analysis of thirteen 2,4-DNPH-derivatized aldehyde and ketone standards run on a conventional 5 µm particle column, and an example of rapid analysis with a 2.2 µm particle column.

Simultaneous Analysis of 2,4-DNPH-Derivatized Aldehydes and Ketones

Fig. 1 shows the chromatogram obtained from simultaneous analysis of thirteen 2,4-DNPH-derivatized aldehydes and ketones using the analytical conditions shown in Table 1. With the conventional VP-ODS column, separation of the thirteen components is complete within a 30 minute run time. The detection limit and quantitation limit for formaldehyde were determined to be 13 pg and 41 pg, respectively.

Table 1 Analytical Conditions

Column	: Shim-pack VP-ODS (150 mm L. × 4.6 mm I.D., 5 µm)
Mobile Phase	: A: Water/THF = 8/2 B: Acetonitrile
Time Program	: B Conc. 20 % → 60 % (30 min) → 20 % (30-35 min)
Flowrate	: 1.5 mL/min
Injection Volume	: 10 µL
Column Temp.	: 40 °C
Detection	: UV 360 nm

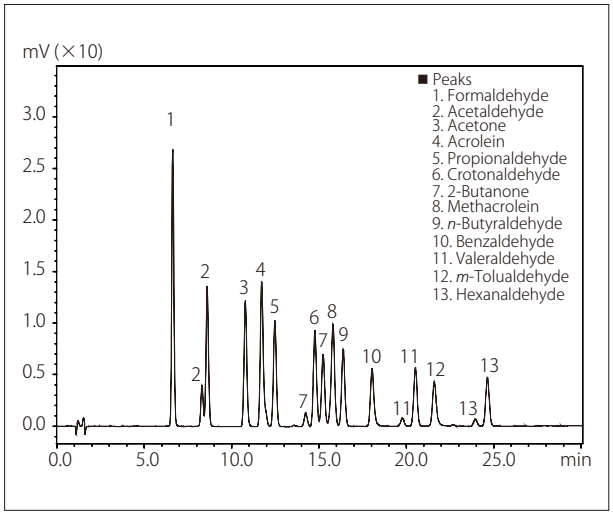

Fig. 1 Chromatogram of Standard Sample (each at 1.0 mg/L as carbonyl compounds)

Linearity

Calibration curves were generated for standard solutions of thirteen 2,4-DNPH-derivatized aldehydes and ketones (each at 0.03 – 3 mg/L, 10 µL injected) using the Shim-pack VP-ODS. Fig. 2 shows the calibration curves for formaldehyde, 2-butanone, and hexanaldehyde. All three compounds produced a linear plot, with an R^2 value of greater than 0.9999 for each. Table 2 shows the area reproducibility values (n=3), using a concentration of 1.0 mg/L.

The area %RSD values were within 1 % for all components, indicating very good repeatability.

Table 2 Repeatability of Peak Area (n=3)

	Area %RSD		Area %RSD
Formaldehyde	0.429	Methacrolein	0.186
Acetaldehyde	0.209	*n*-Butyraldehyde	0.095
Acetone	0.728	Benzaldehyde	0.979
Acrolein	0.108	Valeraldehyde	0.580
Propionaldehyde	0.222	*m*-Tolualdehyde	0.355
Crotonaldehyde	0.765	Hexanaldehyde	0.644
2-Butanone	0.969		

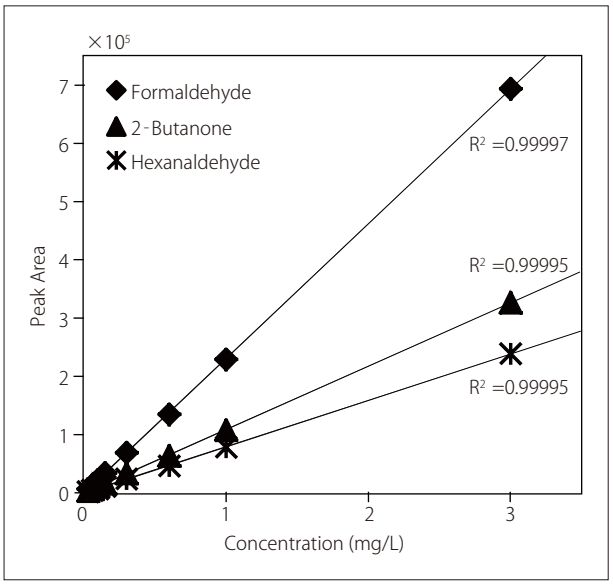

Fig. 2 Linearity

Rapid Analysis of 2,4-DNPH-Derivatized Aldehydes and Ketones

Next, we investigated the use of the Shim-pack XR-ODS (75 mm × 4.6 mm, 2.2 µm) column to shorten the run time. Fig. 3 shows the resulting chromatogram, and Table 3 shows the analytical conditions used. The detection limit of formaldehyde was determined to be 12 pg, and the limit of quantitation, 37 pg. Even with the analysis time shortened by half, from 30 minutes to 15 minutes, the chromatogram showed no adverse effects on resolution or peak shape.

Table 3 Analytical Conditions

Column	: Shim-pack XR-ODS (75 mm L. × 4.6 mm I.D., 2.2 µm)
Mobile Phase	: A : Water/THF = 8/2
	B : Acetonitrile
Time Program	: B Conc. 20 % → 60 % (15 min) → 20 % (15-17 min)
Flowrate	: 1.5 mL/min
Injection Volume	: 10 µL
Column Temp.	: 40 °C
Detection	: UV 360 nm

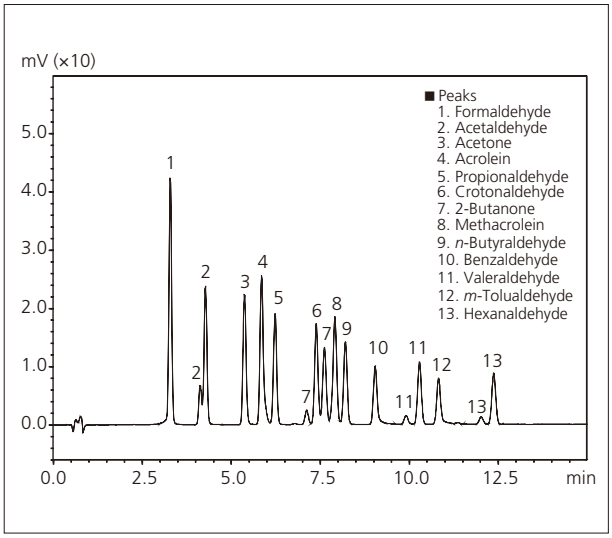

Fig. 3 Chromatogram of Standard Sample (each at 1.0 mg/L as carbonyl compounds)

Linearity in Rapid Analysis

Calibration curves were also generated using the Shim-pack XR-ODS for high-speed analysis of solutions at concentrations ranging from 0.03 to 3 mg/L (10 µL injected) prepared by stepwise dilution of the stock standard solutions of the thirteen 2,4-DNPH-derivatized aldehydes and ketones. Fig. 4 shows the calibration curves for formaldehyde, 2-butanone, and hexanaldehyde. These also showed excellent linearity with R^2 values greater than 0.9999. The area %RSD values at a concentration of 1.0 mg/L for all components were within 1 %, as shown in Table 4.

Table 4 Repeatability of Peak Area (n=3)

	Area %RSD		Area %RSD
Formaldehyde	0.255	Methacrolein	0.229
Acetaldehyde	0.139	*n*-Butyraldehyde	0.333
Acetone	0.139	Benzaldehyde	0.247
Acrolein	0.226	Valeraldehyde	0.101
Propionaldehyde	0.171	*m*-Tolualdehyde	0.917
Crotonaldehyde	0.423	Hexanaldehyde	0.579
2-Butanone	0.333		

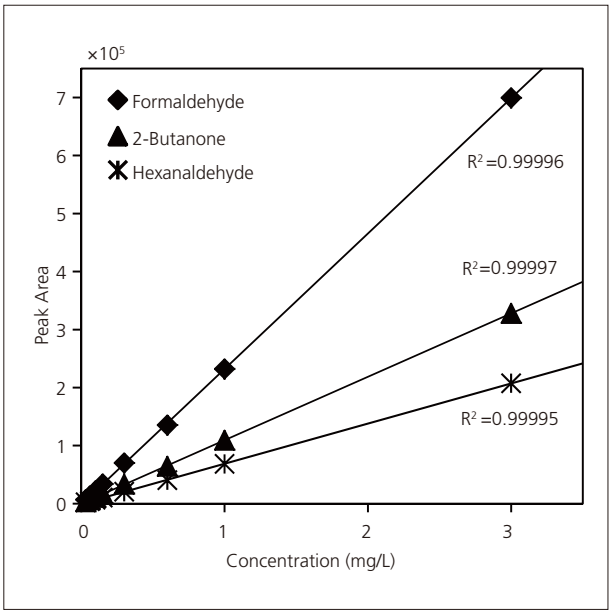

Fig. 4 Linearity

First Edition: Nov. 2014

Shimadzu Corporation
www.shimadzu.com/an/