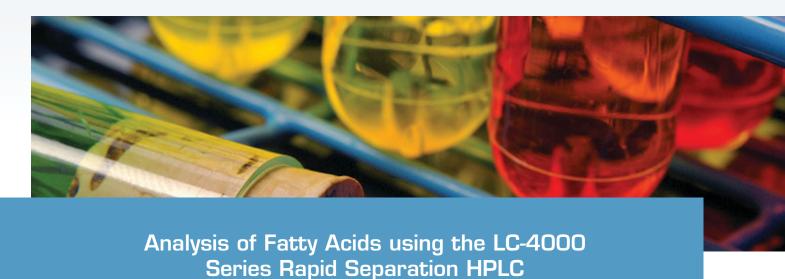
## **Application Note**





## Introduction

An analytical HPLC system using microparticulate packing materials requires a lower system volume, higher operating pressure and high speed response. Rapid separation HPLC (RHPLC) is likely to become the new standard for high performance analysis, covering measurement in a wide range of applications from conventional HPLC to some types of UHPLC. The following is an analysis report for the measurement of fatty acids using a Unifinepak C18 column with the RHPLC system including a refractive index detector (RI-4035) for a semi-micro scale measurement.

**Keyword:** RHPLC, fatty acids, semi-micro, ODS, Refractive index detector

## **Experimental**

[Equipment] [Conditions]

Pump: PU-4185 Column: A) Unifinepak C18 (4.6 mml.D. x 150 mmL, 5 µm)
Pump option: Degasser unit B) Unifinepak C18 (2.0 mml.D. x 150 mmL, 1.9 µm)

Autosampler: AS-4150 C) Unifinepak C18 (2.0 mml.D. x 30 mmL, 1.9 µm)

Column oven: CO-4060 Eluent: 0.1%TFA/Acetonitrile (20/80)

Detector: RI-4035 Flow rate: A; 1.2 mL/min, B-C; 0.6 mL/min

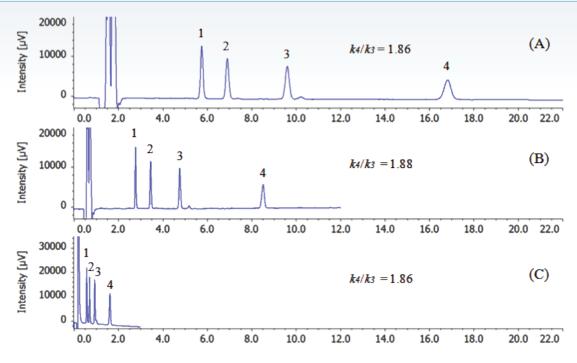
Column temp.: 40°C

Injection volume: A; 10 µL, B-C; 2 µL

Standard Sample: Fatty acids mixture (1 mg/mL per component)

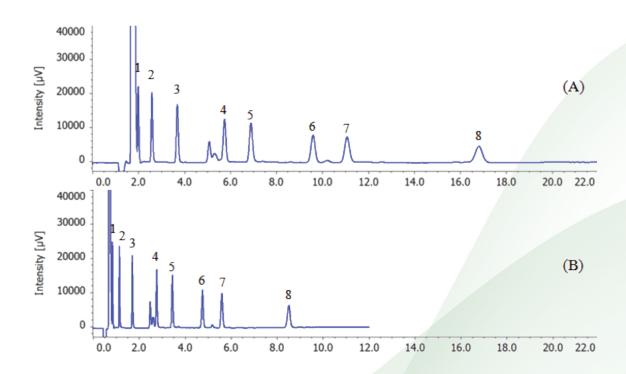
## **Results**

Fig. 1 Chromatograms of fatty acids were measured using the LC-4000 series RHPLC system with conventional HPLC and sub 2  $\mu$ m UHPLC columns. The RI-4035 refractive index detector is optimized for lower flow rates and allows the acquisition of chromatograms with improved peak shape and a higher resolution separation. Unifinepak offers a common packing material in a range of particle sizes, which is independent of the measurement method, such as column size.



**Figure 1.** Chromatogram of fatty acid standard 1: Myristic acid, 2: Linoleic acid, 3: Palmitic acid, 4: Stearic acid

Fig. 2 Chromatograms for an 8-component fatty acid mixture using columns with two different particle sizes (A column: 5 µm, B column: 1.9 µm).



**Figure 1.** Chromatogram of 8-component fatty acid mixture 1: Caprylic acid, 2: Capric acid, 3: Lauric acid, 4: Myristic acid, 5: Linoleic acid, 6: Palmitic acid, 7: Elaidic acid, 8: Stearic acid



P/N	Description
7003-J014A	PU-4185 RHPLC Semi-micro Pump (Base Unit)
7006-H003A	DG-4000-04 4-channel Degasser Unit, for Analytical
7064-J002A	AS-4150 RHPLC Autosampler
7021-J002A	CO-4060 Column Oven
7031-J002A	RI-4035 Refractive Index Detector, for Semi-micro LC
7058-J011A	BS-4000-1 Bottle stand
7059-J012A	ChromNAV Ver.2 Chromatography Data System
6688-H564A	LC-Net CG cable (3x)
7001-H401A	HPLC Start Up Kit for LC-4000
7001-H403A	RHPLC/UHPLC Start Up Kit for LC-400
7001-H405A	Maintenance tool kit
	Unifinepak C18 (4.6 mmID x 150 mmL, 5µm)
	Unifinepak C18 (2.0 mmID x 150 mmL, 1.9µm)
	Unifinepak C18 (2.0 mmlD x 30 mmL, 1.9µm)

