SHIMADZU
Excellence in Science

Application News

Supercritical Fluid Chromatograph Nexera™ UC

Simultaneous Analysis of Carboxylic Anhydrides and Hydrolysates Using Supercritical Fluid Chromatography

Yusuke Masuda

User Benefits

- Supercritical Fluid Chromatography (SFC) allows analysis of carboxylic anhydrides without hydrolysis.
- Efficient column scouting is possible using a package of SFC columns with different separation selectivities.
- Simultaneous analysis of carboxylic anhydrides and hydrolysates is possible using a single method by switching modifiers during the analysis.

■ Introduction

Carboxylic anhydrides are easily decomposed by water or alcohol, making analysis by reversed-phase liquid chromatography difficult. Therefore, normal phase liquid chromatography (NPLC) is often used for direct analysis of carboxylic anhydrides. However, NPLC uses a large amount of organic solvent such as hexane and chloroform, which raises concerns about human health and environmental impact.

In this article, the use of supercritical fluid chromatography (SFC) for the analysis of carboxylic anhydrides is examined. SFC uses non-polar carbon dioxide as the mobile phase, similar to NPLC with lower solvent cost and environmental impact. In addition, SFC does not require water or alcohol as the mobile phase, which is expected to reduce the effect of decomposition on the analytical results.

■ Selecting an SFC Column

For efficient method development, column scouting using six stationary phases (Shim-pack™ UC Series) with varying retention mechanisms was carried out to identify the most suitable column to separate a mixture of four model compounds. Table 1 shows the stationary phase structures and features of the columns.

■ Model Compounds

Four isomers of biphthalic anhydride (BPDA) and oxydiphthalic anhydride (ODPA), known raw materials for polyimide, were used as model compounds for carboxylic anhydride (Fig. 1). These compounds were dissolved in acetonitrile (superdehydrated) to suppress hydrolysis.

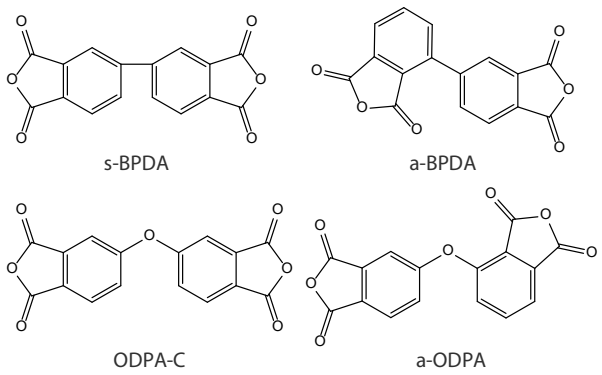
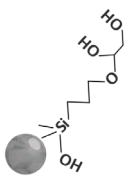
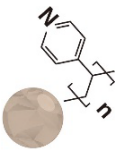
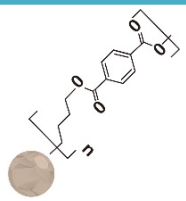
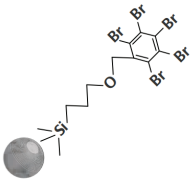
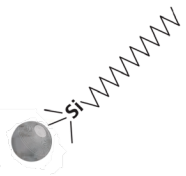


Fig. 1 Structure of Model Compounds

Table 1 Structures and Features of Stationary Phase Used for Column Scouting

	Shim-pack UC-Diol II	Shim-pack UC-Sil II	Shim-pack UC-PolyVP
Chemistry			
Feature	The separation mode is normal phase. This inhibits non-specific interactions.	This is excellent for retention of basic compounds and recognition of their tertiary structures.	A favorable peak shape is obtained even without acid-base additives.
	Shim-pack UC-PolyBT	**Shim-pack UC-PBr**	**Shim-pack UC-ODS**
Chemistry			
Feature	This is excellent for resolving aromatic compounds through π-π interactions.	With ODS, separation of poorly retained compounds is improved.	The separation mode is reverse phase. Retention is provided through hydrophobic interaction

■ Method Development System

The software for analytical method development allows the creation of multiple analytical methods in only five steps, which simplifies and reduces the overall workload for creating a method screening experiment and easily determining optimum analytical conditions (Fig. 2).

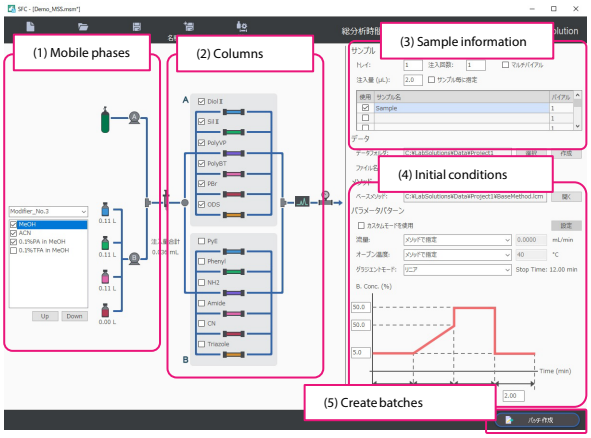


Fig. 2 User Interface of the Software

■ Column Scouting Results

Fig. 3 shows chromatograms obtained in the column screening experiment. The best separation was achieved using the Shim-pack UC-PolyBT column. Table 2 shows column scouting conditions. Acetonitrile was used as a modifier to suppress hydrolysis of the carboxylic anhydride.

Table 2 Column Scouting Conditions

Column:	Shim-pack UC-Diol II, UC-Sil II, UC-PolyVP, UC-PolyBT, UC-PBr, UC-ODS (250 mm × 4.6 mm I.D., 5 µm)*1
Mobile Phase A:	CO_2
Mobile Phase B:	Acetonitrile
Flowrate:	3.0 mL/min
Time Program:	B. Conc. 5 % (0 min) → 50 % (8 - 10 min) → 5 % (10 - 12 min)
Column Temp.:	40 °C
BPR Pressure:	15 MPa
BPR Temp.:	50 °C
Detection:	300 nm (PDA with a high-pressure flow cell)
Injection Volume:	2 µL in acetonitrile
Vial:	SHIMADZU LabTotal™ for LC 1.5 mL, Glass*2

*1 P/N : 227-32606-22, 227-32607-22, 227-32509-02, 227-32503-02, 227-32602-22 and 227-32608-25 in that order

*2 P/N : 227-34001-01

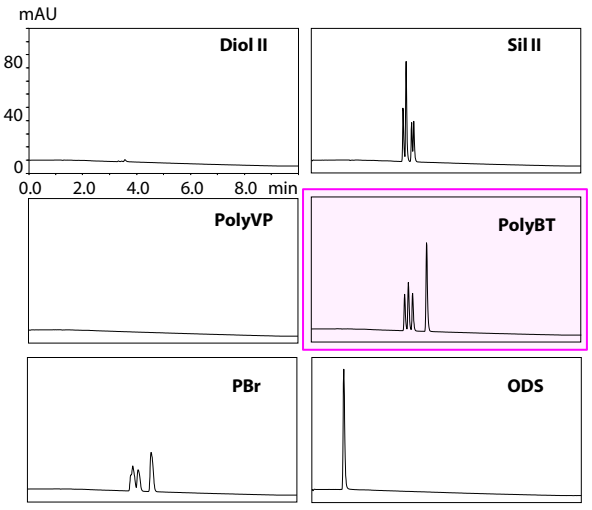


Fig. 3 Chromatograms Obtained by Column Screening Experiment

*3 These scales are identical.

■ Optimization of Analytical Conditions

Based on the results obtained, the analytical conditions were optimized to create a method that offers baseline separation of the four model compounds with similar structures in about 7 min (separation > 2.1) (Table 3, Fig. 4).

Calibration curves created for concentrations of 5 - 125 mg/L showed good linearity and reproducibility with R^2 > 0.999 and %RSD (n = 6) < 1.0 (Table 4, Fig. 5).

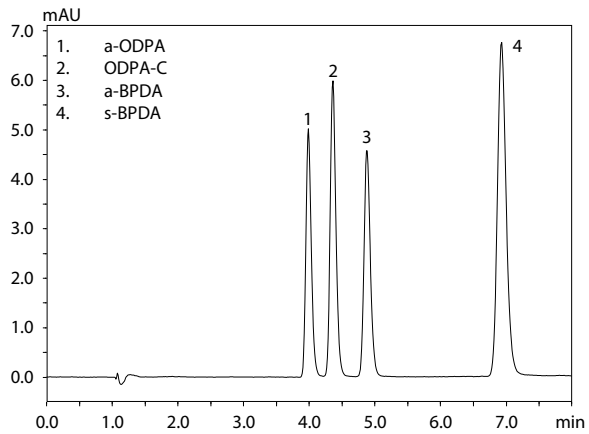


Fig. 4 Chromatogram Using Optimized Conditions

Table 3 Optimized Analytical Conditions

Column:	Shim-pack UC-PolyBT (250 mm × 4.6 mm I.D., 5 µm)
Mobile Phase:	CO_2 : acetonitrile = 90/10
Flowrate:	3.0 mL/min
Column Temp.:	40 °C
BPR Pressure:	15 MPa
BPR Temp.:	50 °C
Detection:	300 nm (PDA with a high-pressure flow cell)
Injection Volume:	2 µL in acetonitrile
Vial:	SHIMADZU LabTotal™ for LC 1.5 mL, Glass

Table 4 Repeatability (%RSD) and Linearity of Calibration Curve (R^2)

Sample	%RSD (peak area, n = 6 (50 mg/L))	R^2 (5, 10, 25, 50, 125 mg/L)
a-OPDA	0.68	0.9999
OPDA-C	0.84	0.9998
a-BPDA	0.85	0.9997
s-BPDA	0.93	0.9993

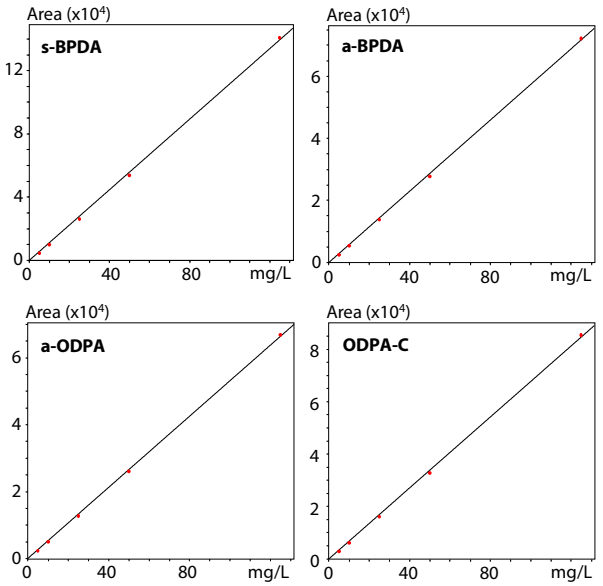


Fig. 5 Calibration Curves for Each Compound

■ Simultaneous Analysis of Carboxylic Anhydrides and Hydrolysates

The simultaneous analytical method for carboxylic anhydrides and its hydrolyzed products was developed by switching modifiers. After elution of the carboxylic anhydride, the valve with the built-in modifier feed pump was switched, and the modifier was changed from acetonitrile to 0.1 % phosphoric acid in methanol (Table 5, Fig. 5). The results show that SFC can be used for simultaneous analysis of compounds with widely different characteristics.

Table 5 Analytical Conditions

Column:	Shim-pack UC-PolyBT (250 mm × 4.6 mm I.D., 5 µm)
Mobile Phase A:	CO_2
Mobile Phase B:	Acetonitrile (0 - 8 min), 0.1 % phosphoric acid in MeOH (8 - 30 min)
Flowrate:	3.0 mL/min
Time Program:	B. Conc. 10 % (0 - 8 min) → 5 % (8 - 50 min)
Column Temp.:	40 °C
BPR Pressure:	15 MPa
BPR Temp.:	50 °C
Detection:	MaxPlot 250 - 300 nm (PDA with a high-pressure flow cell)
Injection Volume:	2 µL
Vial:	SHIMADZU LabTotal™for LC 1.5 mL, Glass

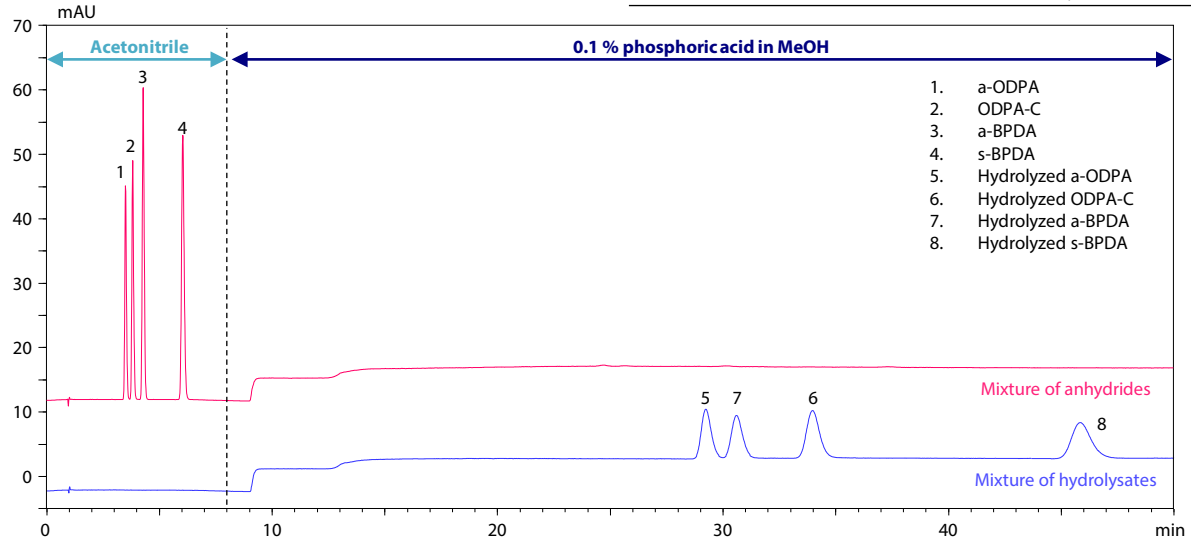


Fig. 6 Chromatograms of Carboxylic Anhydrides (Top) and Hydrolysates (Bottom)

■ Repeatability of Retention Times and Area for Hydrolysates

The retention time and peak area of each compound showed good reproducibility with %RSD (n = 6) < 0.9 (retention time) and %RSD (n = 6) < 1.1 (peak area). Each peak of the hydrolysate was separated well (separation > 1.5) (Table 6).

Table 6 Repeatability of Retention Time and Peak Area (%RSD)*4

Sample	%RSD (retention time, n = 6 (50 mg/L))	%RSD (peak area, n = 6 (50 mg/L))
Hydrolyzed a-ODPA	0.76	0.67
Hydrolyzed ODPA-C	0.80	0.62
Hydrolyzed a-BPDA	0.81	0.88
Hydrolyzed s-BPDA	0.83	1.08

*4 wavelength 254 nm

■ Conclusion

This article introduces SFC analysis of carboxylic anhydrides without hydrolysis. The simultaneous analytical method for carboxylic anhydrides and its hydrolyzed products was achieved by switching modifiers. This method is expected to be applied to the measurement of impurities and sample degradation indicators.

The method development system was used for fast and efficient method development, reducing the risk of human error, by automated creation of multiple screening methods. One big advantage of using SFC is that CO_2 is less expensive and more environmentally friendly than many organic solvents used in LC, and there is no need for waste disposal.

01-00478-EN First Edition: Jul. 2023

SHIMADZU
Shimadzu Corporation
www.shimadzu.com/an/