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Application News

Ultra Fast Preparative and Purification LC System Nexera™ UFPLC

Preparative Purification of Ibuprofen and Related Substances by Nexera UFPLC

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User Benefits

- Fully automated purification process (Fractionation, purification, concentration, recovery, etc.) significantly reduces the operator workload and improves the overall analytical efficiency.
- Target compounds can be recovered after removing the salt contained in the mobile phase solvent.
- The time required for the drying process of the fraction can be reduced because the fraction with a high content of organic solvent can be recovered.

■ Introduction

Preparation and purification by liquid chromatography is a widely-used technique in the pharmaceutical, food, and chemical industries for drug synthesis, finding effective compounds in natural products, and for structural analysis of unknown trace compounds. Nexera UFPLC*1 enables substantial labor savings in preparative purification by automating not only the fractionation of the target compound but also related processes such as concentration, purification, and recovery. This article describes an example of preparative purification of a mixed sample of the pharmaceutical ibuprofen and its analogues using Nexera UFPLC (Fig. 1).

*1 UFPLC: Ultra Fast Preparative and Purification Liquid Chromatograph

Fig. 1 Ultra Fast Preparative and Purification LC system Nexera™ UFPLC

■ Preparative Purification by UFPLC

UFPLC automatically performs the various processes related to preparative isolation of target compounds using a combination of preparative LC and trapping columns. The details of those processes are as follows.

1. Separate target compounds in a complex sample by preparative LC and introduce them into trapping columns (fractionation and concentration)
2. Wash the trapping columns online to remove impurities and counter ions (purification)
3. Elute target compounds from trapping columns using organic solvent (elution)

An outline of the respective processes is shown in Fig. 2, and a simplified flow chart of UFPLC is shown in Fig. 3.

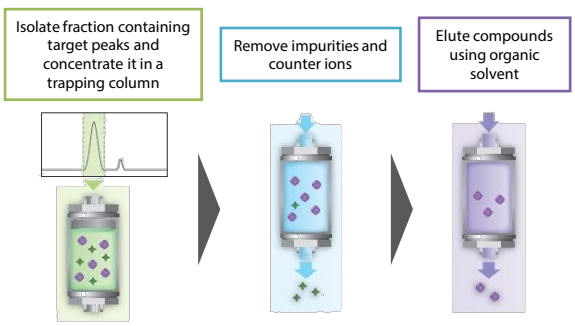


Fig. 2 Flow of Fractionation, Concentration, Purification, and Elution by UFPLC

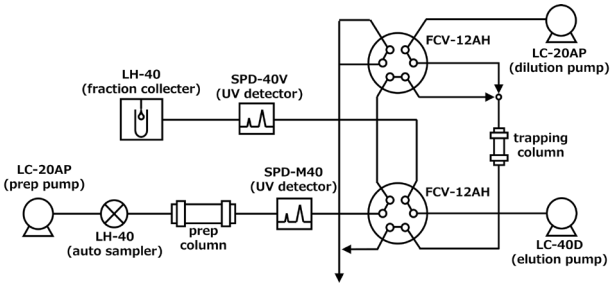


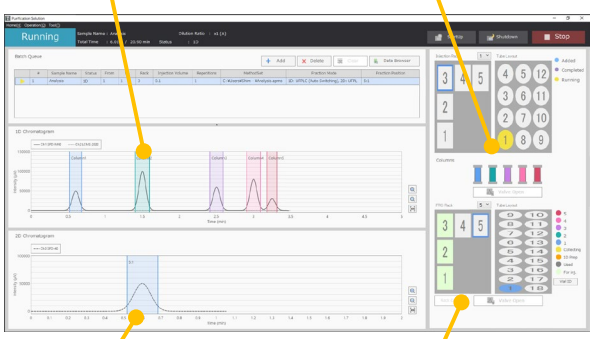
Fig. 3 A simplified flow chart of UFPLC
(Display the flow path while introducing the target compound into the trapping column)

■ UFPLC Dedicated Software Purification Solution™

For preparation and purification by UFPLC, the dedicated Purification Solution software is used. Purification Solution facilitates the entire process, from setting the preparative conditions to the liquid recovery of the target compound on a simple user interface. The Purification Solution operation screen is shown in Fig. 4. The chromatogram of preparative separation, the trapping column into which fractions were introduced, and the elution chromatogram after purification are displayed on a single screen. It allows the user to confirm the entire process of preparative purification for the target compounds at a glance.

Preparative LC Chromatogram
The peaks corresponding to each fraction are color-coded

Trapping Column
Trapping column is displayed in the same color as fractionated peak



Elution Chromatogram
Color-coded display of the area collected in the fraction collector in the same color as the trapping column

Fraction Collector
Shows location of final recovery solution on fraction collector

Fig. 4 Purification Solution™ Operation Screen

■ Preparative Purification of Ibuprofen and its Analogues

Ibuprofen is a non-steroidal anti-inflammatory drug (NSAID) used as a fever-reducing drug and analgesic. The United States Pharmacopeia (USP) provides analytical methods for ibuprofen and its analogue, 4-isobutylacetophenone, using valerophenone as an internal standard. Each compound was separately purified to high purity from a mixed solution of these three compounds using Nexera UFPLC.

Fig. 5 shows the structural formulas of ibuprofen and its analogues, and Table 1 shows the preparative purification conditions. The mixed solution was prepared by dissolving the three target compounds in the mobile phase to adjust the content of each compound to 10 mg/mL.

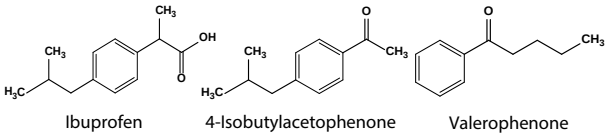


Fig. 5 Structural Formulas of Ibuprofen and its Analogues

Table 1 Preparative and Purification LC

System:	Nexera UFPLC
Preparative LC Conditions	
Column:	Shim-pack™ Scepter C18-120*1 (150 mm × 20 mm I.D., 5 µm)
Mobile Phase:	A) 1 % (wt/v) Chloroacetic acid in water (pH3*2) B) Acetonitrile A/B = 40 : 60 (v/v)
Flowrate:	20 mL/min
Column Temp.:	Ambient
Injection Vol.:	200 µL (Fig. 5) 2000 µL (Fig. 8)
Detection:	230 nm (SPD-M40, High pressure preparative flow cell)
Rinsing Conditions	
Column:	Shim-pack UFPLC 20×30*3 (30 mm × 20 mm I.D., 20-30 µm)
Rinse Solvent:	A) Acetonitrile/water = 2 : 98 (v/v) B) 0.2 % Formic acid in water C) Water
Time Program:	A 40 mL/min (0.01-3 min) → B 15 mL/min (3.01-11 min) → C 40 mL/min (11.01-15.9 min)
Elution Conditions	
Eluent:	Acetonitrile
Flowrate:	9 mL/min
Detection:	230 nm (SPD-40V, Preparative flow cell)

*1 P/N: 227-31102-03 *2 pH 3.0 adjusted with ammonium hydroxide
*3 P/N: 228-80220-41

■ Verification of Purity of Ibuprofen and its Analogues

Fig. 6 shows the preparative LC chromatogram of the mixed solution. The injection volume was 200 µL. The fractions of ibuprofen, valerophenone and 4-isobutylacetophenone collected by Nexera UFPLC were analyzed by Nexera XR to verify the purity of the compounds. Table 2 shows the analytical conditions, and Fig. 7 shows the chromatograms. Table 3 shows the purities of the target compounds in each fraction by area percentage (peak detection range: 2.5-15 min).

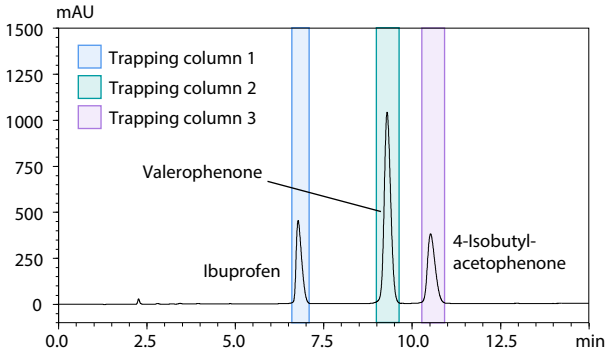


Fig. 6 Preparative LC Chromatogram of Ibuprofen and its Analogues (Nexera UFPLC)

Table 2 Analytical Conditions for Purity Verification

System:	Nexera XR*1
Column:	Shim-pack Scepter C18-120*2 (150 mm × 4.6 mm I.D., 5 µm)
Mobile Phase:	A) 1 % (wt/v) Chloroacetic acid in water (pH 3*3) B) Acetonitrile A/B = 40 : 60 (v/v)
Flowrate:	0.8 mL/min
Column Temp.:	30 °C
Injection Vol.:	10 µL
Detection:	230 nm (SPD-40V, Standard flow cell)

*1 600 mm × 0.3 mm I.D. tubing was used to connect the SIL-40C XR autosampler to the column inlet
*2 P/N: 227-31020-05
*3 pH 3.0 adjusted with ammonium hydroxide

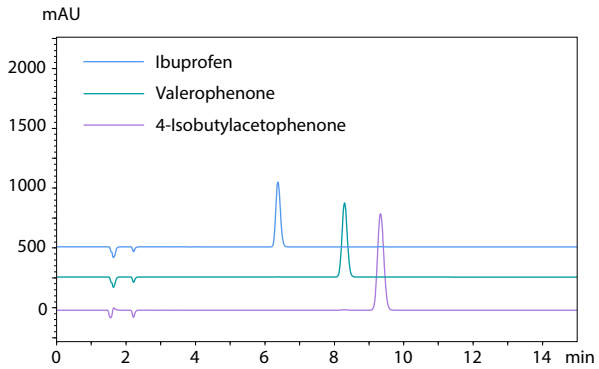


Fig. 7 Chromatograms of Fraction Obtained by Nexera UFPLC (Nexera XR)

Table 3 Purities of Target Compounds Contained in Collected Fractions (Area Percentage, Peak detection range : 2.5-15 min)

Compound	Area %
Ibuprofen	99.5
Valerophenone	99.8
4-Isobutylacetophenone	99.6

■ Concentration and Drying of Ibuprofen Fraction

When a complex sample is separated and collected by preparative LC, the solvent may be dried in order to use the collected fraction in the next step. However, the fraction obtained in reversed-phase mode contains water, so drying takes a long time. Moreover, if the mobile phase contains non-volatile compounds, these compounds may be precipitated together with the target compound during drying, resulting in a decrease in the purity of the recovered target compound. In preparative purification using UFPLC, the fraction containing the target compound is introduced and concentrated into a trapping column and then purified on the trapping column by washing the mobile phase components during preparation process. In addition, the drying time is shortened substantially due to the use of an organic solvent for sample recovery from the trapping columns.

UFPLC can also concentrate target compounds in a trapping column by repeatedly injecting the sample and introducing the fraction containing the target compound into the same trapping column. The trapping column used in this paper, Shim-pack UFPLC 20×30, has a maximum loading capacity of 100 mg.

The concentration, purification, and recovery processes can be carried out efficiently by introducing the target compound repeatedly into this trapping column, even if the concentration of the target compound in the analysis sample solution is low.

Here, after injecting 2000 µL of the mixed solution and separating ibuprofen by preparative LC under the conditions shown in Table 1, the ibuprofen fraction was introduced into a Shim-pack UFPLC 20×30. The procedure was repeated 5 times, and after concentrating and purifying the sample in the trapping column, the target compound was eluted with an organic solvent (sample load to the trapping column: 100 mg). The preparative purification flow is shown in Fig. 8, and the preparative chromatogram of the mixed solution is shown in Fig. 9.

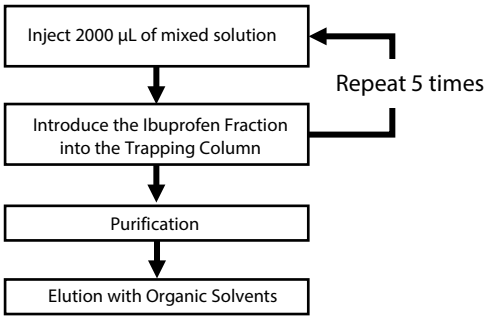


Fig. 8 Flow of Recovery of High Concentration Fraction of Ibuprofen

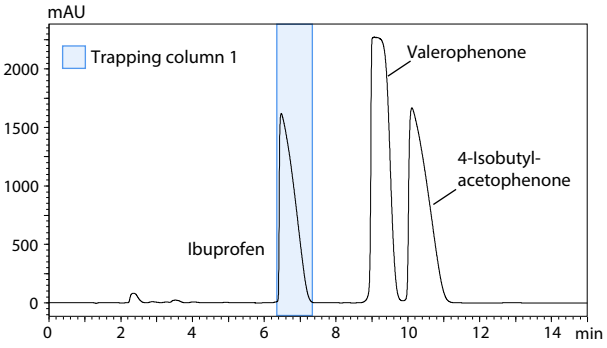


Fig. 9 Preparative Chromatogram of Ibuprofen (2000 µL Injection, Nexera UFPLC)

To confirm the reduction in drying time, we compared the time taken to dry the ibuprofen fractions collected from the standard preparative LC and Nexera UFPLC using a centrifugal concentrator. The comparison results are shown in Table 4. Drying of the standard preparative LC fraction required approximately 260 min, whereas the UFPLC fraction was dried in about 120 min. When the respective dried yields were checked, compounds derived from the mobile phase were found in the standard preparative fraction. Consequently, it could not be used as-is for the next step. In the case of the UFPLC fraction, just ibuprofen was confirmed after drying due to purification on the trapping column (Fig. 10).

Table 4 Comparison of Fractions of Standard Preparative LC and Nexera UFPLC

Method	Sample loading amount (mg)	Drying time (min)
Standard Preparative LC	20	260
Nexera UFPLC	100*1	120

*1 Introducing a Fraction of Ibuprofen into the Trapping Column was repeated 5 times

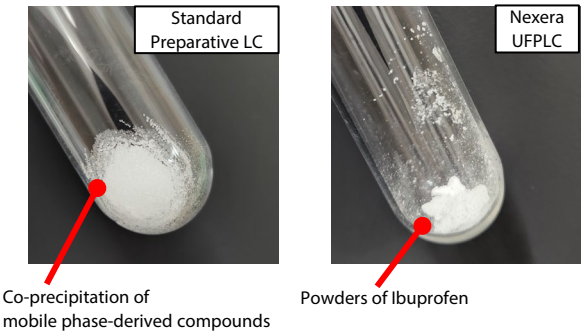


Fig. 10 Conditions of Ibuprofen Fractions after Drying

■ Conclusion

This article introduced an example of preparative purification of a mixed sample of the pharmaceutical ibuprofen and its analogues by using UFPLC. By concentrating, purifying, and recovering the fractionation with UFPLC, the drying time of the highly concentrated fraction of ibuprofen was reduced to about half compared with standard preparative LC.

Automating the concentration and purification processes online, enables labor and time savings in the preparative purification process.

Moreover, the Nexera UFPLC is extremely flexible and can be used for both standard preparative LC and UFPLC.

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